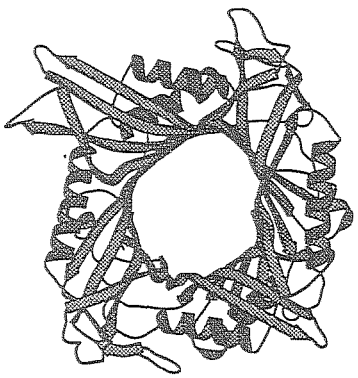
MS04.01.05 AN EVOLUTIONARY FUSION OF TWO ENZYMES: STRUCTURE OF THE UNIQUE BIFUNCTIONAL ENZYME 6-PHOSPHOFRUCTO-2-KINASE/FRUCTOSE-2,6-BISPHOSPHATASE. Charles A. Hasemann*, E. Istvan#, K. Uyeda#, and Johann Deisenhofer#t, Depts. of Internal Medicine, Biochemistry#, and the Howard Hughes Medical Inst, UT Southwestern Medical Center, Dallas, Texas 75235-8884

The evolutionary fusion of two protein domains with antagonistic catalytic activities has led to the unique bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6-PF-2-K/Fru-2,6-P_2ase). This enzyme indirectly controls the rate of glycolysis via the synthesis and degradation of fructose-2,6-bisphosphate (Fru-2,6-P_2), a potent activator of phosphofructokinase (PFK). 6-PF-2-K/Fru2,6P_2ase is found as a homodimer of ~56 kD subunits in the cytosol of all eukaryotes. The need for glycolytic activity varies both with time and from tissue to tissue, and thus the activity of 6-PF-2-K/Fru2,6P_2ase regulated both in time, and in a tissue specific manner. Consequently, tissue specific isoforms of 6-PF-2-K/Fru2,6P_2ase have been identified (liver, muscle, heart, testis, and brain), each with a characteristic relative ratio of kinase to phosphatase activity, and each regulated by inhibitors (i.e., PEP, citrate, phosphoglycerate) which vary in concentration with the metabolic needs of the cell. The activities of the liver and heart COOH-terminal regulatory domains. The phosphorylation of the liver isozyme has been shown to coordinately modulate both the 6-PF-2-K and 6-PF-2-K/Fru2,6P_2ase activities, are fused in a single polypeptide.

We have recently solved by X-ray diffraction the 2.0Å resolution structure of the rat testis isozyme of 6-PF-2-K/Fru2,6P_2ase. As predicted by sequence homology, the Fru2,6P_2ase domain resembles the structure of yeast phosphoglycerate mutase, and these enzymes no doubt share a common catalytic mechanism involving a phosphohistidine intermediate. Surprisingly, the 6-PF-2-K domain does not resemble PFK as had been predicted, but instead has clearly evolved from the adenylate kinase family. This homology, coupled with the apparent lack of a nucleophile to activate the 2-hydroxyl of F6P, points to a mechanism of catalysis distinct from that of PFK. The domain interface in the monomer, and the orientation of the domains in the functional dimer, provide clues to the regulation by phosphorylation observed in the liver and heart isozymes.

MS04.01.06 STRUCTURE OF URATE OXIDASE OF *Aspergillus flavus* : AN ORIGINAL FOLD AND A NEW MECHANISM. N. Colloc'h[1], M. El Hajji[2], B. Castro[3], T. Prangé[4], M. Schiltz[4], B. Bachet[1] and J.P. Mornon[1]. [1]Laboratoire de Minéralogie-Cristallographie, 4 place Jussieu, 75252 Paris cedex 05, France; [2]SANOFI Chimie, route d'Avignon, 30390 Aramon, France; [3]SANOFI Chimie, 82 avenue Raspail, 94255 Gentilly cedex, France; [4]LURE, Université Paris-Sud, Bat. 209d, 91405 Orsay cedex, France



The crystal structure of urate oxidase from *Aspergillus flavus* (UoAf) has been solved at 2Å resolution using MIR. The dimer of this first structure of an urate oxidase displays an original fold described by a barrel of 16 antiparallel strands surrounded by 8 helices. The active form of UoAf is a tetramer (301 residues per chain), made up with 2 dimers above each other. A tunnel (diameter : 15Å, length: 50Å) run accross the tetramer. Urate oxidase is an enzyme that catalyses the conversion of uric acid to allantoin. UoAf has been crystallized with an inhibitor, the azaxanthine. This inhibitor is localized between 2 monomers, in a pocket looking toward the exterior of the barrel. The mechanism of oxidation of acid uric to allantoin is currently under investigation, since no copper, nor any similar ion, have been found in the structure. UoAf is produced and marketed by Sanofi. Direct injection of UoAf helps to prevent or resolve hyperuricemia disorders that may occur during chemiotherapeutic treatment, or in the case of gout. UoAf crystallized in the space group I222 with a=81.33Å, b=96.28Å, c=105.63Å with one monomer in the asymmetric unit. The structure has been solved by isomorphous replacement using 3 derivatives (lead, mercury, xenon under pressure). Refinement is in progress using X-Plor and the current R-factor is 0.22 for data to 2Å resolution.

MS04.01.07 ARGININOSUCCINATE LYASE & δ II CRYSTALLIN: INTRAGENIC COMPLEMENTATION AND CATALYSIS. P. Lynne Howell[1,2], Mary A. Turner[1], Mona Abu Abed[1], Rod R. McInnes[1,3], [1]Hospital for Sick Children, Toronto, Ontario, M5G 1X8, [2]Departments of Biochemistry and [3]Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, M5S 1A8

Argininosuccinate lyase (ASL) and δ II crystallin are homologous proteins with different biological functions but identical enzymatic activities. Both proteins catalyze the reversible cleavage of argininosuccinate into arginine and fumarate. Genetic defects in ASL result in *argininosuccinic aciduria*, the second most common urea cycle disorder. In addition to the 13 unique ASL mutations that have been identified, intragenic complementation has also been demonstrated at the ASL locus. This is a phenomenon that occurs when a multimeric protein is formed from monomers produced by two different mutant alleles of the same gene. Of the complementing alleles found to date, a combination of the Q286R allele with the D87G allele is the most successful with the greatest restoration of activity. While the homotetrameric mutant proteins were inactive, the hybrid multimers exhibit approximately 30% of wild-type activity.

In order to understand how the enzymatic activity is restored and to elucidate the catalytic mechanism for the reaction, we have determined the X-ray crystal structures of human ASL and wild-type and several duck δ II crystallin mutants. The structural results suggest that the restoration of enzymatic activity in the hybrid tetramers is due to the reconstruction of one or more "native" like active sites and that Asp 87 and His 89 are involved in binding the arginine end of the substrate molecule.

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