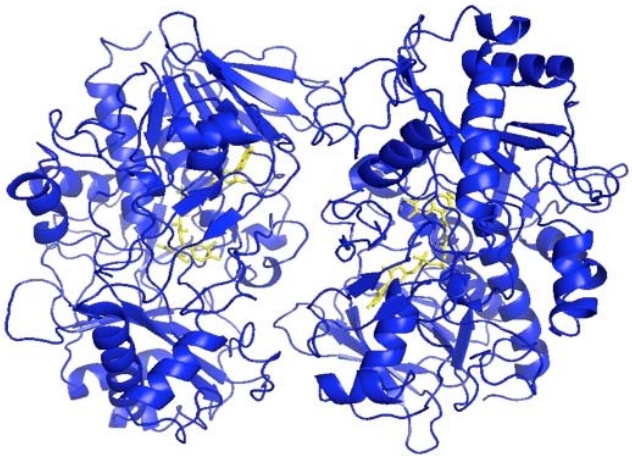
First Southeast Enzyme Conference



Saturday, April 10, 2010

Georgia State University
Atlanta, GA

Talks:
Library South, Room 102
103 Decatur Street

Lunch / Poster Session:
Natural Science Center, 5th Floor
50 Decatur Street

First Southeast Enzyme Conference

Saturday, April 10, 2010

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Southeast Enzyme Conference (SEC)

Meeting	Year	Program Chair	Site Chair	Site
I	2010	Giovanni Gadda	Will Lovett	GSU
II	2011			

Table of Contents:

Schedule:

Location: Library South Room 102

All Talks 20-25 min plus Q&A up to 30 min total!

7:45-8:30 Breakfast

8:30-8:40 Welcome and Introductory Remarks - **Giovanni Gadda**, Georgia State University, Atlanta

Session 1 - *Chair*, **Aimin Liu**, Georgia State University, Atlanta

8:40-9:10 Moonsung Choi, University of Mississippi Medical Center, Jackson
"Defining the role of the axial ligand of the type 1 copper site in amicyanin by replacement of Met"

9:10-9:40 Erika Milczek, Emory University, Atlanta
"Investigation of the 'gating' functions of the Ile199 and Tyr326 in human MAO B"

9:40-10:10 Anne-Frances Miller, University of Kentucky, Lexington
"Nitroreductase undergoes a dynamic transition at physiological temperature that affects substrate analog binding"

10:10-10:30 Coffee Break

Session 2 - *Chair*, **Liz Howell**, University of Tennessee, Knoxville

10:30-11:00 Michelle Oppenheimer, Virginia Tech, Blacksburg
"Identification of active site residues in Trypanosoma cruzi UDP-galactopyranose mutase"

11:00-11:30 Shalley N. Kudalkar, Auburn University, Auburn
"Impact of intersubunit interactions on catalytic versatility of catalase-peroxidase"

11:30-12:00 Irene T. Weber, Georgia State University, Atlanta
"Serendipitous reaction intermediates in crystal structures of enzymes"

Location: Natural Science Center 5th Floor

12:10-1:00 Lunchbox and Poster set-up

1:00-2:45 Poster Session
2:00 Coffee in the 5th Floor Lobby

Location: Library South Room 102

Session 3 - *Chair*, **Patrick A. Frantom**, University of Alabama, Tuscaloosa

3:00-3:30 Ellen W. Moomaw, Gainesville State College, Oakwood
"Expression, purification, and characterization of Ceriporiopsis subvermispora bicupin oxalate oxidase expressed by Pichia pastoris"

3:30-4:00 Robert S. Phillips, University of Georgia, Athens
"Hydrostatic pressure as a probe of enzyme conformational changes"

4:00-4:10 Concluding Remarks - **Giovanni Gadda**, Georgia State University, Atlanta

See you next year in Atlanta on April 16, 2011 for the **Second Southeast Enzyme Conference!**

Session 1:

Aimin Liu

Chair

Defining the role of the axial ligand of the type 1 copper site in amicyanin by replacement of Met.

Moonsung Choi[1], Narayanasami Sukumar[2], Aimin Liu[3] and Victor L. Davidson[1]

[1]Department of Biochemistry, The University of Mississippi Medical Center, Jackson,Mississippi 39216-4505, [2]NE-CAT and Department of Chemistry and Chemical Biology,Cornell University, Building 436E, Argonne National Laboratory, Argonne, IL, 60439, [3]Department of Chemistry, Georgia State University, Atlanta, GA, 30302

The effects on the structure and function of amicyanin of replacing the axial methionine ligand of the type 1 copper site have been characterized. Changing or eliminating the axial ligand of amicyanin alters the ligation geometry, but the absorption and EPR spectra of Met98 mutants are only slightly altered and still consistent with that of a type 1 site. The axial ligand strongly influences the specificity of the type 1 site for copper rather than zinc. Met98 mutants are not fully loaded with Cu^{2+} as isolated and must be unfolded to remove metals then refolded in the presence of Cu^{2+} to fully load the type 1 copper site. The M98Q and M98A mutations have little effect on E_m value suggesting the identity and position of the axial ligand alone does not significantly influence the E_m value of amicyanin. However loss of the axial ligand in the M98L amicyanin results in a more positive E_m value most likely due to the increased hydrophobicity around type 1 site. The most dramatic effect of the mutation is on the electron transfer (ET) reaction from reduced M98L amicyanin to cytochrome *c*-551i within the protein ET complex. The rate decreased 435-fold, which is much more than expected from the change in *Em* value. Examination of the temperature dependence of the ET rate (k_{ET}) revealed that the mutation caused a 13.6 fold decrease in the electronic coupling (H_{AB}) for the reaction. The most direct route of ET for this reaction is through the Met98 ligand. Inspection of the structures suggests that the major determinant of the large decrease in the experimentally determined values of H_{AB} and k_{ET} *is* the increased distance from the copper to the protein within the type 1 site of M98L amicyanin.

Investigation of the "Gating" Functions of Ile199 and Tyr326 in Human MAO B

Erika M. Milczek[1] and Dale E. Edmondson[2],

[1]Department of Chemistry and Biochemistry, Emory University, Atlanta, GA 30322
[2]Department of Biochemistry, Emory University, Atlanta, GA 30322

A major structural difference in human MAO A and MAO B is that MAO A has a monopartite substrate cavity of ~550Å^3 and MAO B is dipartite with a 290 Å^3 entrance cavity and a 400 Å^3 substrate cavity. Ile199 and Tyr326 function to separate these two cavities. The Ile199 side chain is in a required "open" conformation with large inhibitors and is "closed" with smaller inhibitors. To probe the function of these gating residues, Ile199Ala and Ile199Ala–Tyr326Ala mutant forms of MAO B were constructed, expressed in *Pichia pastoris*, and purified. Both mutants exhibit catalytic activities that are altered with increased K_m values. These mutant enzymes also exhibit lowered binding affinities relative to WT enzyme for small inhibitors. These observations demonstrate the Ile199 gate in MAO B plays an important role in its function. Preliminary structural data on the Ile199Ala MAO B mutant shows no alterations in active site geometries.

Acknowledgements
This work was supported by NIGMS 29433 and by an NIH predoctoral fellowship F31 NS063648-01.

Nitroreductase undergoes a dynamic transition at physiological temperature that affects substrate analog binding.

Anne-Frances Miller, Peng Zhang, Dongtao Cui and Mallory Mueller.
Department of Chemistry, University of Kentucky, Lexington KY 40504.

Nitroreductase (NR) catalyzes the reduction of nitrated aromatics, including TNT, via a ping-pong mechanism involving alternating two-electron oxidation and rereduction of a non-covalently bound FMN.[1] We find that the enzyme exhibits two completely different HN-HSQC NMR spectra of the backbone NH groups, depending on the temperature at which the enzyme is observed. The two different states interconvert slowly on the NMR timescale and both appear to be needed for optimal catalytic activity, which peaks at 25 °C, near the midpoint of the transition between states. One state appears to have a unique persistent structure based on the well-dispersed HSQC, whereas the other state appears subject to rapid conformational averaging. The conformationally-averaged state of the enzyme does not appear to be unfolded, nor does it appear to be a molten globule. We can also rule out dissociation of the NR dimer, as well as flavin dissociation, as causes of the transition. However, saturation transfer and magnetization transfer NMR experiments indicate that the conformationally-averaged state is more hydrated than the persistently-structured state, and we find that binding of substrate analogs favours the conformationally-averaged state. Thus, we propose that conformational plasticity mediated by 'lubricating' water molecules contributes to substrate binding in NR, and may underlie NR's capacity to bind such a very large range of substrates. NR's significance as a TNT reductase must be relatively recent. Promiscuity has been proposed as a resource for evolution of new enzymes. Protein dynamics provides a general molecular mechanism that could facilitate evolution of modified enzyme activities.

Acknowledgements: This work was supported in part by funding from the N.I.H. (GM-063921) and the ACS-PRF 44321-AC4.

1. Koder, R.L., Jr. and A.-F. Miller, *Steady state kinetic mechanism, stereospecificity, substrate and inhibitor specificity of Enterobacter cloacae nitroreductase.* Biochim. Biophys. Acta, 1998. **1387**: p. 394-405.

Session 2:

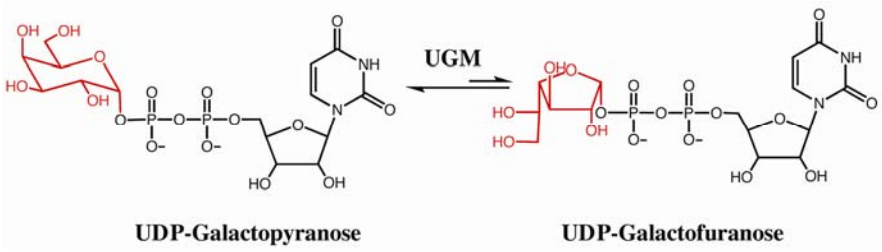
Liz Howell

Chair

Identification of active site residues in *Trypanosoma cruzi* UDP-galactopyranose mutase

Michelle Oppenheimer, Allison Blumer, Pablo Sobrado
Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061

UDP-Galactopyranose mutase (UGM) is a flavoprotein that catalyzes the conversion of UDP-galactopyranose (UDP-Galp) to UDP-galactofuranose (UDP-Galf) (Scheme 1). UGM serves as the sole biosynthetic source of galactofuranose, which is found in the cell wall of many bacteria, fungi, and parasites. The function of UGM has been shown to be essential in *M. tuberculosis* and it is important for virulence in *A. fumigatus* and in human parasites such as *L. major* and *T. cruzi*. Residues important for catalysis in the bacterial enzymes have been identified by x-ray structure, sequence alignments, and site-directed mutagenesis. In contrast, little is known about residues important for catalysis in the eukaryotic UGMs.



Scheme 1. Reaction catalyzed by UDP-galactopyranose mutase

We have used a combination of sequence alignments and structural models to identify residues that might be important for catalysis in *Trypanosoma cruzi* UGM (*Tc*UGM). We have mutated several of these residues and have determined the activity with UDP-Galp as substrate (Figure 1). Comparison of the effects in catalysis in the *Tc*UGM mutants to the corresponding mutants in the bacterial UGMs, suggests that the putative active site residues in *Tc*UGM play different roles in catalysis. We also show that *Tc*UGM functions as a monomer, while the bacterial enzymes are all homodimers. Structural differences are also demonstrated by the inability to measure binding of substrate or substrate analogs by FAD and tryptophan fluorescence in *Tc*UGM, unlike its prokaryotic counterpart *K. pneumonia* UGM (*Kp*UGM) where fluorescence studies were successfully used to determine a K_D of UDP and UDP-Galp.

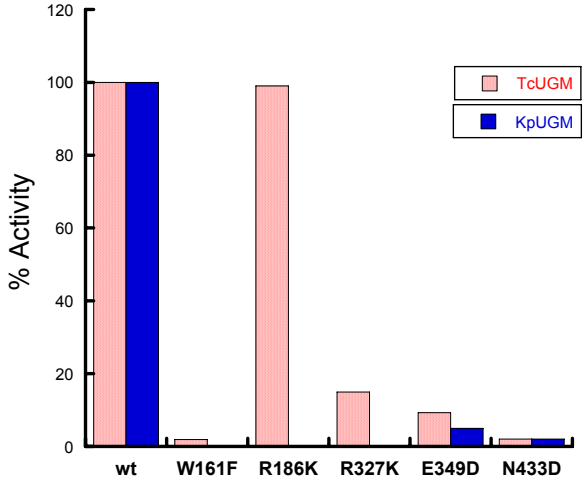


Figure 1. Relative activity of active site residues in *Tc*UGM and *Kp*UGM (*Kp*UGM values obtained from *Biochemistry*, 43, 23, 6723-6732.)

Impact of Intersubunit Interactions on Catalytic Versatility of Catalase-peroxidase

Shalley N. Kudalkar, Douglas C. Goodwin

Department of Chemistry and Biochemistry, Auburn University, Auburn, Al-36830

Catalase-peroxidases (KatGs) provide a useful model to investigate the important role of distant protein structures and their interactions on enzyme active site structure and catalysis. The active sites of KatG enzymes are nearly superimposable on those of classical heme peroxidases (e.g., cytochrome c peroxidase). However, typical heme peroxidases have very poor catalase activity while KatGs are able to efficiently facilitate H_2O_2 decomposition by both catalase and peroxidase mechanisms. The primary differences in structure between these two groups of enzymes are external to the active site. Two interhelical insertions and a second (C-terminal) domain are present in the bifunctional KatG enzymes but absent from typical peroxidases. Although quite distant from the active site (e.g., 30 – 35 Å), deletion of any of these structures have profound impact on KatG active site structure and catalytic function (e.g., selective loss of catalase activity). We applied site-directed and deletion mutagenesis to investigate the role of a remote interaction between two of these KatG-unique features. For variants produced by site-directed mutagenesis, modest modulation of active-site structure and function was determined by UV-Vis, EPR, and MCD spectroscopic techniques and steady-state kinetic analyses of catalase and peroxidase activities. Strikingly, variants produced by deletion mutagenesis showed the near complete loss of catalase activity but retention of peroxidase activity comparable to the wild-type enzyme. Concomitant with these shifts in activity was a change in heme coordination from predominantly hexacoordinate high-spin to predominantly pentacoordinate high-spin. These results highlight the importance of distant protein structures and their interactions for fine-tuning active site function, and in addition, point to factors beyond simple sequence differences which control active site function even some 30 Å removed from the active site.

Acknowledgement: This project was supported by National Science Foundation (MCB-0641614).

Serendipitous Reaction Intermediates in Crystal Structures of Enzymes

Irene T. Weber[1,2], Guoxing Fu[1], Chen-Hsiang Shen[1], Ping Liu[4], Andrey Kovalevsky[5], Yuan-Fang Wang[1] and Robert W. Harrison[3,1]

[1]Departments of Biology, [2]Chemistry, and [3]Computer Science, Molecular Basis of Disease Program, Georgia State University, Atlanta, GA.
[4]Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD.
[5]Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM.

High to atomic resolution (1.0-1.5 Å) crystal structures of enzymes can reveal unexpected reaction intermediates bound at the active site. Examples will be presented from our crystal structures of the bacterial carboxylesterase Est30, HIV-1 protease, and a novel bacterial dehydrogenase. The structure of Est30, a member of the serine hydrolase family, was discovered to include a tetrahedral reaction intermediate (Liu et al., 2004). HIV-1 protease is a retroviral aspartic protease and drug target for HIV/AIDS. The crystal structure in the absence of inhibitor was observed to include a gem-diol tetrahedral intermediate of a peptide (Kovalevsky et al., 2007). The detailed geometry of the intermediates will assist in the future design of mechanism based inhibitors. The intermediates are assumed to be quasi-stable when bound to the enzyme and presumably represent slow steps in the reaction. Therefore, crystallographic analysis at high resolution has helped to understand the catalytic mechanisms of the enzymes.

Liu, P., Wang, Y.-F., Ewis, H., Abdelal, A., Lu, C.D., Harrison, R.W., Weber, I.T. Covalent Reaction Intermediate Revealed in Crystal Structure of the *Geobacillus stearothermophilus* Carboxylesterase Est30. (2004) J. Mol. Biol. 342, 551-556.

Kovalevsky, A.Y., Chumanevich, A.A., Liu, F., Weber, I.T. Caught in the Act: 1.5 Å Resolution Crystal Structures of the HIV-1 Protease and the I54V Mutant Reveal a Tetrahedral Reaction Intermediate. (2007) Biochemistry, 46, 14854-14864.

Acknowledgements: This research was supported in part by the NIH grant GM062920, the Georgia Cancer Coalition, and the Georgia State University Molecular Basis of Disease Fellowships (G.F., P.L.).

Session 3:

Patrick A. Frantom

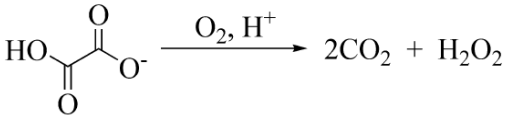
Chair

Expression, Purification, and Characterization of *Ceriporiopsis subvermispora* Bicupin Oxalate Oxidase Expressed By *Pichia Pastoris*

Patricia Moussatche[1], Christopher Brooks[2], Crystal Bruce[2], Daniel Sledge[2], Alexander Angerhofer[1], Nigel G. J. Richards[1], Ellen W. Moomaw[2*]

[1] Department of Chemistry, University of Florida, Gainesville, FL 32611-7200
[2] Department of Chemistry, Gainesville State College, 3820 Mundy Mill Road, Oakwood, GA 30566-3414
[*] Department of Chemistry and Biochemistry, Kennesaw State University, 1000 Chastain Road, Kennesaw, GA 30144-5588 after June 1, 2010.

Oxalate oxidase (E.C. 1.2.3.4) catalyzes the oxygen-dependent oxidation of oxalate to carbon dioxide in a reaction that is coupled with the formation of hydrogen peroxide (Scheme 1). Although there is currently no structural information available for oxalate oxidase from *Ceriporiopsis subvermispora* (CsOxOx), sequence data and homology modeling indicate that it is the first manganese-containing bicupin enzyme identified that catalyzes this reaction. The best characterized oxalate oxidases are from barley and wheat. These enzymes, also known as germins, contain a single cupin domain and are therefore classified as monocupins. Interestingly, CsOxOx shares greatest sequence homology with the bicupin microbial oxalate decarboxylases. The expression, purification, and characterization of CsOxOx will be presented in the poster.



Scheme 1: Reaction catalyzed by oxalate oxidase

Acknowledgements
This work was supported by a National Science Foundation Research in Undergraduate Institutions (RUI) Award (MCB-0919908) to EWM.

Hydrostatic Pressure As a Probe of Enzyme Conformational Changes

Robert S. Phillips[1], Reinhard Lange[2], Stephane Marchal[2], Edith W. Miles[3], and Peter McPhie[3].
[1]Departments of Chemistry and Biochemistry and Molecular Biology, University of Georgia, Athens, GA 30602
[2]INSERM, U710, F-34095 Montpellier, France
[3]NIADDK, NIH, Bethesda, MD 20892

Conformational changes are often proposed to occur during the catalytic cycle of enzymes. We have been using hydrostatic pressure perturbation as a probe for conformational changes in catalysis by pyridoxal-5'-phosphate (PLP) dependent enzymes. Tryptophan synthase is a PLP dependent multienzyme complex which exhibits an allosteric equilibrium between open (low activity) and closed (high activity) conformations, affected by monovalent cations, ligands, pH, solvents, temperature and hydrostatic pressure. Increasing hydrostatic pressure up to 2000 bar shifts the Trp synthase-Serine aldimine-aminoacrylate equilbrium from the closed to open form, with concomitant changes in the cofactor visible absorption and fluorescence spectra. These spectral changes are fully reversible upon decompression. The open conformation is favored at high pressure due to the negative volume change of solvation. The effects of various monovalent cations and ligands on the conformational equilibrium were quantitatively determined by hydrostatic pressure perturbation. Bidirectional pressure-jump experiments demonstrated that there is microheterogeneity of the open and closed conformations in Trp synthase. In another enzyme, tryptophan indole-lyase, increasing pressure shifts the spectrum of the PLP in the resting enzyme from the 420 nm ketoenamine to the 338 nm form, suggesting that there is a conformational change associated with the different species. Pressure also affects the spectrum of the PLP in the aspartate β-decarboxylase-succinate complex, shifting it from the protonated 420 nm form to the unprotonated 360 nm form. This suggests that the binding of ligands to aspartate β-decarboxylase triggers a conformational change which lowers the pK_a of the Lys-PLP Schiff's base, activating it to form the external aldimine. Thus, hydrostatic pressure is a useful technique to study and quantify conformational equilibria in enzymes.

Abstracts for Poster Presentations:

The Poster Session will be held on the 5th floor of the Natural Science Center, starting at 1:00 and ending at 2:45.

With two exceptions, the abstracts are arranged alphabetically by the last name of the first person in the list of authors. The following abstracts are also in this order.

1. Blum, Janna K.
2. Burns, J. Nicole
3. Chen, Yan
4. Chenna, Balachandra
5. Childers, W. Seth
6. Choi, Moonsung
7. Chou, Han-Ting
8. Clark, Lorraine
9. Combs, Steven
10. Cui, Dongtao
11. Dalton, Amy
12. Davis, Ian
13. Dornevil, Kednerlin
14. Feng, You
15. Finnegan, Steffan
16. Francis, Kevin
17. Frantom, Patrick A.
18. Fu, Guoxing
19. Fu, Rong
20. Gannavaram, Swathi
21. Gault, Jordan
22. Grubbs, Jordan
23. Guo, Ran
24. Hall, Mélanie
25. Hoang, Linh
26. Horton, John R.
27. Hsieh, Steve A.
28. Huo, Lu
29. Kudalkar, Shalley N.
30. Rungsrisuriyachai, Kunchala
31. Lavender, Kevin
32. Law, Lydia
33. Lee, Sangbae
34. Lutz, Stefan
35. Mei, Xiaonan
36. Meyer, Megan E.
37. Milczek, Erika M.
38. Miller, Anne-Frances
39. Mishra, Subrata H.
40. Moussatche, Patricia
41. Naik, Sunil G.
42. Njeri, Catherine
43. Norris, Adrianne L.
44. Oppenheimer, Michelle
45. Ortillo, Danilo O.
46. Parks, Samantha T.
47. Pennati, Andrea
48. Phillips, Robert S.
49. Pulley, Christopher
50. Qi, Jun
51. Robbins, John M.
52. Robinson, Reeder
53. Saylor, Benjamin T.
54. Shin, Sooim
55. Sobrado, Pablo
56. Abu Tarboush, Nafez
57. Thompson, Lawrence C.
58. Timson, Mary
59. Wang, Jin
60. Wang, Juxian
61. Wang, Ting
62. Weber, Irene T.
63. Wei, Xiaoxi
64. Woldman, Yakov
65. Wu, Jiang
66. Wu, Jin
67. Xiong, Jingyuan
68. Yang, Chao
69. Yang, Yutao
70. Yuan, Hongling
71. Zhang, Ying

Improved Thermostabilty of an Amino Ester Hydrolase for use in the Enzymatic Synthesis of Semi-synthetic β-lactam Antibiotics

Janna K. Blum, Michael D. Ricketts, and Andreas S. Bommarius

Amino ester hydrolases (AEH) can be used in the enzymatic synthesis of semi-synthetic antibiotics such as ampicillin and amoxicillin. In comparison to the industrial used enzyme for antibiotic synthesis, penicillin G acylases, the AEH from *X. campestris pv. campestris* has a low thermostability with an optimal temperature of only 25°C and a T_{50} (30 min) value, the incubation temperature at which one-half of the initial activity is retained after 30 min, of only 27°C, similar to the other AEHs. In order to improve the industrial relevance of these enzymes the thermostability needs improvement. Additionally, improved thermostability has been linked to improved evolubility, which is of critical importance since our project goal is to alter the substrate specificity of our enzyme. In order to render our new protein useful for processing, thermostability has to be improved to at least about 35°C. We tackle this issue by combining two novel techniques: i) a consensus model of eight homologous amino acid sequences of active enzymes was generated along with ii) analysis of the B factors from the available crystal structures of the known AEHs from *Xanthomonas citri* and *Acetobacter turbidans*. Based on this analysis we have generated several mutants with improved thermostabilty by a T_{50}(30 min) 5^{o}C-10^{o}C. Additionally the A275P mutation improved the WT activity by 1.4-fold but did not effect the thermostability.

Stability Studies of Glaucoma-causing Myocilin Mutants and Rescue by Chemical Chaperones

J. Nicole Burns[1], Susan D. Orwig[1], Julia L. Harris[1], J. Derrick Watkins[1], Douglas Vollrath[2], and Raquel L. Lieberman[1]

[1]School of Chemistry & Biochemistry, Georgia Institute of Technology, 901 Atlantic Drive NW, Atlanta, GA 30332

[2]Departments of Genetics of Ophthalmology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305

Glaucoma is a major contributor of blindness worldwide, affecting about 70 to 80 million people. Myocilin, expressed in the trabecular meshwork of the eye, has been linked to one major form of inherited primary open-angle glaucoma (POAG). Missense mutations in the olfactomedin (OLF) domain of myocilin are correlated with inherited POAG, increasing the intraocular pressure of the eye. The biological function of myocilin is unknown, but mutant myocilin exhibits a gain-of-function mechanism, aggregating within the endoplasmic reticulum, causing cell stress and eventually apoptosis. We have expressed and purified the wild-type OLF domain and four disease-causing mutants. We developed a facile thermal stability assay using differential scanning fluorimetry, which follows the unfolding of a protein through the fluorescence of a dye. We have determined melting temperatures for the wild-type and for each of the mutants, as well as the ability of chemical chaperones to stabilize the mutant protein. The long-term goal is to find tailored small molecules that could increase stability of mutant myocilin and enable their secretion from the trabecular meshwork cell, which will help delay the onset of glaucoma.

Isolation, Biochemical and Spectroscopic Characterization of Cysteamine Dioxygenase

Yan Chen,[†] Sunil G. Naik, [†] Kednerlin Dornevil,[†] William Morrios, Martha H. Stipanuk,[‡] and Aimin Liu[†]

[†]Department of Chemistry, Georgia State University, Atlanta, GA 30303

[‡] Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853

Cysteamine dioxygenase (ADO) is a non-heme iron-dependent enzyme responsible for the oxidation of cysteamine with molecular oxygen to form hypotaurine. ADO is a homologue enzyme of cysteine dioxygenase (CDO) which employs a non-heme Fe (II) ion to activate O2 and insert the two oxygen atoms into cysteine to yield cysteinesulfinate. ADO and CDO, the only known thio dioxygenase enzymes, belong to the cupin superfamily. The characterization of as-isolated ADO by EPR and Mössbauer spectroscopy indicates that the majority iron in the protein is ferrous with a high coordination number (five or six), which is different from the four coordinated Fe(II) center found in CDO. EPR and Mössbauer spectroscopic study of substrate binding ADO is being conducted to better understand the iron center in this enzyme.

ACKNOWLEDGEMENT

This work is supported by the Department of Chemistry of GSU, National Institutes of Health grant R01DK056649 (to MHS and AL), NSF grant MCB 843537 (AL), the Molecular Biology of Diseases (MBD) program of Georgia State University (KD), the Georgia Cancer Coalition (AL), and the Department of Chemistry, Georgia State University (YC & WM). We thank Professor Boi Hanh (Vincent) Huynh for access to the Mössbauer spectrometer.

Sortase A: A novel therapeutic target for *Staphylococcus aureus*

Balachandra Chenna[1], Aaron L. Lucius[1] and Sthanam V. L. Narayana[2] and Sadanandan E. Velu*[1]

[1]Department of Chemistry, The University of Alabama at Birmingham, 901 14th Street S, Birmingham, AL 35294 – 1240.
[2]Center for Biophysical Sciences and Engineering, The University of Alabama at Birmingham, 1530 3rd Ave S, Birmingham, AL 35294-4400.

Staphylococci are responsible for more than a million hospital-acquired bacterial infections every year. There has been a recent steep increase in multi-drug resistance found in bacteria. Hospital strains of *Staphylococcus aureus* (*S. aureus*) are usually resistant to a variety of different antibiotics. A few strains are resistant to all clinically useful antibiotics except vancomycin, and vancomycin-resistant strains are increasingly-reported. Considering this, as well as the potential of 'drug resistant bacteria' to be used in biowarfare and bioterrorism, development of new antibacterial agents is a high priority. In an effort to identify new antibacterial agents against *S. aureus* that act through novel mechanisms we have pursued inhibitors of the bacterial surface enzyme *S. aureus* Sortase A (SrtA). SrtA plays a crucial role in pathogenesis of Gram-positive bacteria by modulating the ability of bacterium to adhere to host tissue *via* a covalent anchoring of surface proteins to cell wall peptidoglycan. SrtA cleaves the amide bond between the threonine and glycine of the LPXTG motif of surface proteins during the surface anchoring. Genetic knockout experiments have demonstrated that mutants of Gram-positive bacteria lacking SrtA fail to display surface proteins and are defective in establishing infection in animal models. Thus, inhibitors of SrtA are promising candidates for treatment and prevention of Gram-positive bacterial infections. Our collaborators have recently determined the crystal structures of recombinant *S. aureus* $SrtA_{\Delta 59}$, a fully active variant of *S. aureus* SrtA, and its complex with its substrate LPETG. Utilizing the crystal structure of *S. aureus* $SrtA_{\Delta 59}$ we have initiated structure based inhibitor design studies. In our preliminary studies, we have identified low micromolar inhibitors of *S. aureus* SrtA by using virtual screening of commercial compound libraries against the *S. aureus* $SrtA_{\Delta 59}$ active site using the FlexX software package and testing the activity *in vitro* using a fluorescence resonance energy transfer (FRET) activity assay. Lead identification and Structure Activity Relationship studies will be presented.

Amyloid: Combing the Physiochemical Properties of Membranes and Proteins

W. Seth Childers[1,2], Anil K. Mehta[1,2], Yan Liang[1,2], Thinh Bui[1,2], and David G. Lynn[1,2]
[1]Center for Fundamental and Applied Molecular Evolution
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Within the vast protein sequence space, there maybe only a small fraction that fold into functional enzymes seen today[1] . In contrast, an increasing population of protein sequence space appear to be able to aggregate as β-sheet rich amyloid assemblies[2]. From the neurodegenerative plaques of Alzheimer's disease to the organizing medium of bacterial biofilms the precise physiochemical nature of amyloid remains unclear. To better understand the material properties and catalytic capacity of amyloid, we have performed a detailed structural characterization of one of the shortest amyloid forming peptides, KLVFFAE, derived from Alzheimer's Disease Aβ peptide. Recent structural characterization by solid-state NMR indicates that peptides organize like a bilayer membrane and respond dynamically to their chemical environment, undergoing structural phase transitions.

This bilayer organization creates an amphiphilic solvent exposed surface composed of organized arrays of positively charged lysines and hydrophobic leucines with the capacity to template the organization of the histochemical dye Congo Red into linear arrays[3]. Here we demonstrate that amyloid's surface can interact with a wide range of co-factors and characterize the unique chemical environment amyloid presents for catalysis. In order to compare with known catalysts (natural enzymes, de novo designed enzymes, catalytic antibodies, etc), we examined the ability KLVFFAL amyloid surface to catalyze the hydrolysis of para-nitrophenol acetate. KLVFFAL exhibited a kcat/Km of 0.013 M-1 s-1, much less efficient that Nature's best enzymes but comparable to some de novo design enzymes. Secondly, we have also discovered that the amyloid peptide surface presents a desolvating environment capable of driving imine formation from an amine and aldehyde. Using these examples, amongst our latest findings, we will structurally and functionally compare amyloids to modern enzymes and phospholipid membranes.

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Acknowledgements

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Defining the role of the axial ligand of the type 1 copper site in amicyanin by replacement of Met.

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The effects on the structure and function of amicyanin of replacing the axial methionine ligand of the type 1 copper site have been characterized. Changing or eliminating the axial ligand of amicyanin alters the ligation geometry, but the absorption and EPR spectra of Met98 mutants are only slightly altered and still consistent with that of a type 1 site. The axial ligand strongly influences the specificity of the type 1 site for copper rather than zinc. Met98 mutants are not fully loaded with Cu^{2+} as isolated and must be unfolded to remove metals then refolded in the presence of Cu^{2+} to fully load the type 1 copper site. The M98Q and M98A mutations have little effect on E_m value suggesting the identity and position of the axial ligand alone does not significantly influence the E_m value of amicyanin. However loss of the axial ligand in the M98L amicyanin results in a more positive E_m value most likely due to the increased hydrophobicity around type 1 site. The most dramatic effect of the mutation is on the electron transfer (ET) reaction from reduced M98L amicyanin to cytochrome *c*-551i within the protein ET complex. The rate decreased 435-fold, which is much more than expected from the change in *Em* value. Examination of the temperature dependence of the ET rate (k_{ET}) revealed that the mutation caused a 13.6 fold decrease in the electronic coupling (H_{AB}) for the reaction. The most direct route of ET for this reaction is through the Met98 ligand. Inspection of the structures suggests that the major determinant of the large decrease in the experimentally determined values of H_{AB} and k_{ET} *is* the increased distance from the copper to the protein within the type 1 site of M98L amicyanin.

L-Lysine Catabolism is Controlled by L-Arginine and ArgR in *Pseudomonas aeruginosa* PAO1

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In comparison to other pseudomonads, *P. aeruginosa* can only utilize L-lysine as poor nitrogen source. In this study, the *ldcA* (*l*ysine *de*carboxylase A; E.C. 4.1.1.18) gene, previously identified as a member of the ArgR regulon of L-arginine metabolism, was found essential for lysine catabolism in this organism. LdcA was purified to homogeneity from a recombinant strain of *E. coli*, and the results of enzyme characterization revealed that this pyridoxal-5-phosphate dependent decarboxylase takes L-lysine, but not L-arginine, as substrate. At optimal pH 8.5, cooperative substrate activation by L-lysine was depicted from kinetics studies, with calculated K_m and V_{max} values of 0.73 mM and 2.2 μmole/mg/min, respectively. Contrarily, the *ldcA* promoter was induced by exogenous L-arginine but not L-lysine in the wildtype strain PAO1, and binding of ArgR to this promoter region was demonstrated by electromobility shift assays. Growth on L-lysine was enhanced in a mutant devoid of the main arginine catabolic pathway, resulting in a higher basal level of intracellular L-arginine pool and hence an elevated ArgR-responsive regulon including *ldcA*. Growth on L-lysine can also be enhanced when the *aruH* gene encoding an arginine/lysine:pyruvate transaminase was expressed constitutively from plasmids; however, no growth of the ldcA mutant on L-lysine suggests minor role of this transaminase in lysine catabolism. In summary, this study reveals a tight connection of lysine catabolism to arginine regulatory network, and the lack of lysine-responsive control on ldcA provides an explanation of L-lysine as poor nutrient for *P. aeruginosa*.

Biosynthesis of azasugars

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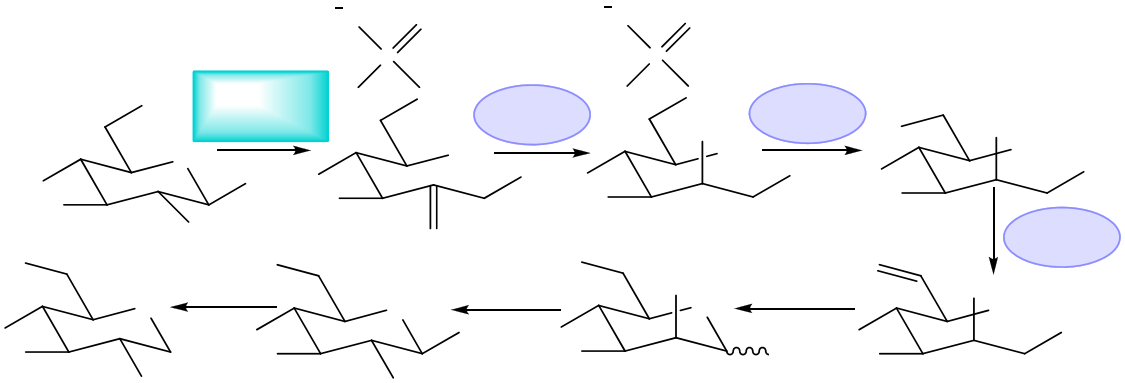
The azasugar 1-deoxynojirimycin (DNJ) is a glucose derivative in which the ring oxygen is substituted with a nitrogen atom. Natural producers of DNJ include microbes, such as *Bacilli* and *Streptomyces* species, as well as various plants, such as *Morus alba* (Mulberry) and the dayflower *Commelina communis*. As potent inhibitors of glycosidases, DNJ and analogs have been used in Asian folk medicine for centuries and nowadays function as important medicinal compounds for the treatment of diabetes and Gaucher's disease. Labeling studies in the early 1990s by Hardick et al. established that glucose is a precursor of DNJ in *Bacilli* and *Streptomyces* species and have led to a proposed biosynthetic pathway. No enzymes responsible for azasugar biosyntheses have yet been identified.

The genome of the known DNJ producer *Bacillus amyloliquefaciens* was searched for gene products with transaminase activity, and potential recognition of carbohydrate-like functionality. Three candidate genes were identified referred to as GabT1, Yktc1, and GutB1, which exhibit putative transaminase, fructose phosphatase and dehydrogenase functions, respectively. Taking this observation and the previous labeling studies into consideration, a possible biosynthetic pathway of DNJ in *Bacillus amyloliquefaciens* could proceed via the following scheme:



We present kinetic and spectroscopic data confirming loss of DNJ production in a GabT1 *B. amyloliquefaciens* knockout, and describe progress in the cloning and expression of putative DNJ biosynthesis enzymes.

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Characterization of a computationally designed sequence and fold symmetric TIM barrel enzyme.

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The $(\beta\alpha)_8$ barrel proteins are one of the most common folds seen in nature. Their structural symmetry is believed to have arisen due to gene duplication events. Gene duplication events are responsible for the propagation and diversity of enzymes that perform biological functions however, an enzyme that is symmetric in both fold and in sequence has not yet been identified. HisF, a $(\beta\alpha)_8$ barrel enzyme in the histidine biosynthesis pathway, has an internal two-fold symmetry. We present a computationally designed variant of HisF that is two-fold symmetric in both fold and in sequence that represents the "missing link" in enzyme evolution. The catalytic activity, stability, and crystal structure are presented in this study.

^{15}N Solid-State NMR Detection of Flavin Perturbation by H-bonding in Models and Enzyme Active Sites

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Massey and Hemmerich proposed that the different reactivities displayed by different flavoenzymes could be achieved as a result of dominance of different flavin ring resonance structures in different binding sites[1]. Thus, the FMN cofactor would engage in different reactions when it had different electronic structures. To test this proposal and understand how different protein sites could produce different flavin electronic structures, we are developing solid-state NMR as a means of characterizing the electronic state of the flavin ring, via the ^{15}N chemical shift tensors of the ring N atoms. These provide information on the frontier orbitals[2]. We propose that the ^{15}N chemical shift tensors of flavins engaged in different hydrogen bonds will differ from one another. Tetraphenylacetyl riboflavin (TPARF) is soluble in benzene to over 250 mM, so, this flavin alone and in complexes with binding partners provides a system for studying the effects of formation of specific hydrogen bonds[3]. For N5, the redox-active N atom, one of the chemical shift principle values (CSPVs) changed 12 ppm upon formation of a hydrogen bonded complex, and the results could be replicated computationally. Thus our DFT-derived frontier orbitals are validated by spectroscopy and can be used to understand reactivity. Indeed, our calculations indicate that the electron density in the diazabutadiene system diminishes upon H-bond complex formation, consistent with the observed 100 mV increase in reduction midpoint potential. Thus, the current studies of TPARF and its complexes provide a useful baseline for further SSNMR studies aimed at understanding flavin reactivity in enzymes.

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"One Catalytic Reaction: Why Two Metal Centers in 3-Hyroxyanthranilate 3,4-Dioxygenase?"

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Abstract:

3-Hydroxyanthranilate 3,4-Dioxygenase, also known as HAD, is an extradiol dioxygenase that is part of tryptophan catabolism, otherwise known as the kynurenine pathway. This pathway is of significance since it is the metabolic route from tryptophan to quinolinate, a neurotoxin that is linked to inflammatory diseases such as Huntington's disease, AIDS-related dementia, etc. HAD has two metal centers, one being the catalytic site, and another, which is an iron-sulfur FeS_4 class that is close to the solvent surface. The significance of the rubidoxin-like FeS_4 center is yet unknown. Relative activity will be determined by titrating HAD with divalent metals, such as Zn^{+2} and Cu^{+2}. Mossbauer spectroscopy and redox potentials will also be measured to determine how the iron is distributed among the two metal centers.

An Enzyme Trio in the Kynurenine Pathway of Tryptophan Catabolism Prevents Neurotoxin Formation

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In mammals, there are two separate pathways by which tryptophan can be metabolized, into serotonin and through the Kynurenine pathway respectively. Only a small portion of ingested tryptophan is converted to serotonin and then melatonin. Over ninety-five percent is catabolized through the Kynurenine pathway into a number of neuroactive molecules such as kynurenic acid, quinolinic acid, a NAD+ precursor, picolinic acid, or to acetyl-CoA which can enter glycolysis. The Kynurenine pathway is of interest to study because elevation all of the aforementioned neuroactive compounds is associated with several disease states: Alzheimer's disease, anxiety, depression, epilepsy, AIDS dementia, and Huntington's disease. This research will focus on the enzymatic activity of 2-Aminomuconic semialdehyde dehydrogenase, AMSH, and how it interacts with 3-Hydroxyanthranilate 3,4-dioxygenase, HAD, and 2-Amino-3-carboxymuconic semialdehyde decarboxylase, ACMSD.

Trapping and Characterizing Reactive Intermediates in Tryptophan 2,3-Dioxygenase

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A**BSTRACT**

Tryptophan 2,3-dioxygenase (TDO) is a ferrous heme enzyme that catalyzes the first and rate-limiting step of the kynurenine metabolic pathway. Here we report that the ferric form of TDO possesses two previously unknown catalytic activities with hydrogen peroxide as a substrate. In the presence of L-tryptophan, ferric TDO utilizes peroxide as the oxygen donor and produces the dioxygenase product N-formylkynurenine. In the absence of L-tryptophan, TDO displays a catalase activity and produces O2 from peroxide. A thorough mass spectrometry analysis employing ESI-MS, LC-MS, and ESI-MS-MS methods shows that both oxygen atoms inserted in N-formylkynurenine are derived from H2O2 and one of the inserted oxygen atoms is solvent exchangeable. These observations are further elaborated by the isotope-labeling experiments using ^{18}O-enriched H_2O_2 and water, respectively, in the enzyme-mediated reaction. Notably, a monooxygenated product of L-tryptophan with m/z of 221 in the 16O experiments, or 223 with ^{18}O, is also observed. ESI-MS-MS experiments confirm that this monooxygnated product is not a fragment of the substrate or N-formylkynurenine. Collectively, these results support a catalytic mechanism for the peroxide-dependent sequential addition of two oxygen atoms to L-tryptophan, as opposed to a one-step concerted dioxygenation reaction. These findings are pertinent to the chemical properties, particularly the reactivity of the ferric heme Fe in TDO. They also have important implications for the biological role of this enzyme under oxidizing environments.

Inhibition of Protein Arginine Methyltransferase 1

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Protein arginine methyltransferases (PRMTs) catalyze the transfer of methyl groups from S-adenosyl-L-methionine (AdoMet, SAM) to the guanidino group of arginines in histone and nonhistone protein substrates. Thus far, eleven human PRMT members have been identified at the proteomic level and categorized into two major types, type I and type II, according to substrate and product specificity. In the PRMT family, PRMT1 is found as the predominant type I PRMT in mammalian cells, accounting for 85% of cellular PRMT activity. PRMT1 is a key player in transcriptional control and is a potential target for therapeutic treatment of cancer. Herein, we present the recent work in our laboratory about PRMT1 study, including protein expression, enzymatic catalysis, chemical probe design, and inhibitor development. This work is significant for elucidating the molecular basis of protein arginine methylation and provides useful chemical tools for PRMT research. The small molecule inhibitors may be potential lead compounds for further drug development to target PRMT deregulation in tumors.

The nature of the kinetically slow equilibrium between two conformers of the Val464Ala variant choline oxidase

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The importance of residues in the active site that do not directly participate in catalysis is often overlooked in terms of studies trying to pinpoint key residues for enzymatic turnover, as they would likely only exert a minimal effect on the overall catalysis. However, studies in which valine 464 in the active site of choline oxidase has been replaced with either alanine or threonine have revealed that this hydrophobic residue lining the active site cavity close to the N(5) atom of the flavin plays several important roles. The replacement of Val464 with threonine or alanine in choline oxidase resulted in a significantly lowered oxygen reactivity and establishment of a kinetically slow equilibrium between a catalytically competent and incompetent form of enzyme. It is the nature of this equilibrium that is the subject of investigation in the present study. Rapid kinetic approaches revealed that the interconversion of the two forms of enzyme is independent of the isotopic composition of the substrate and that a protonated group is needed for maximum rate of interconversion.

A Novel Function of Nitronate Monooxygenase: Detoxification of the Plant Toxin Propionate-3-Nitronate

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Nitronate monooxygenase (NMO; E.C. 1.13.12.16) is an FMN dependent enzyme that catalyzes the oxidative denitrification of akyl nitronates to their corresponding carbonyl compounds and nitrite. The enzyme has been most extensively characterized from the yeasts *Neurospora crassa* and *Hansenula mrakii* both of which have been shown to effectively utilize primary alkyl nitronates as substrate. Given that these compounds are not prevalent in the natural environments of the yeasts and that the K_D values for nitronate binding are large ($\geq$ 5 mM), the oxidation of primary nitronates by NMO is likely non-physiological. The current study describes a novel and likely physiological activity of the enzymes from *N. crassa* and *H. mrakii*, as well as a biochemical characterization of a recently discovered NMO from *Pseudomonas aeruginosa.* Each enzyme effectively oxidizes propionate-3-nitronate (P3Nate), which is a potent and irreversible inhibitor of succinate dehydrogenase produced by many plants. The kinetic parameters of each NMO with P3Nate as substrate at pH 7.4 and 30 ^{o}C were significantly larger than those previously determined with ethylnitronate. Turnover of *P. aeruginosa* and *H. mrakii* NMO occurs without the release of hydrogen peroxide or superoxide, as evident from the lack of effects of catalase or superoxide dismutase on rates of oxygen consumption. Growth curves of *E. coli* measured in the either the absence or presence of the nitronate and enzyme suggest a physiological role for P3Nate oxidation. The nitronate prevents the growth of *E. coli*, but these bacteriostatic effects are overcome when cultures are supplied NMO either through induction of the recombinant gene with IPTG or through addition of the purified enzyme to the cultures. The results presented herein indicate that P3Nate is the physiological substrate of NMO.

Allosteric Regulation of α-Isopropylmalate Synthase from *Mycobacterium tuberculosis*.

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Understanding the mechanism of allosteric regulation of proteins, where binding of an effector molecule influences the environment at a distal site, has been a central pursuit in the field of biochemistry for over 40 years. As the role of enzyme dynamics in catalysis has received a great deal of attention over the past ten years, it is not surprising that altered dynamics may play a role in allosteric regulation as well. Recently, we have reported one of the first complete maps of an inter-domain allosteric network described by changes in local dynamics for the enzyme α-isopropylmalate synthase from *Mycobacterium tuberculosis* (MtIPMS) utilizing backbone amide hydrogen/deuterium exchange (Frantom, et al. *Biochemistry* **2009**, *48*, 7457). MtIPMS catalyzes the first step in the biosynthesis of leucine. As such it is subject to *V*-type feedback inhibition by leucine. As seen in Figure 1, the enzyme is a homodimer with each monomer consisting of three domains, an *N*-terminal catalytic domain (red/blue), a flexible linker domain (orange/cyan), and a *C*-terminal regulatory domain (yellow/green). Kinetically, leucine has been shown to act as a slow-onset inhibitor (K_i* = 2.3 μM) despite binding over 50 Å from the active site of the enzyme. The hydrogen/deuterium exchange results provided a map for the inhibition of the enzyme by leucine consistent with a global change in dynamics in the regulatory domain coupled to the perturbation of a single peptide in the active site of the catalytic domain.

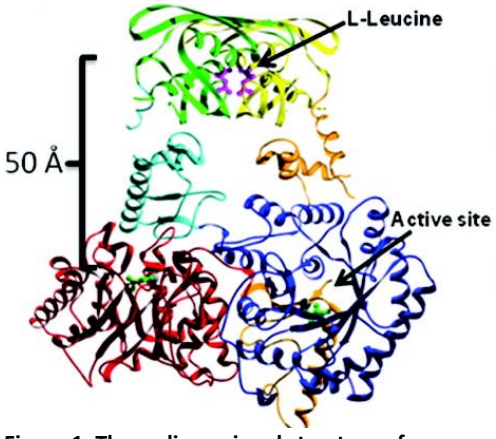

Figure 1. Three-dimensional structure of MtIPMS. The location of the active site in the catalytic domain and the binding site for leucine in the regulatory domain are indicated.

Here, we describe the inhibition kinetics of MtIPMS by several alternate amino acids. The alternate inhibitors also act as *V*-type inhibitors with inhibition constants ranging from 200 μM to several millimolar, suggesting that they are all capable of promoting an inhibited conformation of the enzyme. Surprisingly, none of the alternate inhibitors display slow-onset kinetics suggesting either that there is a change in the microscopic rate constants that obscures the detection of the slow-onset mechanism or that these inhibitors act through a different physical mechanism relative to leucine inhibition. The results can also tell us something about the plasticity of the inhibitor binding site. We can conclude that substitution at the β-carbon is not well tolerated due to steric conflicts in the inhibitor binding site. However, additions to the length of the side chain can be accommodated by the binding pocket. We also describe initial experiments aimed at constructing an MtIPMS variant suitable for labeling with site-directed spin labels. These spin labels contain a stable unpaired electron and their motions can be observed utilizing electron paramagnetic resonance spectroscopy. The results from these experiments will help us to quantify the changes in backbone dynamics upon leucine binding.

Structural basis for substrate specificity of executioner caspases

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Caspase-3, -6 and -7 are executioner caspases and cleave many proteins at specific sites to induce apoptosis, which is disrupted in many diseases including cancer, heart disease, and neurodegenerative diseases. Their recognition of the P5 position in substrates has been investigated by kinetics, modeling and crystallography. Caspase-3 and -6 recognize P5 in pentapeptides as shown by enzyme activity data and interactions observed in the crystal structure of caspase-3/LDESD and in a model for caspase-6/LDESD. In caspase-3 the P5 main-chain was anchored by interactions with Ser209 in loop-3 and the P5 Leu side-chain interacted with Phe250 and Phe252 in loop-4 consistent with 50% increased hydrolysis of LDEVD relative to DEVD. Caspase-6 formed similar interactions and showed a preference for polar P5 in QDEVD likely due to interactions with polar Lys265 and hydrophobic Phe263 in loop-4. Caspase-7 exhibited no preference for P5 residue in agreement with the absence of P5 interactions in the caspase-7/LDESD crystal structure. Initiator caspase-8, with Pro in the P5-anchoring position and no loop-4, had only 20% activity on tested pentapeptides relative to DEVD. Therefore, caspases-3 and -6 bind P5 using critical loop-3 anchoring Ser/Thr and loop-4 side-chain interactions, while caspase-7 and -8 lack the P5-binding residues. These discoveries will be valuable for the future design of novel inhibitors that are more specific for target caspase members. The distinct preferences observed for P5 residue in substrates will help define the particular cellular signaling pathways associated with each executioner caspase.

A Catalytic Shunt Pathway is Operative in Heme-Based Tryptophan Dioxygenase

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Tryptophan 2,3-dioxygenase (TDO) is a ferrous heme enzyme that catalyzes the first and rate-limiting step of the kynurenine metabolic pathway of L-Tryptophan. Here we report that the ferric form of TDO possesses two previously unknown catalytic activities with hydrogen peroxide as a substrate. In the presence of L-tryptophan, ferric TDO utilizes peroxide as the oxygen donor and produces the dioxygenated product *N*-formylkynurenine. In the absence of L-tryptophan, TDO displays a catalase-like activity and produces O_2 from peroxide. A thorough mass spectrometry analysis employing ESI-MS, LC-MS, and ESI-MS-MS methods shows that both oxygen atoms inserted in *N*-formylkynurenine are derived from H_2O_2 and one of the inserted oxygen atoms is solvent exchangeable. These observations are further elaborated by the isotope-labeling experiments using ^{18}O-enriched H_2O_2 and water, respectively, in the enzyme-mediated reaction. Notably, a monooxygenated product of L-tryptophan with *m*/*z* of 221 in the ^{16}O experiments, or 223 with ^{18}O, is also observed. ESI-MS-MS experiments confirm that this monooxygnated product is not a fragment of the substrate or *N*-formylkynurenine. An Fe(IV)=O species and a protein-bound free radical intermediate are observed and characterized by EPR and Mössbauer spectroscopy. These findings are pertinent to the chemical properties, particularly the reactivity of the ferric heme Fe in TDO.

Kinetic Isotope effect of CHO-wt from *Arthrobacter globiformis*

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Choline oxidase (CHO), a homodimer of 120 kDa, is a prototype for alcohol oxidation in flavin dependent enzymes. Each subunit binds one molecule of FAD covalently. CHO catalyzes the two-step oxidation of choline to glycine betaine with betaine aldehyde as intermediate. It has been shown that accumulation of glycine betaine in pathogens and plants increase their resistance to stress in hyperosmotic conditions. Therefore, characterization of CHO enables to develop therapeutic agents that can prevent the synthesis of glycine betaine and thus making the pathogens susceptible to medication or sensitive to the immune system.

The $^{D}(k_{cat}/K_{m})$ and $^{D}k_{cat}$ of CHO were reported to be 10.7 ± 2.6 and 7.3 ± 1.0 respectively. Later studies did not however agree with the reported values. By sequencing the gene encoding CHO we excluded the possibility of additional mutations in the gene. In order to have a better understanding of what might have caused a lower KIE, current research prompts on retransforming Rosetta(DE3)pLysS competent cells with pET*CodA* , the plasmid encoding CHO and measure KIE for choline and 1,2-[$^{2}H_{4}$]-choline using the new enzyme by stopped-flow techniques.

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A Density Functional Theory Study of the Heterolytic Bond Dissociation Energy of Water in First-Row d-Block Biomimics of the α-Carbonic Anhydrase Active Center

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The active center of α-Carbonic anhydrase is a Zn^{2+} ion bound by three histidine residues. α-Carbonic anhydrase (E.C. 4.2.1.1) catalyzes the conversion of carbon dioxide to bicarbonate ion via heterolytic bond dissociation of water to hydroxyl anion and proton and subsequent nucleophilic attack on carbon dioxide by the Zn-bound hydroxyl group. Because α-Carbonic anhydrase is capable of rapid conversion of carbon dioxide to another form (operating at the diffusion-controlled limit), studying the energetics of the naturally occurring metalloenzyme active center and its biomimics can provide valuable insight into artificial carbon dioxide catalysis. The active site of α-Carbonic anhydrase was modeled with metals other than Zn and using imidazole in place of histidine to calculate the heterolytic bond dissociation energy (HBDE) of the H_2O ligand. Density Functional Theory (DFT) was used to calculate geometry optimizations and single point energies for the metal complexes. A correlation between charge-to-radius ratio and HBDE was found, suggesting that the catalytic activity of the enzyme active center can be maximized in an artificial setting with an active center biomimic. While the naturally occurring form of the active center lowers the HBDE of water by roughly 70%, some biomimics of this active center with first-row d-block metals, (with varying oxidation states and coordination environments) show even greater reduction in the HBDE of water. The results of the DFT study demonstrating that some biomimics of the α-Carbonic anhydrase active center are potentially better artificial catalysts will be presented in this poster.

Use of Isothermal Titration Calorimetry to Probe Proton Uptake/Release and the Role of Water During Ligand Binding to *E. coli* Chromosomal Dihydrofolate Reductase

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Dihydrofolate reductase (DHFR) catalyzes the conversion of 7,8-dihydrofolate (DHF) into 5, 6, 7,8-tetrahydrofolate (THF) by addition of a hydride from its cofactor, nicotinamide adenine dinucleotide phosphate (NADPH) and a proton from solvent. The product, THF, is necessary for the synthesis of methionine, purine nucleotides, thymidylate, and other compounds. Thus, the inhibition of DHFR leads to interruption of DNA synthesis and consequently cell death, making this enzyme a crucial target in the treatment of cancer and other diseases.

Upon binding its ligands, chromosomal DHFR from *E. coli* (EcDHFR) undergoes a conformational change in which the active site loop (Met20) closes against the nicotinamide ring of NADPH, aiding hydride transfer by orienting the nicotinamide ring and the pteridine ring of DHF in close proximity.

Isothermal titration calorimetry (ITC) was used to determine whether proton uptake or release occurs upon substrate and cofactor binding. Here, the observed enthalpy was monitored using buffers with differing heats of ionization. A plot of the observed enthalpy versus the enthalpy of buffer ionization upon $NADP^+$ binding to enzyme-DHF revealed a slope of 0.9, corresponding with proton release. Conversely, a plot of the observed enthalpy versus the enthalpy of ionization upon DHF binding to enzyme-$NADP^+$ revealed a slope of -0.1, indicating a low level of proton uptake.

The role of water in ligand binding was monitored using ITC by addition of increasing concentrations of neutral osmolytes. For each osmolyte, a net uptake of water was observed for DHF binding to enzyme-$NADP^+$ and a net release of water was observed for $NADP^+$ binding to enzyme-DHF. However, the slopes associated with the various osmolyte were different, consistent with interaction of the osmolytes with the protein and/or ligand.

Uptake of water upon DHF binding has also been observed in a non-homologous R-plasmid encoded R67 DHFR (Chopra et al. (2008) JBC 283, 4690-4698). These similar results potentially implicate osmolyte effects on free DHF.

Acknowledgements
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Enzymatic Study of Synthetic Human Protein Arginine Methyltransferase 5

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Protein arginine methyltransferases (PRMTs) mediate the transfer of methyl groups (CH_3) to arginines in histones and non-histone proteins. PRMT5 is a member of PRMTs which methylates H3R8 and H4R3. PRMT5 was report to suppress the RB family of Tumor suppressors in leukemia and lymphoma cells and regulates p53 gene, which acts an important role in apoptosis of tumor cells, through affecting the promoter of p53. In addition, knockdown of PRMT5 can induce G1 arrest, then causing the inhibition of cell proliferation. Through methylation of H4R3, PRMT5 can recruit DNMT3A which is a DNA methyltranferase. All above suggest that PRMT5 enzyme has an important function of suppressing cell apoptosis. However, the enzymatic activities of PRMT5 are not clearly demonstrated so far. In our study, we improved the protein expression methodology and greatly enhanced the yield and quality of the recombinant PRMT5. Further, mutagenesis study reveals an interesting mechanism of PRMT5 activity regulation.

Enhancing (Ligno)Cellulose Hydrolysis Rates through Crystallinity Reduction using Cel7A Cellulose-Binding Domains

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The enzymatic hydrolysis of crystalline cellulose encounters various limitations that are both substrate- and enzyme-related. One major feature impeding the activity of the enzymes is the recalcitrance of cellulose to enzymatic attack. Its high crystallinity content appears to be one of the major reasons for overall slow hydrolysis rate, amorphous cellulose being converted one order of magnitude faster than crystalline cellulose.

Cellulose-binding domains (CBDs) isolated from the major cellobiohydrolase Cel7A from *Trichoderma reesei* were found to display "decrystallase" activity: incubation of cellulose with CBDs resulted in a decrease in the degree of crystallinity of cellulose.

A pretreatment protocol was therefore investigated where CBDs were allowed to react with the substrate (cellulosic or lignocellulosic material), followed by the addition of full-length cellulases to carry out the hydrolysis. The hydrolysis rates obtained thereof were found to be significantly higher than those when no pretreatment was performed, pointing at the reduction in crystallinity as major reason for rate enhancement. Incubation of dry cellulose in buffer alone resulted in increased rates as well (hydration effect), however to a lesser extent compared to the pretreatment with CBDs.

The effect on lignocellulose was compared to BSA treatment where non-specific competitive and irreversible adsorption of BSA on lignin has been shown to increase rates as well. The actual role of CBD on lignocellulose (adsorption on lignin vs cellulose crystallinity reduction) is being investigated to explain the higher conversion levels observed after treatment.

Investigation of the Molecular Interrelationship of Arginine Methylation with Other Posttranslational Modifications on Histone H4

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Abstract

Protein arginine methylation has emerged as an important regulatory mechanism for gene expression and cellular signaling. The methylation is catalyzed by protein arginine methyl-transferases (PRMTs) that transfer the methyl group from *S*-adenosyl-L-methionine (AdoMet, SAM) to specific arginine residues in histone and nonhistone protein substrates, resulting in mono and di-methylated arginine residues and *S*-adenosyl-L-homocysteine (AdoHcy, SAH). It has been shown that PRMTs are involved in the regulation of diverse biological processes such as DNA transcription, RNA processing, DNA repair, and cell differentiation. In the PRMT family, PRMT1 is found as the predominant type I PRMT in mammalian cells, accounting for 85% of cellular PRMT activity. A major target of PRMT1 on the chromatin in the cell is histone H4. Herein, we address how PRMT1 catalysis in H4R3 methylation communicates other posttranslational modifications on the H4 N-terminal tail. The selected peptides are synthesized by solid phase peptide synthesis (SPPS) with the Fmoc strategy using an automatic peptide synthesizer. The peptides are cleaved from solid resin using trifluoroacetic acid. The final compounds are purified by reverse phase high performance liquid chromatography (RP-HPLC). After synthesis and purification, the peptides are subject to methylation assay by PRMT1. The Recombinant PRMT1 is expressed from E. coli. Radioactive AdoMet is used in the methylation assay. Phosphorimaging analysis is used to analyze the methylated products. These studies provide molecular insight into the understanding of the histone codes in transcriptional regulation and signal transduction.

Acknowledgments:

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Enzymatic and Structural Basis for Substrate Specificity of Jumonji Histone Lysine Demethylases

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Reversible methylation of specific lysine (K) residues in histone tails constitutes explicit epigenetic codes which regulate wide range of biological processes. Proper establishment, reading and removal of methyl marks on specific histone lysines are thus important in regulating chromatin dynamics. Human proteins PHF8 and KIAA1718 belong to an emerging class of jumonji C (JmjC) domain containing histone demethylases, which requires Fe(II) and α-ketoglutarate for their activity. Both of these enzymes harbor two conserved domains in their N-terminal halves: a PHD domain that binds to tri-methylated histone H3K4 (H3K4me3) ('reader') and a catalytic JmjC domain that removes ('eraser') mono- or di- (but not tri) methylation marks on target lysines. The two domains in these enzymes are separated by a small linker region, which differs in length and flexibilities between the two enzymes. Here we show that the presence of H3K4me3 (an activating mark) on the same peptide as H3K9me2 (a repressive mark) stimulates the H3K9me2 demethylase activity of PHF8 but abrogates the H3K9me2 activity of KIAA1718. On the contrary, the same activating H3K4me3 mark stimulates demthylase ativity of KIAA1718 on H3K27me2. This difference in specificities between the two enzymes is attributed to the shorter and conformationally more flexible linker in PHF8, which allows cooperative binding of PHD and JmjC domains to H3K4me3 and H3K9me2, respectively. In contrast, the extended rigid linker of KIAA1718 renders it specific to repressive H3K27me2 marks distal to H3K4me3. The structural properties of the linkers between PHD and JmjC domains thus play crucial role in determining the specificities of the two demethylases towards bivalent (containing both activating and repressive lysine-methylation marks) substrates.

Generation of a Mutant Core Streptavidin for Complexation with and Crystallization of Biotinylated Membrane Proteins

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There is a great need to facilitate the crystallization of membrane proteins, because once the crystals are grown, the molecular structure can be determined by X-ray crystallography. However, membrane proteins prove difficult to isolate and crystallize. Hydrophobic membrane proteins have limited hydrophilic interactions to stabilize the protein in its crystalline form, and the lipid membrane surrounding the protein must be replaced with a non-denaturing detergent. The overall goal of this project is to develop a new generalizable procedure to facilitate the crystallization of membrane proteins.

For our approach, we take advantage of the high affinity biotin for streptavidin, a water soluble protein that crystallizes easily. We will crystallize the complex between streptavidin and a biotinylated membrane protein. To achieve this goal, we introduced four key mutations disrupting the streptavidin tetramer to generate a dimeric streptavidin construct with two biotin binding sites.

Site directed mutagenesis, which encompassed primer design, PCR, DPN1 digest, transformation, cell culture, plasmid isolation, and sequencing, were used to introduce each mutation. The mutant streptavidin was then expressed and purified.

Our next step was to form the streptavidin-biotinylated membrane protein complex. We are testing our method on a biotinylated form of signal peptide peptidase. We isolated the complex by size exclusion chromatography and then conducted crystallization trials.

Compared with the importance of membrane proteins in Nature, our molecular understanding of membrane proteins is limited. If our approach works, it will be widely applicable to the structure determination of other membrane proteins.

α-Amino-β-Carboxymuconic-ε-Semialdehyde Decarboxylase (ACMSD): Structure, Function, Mechanism

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Abstract:

α-Amino-β-Carboxymuconic-ε-Semialdehyde Decarboxylase (ACMSD) is a zinc-dependent enzyme that catalyzes a nonoxidative decarboxylation reaction. In this presentation, the structure, function and mechanism of ACMSD will be discussed.

ACMSD determines the partitioning of the metabolic fates in both the 2-nitrobenzoic acid degradation pathway and the kynurenine pathway. This enzyme competes with a spontaneous reaction so that directs the metabolites to an energy-production route as opposite to the biosynthesis of neurotoxin quinolinic acid. Several metabolic intermediates including quinolinic acid in these pathways are involved in various physiological and pathological conditions, including immunologic, neurodegerative, and neuropsychiatric disorders. Thus, ACMSD is an important drug target.

Impact of Intersubunit Interactions on Catalytic Versatility of Catalase-peroxidase

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Catalase-peroxidases (KatGs) provide a useful model to investigate the important role of distant protein structures and their interactions on enzyme active site structure and catalysis. The active sites of KatG enzymes are nearly superimposable on those of classical heme peroxidases (e.g., cytochrome c peroxidase). However, typical heme peroxidases have very poor catalase activity while KatGs are able to efficiently facilitate H_2O_2 decomposition by both catalase and peroxidase mechanisms. The primary differences in structure between these two groups of enzymes are external to the active site. Two interhelical insertions and a second (C-terminal) domain are present in the bifunctional KatG enzymes but absent from typical peroxidases. Although quite distant from the active site (e.g., 30 – 35 Å), deletion of any of these structures have profound impact on KatG active site structure and catalytic function (e.g., selective loss of catalase activity). We applied site-directed and deletion mutagenesis to investigate the role of a remote interaction between two of these KatG-unique features. For variants produced by site-directed mutagenesis, modest modulation of active-site structure and function was determined by UV-Vis, EPR, and MCD spectroscopic techniques and steady-state kinetic analyses of catalase and peroxidase activities. Strikingly, variants produced by deletion mutagenesis showed the near complete loss of catalase activity but retention of peroxidase activity comparable to the wild-type enzyme. Concomitant with these shifts in activity was a change in heme coordination from predominantly hexacoordinate high-spin to predominantly pentacoordinate high-spin. These results highlight the importance of distant protein structures and their interactions for fine-tuning active site function, and in addition, point to factors beyond simple sequence differences which control active site function even some 30 Å removed from the active site.

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Role of Asparagine 510 in the Relative Timing of Substrate Bond Cleavages in the Reaction Catalyzed by Choline Oxidase

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The flavoprotein choline oxidase catalyzes the oxidation of choline to glycine betaine with transient formation of an aldehyde intermediate, and molecular oxygen as final electron acceptor. The enzyme has been grouped in the Glucose-Methanol-Choline oxidoreductase enzyme superfamily, which shares a highly conserved His-Asn catalytic pair in the active site. In this study, the conserved asparagine residue at position 510 in choline oxidase was replaced with alanine, aspartate, histidine, or leucine by site-directed mutagenesis and the resulting mutant enzymes were purified and characterized in their biochemical and mechanistic properties. All of the substitutions resulted in low incorporation of FAD into the protein. The Asn510Asp enzyme was not catalytically active with choline and had 75% of the flavin associated non-covalently. The most notable changes in the catalytic parameters with respect to wild-type choline oxidase were seen in the Asn510Ala enzyme, with decreases of 4,300-fold in the $k_{cat}/K_{choline}$, 600-fold in the k_{red}, 660-fold in the k_{cat}, and 50-fold in the k_{cat}/K_{oxygen} values. Smaller, but nonetheless similar, changes were seen also in the Asn510His enzyme. Both the K_d and K_m values for choline changed ≤7-fold. These data are consistent with Asn510 participating in both the reductive and oxidative half-reactions, but having minimal role in substrate binding. Substrate, solvent and multiple kinetic isotope effects on the k_{red} values indicated that the substitution of Asn510 with alanine, but not with histidine, resulted in a change from stepwise to concerted mechanisms for the cleavages of the OH and CH bonds of choline catalyzed by the enzyme.

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Microwave Assisted Enzymatic Digestion of Multiple Proteins with Peptide Fluorescence Labeling Using Pyridoxal and Sodium Borohydride

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Enzymatic digestions are a very time consuming and tedious task that nonetheless fulfills a very important role in protein analysis. Most digestions are carried out over a period of hours with incubation at 37°C. Microwave assisted enzymatic digestion can be accomplished in 15 minutes instead of the traditional three to six hours. Peptides can then be analyzed using HPLC with uv-visible and fluorescence detection. Labeling peptides with pyridoxal gives a more sensitive method of detection using the natural fluorescence of pyridoxal which binds to the amino termini of the hydrolyzed peptides. Microwave assisted tryptic digestion and pyridoxal labeling has been attempted with cytochrome C, myoglobin, hemoglobin, and bovine serum albumin.

Investigation of D-arginine Dehydrogenase Tyr249 Mutants

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D-arginine dehydrogenase (DADH) is a flavin dependent enzyme that catalyzes the oxidation of D-arginine to iminoarginine whereas the final electron acceptor is unknown. Characterization of the wild type shows that the catalytic reaction is carried out using a ping pong mechanism with D-arginine as the substrate. The crystal structure of DADH in complex with iminoarginine, recently solved to a resolution of 1.3 Å, reveals that Y249 may be important for the positioning of the substrate in the active site since its hydroxyl group is 2.85 Å from the carboxylate group of the substrate. To better understand the role of Y249 in the binding of the substrate, we replaced the residue by site-directed mutagenesis to phenylalanine, methionine, lysine, alanine, or histidine. The results of this ongoing project are presented here.

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Sampling Long Timescale Protein Motions: OSRW Simulation of Active Site Loop Conformational Free Energies in Formyl-CoA:Oxalate CoA Transferase

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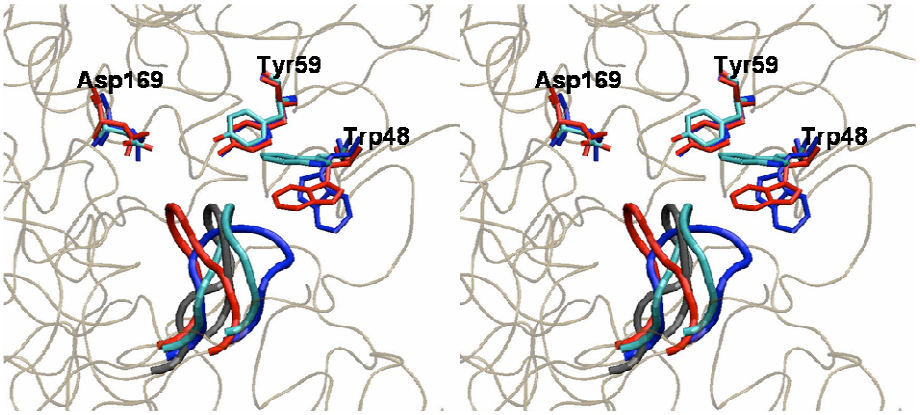
We report the application of the orthogonal space random walk (OSRW) method to computing the free energy difference of the "open" and "closed" conformational states of a tetraglycine loop in the active site of formyl-CoA: oxalate CoA transferase (FRC). X-ray crystallographic "snapshots" have shown that these conformational changes, which are coupled to motions of proximal active site side chains, are essential to catalysis. The OSRW calculations not only show that the "closed" conformation has a lower free energy in apo-FRC, but they predict a barrier to inter-conversion that is consistent with the observed turnover number of the enzyme. In addition, OSRW simulations have been carried out on models of four FRC variants in which each of the loop glycine residues is replaced by alanine in order to evaluate the energetic effects of altering the accessibility of allowed backbone torsion angles in the loop. The computational results are consistent with the steady-state kinetic properties of FRC and known FRC loop variants, and demonstrate the feasibility of using OSRW sampling to obtain quantitative information on the conformational preferences of loops within enzyme active sites.



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Directed evolution and enzyme design of orthogonal nucleoside analog kinases

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Nucleoside analogs (NAs) represent an important category of prodrugs for the treatment of viral infections and cancer, yet the biological potency of many analogs is compromised by their inefficient activation through cellular 2'-deoxyribonucleoside kinases (dNKs). In the absence of specific NA kinases in nature, the only route to identify effective biocatalysts is through enzyme engineering. In two separate experiments, we have applied traditional directed evolution, as well as computational enzyme design by the Rosetta Design software to generate orthogonal NA kinases for 3'-deoxythymidine (ddT).

Combinatorial enzyme libraries were analyzed, using a new FACS-based screening protocol with a fluorescent analog of ddT [1] and selected candidates were characterized by enzyme kinetics. Four rounds of random mutagenesis and DNA shuffling of *Drosophila melanogaster* 2'-deoxynucleoside kinase, followed by FACS analysis, yielded an orthogonal ddT kinase with a 6-fold higher activity for ddT and an overall 10,000-fold change in substrate specificity. The contributions of individual amino acid substitutions in the ddT kinase were evaluated by reverse engineering, enabling a detailed structure–function analysis to rationalize the observed changes in performance.

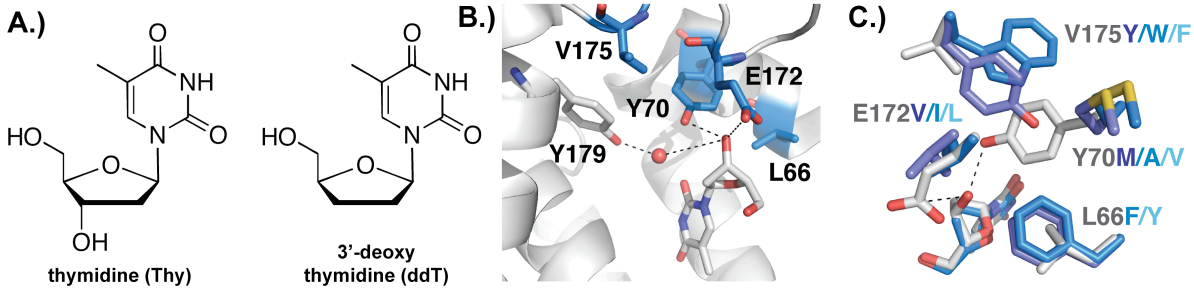

Figure 1: Enzyme engineering of a kinase selective for ddT (A) identifies several key residues in the active site (B) and computational models can guide the redesign (C).

Separately, computational studies have suggested an alternative solution for the desired change in substrate specificity [2]. Experimental data confirmed the validity of the in silico design and, together with the previous results, have identified a small number of active site residues which define the enzyme's binding specificity for the sugar moiety of the nucleoside substrate. Based on our results, engineering by computational design and directed evolution should prove a highly versatile strategy for evolving selective kinase:NA pairs and for studying fundamental aspects of the structure–function relationship in dNKs.

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[1] L. Liu, Y. Li, D. Liotta, S. Lutz (2009) *Nucleic Acids Res.* **37**(13) 4472-4481
[2] L. Liu, P. Murphy, D. Baker, S. Lutz (unpublished results)

Time matters: different growth time affects wild-type iron SOD protein activity and yield

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Overexpression of proteins is increasingly routine, based on modern overexpression vectors and strains. However for enzymes that rely on bound cofactors for activity and integrity, protein production must be coordinated with cofactor production and insertion. In overexpressing Fe-containing superoxide, issues related to Fe insolubility, storage and mobilization can all limit the Fe content and quality of the overexpressed enzyme. Therefore we have been optimizing our protocols for overexpression cultures.

E. coli with wild-type iron superoxide dismutase (FeSOD) gene has been grown in 2L LB culture and harvested at two different post-IPTG intervals, 5 hours vs. overnight. 8.87g cell paste were obtained from the overnight growth, almost 2 times the yield of the 5-hour growth. After a heat cut and an ammonium sulfate cut, crude protein solution was passed over a G25 column and a DE52 column to purify FeSOD. SDS PAGE and the nitroblue tetrazolium (NBT) assay were used to qualitatively identify FeSOD in complex mixtures. The cytochrome C-reduction interference assay and the Nishikimi assay were used to measure the specific activity of the FeSOD and the ferrozine assay was used to quantify total Fe. To characterize the distribution of iron among different FeSOD variants, we used the in-gel stain of Che-fu Kuo et al (1988).

Yields, specific activities and metal ion contents for the two growths will be compared, along with profiles of different forms of FeSOD obtained from different harvesting times.

Acknowledgements
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Site-directed Mutagenesis and ^{18}O Isotopic Labeling Studies Reveal the Functional Role of an Active Site Glutamate in the Glutamine-Dependent Asparagine Synthetase from *Escherichia coli*

Author List: Megan E. Meyer,[†] Jemy A. Gutierrez-Amos, [†] Yun Ding, [†] Frank M. Raushel,[‡] Nigel G. J. Richards[†]

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Our group has identified a transition state analog that suppresses the proliferation of a drug-resistant MOLT-4 leukemia cell line, presumably by inhibiting glutamine-dependent asparagine synthetase. As part of work to obtain more potent compounds with better cell permeability and pharmacological properties, we have constructed a computational model of how this inhibitor binds to the synthetase site of the enzyme. This poster will outline studies in which site-directed mutagenesis, ^{18}O-transfer, and positional isotope exchange (PIX) experiments provide evidence to suggest that a conserved glutamate residue (Glu-348) in the synthetase active site plays a critical role in catalyzing the reaction of aspartate and ATP to form an acyl-AMP intermediate.

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Investigation of the "Gating" Functions of Ile199 and Tyr326 in Human MAO B

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A major structural difference in human MAO A and MAO B is that MAO A has a monopartite substrate cavity of ~550Å^3 and MAO B is dipartite with a 290 Å^3 entrance cavity and a 400 Å^3 substrate cavity. Ile199 and Tyr326 function to separate these two cavities. The Ile199 side chain is in a required "open" conformation with large inhibitors and is "closed" with smaller inhibitors. To probe the function of these gating residues, Ile199Ala and Ile199Ala–Tyr326Ala mutant forms of MAO B were constructed, expressed in *Pichia pastoris*, and purified. Both mutants exhibit catalytic activities that are altered with increased K_m values. These mutant enzymes also exhibit lowered binding affinities relative to WT enzyme for small inhibitors. These observations demonstrate the Ile199 gate in MAO B plays an important role in its function. Preliminary structural data on the Ile199Ala MAO B mutant shows no alterations in active site geometries.

Acknowledgements
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Nitroreductase undergoes a dynamic transition at physiological temperature that affects substrate analog binding.

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Nitroreductase (NR) catalyzes the reduction of nitrated aromatics, including TNT, via a ping-pong mechanism involving alternating two-electron oxidation and rereduction of a non-covalently bound FMN.[1] We find that the enzyme exhibits two completely different HN-HSQC NMR spectra of the backbone NH groups, depending on the temperature at which the enzyme is observed. The two different states interconvert slowly on the NMR timescale and both appear to be needed for optimal catalytic activity, which peaks at 25 °C, near the midpoint of the transition between states. One state appears to have a unique persistent structure based on the well-dispersed HSQC, whereas the other state appears subject to rapid conformational averaging. The conformationally-averaged state of the enzyme does not appear to be unfolded, nor does it appear to be a molten globule. We can also rule out dissociation of the NR dimer, as well as flavin dissociation, as causes of the transition. However, saturation transfer and magnetization transfer NMR experiments indicate that the conformationally-averaged state is more hydrated than the persistently-structured state, and we find that binding of substrate analogs favours the conformationally-averaged state. Thus, we propose that conformational plasticity mediated by 'lubricating' water molecules contributes to substrate binding in NR, and may underlie NR's capacity to bind such a very large range of substrates. NR's significance as a TNT reductase must be relatively recent. Promiscuity has been proposed as a resource for evolution of new enzymes. Protein dynamics provides a general molecular mechanism that could facilitate evolution of modified enzyme activities.

Acknowledgements: This work was supported in part by funding from the N.I.H. (GM-063921) and the ACS-PRF 44321-AC4.

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Structural studies of HIV I and HTLV II Long Terminal Repeat substrates investigating retroviral Integrase promiscuity.

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The integration of retroviral DNA to the host genome is a crucial part of all retroviral life cycles. The retroviral protein Integrase (IN) facilitates the covalent insertion of the proviral DNA, transcribed from the viral RNA by reverse transcriptase, into the host genome. Integration is a 3-step process with the first 2 steps solely done by the IN while the final step is carried out with the aid of host proteins. IN specifically recognizes the ends of the linear proviral DNA (Long terminal repeats or LTR) and cleaves two nucleotides from each 3' end, exposing the 3' hydroxyl group of the conserved CA dinucleotide. This is termed as 3' processing and is followed by strand transfer; where IN joins each 3' recessed end to the opposing strands of the host DNA by a single transesterification reaction. The last step involving the removal of the unpaired 5' nucleotides of the viral DNA, filling in gaps and ligating the viral DNA to the host DNA is carried out in conjunction with the host enzymes.

Studies have shown that IN of a retrovirus can catalyze the 3' processing of other retroviral LTR's although there is little sequence conservation between them, except for the conserved CA dinucleotide step. More specifically, the IN's of HIV 1 and HTLV II process the LTR substrate of the other with similar efficiency. We are interested in evaluating whether such promiscuity results from unique structural features common to both retroviral LTR terminals at the CA step despite different flanking sequences. To this effect we are determining the NMR solution structures of 2 DNA hairpins (7 bp long) representing the U5 LTRs of HIV I and HTLV II. ^{1}H and ^{31}P chemical shifts for both hairpins have been assigned and comparison of their CA/GT steps did not show any discernible trend. Distance restraints (NOESY experiments) have been obtained and sugar puckering analysis has been performed for both hairpins. We are currently incorporating these structural restraints to obtain high-resolution structures of these hairpins to determine the presence of potentially shared unique structural features at the CA/GT step.

Expression, Purification, and Characterization of *Ceriporiopsis subvermispora* Bicupin Oxalate Oxidase Expressed By *Pichia Pastoris*

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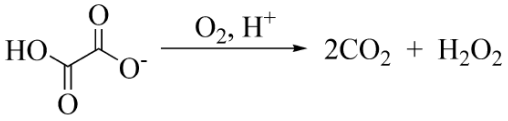
Oxalate oxidase (E.C. 1.2.3.4) catalyzes the oxygen-dependent oxidation of oxalate to carbon dioxide in a reaction that is coupled with the formation of hydrogen peroxide (Scheme 1). Although there is currently no structural information available for oxalate oxidase from *Ceriporiopsis subvermispora* (CsOxOx), sequence data and homology modeling indicate that it is the first manganese-containing bicupin enzyme identified that catalyzes this reaction. The best characterized oxalate oxidases are from barley and wheat. These enzymes, also known as germins, contain a single cupin domain and are therefore classified as monocupins. Interestingly, CsOxOx shares greatest sequence homology with the bicupin microbial oxalate decarboxylases. The expression, purification, and characterization of CsOxOx will be presented in the poster.



Scheme 1: Reaction catalyzed by oxalate oxidase

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Mössbauer spectroscopy and its application to biomolecules

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Abstract: Mössbauer spectroscopy is a unique spectroscopic technique that employs recoilless emission and absorption of γ-rays by the nuclei in solids or the frozen solutions. The underlying phenomenon used in this technique is the Mössbauer Effect, wherein certain nuclei, in a solid or frozen solution, emit and absorb γ-rays without any loss of energy due to recoil. The result is the resonant absorption with an extremely high precision. This can be used to investigate the slightest variation in the nuclear energy level resulting from the hyperfine interaction between the nucleus and its electronic environment. Thus, the Mössbauer nucleus in a solid helps to reveal the chemical and physical state of the atom, molecule, and solid in which the nucleus is located. We applied ^{57}Fe-Mössbauer spectroscopic technique to study the Fe centers in proteins. Since many biological molecules contain iron, ^{57}Fe-Mössbauer spectroscopy can be used as a scientific tool for gaining the details of the iron centers. Here we have focused on the application of the ^{57}Fe-Mössbauer spectroscopy mainly on the iron centers of cysteamine dioxygenase, tryptophan 2,3-dioxygenase, methylamine utilization (*mau*) gene product MauG protein and a nuclear metalloprotein Pirin. The sensitivity of the Mössbauer spectroscopy to the iron centers in these biomolecules has been exploited to obtain a clear understanding of the spin state, oxidation state and coordination environment of the iron atoms. The wealth of these details of the iron centers make Mössbauer spectroscopy a unique technique which plays an important role to provide molecular-level insight into the structure and function of the biomolecules.

ANALYSIS OF THE CYSTEINE-TYROSINE LINKAGE WITHIN THE ACTIVE SITE OF MAMMALIAN CYSTEINE DIOXYGENASE

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ABSTRACT

Cysteine dioxygenase (CDO) is necessary in the regulation of intracellular levels of cysteine in mammals and it catalyzes the first step in the biosynthesis of sulfate and taurine. CDO is a member of the cupin superfamily and it has been identified in both prokaryotic and eukaryotic organisms. Unlike many non-heme Fe (II) dioxygenases which coordinate the iron in a 2-His-1-carboxylate facial triad, CDO coordinates its metal in a 3-His facial triad. This deviation is expected to affect the activation of dioxygen in the catalytic site. The three-dimensional structure of CDO reveals a cysteine-tyrosine linkage within the active site. Previous studies have shown that CDO protein expressed by mammalian tissues resolves as two bands on SDS-PAGE suggesting there are two isoforms of CDO. Variants of CDO that disrupt crosslink formation indicate that the lower band represents the crosslinked isoform.

The main focus of this study is to elucidate the mechanism that leads to the formation of this thioether linkage and its role in catalysis. The crosslinked isoform of CDO was investigated by EPR spectroscopy and metal analysis to determine the degree of iron incorporation within the active site. The contribution of the substrate, iron (II) and dioxygen towards the formation of the crosslinked form of CDO was tested to evaluate the mechanism of crosslink formation. Steady state kinetics assays were performed to determine the activity of the crosslinked isoform relative to the non crosslinked wild-type CDO. The results from these experiments have provided important information on the mechanism of crosslink formation and the role of the crosslink in catalysis.

The Aminoglycoside Antibiotic Recognition by Resistance Enzymes

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Aminoglycoside antibiotics are one of the oldest and largest groups of natural and semi-synthetic antibiotics that are used to treat serious infections. The main mode of bacterial resistance to the action of these antibiotics is enzymatic modification by a large group of enzymes that catalyze *N*-acetylation, *O*-nucleotidylation, and *O*-phosphorylation. Our laboratory employs a variety of biophysical techniques to study enzyme-antibiotic interactions using representatives from each catalytic group to understand the molecular reasons that allow these enzymes to be highly promiscuous and show significant substrate overlap irrespective of sequence homology among them.

Isothermal titration calorimetry (ITC) experiments revealed that binding of aminoglycosides to aminoglycoside-modifying enzymes (AGMEs) occurs with favorable enthalpy ($\Delta H<0$) and unfavorable entropy ($\Delta S<0$). The presence of co-substrate has opposite effects on the thermodynamic parameters such that enzymes that utilize MgATP as the cosubstrate (nucleotidyl- and phosphoryl-transferases) show that binding enthalpy is less favored while binding entropy is less disfavored. However, the presence of cosubstrate causes binding enthalpy of aminoglycosides to the acetyltransferase enzyme to be more favored while entropy becomes more disfavored.

NMR spectra of the aminoglycoside phosphotransferase(3′)-IIIa (APH) showed that the enzyme was intrinsically unstructured and the binding of the aminoglycoside substrate (not MgATP) caused the enzyme to adopt a well defined structure in solution. H/D exchange studies, detected by NMR, paralleled these observations and showed that the apoenzyme can exchange all of its backbone amide protons while 40% of them remained unexchanged when aminoglycoside is bound. The H/D exchange pattern was also dependent on the nature of the aminoglycoside and affected hydrophobic residues away from the ligand binding site thus indicating the importance of dynamics in substrate recognition and the enzyme's ability to adapt its conformation for different ligands. These studies also explained the dramatic differences in the enthalpy of formation (> 20 kcal/mol) observed between different complexes of the enzyme with antibiotic and nucleotide.

NMR studies of AAC showed that this enzyme was also highly dynamic in solution suggesting that dynamic properties of these enzymes may be the common behavior for the entire family allowing them to be promiscuous towards substrates.

Acknowledgments

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Identification of active site residues in *Trypanosoma cruzi* UDP-galactopyranose mutase

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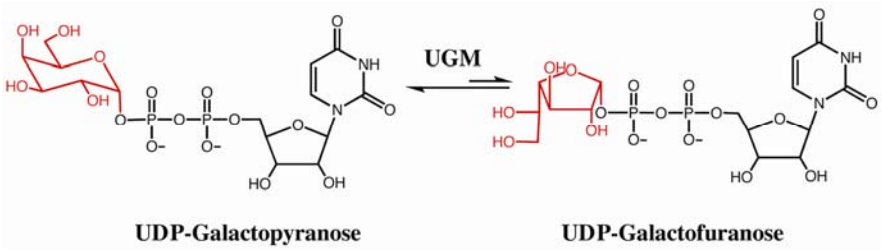
UDP-Galactopyranose mutase (UGM) is a flavoprotein that catalyzes the conversion of UDP-galactopyranose (UDP-Galp) to UDP-galactofuranose (UDP-Galf) (Scheme 1). UGM serves as the sole biosynthetic source of galactofuranose, which is found in the cell wall of many bacteria, fungi, and parasites. The function of UGM has been shown to be essential in *M. tuberculosis* and it is important for virulence in *A. fumigatus* and in human parasites such as *L. major* and *T. cruzi*. Residues important for catalysis in the bacterial enzymes have been identified by x-ray structure, sequence alignments, and site-directed mutagenesis. In contrast, little is known about residues important for catalysis in the eukaryotic UGMs.



Scheme 1. Reaction catalyzed by UDP-galactopyranose mutase

We have used a combination of sequence alignments and structural models to identify residues that might be important for catalysis in *Trypanosoma cruzi* UGM (*Tc*UGM). We have mutated several of these residues and have determined the activity with UDP-Galp as substrate (Figure 1). Comparison of the effects in catalysis in the *Tc*UGM mutants to the corresponding mutants in the bacterial UGMs, suggests that the putative active site residues in *Tc*UGM play different roles in catalysis. We also show that *Tc*UGM functions as a monomer, while the bacterial enzymes are all homodimers. Structural differences are also demonstrated by the inability to measure binding of substrate or substrate analogs by FAD and tryptophan fluorescence in *Tc*UGM, unlike its prokaryotic counterpart *K. pneumonia* UGM (*Kp*UGM) where fluorescence studies were successfully used to determine a K_D of UDP and UDP-Galp.

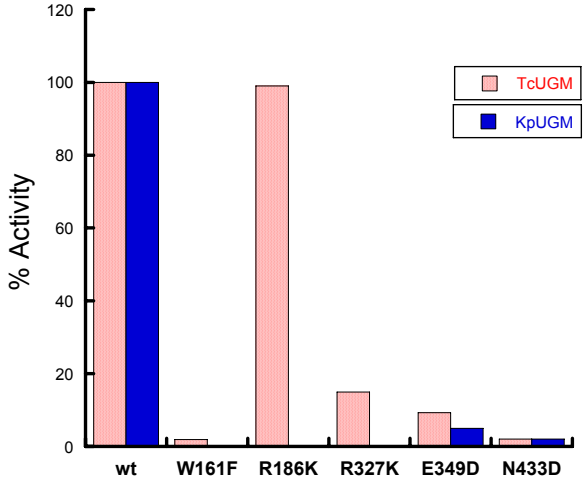


Figure 1. Relative activity of active site residues in *Tc*UGM and *Kp*UGM (*Kp*UGM values obtained from *Biochemistry*, 43, 23, 6723-6732.)

Determining the Role of the Carboxylate Groups of Dihydrofolate in R67 Dihydrofolate Reductase

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Dihydrofolate reductase (DHFR) catalyzes the reduction of dihydrofolate to tetrahydrofolate using NADPH as the reducing agent. R67 DHFR, encoded by an R-plasmid, is a homotetramer with a monomer length of 78 amino acids. The structure of R67 DHFR displays 222 symmetry and a single active site pore that traverses the middle of the enzyme. R67 binds a total of two molecules, either 2 NADPH, 2 folate/DHF or 1NADPH plus 1DHF where the latter complex yields the reaction products. R67 is not homologous in sequence or structure with chromosomal DHFRs.

A crystal structure of the ternary enzyme•$NADP^+$•DHF complex shows $NADP^+$ interacting with R67 via specific contacts (Krahn et al., (2007), Biochemistry 46, 14878-14888). The pteridine ring of DHF also interacts specifically with R67 DHFR, but the p-amino-benzoyl-glutamate (pABA-glu) tail is disordered. NMR experiments and the crystal structure of an R67 DHFR•2 folate complex also provide additional evidence for a bound, disordered tail.

Lysine 32 (K32) plays an important role in the binding of cofactor as the crystal structure shows K32 interacting with the 2'phosphate and pyrophosphate moieties of $NADP^+$. For bound DHF, modeling studies predict that the side chains of symmetry related K32 residues project into the active site pore and interact with DHF via ion pairs with the carboxylates in the p-ABA-glu tail. However, the carboxylates cannot span the diameter of the pore and further studies suggest the glutamate tail of DHF moves back and forth between two symmetry related K32s.

To determine how the α- and γ-carboxyl groups in the p-ABA-glu tail interact with K32, the carboxylate groups of folate have been selectively modified. Synthesis and purification of the ethylene diamine (EDA) derivatives of folic acid are described and inhibition kinetics of the α- and γ- isomers of the EDA-folate derivatives are reported.

Acknowledgements

This work was supported in part by a National Science Foundation Award (MCB-0817827) to LH.

First Southeast Enzyme Conference

Title: Identification of 4-hydroxy-3-nitro-phenylacetate denitrase in *Variovorax* sp. JS669

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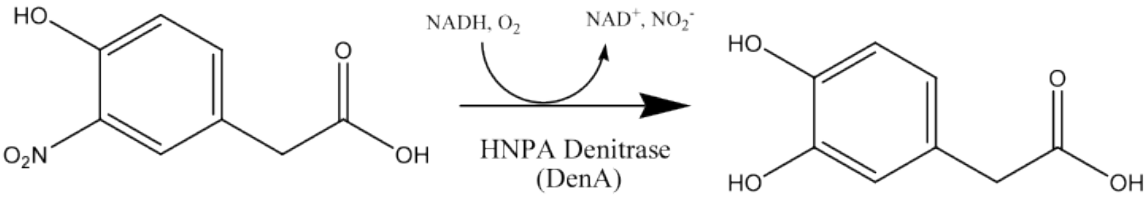
3-Nitrotyrosine (3NTyr) is produced by a common post-translational protein modification that occurs ubiquitously at sites of infection and inflammation in plants and animals. The biodegradation pathway for 3NTyr was recently described, however the genetics of 3NTyr degradation remained a mystery. The key enzymatic step in the biodegradation of 3NTyr is the removal of the nitro group from 4-hydroxy-3-nitro-phenylacetate (HNPA). We have identified and characterized the HNPA denitrase (DenA) from *Variovorax* sp. JS669. Assays with whole cells reveal that nitrite is released from HNPA.



4-hydroxy-3-nitro-phenylacetate homoprotocatechuate

DenA was identified using a combination of degenerate primers and genome walking. The gene is transcriptionally upregulated twenty-fold when cultures are grown on 3NTyr or HNPA. An insertional mutant in *denA* lost activity against HNPA, confirming the role of DenA.

In silico analysis of DenA revealed that the protein is a Class A FAD monooxygenase that represents a unique clade of such enzymes. Several conserved residues common to FAD monooxygenases were identified in DenA including the FAD binding fingerprints 1 and 2, a dual FAD/NAD(P)H binding domain, GD and DG motifs (involved in hydrogen bonding to the FAD ribose and recognition of the NAD(P)H cofactor, respectively) and a second NAD(P)H binding site. DenA homologs with hypothetical denitration functions were found via BLAST analysis. DenA from JS669 along with the DenA homologs form a separate phylogenetic grouping among FAD monooxygenases. Heterologous expression of the DenA homologs revealed that several such homologs release nitrite from HNPA.

HNPA denitration is a newly described metabolic activity that extends the known diversity of FAD monooxygenases. There are many naturally occurring analogs to both 3NTyr and HNPA, therefore it is plausible that members of the DenA clade that do not exhibit denitration with HNPA may instead catalyze denitration of one or more of the natural analogs to 3NTyr or HNPA.

Stabilization of a Flavosemiquinone in the Oxidative Half Reaction of Human Liver Glycolate Oxidase

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Human liver glycolate oxidase is a flavin-dependent enzyme that catalyzes the oxidation of glycolate to glyoxylate. Oxygen is enzymatically reduced by the flavin to hydrogen peroxide. A number of probes have been used here to investigate the oxidative half reaction catalyzed by the enzyme, including pH studies, rapid reaction techniques, solvent kinetic isotope effects and viscosity effects.

In the oxidative half reaction, k_{ox} increased upon increasing pH between limiting values at low and high pH. A deuterium kinetic isotope effect of 1.4 ± 0.1 was observed at pL 5.0 on the k_{cat}/K_{oxygen}, accompanied by lack of solvent viscosity effects. At pL 9.0, a negligible solvent viscosity effect of 1.15 ± 0.10 was observed. The solvent kinetic isotope effect observed at pD 5.0 was further investigated by stopped-flow experiments carried out in deuterium oxide. At pD 5.0, the oxidation of the enzyme-bound reduced flavin showed a biphasic pattern. The UV-visible absorbance spectrum recorded immediately after mixing (~2.2 ms) displayed an absorbance at 390 nm higher than that expected for the reduced GOX and a broad absorbance band in the 530-600 nm region, similar to the one typically associated with flavosemiquinones. The 450-nm band subsequently increased, with a parallel decrease of the long wavelength absorbance. The data presented are the first evidence for the direct observation of a flavosemiquinone intermediate in the oxidative half reaction of a flavoprotein oxidase.

The study was supported by NSF-CAREER MCB-0545712.

Hydrostatic Pressure As a Probe of Enzyme Conformational Changes

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Conformational changes are often proposed to occur during the catalytic cycle of enzymes. We have been using hydrostatic pressure perturbation as a probe for conformational changes in catalysis by pyridoxal-5'-phosphate (PLP) dependent enzymes. Tryptophan synthase is a PLP dependent multienzyme complex which exhibits an allosteric equilibrium between open (low activity) and closed (high activity) conformations, affected by monovalent cations, ligands, pH, solvents, temperature and hydrostatic pressure. Increasing hydrostatic pressure up to 2000 bar shifts the Trp synthase-Serine aldimine-aminoacrylate equilbrium from the closed to open form, with concomitant changes in the cofactor visible absorption and fluorescence spectra. These spectral changes are fully reversible upon decompression. The open conformation is favored at high pressure due to the negative volume change of solvation. The effects of various monovalent cations and ligands on the conformational equilibrium were quantitatively determined by hydrostatic pressure perturbation. Bidirectional pressure-jump experiments demonstrated that there is microheterogeneity of the open and closed conformations in Trp synthase. In another enzyme, tryptophan indole-lyase, increasing pressure shifts the spectrum of the PLP in the resting enzyme from the 420 nm ketoenamine to the 338 nm form, suggesting that there is a conformational change associated with the different species. Pressure also affects the spectrum of the PLP in the aspartate β-decarboxylase-succinate complex, shifting it from the protonated 420 nm form to the unprotonated 360 nm form. This suggests that the binding of ligands to aspartate β-decarboxylase triggers a conformational change which lowers the pK_a of the Lys-PLP Schiff's base, activating it to form the external aldimine. Thus, hydrostatic pressure is a useful technique to study and quantify conformational equilibria in enzymes.

A Comparative Mechanistic Study of the ACMSD Enzyme Family: Decarboxylase vs. Hydratase

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α-amino-β-carboxymuconic-ε-simialdehyde decarboxylase (ACMSD) was the first decarboxylase to be classified under the amidohydrolase super family, and exhibits the characteristic TIM-barrel fold. It is a member of the kynurenine pathway which converts excess tryptophan to serotonin or NAD^+. ACMSD regulates the formation of quinolinic acid, a chemical with neuropsychiatric side effects. Quinolinic acid has been linked to cases of Alzheimer's disease, Huntington's disease, anxiety, depression, epilepsy, and asphyxiation in newborns. The ACMSD family of enzymes is also the only known group of non-oxidative, metal dependant decarboxylases.

Previous work showed that it is very similar to 4-oxalomesaconate hydratase (OMAH). The general reaction for a decarboxylase and a hydratase can be seen in figure 3. The catalytic site of ACMSD is nearly identical to OMAH. OMAH is a part of a bacterial catabolic pathway that breaks down aromatic compounds into oxaloacetate and pyruvate. OMAH is responsible for the conversion of 4-oxalomesaconate to I-4-carboxy-4-hydroxy-2-oxoadipate. In order for these two enzymes to have different functions, something must be different about their active sites.

One dramatic difference can be seen by studying the crystal structures. The unligated carboxylate oxygen is rotated roughly 100° when compared to ACMSD! The E294 in ACMSD is hydrogen bound to the water ligand and may assist in activation. This ring does not exist in OMAH, which may be the cause of the dramatic change in catalytic efficiency ($4x10^5$ $s^{-1}M^{-1}$ for ACMSD, and $7x10^{-6}$ $s^{-1}M^{-1}$ for OMAH). Previous mutations performed on ACMSD showed that when this E294 is changed to D, as it exists in OMAH, catalytic efficiency is significantly decreased. Currently, mutation studies are being performed on this residue in OMAH to further explore this phenomenon.

As mentioned previously, a proton source must be provided to validate the proposed mechanism. Two strictly conserved residues in ACMSD are located very near to the active site. W194, and R249 are located roughly six Å apart, very close to the active site. This area of the enzyme is also highly solvent exposed and is in close proximity to several ordered water molecules according to crystallographic data. When the substrate enters, it is possible that a water molecule may hydrogen bond W194 and R249. This may result in the deprotonation of the water molecule and provide the necessary proton for the mechanism. Mutation experiments are being performed to investigate this possibility. This R249 is also part of the other subunit and may play the role of a communication residue.

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Trapping of a covalent intermediate in the non redox reaction catalyzed by UDP-galactopyranose mutase from *Trypanosoma cruzi*

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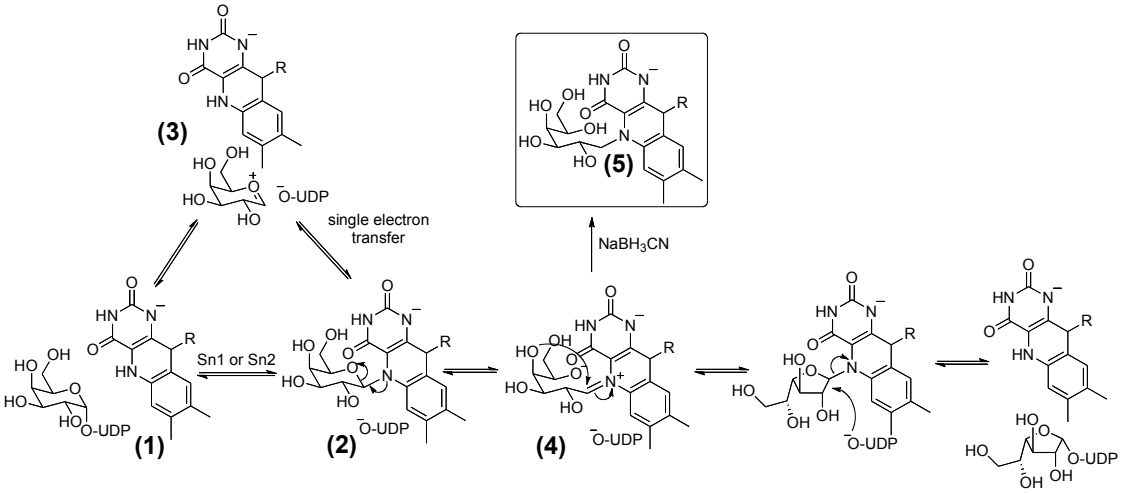
In the *Trypanosoma cruzi* parasite, the flavoenzyme uridine 5'-diphosphate galactopyranose mutase (UGM) catalyzes the transformation of UDP-galactopyranose (UDP-Gal*p*) to UDP-galactofuranose (UDP-Gal*f*). UDP-Gal*f* is a key intermediate in the biosynthesis of galactofuranose, which is an essential component of the cell surface and plays an important role in pathogenesis. The reaction does not involve a net redox change, however, the reduced form of the flavin cofactor is required for catalysis. There is no mechanistic information about the eukaryotic UGMs. In contrast, significant structural and mechanistic information is available for the bacterial enzymes, which have led to two main mechanistic proposals (Scheme 1).



One mechanism involves a nucleophilic attack **(1)** by reduced FAD, via either an Sn1 or Sn2 reaction, leading to the cleavage of the glycosidic bond and the formation of a flavin-sugar adduct **(2)**. Alternatively, it has been proposed that the cleavage of the glycosidic bond might lead to the formation of an oxocarbenium ion **(3)**. This will be followed by a single electron transfer step, leading to flavin and sugar radicals that can form a sugar-flavin adduct **(2)**. From here, both mechanisms are predicted to proceed through the opening of the pyranose and the formation of furanose. This occurs via the formation of a flavin imminium ion **(4)** as a common intermediate. We tested the presence of a flavin imminium ion in the reaction of TcUGM by assaying the enzyme in the presence of sodium cyanoborohydride. The flavin was extracted and analyzed by HPLC. Two peaks were observed, one peak contained free flavin and the other showed characteristic features of an N5-flavin adduct **(5)**, demonstrating the existence of imminium ion intermediate in the reaction of TcUGM. We also present, the synthesis and application of two fluorescently labeled UGM ligands that will be used to develop a high-throughput assay for inhibitor screening.

Acknowledgements
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Elucidating the Catalytic Mechanism of a Two-Component Flavin-Dependent Monooxgenase

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In recent years, two-component flavin-dependent monooxygenases have emerged as enzymes of increasing interest involved in various metabolic and biosynthetic processes in microorganisms. Efforts to elucidate the details governing the catalytic mechanisms of these systems continue to be an area of active investigation. The two-component alkanesulfonate monooxygenase reaction found in various bacterial organisms is being studied as a model for this group. The system is composed of a NAD(P)H-dependent FMN reductase (SsuE) and an $FMNH_2$-dependent monooxygenase (SsuD) that alleviate periods of limited sulfur bioavailability by converting environmental alkanesulfonates into aldehydes and metabolically active sulfite.

Active site amino acid residues have been proposed to play a direct role in catalysis at specific steps in the reaction pathway. Sequence and structural analyses of the monooxgenase enzyme were used to identify several conserved residues near the proposed active site with the potential to contribute to catalytic function. Variants of these residues were constructed and evaluated using various kinetic approaches including rapid reaction kinetics, deuterium solvent isotope effects, and pH dependence studies. Although studies on related enzymes implicated His228 serving as an active site base in SsuD, the present study suggests Arg226 plays a more substantial role in catalysis as any mutation to this residue results in complete inactivation of the enzyme. These combined results illuminate critical catalytic features of this complex system.

Acknowledgements This work was supported by the National Science Foundation (MCB-0545048)

The lysine N^6-monooxygenase MbsG from *Mycobacterium smegmatis* is regulated by substrate binding

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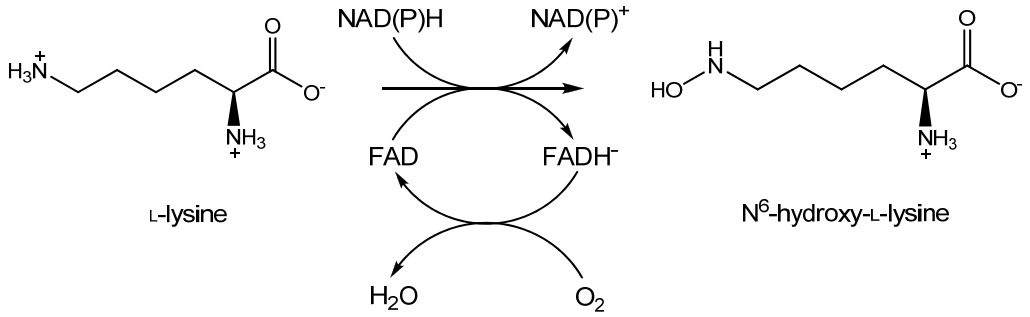
M. smegmatis MbsG is a flavin-containing monooxygenase that catalyzes the NAD(P)H and oxygen dependent hydroxylation of the amino group on the side chain of L-lysine in the biosynthetic pathway of the siderophore mycobactin (Scheme 1). *Ms* MbsG is a homolog of an enzyme found in *Mycobacterium tuberculosis* and represents a potential drug target as siderophores are critical for the survival of microbes via the acquisition of iron in hosts.



Scheme 1: Reaction catalyzed by *Ms* MbsG

Ms MbsG was cloned in *E. coli* and expressed as a fusion to Maltose Binding Protein (MBP). The enzyme was purified to homogeneity by the use of interactive metal affinity and ionic exchange chromatographies. When measuring the rate of hydroxylated L-lysine formation under steady-state conditions, a k_{cat} value of 7.8 min^{-1} and K_m values for L-lysine, NADH, and NADPH of 0.633 mM, 1.1 mM, and 2.1 mM were calculated, respectively. The activity of *Ms* MbsG can be measured by monitoring oxygen consumption. In the presence of L-lysine and NADH, a k_{cat} value of 19.6 min^{-1} and a K_m value of 1.7 mM for NADH were calculated. In the presence of L-lysine and NADPH, a k_{cat} value of 46.7 min^{-1} and a K_m value of 5.2 mM for NADPH were measured. In the absence of L-lysine, *Ms* MbsG effectively reacts with NAD(P)H producing hydrogen peroxide; this is the enzyme's oxidase activity. Under these conditions the reaction proceeds with first-order kinetics and does not appear to saturate. When L-lysine is present, the enzyme is regulated and the reaction becomes more coupled, producing hydroxylated L-lysine. The results suggest a mechanism of regulation in *Ms* MbsG where substrate binding decreases the oxidase activity and increases the monooxygenase reaction. This is a unique mechanism among the flavin-containing monooxygenase family of enzymes.

Acknowledgements
This work was supported in part by the Chemistry award from the Virginia Academy of Sciences, by the Ralph Powe Junior Faculty Enhancement award from the Oak Ridge Universities, and the Fralin Life Science Institute.

^{13}C and ^{18}O Isotope Effects and Spin Labeling Experiments Reveal the Functional Importance of an Active Site Loop in *Bacillus subtilis* Oxalate Decarboxylase

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Oxalate decarboxylase catalyzes a remarkable transformation in which the C-C bond of monoprotonated oxalate is *non-oxidatively* cleaved to give carbon dioxide and formate. Recent work has identified a mobile loop segment (Ser-161 to Thr-165) that can adopt "open" and "closed" conformations that are important in the catalytic mechanism. In the latter structure, this loop not only correctly positions the Glu-162 side chain carboxylate for (putative) proton transfer but also shields the active site from solvent. Analysis of the X-ray structure shows an intriguing hydrogen bonding interaction between the Thr-165 and Arg-92 side chains, which is of particular interest given the importance of this arginine residue for catalysis. In this poster, we will describe the kinetic and EPR characterization of a series of His_6-tagged OxDC mutants prepared to probe the functional importance of the Arg-92/Thr-165 interaction. These studies provide the first direct evidence for the existence of a formyl radical anion intermediate in the decarboxylation reaction, and heavy atom isotope effect measurements implicate the arginine side chain as playing an important role in a putative proton-coupled electron transfer step that takes place prior to C-C bond cleavage in the substrate.

This work was supported by the NIH (DK61666 to N.G.J.R and GM18938 to W.W.C) and the NSF (CHE-0809725 to A.A.).

Kinetic characterization of the initial and postulated final steps in MauG-dependent tryptophan tryptophylquinone biosynthesis

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MauG is a diheme protein that catalyzes the six-electron oxidation of a biosynthetic precursor of methylamine dehydrogenase (preMADH) containing a partially synthesized tryptophan tryptophylquinone (TTQ) to yield the mature protein with the functional protein-derived TTQ cofactor. The kinetic s of the initial two-electron oxidation of preMADH and the two-electron oxidation of quinol MADH are characterized. The biosynthetic reaction proceeds via a relatively stable high valent *bis*-Fe(IV) intermediate formed by oxidizing equivalents [O] and at a rate of > 300 s^{-1}. The reaction of *bis*-Fe(IV) MauG with preMADH exhibits a limiting first-order rate constant is 0.8 s^{-1} and a K_d less than 1.5 μM. For the first two-electron oxidation reaction, the order of addition of [O] and preMADH does not matter. This random kinetic mechanism is consistent with the structure of the MauG-preMADH complex. In the absence of substrate *bis*-Fe(IV) MauG spontaneously returns to the diferric state at a rate of 2×10^{-4} s^{-1}, but repeated reaction of MauG with [O] in the absence of substrate leads to loss of activity and inactivation of heme. In the two-electron oxidation, reaction of *bis*-Fe(IV) MauG with reduced quinol MADH a rate of 20 s^{-1} and K_d of 11 μM are observed. A current working model of MauG-dependent TTQ biosynthesis that include these data is presented.

Acknowledgement

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Aspergillus fumigatus SidA is a highly specific ornithine hydroxylase with bound flavin cofactor

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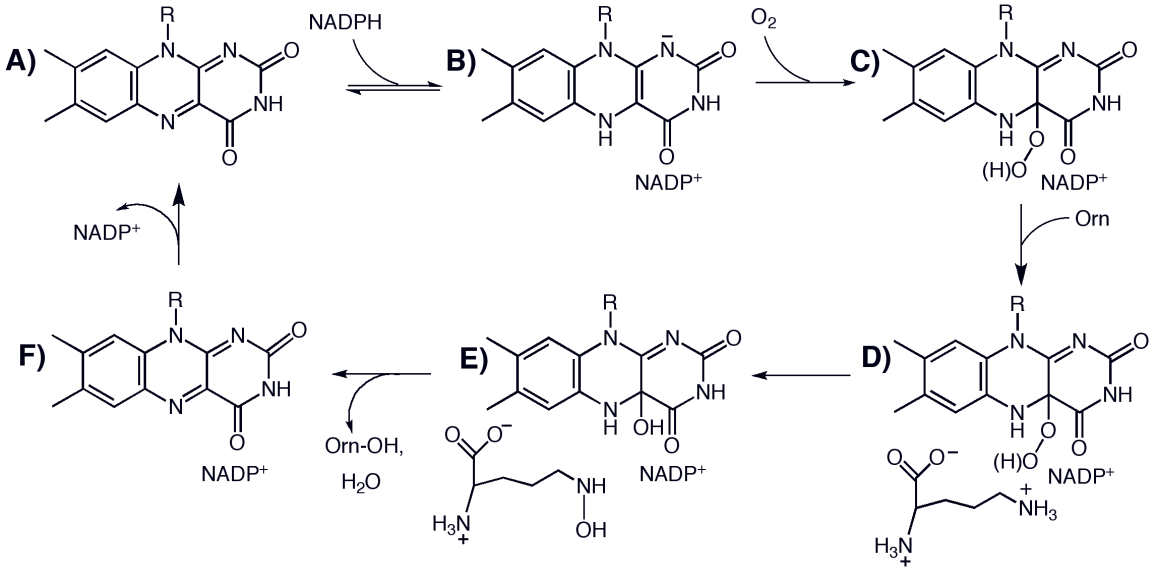
Ferrichrome is a hydroxamate-containing siderophore produced by the pathogenic fungus *Aspergillus fumigatus* during iron limiting condition. This siderophore contains N^5-hydroxylated L-ornithines that are essential for iron binding. *Af* SidA catalyzes the flavin- and NADPH-dependent hydroxylation of L -ornithine in ferrichrome biosynthesis. *Af* SidA was recombinantly expressed and purified as a soluble tetramer with a bound FAD cofactor. *Af* SidA is the first member of this class of flavin monooxygenases to be isolated with a tightly bound flavin cofactor. The enzyme showed typical saturation kinetics with respect to L-ornithine, while substrate inhibition was observed at high concentrations of NADPH or NADH. Increasing amounts of hydrogen peroxide were measured as a function of reduced nicotinamide coenzyme concentrations, indicating that inhibition was caused by an increase in uncoupling.



Scheme 1: Mechanism of *Af* SidA

Af SidA is highly specific for its amino acid substrate, only hydroxylating L -ornithine. An 8-fold preference in the catalytic efficiency was determined for NADPH as compared to NADH. In the absence of substrate, *Af* SidA can be reduced by NADPH and a stable C4a-(hydro)peroxyflavin intermediate is observed. The decay of this intermediate is accelerated by L -ornithine binding. This intermediate was only stabilized by NADPH and not by NADH, suggesting a role for $NADP^+$ in the stabilization of intermediates in the reaction of *Af* SidA. $NADP^+$ is a competitive inhibitor with respect to NADPH, demonstrating that *Af* SidA forms a ternary complex with $NADP^+$ and L-ornithine during catalysis. Biochemical comparison of *Af* SidA to other flavin-containing monooxygenases suggests that the catalytic cycle of *Af* SidA likely proceeds by a sequential kinetic mechanism (Scheme 1).

Acknowledgements
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Critical roles for a unique tyrosine (Tyr294) and a surface tryptophan (Trp199) in MauG and tryptophan tryptophylquinone biosynthesis

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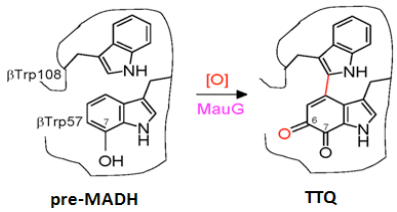
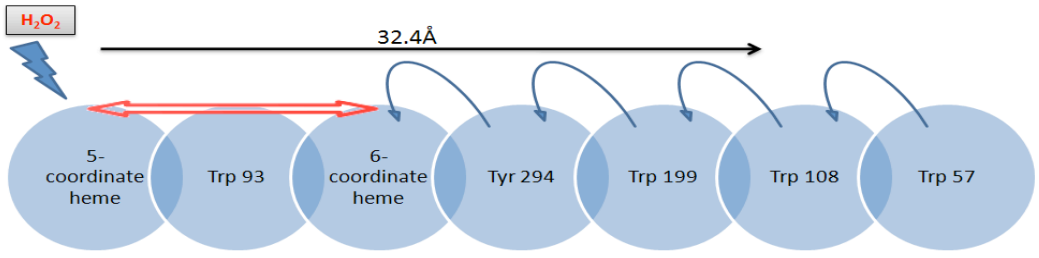
MauG is a diheme enzyme catalyzes the three-step, six-electron oxidation of methylamine dehydrogenase (MADH) biosynthetic precursor which harbors the partially formed TTQ cofactor (Scheme 1).



In more than one aspect, MauG expresses very unusual properties; it utilizes *c*-type hemes for an oxygenation reaction reaction; the substrate is 3-times larger than MauG itself; heme irons are 21 Å apart yet behave as a di-heme cofactor; and when reacts with H_2O_2 yields a *bis*-Fe(IV) state.

Recent crystallographic studies have pointed out the possible importance of two amino acid residues in MauG and TTQ biosynthesis mechanism. First; tyrosine (Tyr294), which has not previously been observed as a heme *c* ligand, provides the distal ligand for the six coordinate heme. Second; a tryptophan (Trp199), which lies equidistant between the six coordinate heme and MADH-TTQ active site, and sits at the MauG/pre-MADH interface.

Both residues have been investigated through site directed mutagenesis studies to unravel their role in MauG and TTQ cofactor biosynthesis. Results are promising and exciting; Tyr294 most probably has an essential role in *bis*-Fe(IV) state formation and stabilization in MauG which is essential for TTQ biosynthesis. And Trp199 studies indicated the possible involvement of a long range hopping electron transfer mechanism throughout MauG (Scheme 2).



Results that support the aforementioned conclusions will be presented in this poster.

Acknowledgements

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Metals Exert an Unexpected Role in Regulating Enzymes in the Plasminogen Activation Cascade

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Proteases regulate many processes in biology, including blood coagulation and clot lysis, the immune response, and processes that remodel the extracellular matrix (ECM) during wound healing and cell migration. Because of the importance of proteases in such a wide realm of biology, their activity must be closely checked. This is accomplished in part by the serine protease inhibitors (serpins), which form inactive covalent complexes with their target proteases. Often accessory factors can modulate this inhibition process. Plasminogen activator inhibitor-1 (PAI-1) is the major inhibitor of both urokinase (uPA) and tissue plasminogen activator (tPA) that lyse fibrin clots and also cleave ECM components. PAI-1 forms a stable complex with these proteases in a suicide fashion. The active conformation of PAI-1 is metastable and spontaneously converts to a latent form with a half-life of 1.1 hours at 37^0C. The half-life of the active conformation is extended to 1.5 hours when PAI-1 is bound to the plasma protein vitronectin. Our recent work demonstrates that the observed stabilization of PAI-1 by vitronectin is metal-dependent. Human PAI-1 more rapidly converts to the latent conformation in the presence of cobalt, nickel, and copper. Strikingly, the half-life is much longer in the presence of vitronectin and these metals, increasing to nearly 6 hours in the presence of copper. Steady-state binding measurements using surface plasmon resonance have been used to determine a dissociation constant for the interaction with nickel. Stopped-flow measurements of approach-to-equilibrium changes in intrinsic protein fluorescence have been used in a complementary fashion to characterize binding to a broader range of metals. These studies reveal distinct steps in the mechanism for metal interaction with PAI-1, with a binding step followed by a series of conformational changes. Measurements of the observed rate constants of binding as a function of varying metal concentrations allowed accurate determination of binding affinities for cobalt, nickel, and copper, yielding dissociation constants of about 40, 30, and 0.09 μM respectively. Identification of binding sites for copper is being pursued using a structural informatics approach. The function of copper binding in regulation of the plasminogen activation system is being tested using model systems for inflammation and extracellular signal processing.
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The Role of Water in R67 Dihydrofolate Reductase

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Chromosomal dihydrofolate reductase (DHFR) reduces dihydrofolate (DHF) to tetrahydrofolate (THF) using NADPH as a cofactor. R67 DHFR is a type II, R-plasmid encoded enzyme that confers resistance to trimethoprim (TMP), an antibacterial drug. It shares no structural or sequence homology with chromosomal DHFRs. R67 DHFR has been proposed to be a primitive enzyme as it shows promiscuity in binding of substrate/cofactor, forms non-productive complexes, does not possess a conserved acid in its active-site and displays a k_{cat}/K_m value (~$3x10^5$ $s^{-1}M^{-1}$) lower than the rate of diffusion.

Previous osmolyte studies in our lab have shown that DHF binding to R67 DHFR is accompanied by water uptake and NADPH binding is accompanied by water release (Chopra et al. (2008) JBC 283, 4690-4698). These data suggest that water plays a role in balancing the binding affinity. This may occur as R67 DHFR has a generalized binding surface and may use differential water effects to accommodate both ligands.

As water reorganization may be important in ligand binding, isothermal titration calorimetry (ITC) was utilized to determine the enthalpy of solvent reorganization by monitoring binding in both H_2O and D_2O. The observed enthalpy (ΔH_{obs}) of the interaction between protein and substrate can be broken down into ΔH_i (enthalpy of interaction) and ΔH_s (enthalpy of solvent reorganization) components. Since the enthalpy of a hydrogen bond in D_2O is approximately 10% greater than in H_2O, a value for ΔH_s can be estimated. Titration of cofactor into apo-enzyme yields a less negative ΔH_{obs} in D_2O than H_2O, while titration of folate into apo-R67 shows a more negative ΔH_{obs} in D_2O than H_2O. These results suggest D_2O is either destabilizing the unbound state for NADPH or stabilizing its enzyme bound state relative to H_2O, with the opposite being true for folate. The change of enthalpy associated with ternary complex formation is less pronounced.

To examine further the role of water in binding and catalysis, we have begun to collect binding and steady state kinetic data under conditions of high hydrostatic pressure (HPP). Increasing HPP hydrates molecules and tests the effect of increasing water concentration upon binding. Hydrostatic pressure can also affect the volume of the active site. An activation volume, defined as the change in molar volume associated with the ternary R67•NADPH•DHF complex going to the transition state, can be determined from a plot of the natural log of k_{cat} vs. pressure. The slope of this line is equal to $-\Delta V/R_pT$. A small slope yielding an activation volume of 0.5 ± 0.9 cm^3/mol is observed until 200 Mpa. A second activation volume describing the effect of pressure going from 200 Mpa to 500 Mpa was found to be 8.06 ± 0.76 cm^3/mol. Positive activation volumes are associated with a volume increase. As the pressure increases from 0-250 Mpa, only a small change in k_{cat} is observed, similar to osmotic stress results. As the pressure is increased past this point, a larger decrease in k_{cat} is observed, which may be due to dissociation of the active tetramer and/or protein unfolding. Studies of NADPH binding to the apo-enzyme were also carried out in the 0-250 Mpa range and increasing HPP weakens NADPH binding.

Acknowledgements
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Functional comparison of human MAO A and rat MAO A

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Monoamine oxidase (MAO, E.C. 1.4.3.4) is a flavin-containing mitochondrial membrane-bound protein which functions in the oxidative degradation of biogenic and xenobiotic amines to their corresponding aldehydes. Human MAO A is ~90% identical in sequence with rat MAO A which is the premise for using rat MAO A as a model for the human enzyme in drug development studies. With the recent demonstration that human MAO A is monomeric perhaps due to a human selection mutation and rat MAO A dimeric, it is of interest to determine whether any functional consequences result from this proposed difference in oligomerization. In a separate study, human and rat MAO A's are found to be dimeric in their membrane-bound forms and ~50% dimeric in their purified forms in detergent solutions. In this study, rat MAO A is expressed in *Pichia pastoris* and purified using a modification of the human MAO A procedure, producing ~700 units of pure rat MAO A activity/liter of cell culture. Recombinant rat MAO A is purified in a 43% yield and exhibits a molecular weight of ~60,000 kD on SDS-PAGE. The purified rat enzyme exhibits a much higher temperature stability than does human MAO A. A Hammett ρ value of +1.8 is shown from oxidation of a series of *para*-substituted benzylamine analogs which is similar to that observed (ρ=+1.9) with human MAO A. Rat MAO A oxidizes serotonin or kynuramine with a 2-fold higher k_{cat}/Km values, and oxidizes phenylethylamine with a 6.7 fold higher catalytic efficiency and benzylamine analogs with ~40-fold higher catalytic efficiency than does the human enzyme. The reversible MAO A selective inhibitors harmane, pirlindole mesylate and tetrindole mesylate show similar inhibitory properties with human and rat MAO As. These studies show that the property of rat MAO A, relative to human, is structurally more stable and exhibits similar substrate reactivities. These functional differences are suggested to result from differences in active site structures of the two enzymes and not due to change in oligomeric state.

Acknowledgments
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COMPLEX INTER-CROSSTALK OF MODIFICATIONS ON HISTONE H4

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Histone H4 undergoes extensive posttranslational modification by a variety of epigenetic enzymes. We investigate the intercrosstalk between posttranslational modifications on the amino-terminal tail of histone H4. Our work provide important insight into the interplay between HATs and PRMTs. Arginine 3 of H4 can be methylated by PRMT1. The potential effect of acetylation at K5, 8, 12, and 16 on R3 methylation is still not clear. Previously, it was shown that methylation of R3 enhances H4 acetylation, while H4 acetylation reduces the R3 methylation by acetylation. However, the four acetylation at K5, 8, 12, 16 has 16 possible combination numbers. It is unknown whether individual combinations affect R3 methylation differentially or in a similar manner. From the experimental data, it was shown that K5 acetylation is detrimental to R3 methylation, which alone decrease R3 methylation by 30%. In combination with other lysine acetylations, e.g. with K8, or K8 and K12 acetylation, the repressive effect is even stronger; the degree of repression can be 2-3 folds. On the other hand, K16 acetylation is favorable to R3 methylation. K16 acetylation alone increases the PRMT activity by 30%. If K16 acetylation coexist together with K5, K8 or K12 acetylation, the activation effect is dominant. The acetylations at K8 and K12 in regulating R3 methylation seems quite minor by themselves, in comparison to K5 and K6 acetylation. K8 acetylation can further enhance the repressive effect of K5 acetylation, suggesting that the positive charges at K5 and K8 are needed for the recognition of H4 by PRMT1. This is seen, for all the modified peptides containing K5 and K8 acetylations, R3 methylation is always weaker than the wt H4 peptide. Overall, K5 acetylation and K16 acetylation are two counteractive modifications that affect R3 methylation. K5 is the dominant factor that negatively impacts R3 methylation, and K16 acetylation dominates the positive regulation of R3 methylation. These results demonstrate epigenetic enzymes modulate histone substrates in complex but fine tuned mechanisms.

Purification of Mn-Superoxide Dismutase from Escherichia coli: Foundations for Future Work

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As a foundation for my thesis project, I have characterized the course of MnSOD purification from E. coli. The classical purification protocols employ salt and heat cut, and ion-exchange chromatography. In addition, the very high solubility of MnSOD permits isolation of substantial quantities of MnSOD without recourse to any columns at all, following its exclusive release from frozen cells upon thawing and osmotic shock.

For MnSOD, the classical column purification causes the loss of some SOD due to some unspecific binding between the resin and protein. The column-free method does not suffer from these losses and could eliminate the damage to the protein during the heat and salt cut as well. Specific activities and metal ion contents of the MnSOD purified both ways have been compared. The MnSOD purified by traditional means has less activity than the MnSOD isolated directly from shocked bacteria. Also the metal content of the no-columns MnSOD is within error of the ideal value of 2.0 per dimer, whereas MnSOD purified by the traditional protocol does not. Therefore the new purification protocol using the cell wash buffer to rupture cells and release soluble MnSOD into the buffer could result in higher quality MnSOD.

Acknowledgement: We thank NIH for supporting our research via 1R01GM085302.

Serendipitous Reaction Intermediates in Crystal Structures of Enzymes

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High to atomic resolution (1.0-1.5 Å) crystal structures of enzymes can reveal unexpected reaction intermediates bound at the active site. Examples will be presented from our crystal structures of the bacterial carboxylesterase Est30, HIV-1 protease, and a novel bacterial dehydrogenase. The structure of Est30, a member of the serine hydrolase family, was discovered to include a tetrahedral reaction intermediate (Liu et al., 2004). HIV-1 protease is a retroviral aspartic protease and drug target for HIV/AIDS. The crystal structure in the absence of inhibitor was observed to include a gem-diol tetrahedral intermediate of a peptide (Kovalevsky et al., 2007). The detailed geometry of the intermediates will assist in the future design of mechanism based inhibitors. The intermediates are assumed to be quasi-stable when bound to the enzyme and presumably represent slow steps in the reaction. Therefore, crystallographic analysis at high resolution has helped to understand the catalytic mechanisms of the enzymes.

Liu, P., Wang, Y.-F., Ewis, H., Abdelal, A., Lu, C.D., Harrison, R.W., Weber, I.T. Covalent Reaction Intermediate Revealed in Crystal Structure of the *Geobacillus stearothermophilus* Carboxylesterase Est30. (2004) J. Mol. Biol. 342, 551-556.

Kovalevsky, A.Y., Chumanevich, A.A., Liu, F., Weber, I.T. Caught in the Act: 1.5 Å Resolution Crystal Structures of the HIV-1 Protease and the I54V Mutant Reveal a Tetrahedral Reaction Intermediate. (2007) Biochemistry, 46, 14854-14864.

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Is Pirin a Redox Sensor for the NF-κB Signaling?

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NF-κB is a nucleus protein family that could regulate the κB gene expression level in immune system. Pirin as a regulating protein could regulate the binding affinity between NF-κB and κB gene.

Our central hypothesis is that Pirin is a redox sensor in the NF-κB signaling pathway. Based on the crystal structure and our preliminary data of human Pirin, we hypothesize that the metal center fulfills a redox role in the nucleus, sensing the changes in the oxidation/reduction level of the nucleus and transmitting them to the NF-κB complex. This putative redox role may be part of the regulatory mechanisms associated with aerobic life. The long range goal of this research is to discern whether or not Pirin's metal center is a natural redox sensor, which might turns on/off the p50 transcriptional cascades .

Preliminary Electrochemistry and EPR results further demonstrate Pirin's capacity to fulfill its role as a capable redox sensor.

ACKNOWLEDGEMENT

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Chemiluminescent measurement of NO and activity of NO synthase

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We developed a sensitive method that allows to measure subnanomolar concentrations of nitric oxide by using soluble guanylyl cyclase (sGC) as a sensor. Guanylyl cyclase catalyzes the conversion of guanosine triphopshate (GTP) into 2', 5'-cyclo-guanosine monophosphate and inorganic pyrophosphate. NO accelerates the reaction a few hundredfold upon binding to sGC. The product of the reaction, pyrophosphate is then enzymatically converted to ATP, which is detected in luciferin-luciferase reaction. We found that superoxide generated in the reaction mixture (possibly, from oxidation of luciferin) efficiently scavenges NO, preventing the detection of low (nanomolar and lower) concentrations of nitric oxide. Addition of superoxide dismutase dramatically increases the sensitivity of the method: it allows to detect 1nM NO added as a bolus, or 0.1 nM/min generated by NO donor. Activity of inducible nitric oxide synthase measured by the suggested method agreed with the same activity measured by the reaction with oxyhemoglobin and required about 20 pg of purified protein per one experiment.

Chemical Inhibitors of Histone Acetyltransferase tip60

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Tip60 (HIV-1 TAT-interactive protein, 60 kDa) is a key member of the MYST family of histone acetyltransferases (HATs) and plays important functions in many cellular processes and human diseases. We report here both substrate-based analog inhibitors and small molecule inhibitors for Tip60. In the first strategy, we designed, synthesized and evaluated a series of substrate-based analogs for the inhibition of Tip60. The structures of these analogs feature that coenzyme A is covalently linked to the side chain amino group of the acetyl lysine residues in the histone peptide substrates. These bisubstrate analogs exhibit stronger potency in the inhibition of Tip60 compared to the small molecules curcumin and anacardic acid. The substrate-based analog inhibitors will be useful mechanistic tools for analyzing biochemical mechanisms of Tip60, defining its functional roles in particular biological pathways, and facilitating protein crystallization and structural determination.

We also performed a virtual screening using the crystal structure of Esa1 (the yeast homolog of Tip60) and 105 small molecules were selected as potential hits. Then, the radioactive HAT assay was used for experimental screening. From the first-round screening, we focus on a series of small molecules that bear structure similarity with JWu-10 to identity lead inhibitors. We also tested the selectivity of this new class of Tip60 inhibitors. IC50 was compared to find out the structure-activity relationship. The discovery of new small molecule inhibitors provides important chemical tools for functional study of Tip60 and is of great potential to be developed into valuable anticancer agents.

Characterization of Glutamylpolyamine Synthetase in Polyamine Catabolism of *Pseudomonas aeruginosa* PAO1

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Polyamines (e.g. putrescine, cadaverine, spermidine and spermine) are essential aliphatic amines in all living cells. *P. aeruginosa* and many other bacteria are able to utilize polyamines as sole source of carbon and/or nitrogen. In *E. coli*, the *puuA* gene encoding an ATP-dependent γ-glutamylputrescine synthetase (EC6.3.1.11) was reported to play an essential role in putrescine catabolism. Previous results of transcriptome analysis revealed polyamine-dependent induction of six *puuA* homologues (PA0296, PA0298, PA1566, PA2040, PA3356, and PA5522) scattered on the chromosome of *P. aeruginosa*. The PA0296, PA2040, and PA1566 genes were cloned and overexpressed in *E. coli*, and their products were purified to homogeneity. Polypeptides encoded by PA0296 and PA2040 existed as dimers, while that of PA1566 as tetramer. All these proteins possessed a low intrinsic ATPase activity, and the concomitant addition of L-glutamate and polyamines could significantly stimulated the ATPase activity. Distinct patterns of substrate specificity were observed – putrescine and cadaverine for PA0296/PA2040 and spermidine for PA1566. The results of EMI-MS support the presence of γ-glutamylpolyamines as products in these reactions containing L-glutamate, ATP and Mg^{2+} as essential common components. Mutants with a single knockout of PA0296, PA2040 or P1566 showed no apparent growth defect on polyamines, but the PA0296/PA2040 double mutant exhibited an apparent growth deficiency on putrescine. In summary, these results support the presence of redundant γ-glutamylpolyamine synthetases in *P. aeruginosa*.

Structural and Functional Significance of the Disordered Region of Alkanesulfonate Monooxygenase

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Bacteria utilize alkanesulfonates as an alternate sulfur source during sulfur starvation conditions. An FMN reductase (SsuE) and an $FMNH_2$-dependent monooxygenase (SsuD) are expressed as a two-component system to generate sulfite and aldehyde from alkanesulfonates. SsuD is shown to be a TIM-barrel fold protein with a disordered region located above the active site. Similar to other TIM-barrel proteins, the disordered region of SsuD is proposed to be a flexible loop that closes over the active site with the binding of` substrates.

Three variants containing a partial deletion of the disordered insertion sequence were constructed to examine the structural and functional significance of this region. The deletion variants showed no activity, suggesting the flexible loop is needed for catalysis. While the variants showed similar affinities for $FMNH_2$ and octanesulfonate as wild-type enzyme, indicating substrate binding is not affected by deleting the loop. Pull down assays of the deletion variants showed the interaction with SsuE was not severely disrupted, suggesting the flexible loop may not be directly involved in protein-protein interaction. Rapid reaction kinetic experiments of the deletion variants showed a similar flavin oxidation rate to oxidation of flavin alone, indicating the flexible loop protects reduced flavin and the proposed peroxyflavin intermediate from premature oxidation. The results from these studies provide insight into the role of the disordered region in the mechanism of the alkanesulfonate monooxygenase.

Mechanistic Study of Tip60 Autoacetylation

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Tip60 (HIV1-Tat interacting protein, 60 kDa) belongs to the MYST family of histone acetyltransferase (HATs) and is involved in many important cellular processes, including DNA damage repair, cellular signaling, gene transcription, cell cycle and apoptosis. HATs acetylate the ε-amine of lysine residues by transferring acetyl group from acetyl CoA. HATs are classified into three major families: GNAT family, MYST family and p300 family. Recent data showed HATs from different family had distinct catalytic mechanism. In this work, we focus on the molecular mechanism of histone H4 acetylation by Tip60. Special emphasis is to elucidate the role of autoacetylation in Tip60 catalysis.

Title: Strucutral Basis of Type II Protein Arginine Methyltransferases

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Among different chromatin modifications, histone arginine methylation is particularly catalyzed by PRMTs, which transfer the methyl group from S-adenosyl-L-methionine (AdoMet, SAM) to the guanidino group of arginines in histone and nonhistone protein substrates, resulting in mono and di-methylarginine residues in substrate proteins. Thus far, nine PRMT members have been identified at the protein and genomic levels in human tissues or cells and are categorized into two major types, type I and type II, according to substrate and product specificity. Type I enzymes (PRMT-1, -2, -3, -4, -6, and -8) catalyze the transfer of the methyl group from AdoMet to the guanidino nitrogen atoms of arginine residues to produce ω-N^G monomethylarginines (MMA, L-NMMA) and ω-N^G,N^G-asymmetric dimethylarginines (ADMA). Type II enzymes (PRMT-5 and -7) catalyze the formation of MMA and ω-N^G, N'^G-symmetric dimethylarginines (SDMA). Although several type I PRMTs have known crystal structures, the structural basis of type II PRMT activity remain ambiguous. In order to better understand the functional difference of type I PRMTs versus type II PRMTs, we conducted homology modeling to investigate the structure-activity relationship of PRMT5, a representative member of type II PRMTs. Mechanistic insights have been obtained.

Kinetic and Structural Studies of D-Arginine Dehydrogenase

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D-arginine dehydrogenase (DADH) contains non-covalently bound FAD and catalyzes the oxidation of D-amino acids to the corresponding imino acids, which are non-enzymatically hydrolyzed to α-keto acids and ammonia. In the present study, we have characterized the kinetic behavior of the enzyme and determined the X-ray crystal structures of the recombinant enzyme in complexes with iminoarginine and iminohistidine to 1.3 Å resolutions.

DADH is a true dehydrogenase and it reacts poorly with oxygen. The steady-state kinetic mechanism of the enzyme was determined with D-arginine at pH 8.7 to be ping-pong with bisubstrate inhibition. Based on the 3D structure of the reduced enzyme in complex with iminoarginine, we propose that a proton relay system involving two water molecules and the imidazole side chain of His48 is required for the activation of the D-arginine substrate in the reaction catalyzed by DADH. The analyses of kinetic data and crystal structure with iminohistidine suggested that the iminohistidine binds to the enzyme in two distinct conformations.

This study was supported in part by NSF-CAREER MCB-0545712(G.G.).

HIV-1 Protease: Structural Perspectives on Drug Resistance

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Drug resistance is a major challenge in treatment of the HIV/AIDS pandemic. We are investigating the molecular mechanisms of drug resistance to inhibitors of HIV protease. This knowledge is applied in structure-guided designs for novel antiviral inhibitors with enhanced interactions with conserved regions of the protease structure. New antiviral inhibitors are being analyzed. Crystal structures at high to atomic resolutions (0.84-1.5Å) demonstrate structural changes due to drug resistance mutations and diverse inhibitors or peptides. We have analyzed HIV-1 protease with ~20 different single mutations and selected combinations to reveal the molecular mechanisms of drug resistance. Drug resistance mutations show distinct effects: 1) mutations in the inhibitor binding cavity can directly alter inhibitor binding; 2) mutations at the dimer interface can alter protease stability; 3) other mutations can have indirect effects on protease activity and inhibition by altering the unliganded protease or the interactions with reaction intermediates. In order to design new drugs we have analyzed many protease complexes with clinical and investigational inhibitors. Our drug design strategy of introducing new polar interactions with inhibitors is firmly based on early studies of the conserved pattern of protease-inhibitor hydrogen bonds. Darunavir, which was approved in 2006 for AIDS therapy, and a series of novel antiviral inhibitors have demonstrated the success of this structure-guided strategy to combat resistance. The new insights into the mechanisms of drug resistance and strategies for drug design have wide impact in many diseases. Moreover, the data provide a uniquely valuable resource for analysis of structural variation due to mutations or ligands.

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