is grown on acetate, IDH is in its inactive phosphorylated form, thus inhibiting Krebs' cycle. Alternatively, a change of carbon source to glucose or pyruvate results in the activation of IDH by dephosphorylation, and the initiation of Krebs' cycle. The function of AceK and its involvement in the regulation of Krebs' cycle and the glyoxylate bypass is well-characterized, but its structural and mechanistic qualities have remained relatively unknown. The determination of the crystal structure could provide confirmation of the function of AceK by identifying the various kinase, phosphatase and ATPase domains and insights into the coordination of the kinase and phosphatase activity of AceK. Of note, it is currently unknown how, or if at all, the active site changes conformation as it switches between kinase and phosphatase activity. Three Acek crystal forms were obtained and SAD datasets were collected at CHESS and BNL synchrotron source. The AceK structure is determined at 2.6 Å. The overall AceK structure displays a typical eukaryotic kinase folding.

Keywords: kinase, phosphatase, isocitrate dehydrogenase

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Structure of decameric PLP-dependent acid induced arginine decarboxylase from *Escherichia coli*

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In order to infect a human host, enteric *Escherichia coli* must pass through the stomach, which has a pH of around 2.0, and survive there for approximately two hours before the stomach is emptied. *E. coli* is capable of surviving in the highly acidic environment of the stomach since it possesses systems that make it acid resistant. Three such systems have been identified, one of which is the arginine-dependent acid resistance system (AR3). AR3 requires the presence of arginine and is dependent on the acid induced arginine decarboxylase (AdiA) and the arginine-agmatine antiporter (AdiC). AdiA converts one molecule of arginine into agmatine and AdiC transports the agmatine out of the cell in exchange for arginine. Through this enzymatic cycle, AR3 acts to protect the organism by preventing the accumulation of protons inside the cell. In order to investigate the enzymatic mechanism involved in AR3, we have determined the structure of the acid induced arginine decarboxylase, AdiA, by X-ray crystallography. The AdiA structure, solved by multiple isomorphous replacement (MIR), revealed a ca. 800 kDa decameric assembly with unusual five-fold non-crystallographic symmetry. Presented here is the structure of AdiA from E. coli refined to 2.4 Å resolution, its pyridoxal-5'-phosphate (PLP) containing active site and its large macromolecular assembly.

Keywords: arginine decarboxylase, macromolecular proteins, acid resistance

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The substrate recognition and the catalytic reaction mechanisms of D-3-hydroxybutyrate dehydrogenase

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D-3-Hydroxybutyrate dehydrogenase (HBDH) is an NAD$^+$-dependent enzyme for reversible conversion between D-3-hydroxybutyrate and acetoacetate. These ketone bodies are important energy sources, but their excess level causes ketoacidosis. HBDH can be used as a marker in the assay of diabetes mellitus, and/or ketoacidosis. To reveal the reaction mechanism, the crystal structures of *Alcaligenes faecalis* HBDH in the *apo* form and two different *holo* forms, one with a substrate analogue, acetate (HBDH-NAD-AC) and the other with the reaction product, acetoacetate (HBDH-NAD-AA), have been determined by X-ray crystallography. The physiologically active enzyme is a homo tetramer assembled according to the non-crystallographic 222 point symmetry. Each subunit has a principal domain with a typical Rossmann fold. The two helices, H6 and H7 of the small domain, move to the principal domain to trap NAD$^+$ in a cavity. In HBDH-NAD-AC complex, an acetate ion is directly bound to the residues Q94, H144, K152 and Q196 through hydrogen bonds. A water molecule is bound to S142 and Y155, mimicking the hydroxyl group of the substrate D-3-hydroxybutyrate. The position of the water oxygen atom is near the methyl group of the acetate. These structural features strongly suggest the binding scheme of the true substrate in the enzyme-NAD complex. In the HBDH-NAD-AA crystal, obtained by adding the substrate, the reaction product acetoacetate has been found in the active site. This structure is consistent to our proposed reaction mechanism [1]. The basic structural architecture of the enzyme is highly conserved as a member of the SDR family with the catalytic triad of S142, Y155 and K159.

[1] Hoque *et al*., *Acta Cryst* . D **64**, In press (2008).

Keywords: hydroxybutyrate dehydrogenase, ketone bodies, X-ray structure

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Using natural variations among shikimate dehydrogenases to study modes of substrate selectivity

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In plants, fungi and bacteria, aromatic amino acids are synthesized via the shikimate pathway. The pathway consists of seven enzyme-catalyzed reactions ending with the production of chorismate, a precursor of the amino acids phenylalanine, tryptophan and tyrosine, as well as a number of other aromatic compounds. The fourth step of the pathway, in which dehydroshikimate is reduced to shikimate, is catalyzed by shikimate dehydrogenase (SDH). In bacteria, kinetic and phylogenetic analyses have identified five SDH functional classes, annotated AroE, YdiB, RifI, SdhL and most recently, Ael1. Representative crystal structures have been determined for all classes except RifI. We are the first to present the crystal structure of the novel Ael1 homolog from *Pseudomonas putida*. SDH structures share a high degree of similarity and conservation of key residues involved in catalysis.[1] However, each SDH class has a distinct biochemical profile. While AroE is the archetypal SDH, YdiB has dual substrate specificity, accepting both shikimate and quinate.[2] RifI is predicted to accept amino-dehydroshikimate during the biosynthesis of the antibiotic rifamycin B.[3] The biological substrate of SdhL is unknown. Ael1 can catalyze the reduction of dehydroshikimate with a higher binding affinity but lower turnover rate than the AroE homolog. The natural enzymatic diversity observed among SDH classes provides an ideal system for studying modes of substrate selectivity.

(1) Singh *et al*. (2005). *J. Biol. Chem., 280*: 17101-17108.
(2) Michel *et al*. (2003). *J. Biol. Chem., 278*: 19463-19472.
(3) Yu *et al*. (2001). *J. Biol. Chem., 276*: 12546-12555.

Keywords: dehydrogenases, cocrystals, structure-function relationships

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Structure-function analysis of Eyes absent protein, aspartate dependent protein tyrosine phosphatase

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Protein tyrosine phosphatases (PTP)represent a large family of proteins involved in fundamental cellular signaling pathways. Eyes Absent(Eya) proteins have the peculiar characteristics that they do not contain the classical active site cysteine present in case of classical PTPs. Taking into account that the activity of this enzyme is depending on Mg^{2+} ions, determination of the 3D-structure would consistently help in evidencing the active site nucleophile and the general acid as well as the role played by the essential Mg^{2+} ion. We cloned full length human Eya3 gene from a human cDNA library and inserted it into pHAT2 prokaryotic expression vector. Recombinant protein thus obtained was further purified yielding pure enzymatic preparations. To evidence that the pure protein displays phosphatase activity several typical substrates were tested. pNPP and DiFMUP were found as efficient enzymatic substrates of hEya3. Using highly pure protein preparations various crystallization setups have been performed. Optimization of the promising precipitants led to needle-like microcrystals of hEya3. Further optimization of crystallization conditions as well as application of seeding procedures is expected to produce suitable crystals for diffraction experiments. Human Eya3 a non-typical protein tyrosine phosphatase was cloned from a cDNA library, the recombinant protein was expressed in prokaryotic system, purified to high purity and enzymatic activity was evidenced using typical phosphatase substrates. Optimization of crystallization setups produced microcrystals. Further increase of the microcrystal dimensions using additional crystallographic techniques expectedly will provide well diffracting crystals and finally the 3D- structure of new type of PTP.

Keywords: eyes absent, aspartate PTP, microcrystal

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First crystallographic structure of mammalian phosphofructokinase from rabbit skeletal muscle

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Phosphofructokinase (Pfk) is a key enzyme of the glycolytic pathway, which is present in all organisms. Pfk is the main regulatory point of the pathway and catalyses phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate in the presence of ATP. Its molecular characteristic as well as its allosteric regulation by various effectors depend on the source of the enzyme. In bacteria the enzyme is a homotetramer with subunits molecular weight of about 35kDa, while most of the eukaryotic Pfks consist of “double-size” subunits when compared with the bacterial ones. Mammalian Pfk consists of subunits of about 80-85kDa molecular weight and is active as a tetramer or in more aggregated forms. The crystal structure of Pfk from rabbit muscle was determined to 3.2Å resolution. The protein model has two subunits in the asymmetric unit consisting of 748 amino acid residues. Nucleotides and phosphate ions have been found interacting with the protein molecules. The crystallographic model of Pfk from rabbit muscle is the first model of a eukaryotic Pfk which evolved by duplication and fusion of the genes as well as the first structure of Pfk from higher organisms. Amino acid sequence of the rabbit muscle enzyme shows 96% identity with the sequence of human muscle Pfk, so the knowledge of the rabbit muscle Pfk structure can be important for research on human Pfk and diseases related to this enzyme (Tarui disease).

Keywords: allosteric enzymes, metabolism enzyme, muscle proteins

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Crystal structure of a family I.3 lipase from *Pseudomonas* sp. MIS38 in a closed conformation

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Pseudomonas sp. MIS38 lipase (PML) is a family I.3 lipase, which is secreted by the type I secretion system. PML consists of two domains, an N-catalytic domain and a C-domain that contains a secretion signal and thirteen repeats of RTX (Repeats in ToXin) sequence motif that form β-roll structure(s) in the presence of Ca^{2+} ions. The β-roll structure was proposed to have a chaperone-like function with an unknown mechanism. PML has also been shown to exhibit interfacial activation, which indicate the presence of a lid structure that covers the active site and opens up upon contact