PS04.01.08 X-RAY STUDIES ON THE STRUCTURE, SPECIFICITY AND MECHANISM OF THE OPINE DEHYDROGENASE ENZYME SUPERFAMILY. [1]S.J. Langridge, [1]K.L. Britton, [2]Y. Asano and [1]D.W. Rice, [1]Krebs Institute, University of Sheffield, Sheffield, S10 2TN, UK and [2]Biotechnology Research Center, Toyama Prefectual University, Toyama 939-03, Japan.

The structure of a novel opine dehydrogenase from *Arthrobacter* has been solved by isomorphous replacement and provides insights into the mechanism and substrate specificity of the enzyme superfamily to which it belongs. Crown gall opines are the products of the NAD(P)H-dependent reductive condensation between an α-keto acid and the α- or ω-NH_2 group of an amino acid in a reaction catalysed by a family of enzymes, generically referred to as the opine dehydrogenases (reviewed in Thompson, J. and Donkersloot, J.A. Annu. Rev. Biochem. 1992 61 517-557). The enzymes catalysing this chemistry are encoded on large plasmids resident in virulent strains of *Agrobacterium*. These tumour inducing plasmids are required for crown gall induction and tumourogenesis involves the excision of a segment of the plasmid DNA on which the opine dehydrogenase gene is located. Following integration of the DNA into the plant genome, the plant cell machinery is hijacked to divert resources to the synthesis of opines which permit growth of the tumour. Sequence studies have established that opine dehydrogenases belong to an enzyme superfamily with differential specificity for the keto acid and amino acid partners. Recently, the gene for a novel opine dehydrogenase from *Arthrobacter* has been sequenced and shown to have 30% sequence identity with the octapine dehydrogenase from *Agrobacterium tumefaciens*. This enzyme is a homodimer of subunit Mr 70,000 and has been overexpressed in *E. coli* and crystallised. X-ray analysis shows that the crystals which diffract to beyond 1.8 Å belong to the orthorhombic space group $P2_12_12$ with a = 104Å, b = 79Å and c = 45Å with a single monomer in the asymmetric unit. The structure will be described and insights into the catalytic mechanism and the differential substrate specificity will be discussed.

PS04.01.09 TYR-LYS BASED CATALYSIS : THE FOUNDATION FOR STRUCTURAL HOMOLOGY AMONG SHORT-CHAIN DEHYDROGENASE/REDUCTASES. D. Ghosh*, W. L. Duax, V. Pletnev, B. M. Burkhart, N. Li, Hauptman-Woodward Institute, 73 High St., Buffalo, NY 14203 USA, *also Roswell Park Cancer Inst., Buffalo, NY.

Short chain dehydrogenase/reductases (SDR) constitute a new class of dinucleotide-linked oxidoreductases that use a wide variety of substrates. We have determined the crystal structures of various forms of two members of the SDR family: the bacterial 3α,20β-hydroxysteroid dehydrogenase and the human type 1 estrogenic 17β-hydroxysteroid dehydrogenase. Structures of five other SDR's have been determined elsewhere. These are : rat liver dihydropteridine reductase, oilseed rape and bacterial enoyl acyl carrier protein reductase, mouse lung carbonyl reductase and bacterial 7α-hydroxysteroid dehydrogenase. Despite a low intra-family amino acid sequence identity (15-35%), these enzymes are characterized not only by strikingly similar subunit tertiary structures that include a dinucleotide-binding fold, but also an analogous quaternary association and a strictly conserved Tyr-Lys pair at the catalytic end of the active site. The conserved catalytic residues form a Tyr-Lys-Ser triad in many of the SDR's, owing to a semi-conserved Ser residue. A mechanism in which the Tyr-Lys pair acts as a general acid and an electrophile to the substrate carbonyl has been proposed.

All seven enzymes of the SDR family form homo-dimeric or -tetrameric crystal structures. While the C-terminal residues that provide substrate-specific interactions are situated at the outer periphery of the oligomer, the Tyr-Lys pair is located on the helix that forms the four-helix bundle dimer interface - the so-called Q-axis dimer, the most conserved interface in the family. The formation of the core of the oligomer, a consequence of tertiary structures of subunits and their quaternary association, is thus influenced by the presence of the Tyr-Lys pair. The dinucletide-binding fold may have little effect on the subunit association, since other well-known dinucleotide-binding enzymes, such as long-chain dehydrogenases, have different quaternary structures. In SDR's, unlike the long-chain dehydrogenases, the catalytic end of the active site borders the interfacial structure elements, such as a helices and ß sheets, near the inner core of the functional oligomers. This suggests that the evolution of the SDR functional oligomer may be driven by the requirements of a Tyr-Lys based catalytic mechanism. Supported by Grant No. DK26546.

PS04.01.10 GLUCOSE 6-PHOSPHATE DEHYDROGENASE: LINKS BETWEEN STRUCTURE AND DEFICIENCY. CE Naylor, AK Basak, S Gover, PJ Mason and MJ Adams

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is the world's most common genetic disease. It affects an estimated 400 million people. The symptoms stretch from the minor (acute haemolytic attacks triggered by a variety of sources) to the severe (chronic non-spherocytic haemolytic anaemia).

PS04.01.11 CRYSTAL STRUCTURE OF GLYCOSOMAL GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM LEISHMANIA MEXICANA: MECHANISTIC IMPLICATIONS AND POTENTIAL DRUG BINDING SITES. Hidong Kim[1,2], Christophe L.M.J. Verlinde[1], Alexander Aronov[3], Michael H. Gelb[3], Piet Herdewijn[4], and Wim G. J. Hol[1,2], [1]Departments of Biological Structure and Biochemistry, and Biomolecular Structure Center, University of Washington, Seattle, Washington, [2]Howard Hughes Medical Institute, University of Washington, Seattle, Washington, [3]Department of Chemistry, University of Washington, Seattle, Washington, [4]Laboratory of Medicinal Chemistry, Rega Institute, Catholic University of Leuven, Leuven, Belgium

The structure of glycosomal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with bound NAD+ from the trypanosomatid parasite Leishmania mexicana has been determined by X-ray crystallography. The protein crystallizes in space group $P2_12_12_1$ (a = 99.0 Å, b = 126.5 Å, c = 138.9 Å) with one 156 kDa protein tetramer per asymmetric unit. Density modification by four-fold noncrystallographic symmetry averaging was used during model building and refinement. The overall structure of L. mexicana GAPDH is similar to previously determined structures of GAPDHs from other species, including the closely related trypanosomatid Trypanosoma brucei, the causative agent of African sleeping sickness. A significant structural difference between L. mexicana GAPDH and most other GAPDHs occurs in a loop region located at the active site. This unusual loop conformation in L. mexicana GAPDH occludes the inorganic phosphate binding site which has been seen in previous GAPDH structures, and results in a new inorganic phosphate position in L. mexicana GAPDH. Modelling studies indicate that this new anion binding site is well situated for nucleophilic attack of the inorganic phosphate on the thioester intermediate in the GAPDH-catalyzed reaction. In addition, L. mexicana GAPDH is being used as a target for structure-based design of trypanocidal compounds. The structures of GAPDHs from L. mexicana and T. brucei have been used to develop a new inhibitor which is nearly four orders of magnitude more potent than its lead compound.