Acta Cryst. (2002). A**58** (Supplement), C105

DIAGNOSING THE STRUCTURAL MECHANISM OF APS KINASE

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The sulfate assimilation pathway is an essential pathway found in all organisms to convert inorganic sulfate into a biologically active carrier molecule, 3'-phosphoadenosine-5'-phosphosulfate (PAPS). PAPS is the universal sulfur donor delivering sulfur for all cellular needs. The intermediate in the pathway, adenosine-5'-phosphosulfate (APS), is converted to PAPS by the enzyme APS kinase. This enzyme from the fungi *Penicillium chrysogenum* follows a sequential ordered mechanism in which MgATP binds in the active site before APS and the product PAPS disassociates before MgADP. APS can bind in the activesite before MgADP is released, inhibiting the enzyme. Our lab previously solved the crystal structure of APS kinase. The structure revealed a classic Walker A motif together with a disordered domain adjacent to the active site P-loop. Presented here is the complex of APS kinase co-crystallized with MgADP and APS in the space group $P2_12_12_1$. The crystal structure was solved by bromide MAD phasing to 1.9 Å with phase extension to 1.4 Å. This structure reveals the binding sites of both substrates and allows the identification of essential residues for catalysis. Later it was determined that transferring the crystals into a new mother liquor that did not contain APS caused the substrate to disassociate from the enzyme leaving only ADP bound. Major structural differences revealed by this soak was a shift of 3.0 Å in a loop, which partly bound APS, to the original position in the apo structure.

Keywords: APS KINASE, PHOSPHATE TRANSFER, SULFATE METABOLISM

Acta Cryst. (2002). A**58** (Supplement), C105

CRYSTAL STRUCTURE OF RABBIT PHOSPHOGLUCOSE ISOMERASE COMPLEXED WITH ITS SUBSTRATE D-FRUCTOSE 6-PHOSPHATE IDENTIFIES THE RING OPEING STEP IN CATALYSIS

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Phosphoglucose isomerase (PGI) catalyzes the interconversion of D-glucose-6-phosphate (G6P) and D-fructose-6-phosphate (F6P) and plays important roles in glycolysis and gluconeogenesis. Biochemical characterization of the enzyme has led to a proposed multistep catalytic mechanism. First, the enzyme catalyzes ring opening to yield the open chain form of the substrate. Then isomerization proceeds via proton transfer between C2 and C1 of a cis-enediol (ate) intermediate to yield the open chain form of the product. Catalysis proceeds in both the G6P to F6P and F6P to G6P directions, so both G6P and F6P are substrates. X-ray crystal structure analysis of rabbit and bacterial PGI has previously identified the location of the enzyme active site and Glu357 as a candidate functional group for transfering the proton. However, it was not clear which amino acid residues in active site catalyze the ring-opening step. We report the X-ray crystal structure of rabbit PGI complexed with the cyclic form of its substrate, D-fructose-6-phosphate, at 2.1 Å resolution. The location of the substrate relative to the sidechains of His388 suggest that His388 promotes ring opening by protonating the ring oxygen. Glu216 helps position His388, and a water molecule that is held in position by Lys518. Comparison to a structure of rabbit PGI with 5PAA bound indicates that ring opening is followed by loss of the protonated water molecule and conformational changes in the substrate and the protein so that a helix containing amino acids 513-520 moves in towards the substrate to form additional hydrogen bonds with the substrate.

Keywords: **PHOSPHOGLUCOSE ISOMERASE COMPLEXED WITH SUBSTRATE**

Acta Cryst. (2002). A**58** (Supplement), C105

THE CRYSTAL STRUCTURE OF DESULFURIZATION ENZYME DszB

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Sulfur oxides released from fossil fuel combustion have been suggested as the major cause of acid rain. Dibenzothiophene, one of the major organic sulfur compounds in the higher boiling fraction of crude oil, is the model compound for studying the desulfurization by microorganisms. Desulfurization operon dsz identified from *Rhodococcus sp*. codes DszA, DszC, NADH dependent monooxygenases, and DszB, an aromatic sulfinic acid hydrolase. We report the crystal structure of DszB.

The structure of DszB has been determined by single wavelength anomalous scattering method and refined to the resolution of 1.8 Å. The model contains 347 amino acids and lacks 18 residues in the N-terminal. The overall fold and topology of secondary structures of DszB are similar to those of periplasmic substrate binding proteins (SBPs), which are composed of two αβ domains related by pseudo 2-fold rotation symmetry. The active site of DszB is located in the cleft between the two domains as is the substrate binding sites of SBPs.

The presumed active site of DszB is composed of nucleophile C27 and E192 which is separated about 4Å from C27 and seems to activates C27. G73 from conserved RXGG motif (starts at R70) acts as an oxyanion hole to stabilize the negative charge built in the reaction intermediate. Hydrophobic amino acids contribute to the binding of the biphenyl ring of substrate.

Keywords: **DESULFURIZATION HYDROLASE DSZB**

Acta Cryst. (2002). A**58** (Supplement), C105

STRUCTURE OF PYRUVATE FORMATE-LYASE IN COMPLEX WITH A NATURAL SUBSTRATE, PYRUVATE

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Pyruvate formate-lyase (PFL; E.C., 2.3.1.54) is an anaerobic enzyme that catalyses the conversion of pyruvate and coenzyme A to acetyl-coenzyme A. PFL is a homodimer of 85 kDa subunits displaying half-site reactivity [1]. Active PFL contains a relatively stable glycyl radical located at Gly734 [2]. The structure of PFL is already known [3,4] and now we have solved at 2.7 Å resolution with pyruvate included in the crystallization mixture. Both active sites of homodimeric enzyme are occupied with pyruvate and additional binding sites were not found. Pyruvate was modeled to a cleft close to the active site cysteines 418 and 419 with the carboxyl group in contact with arginines 176 and 435 and methyl group within van der Waals distance to Phe327. The binding site of pyruvate is most likely not the position of pyruvate as the reaction initiates, because there will be conformational changes during the activation of PFL.

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Keywords: **PYRUVATE FORMATE-LYASE, ENZYME, SUBSTRATE COMPLEX**