

wild-type UPPs and the Asp26Ala mutant are crystallized in a new trigonal unit cell with Mg<sup>2+</sup>/IPP/FsPP (farnesyl thiopyrophosphate, an FPP analogue) bound to the active site. Our results here improve the understanding of the prenyltransferases reaction significantly.

**Keywords:** prenyltransferase, crystal structure, metal ion

#### P.04.02.55

*Acta Cryst.* (2005). A61, C193

##### Crystal Structure of Human HMG-CoA lyase

Zhuji Fu<sup>1</sup>, Jennifer A. Runquist<sup>1</sup>, Henry M. Miziorko<sup>2</sup>, Jung-Ja P. Kim<sup>1</sup>, <sup>1</sup>Dept. of Biochemistry, Medical College of Wisconsin, Milwaukee, WI 53226, USA. <sup>2</sup>School of Biological Science, University of Missouri-Kansas City, Kansas City, MO 64110, USA. E-mail: zhuji@mcw.edu

3-Hydroxyl-3-methylglutaryl-CoA (HMG-CoA) lyase (EC 4.1.3.4) catalyzes the divalent cation dependent cleavage of HMG-CoA to form acetyl-CoA and acetoacetate. This reaction is a key step in ketogenesis and the final step in leucine catabolism. Human HMG-CoA lyase has been previously cloned and overexpressed in *Escherichia coli*. Crystals of the lyase containing a competitive inhibitor have been obtained with PEG 8K using sitting-drop vapor diffusion method. The crystals belong to the monoclinic space group C2, with unit cell parameters a=197.0Å, b=116.1Å, c=86.8Å, and β=122.5°. The native data set has been collected which is 99.8 % complete to 2.1 Å. The calculated V<sub>m</sub> is 2.34 Å<sup>3</sup> Da<sup>-1</sup>, with a solvent content of 43.2%, which corresponds to three dimers of the enzyme in the asymmetric unit (each dimer molecular weight, 65 kDa). One heavy atom derivative (Hg) has been obtained and the search for additional derivatives is in progress.

**Keywords:** HMG-CoA lyase structure, fatty acid metabolism, ketogenesis

#### P.04.02.56

*Acta Cryst.* (2005). A61, C193

##### Structural Analysis of Thr342 Mutants of Soybean β-Amylase: The Role of Conformational Changes of Two Loops in the Catalytic Mechanism

Bunzo Mikami, You-Na Kang, Aiko Tanabe, Motoyasu Adachi, Laboratory of Food Quality Design and Development, Graduate School of Agriculture Kyoto University, Japan. E-mail: mikami@kais.kyoto-u.ac.jp

Soybean β-amylase has two mobile loops in the active site, a flexible loop (residue 96-103) and an inner loop (residues 340-346). The flexible loop moves about 10 Å from “open” to “closed” form to make interactions with substrate. Though the movement is relatively small (about 3 Å), two different conformations of the inner loop have been found in the enzyme/substrate complexes. In the “product form”, the Thr 342 residue creates hydrogen bonds with the Glu 186 (catalytic acid) and with the glucose residues at subsites -1 and +1, whereas most of those interactions are lost in the “apo form”. To elucidate the relationship between the structural states of inner loop and the catalytic mechanism, Thr 342 was mutated to Val, Ser, and Ala, respectively, and their crystal structures complexed with maltose were determined together with that of the apo enzyme at 1.27-1.64 Å resolutions. The *k*<sub>cat</sub> values of the T342V, T342S, and T342A mutants decreased by 13-, 360- and 1700-fold, respectively, compared to that of the wild-type enzyme. Whereas the inner loops in the wild-type/maltose and T342V/maltose complexes adopted the product form, those of the T342S/maltose and T342A/maltose complexes showed the apo form. Structural analyses suggested that the side-chain of Thr 342 in product form plays an important role in distorting the sugar ring at subsite -1, stabilizing the deprotonated form of Glu 186, and grasping the glucose residue of the remaining substrate at subsite +1.

**Keywords:** amylases, mutations, enzymatic reaction mechanism

#### P.04.02.57

*Acta Cryst.* (2005). A61, C193

##### Crystal Structure of the Haloalkane Dehalogenase DbjA

Yukari Sato<sup>a,b</sup>, Ryo Natsume<sup>b</sup>, Zbynek Prokop<sup>c</sup>, Miki Senda<sup>b</sup>, Jiri

Damborsky<sup>c</sup>, Yuji Nagata<sup>a</sup>, Masataka Tsuda<sup>a</sup>, Toshiya Senda<sup>d</sup>, <sup>a</sup>Graduate School of Life Sciences, Tohoku University. <sup>b</sup>JBIRC, JBIC. <sup>c</sup>Loschmidt Laboratories, Masaryk University. <sup>d</sup>BIRC, AIST. E-mail: yukari@ige.tohoku.ac.jp

Haloalkane dehalogenases are key enzymes that catalyze hydrolytic conversion of halogenated aliphatic compounds to alcohol and hydrogen halide. The enzymes have good potential for bioremediation, biosensing and biocatalysis. Recently, the haloalkane dehalogenase DbjA from *Bradyrhizobium japonicum* USDA110 was revealed to have a sufficient enantioselectivity for industry scale synthesis of optically active alcohols. This is the first example of enantioselective catalysis among the haloalkane dehalogenases. To reveal the enantioselectivity mechanism of DbjA on the basis of the structure, we determined the crystal structure of DbjA at 1.47 Å resolution by the molecular replacement method. DbjA has an alpha/beta hydrolase fold. The architecture of specificity-determining cap domain is, however, different from three dehalogenases with known crystal structure. The results of structure-function analysis will be presented.

**Keywords:** enantioselectivity, alpha/beta hydrolase, haloalkane dehalogenase

#### P.04.02.58

*Acta Cryst.* (2005). A61, C193

##### Structure-Function Analysis of a Novel Mitochondrial Antioxidant Protein

Zhenbo Cao<sup>a,b</sup>, Louise Gourlay<sup>a</sup>, Neil Isaacs<sup>b</sup>, Gordon Lindsay<sup>a</sup>, <sup>a</sup>Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences <sup>b</sup>Department of Chemistry, University of Glasgow, UK. E-mail: 0108962c@student.gla.ac.uk

Bovine mitochondrial SP-22 is a member of the peroxiredoxin family which is a novel antioxidant enzymes. It belongs to the typical 2-Cys Prxs containing three cysteines at positions 47, 66, and 168. Its co-elution with E3 of PDC indicate possible interaction between them. SP-22 can be reduced by DTT and oxidized by H<sub>2</sub>O<sub>2</sub>. By electronic microscopy study, SP-22 forms a stable decameric toroid consisting of five basic dimeric units. By GFC study, it is found that SP-22 stay as a decamer under different conditions (salt concentration, pH, redox state). Preliminary crystallography study of SP-22 has been done. 3.3Å data of C168S mutation SP-22 were collected.

Oxidized SP-22 needs to be reduced by thioredoxin, thioredoxin reductase system. To finish this pathway and have a better understanding of the function of SP-22, human mitochondrial thioredoxin and thioredoxin reductase were cloned, overexpressed and purified. Due to human mitochondrial thioredoxin reductase is a SeCys protein, fully active protein was under investment.

**Keywords:** peroxiredoxin, mitochondrial, oligomer

#### P.04.02.59

*Acta Cryst.* (2005). A61, C193-C194

##### Structural Studies on Novel Streptococcal Virulence Factors

HaeJoo Kang<sup>a</sup>, Thomas Proft<sup>b</sup>, Fiona Clow<sup>b</sup>, Heather M. Baker<sup>a</sup>, Edward N. Baker<sup>a</sup>, <sup>a</sup>School of Biological Sciences, <sup>b</sup>Department of Molecular Medicine and Pathology, University of Auckland, Auckland, New Zealand. E-mail: h.kang@auckland.ac.nz

*Streptococcus pyogenes* (*S. pyogenes*) is responsible for a variety of illnesses ranging from mild sore throat to life-threatening toxic shock syndrome. The strain most strongly associated with highly invasive infections is *S. pyogenes* M protein serotype 1 (M1).

We have identified a number of putative genes from the *S. pyogenes* M1 genome which possess various sequence motifs that are often present in bacterial toxins. Three of the proteins encoded by these genes have been expressed, purified and crystallized with the aim of determining their structures by X-ray crystallography.

The protein encoded by gene *spy1492*, Spy1492, contains the GDSL-like lipase motif which is commonly found in lipolytic enzymes including some bacterial toxins such as hemolysin. Its lipolytic activity was detected in biochemical assays. Multiwavelength anomalous diffraction (MAD) data to 3.0 Å resolution have been