PS04.02.09 CRYSTALLISATION AND X-RAY DIFFRACTION ANALYSIS OF THE METALLOENZYME, ARGINASE FROM 'BACILLUS CALDOVELOX'. M. C. Bewley, J.S.Lott, M.L.Patchett & E.N. Baker. Department of Biochemistry, Massey University, Palmerston North, New Zealand

Arginine metabolism is central to life. Arginase is the enzyme which not only controls the hydrolysis of arginine to ornithine in the urea cycle but has been implicated in the regulation of nitric oxide signalling. No structure for arginase currently exists.

Arginase has been isolated from a variety of species ranging from bacteria and fungi to plants and animals. The physiological cofactor is Mn^{2+} but activation by a variety of divalent cations, such as VO^{2+}, Fe^{2+}, Co^{2+} and Cd^{2+}, has also been reported, making arginase an attractive target for the study of metal-ion mediated catalysis, and also of the stabilisation of quarternary and tertiary structure by metal-ions.

The thermostable arginase from the thermophilic bacteria, *'Bacillus caldovelox'* has been cloned, sequenced, expressed in *E. coli* and purified utilising the thermostable character of the protein. The amino acid sequence shows limited similarity to other arginases, but the putative metal ion binding motif (L/I)GGDHS-(14X)-DAH has been conserved.

X-ray quality crystals have been obtained which diffract to 2.2Å resolution and crystallise in an orthorhombic cell (a=89.7Å, b=146.1Å, c=154.9Å) with six molecules in the asymmetric unit. Heavy atom derivative screening is currently in progress, the results will be reported.

PS04.02.10 HIGH RESOLUTION CRYSTAL STRUCTURES OF 2,3-DIHYDROXYBIPHENYL 1,2-DIOXYGENASE FROM A PCB-DEGRADING BACTERIUM COMPLEXED WITH SUBSTRATES Jeffrey T. Bolin, Seungil Han, Lindsay D. Eltis[a], Biological Sciences/LILY, Purdue University, W. Lafayette, IN 47907, U.S.A; [a]Biochemistry Dept., Université Laval, Ste-Foy, Quebec, Canada

2,3-dihydroxybiphenyl 1,2-dioxygenase (DHBD) catalyses the ring cleavage step in the aerobic degradation of biphenyl and PCBs using an Fe(II)-dependent, extradiol cleavage mechanism. Crystal structures of DHBD in complexes with 2,3-dihydroxybiphenyl, 3-methylcatechol, and catechol have been determined at 2.3 Å, 1.9 Å, and 1.9 Å resolution, respectively. The structure of the free enzyme has also been determined at 1.9 Å resolution[1].

These studies target the DHBD from *Pseudomonas* LB400, an organism that transforms a broad range of PCBs. The enzyme is a *D*4 symmetric homooctomer of 298 residue subunits and contains one Fe per subunit. Crystallization and diffraction studies were performed under anaerobic conditions so as to maintain the active, Fe(II) form of the enzyme.

These are the first structures of any extradiol dioxygenase in the Fe(II) form. As such they provide a structural framework for discussion of the mechanism of extradiol ring cleavage reactions as well as the potential adaptation of this enzyme and the biphenyl degradation pathway to achieve bioremediation of PCBs.

The structures of substrate complexes show bidentate binding via the vicinal hydroxyl oxygens. However, the two Fe-O bond lengths are markedly different, suggesting monoanionic binding and deprotonation of the 2-hydroxyl in the case of 2,3-dihydroxybiphenyl. The active site structure in the complexes is similar to that of the free enzyme, but there are several important differences including a > 0.5 Å shift of the Fe and its ligands, and subtle changes in the hydrogen bonding pattern that may be important for the mechanism.

1. S. Han *et al.*, *Science* **270**, 976-980, 1995.

PS04.02.11 STRUCTURAL ELEMENTS OF PROTON TRANSFER IN MURINE CARBONIC ANHYDRASE V. P. Ann Boriack-Sjodin, Dept. Of Chemistry, University of Pennsylvania, 231 S. 34th Street, Philadelphia, PA 19104-6323

Possible pathways for the proton transfer in the mechanism of murine carbonic anhydrase V have been studied using X-ray crystallographic methods. Carbonic anhydrase V is the most recent isozyme in the carbonic anhydrase family whose structure has been solved using crystallographic techniques[1]. The proton acceptor of this enzyme has a pK_a of 9.2[2]. The proton shuttle in the prototypical enzyme carbonic anhydrase II is His 64, however, a proton transfer pathway through Tyr 64 in carbonic anhydrase V is compromised due to the adjacent bulky side chain of Phe 65. A variant of murine carbonic anhydrase V which removes the bulky side chain and mimics the proton shuttle of carbonic anhydrase II, Tyr64→His Phe65→Ala, is more active than the wild-type enzyme, while a single mutation at position 64 (Tyr64→His) does not affect proton transfer[2]. Removing the bulky side chain at position 65 allows a second proton transfer pathway to form with a pK_a of 7 while maintaining the original pathway. Structural analysis of the wild-type enzyme reveals other possible proton transfer groups.

Boriack-Sjodin *et al.*, (1995) *Proc. Natl. Acad. Sci. USA* **92**, 10949-10953.
2 Heck *et al.*, (1994) *J. Biol. Chem.* **269**, 24742-24746.

PS04.02.12 THE ROLE OF ZINC IN DEHYDROQUINATE SYNTHASE: COMPARISON OF MECHANISTIC AND STRUCTURAL DATA. K.A. Brown[1], E.P. Carpenter[2], G.G. Dodson[2], A.R. Hawkins[3], J.D. Moore[3] and J.W. Frost[4], Dept of Biochemistry, Imperial College, London, UK[1], National Institute For Medical Research, Mill Hill, London, UK[2], Dept of Biochemistry and Genetics, University of Newcastle, UK[3], Dept of Chemistry, Michigan State University, East Lansing, MI[4]

Dehydroquinate synthase (DHQS) is a shikimate pathway enzyme which utilizes Zn(II) and NAD to convert 3-deoxy- D - arabino-heptulosonate phosphate to 3-dehydroquinate using a diverse reaction mechanism. This mechanism requires an oxidation, a β-elimination, a reduction, a ring-opening and an aldol condensation. Enzymological studies have shown that divalent metal cations are necessary for the enzyme's activity (1-3). Although it is known that metals can be used in catalyzing the various reactions listed above, the precise role of Zn(II) in the DHQS mechanism is still poorly defined. In order to clarify mechanistic details, the crystallographic determination of th structure of DHQS has recently been undertaken. The molecular organization of this enzyme and potential roles for its cofactors will be discussed in light of emerging structural details.

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