Acta Cryst. (2002). A**58** (Supplement), C109

PLASMODIUM FALCIPARUM TRIOSEPHOSPHATE ISOMERASE COMPLEXES: CATALYTIC LOOP IN THE OPEN AND CLOSED CONFORMATIONS

M.R.N. Murthy[1] S. Parthasarathy[1] H. Balaram[2] P. Balaram[1]
[1]Indian Institute of Science Molecular Biophysics Unit BANGALORE KARNATAKA 560 012 INDIA [2]Molecular Biology and Genetics Unit, Jawaharlal Nehru Center for Advanced Scientific Research, Jakkur, Bangalore 560 064, India.

In contrast to the earlier observation of the movement by about 7 Å. towards the active of the catalytic loop (loop 6, residues 166-176) of Triosephosphate Isomerase (TIM) upon ligand binding, in the structures of two *Plasmodium falciparum* TIM (PfTIM)-substrate analog complexes, 3-phosphoglycerate and glycerol-3-phosphate, the loop remains in the 'open' conformation. This loop open form in the PfTIM complexes is presumably a consequence of the S96F mutation, which appears to be specific to the enzymes from malarial parasites. Ser 73 in PfTIM (Ala in other TIMs) appears to provide anchoring water mediated hydrogen bond to the ligand compensating for the loss of a stabilizing hydrogen bond from Gly171 NH in the closed loop liganded TIM structures. Crystal structures of PfTIM complexed to a transition state analog, phosphoglycolate, were determined in an orthorhombic ($P2_12_12_1$) and a monoclinic ($C2$) form in order to probe the effects of the S96F mutation on the nature of inhibitor-enzyme interactions and the orientation of the critical catalytic loop in PfTIM. This is the first example of a TIM-ligand complex wherein the catalytic loop adopts both the open ($P2_12_12_1$ form) and the closed conformations ($C2$). In the $C2$ form (loop closed), Phe96 and Leu167 adopt alternative conformations, permitting loop closure. These structures provide strong support for the view that loop closure is not essential for ligand binding and that dynamic loop movement may occur in both free and ligand bound forms of the enzyme.

Keywords: PLASMODIUM FALCIPARUM, TRIOSEPHOSPHATE ISOMERASE, CATALYTIC LOOP

Acta Cryst. (2002). A**58** (Supplement), C109

CRYSTAL STRUCTURE OF THE THERMOSTABLE PECTATE LYASE FROM *BACILLUS SP.* TS 47

T. Nakaniwa[1] K. Ishii[1] T. Tada[1] M. Takao[2] T. Sakai[2] K. Nishimura[1]
[1]Osaka Prefecture University Reseach Institute For Advanced Science And Technology Gakuen-Cho 1-2 SAKAI OSAKA 599-8570 JAPAN [2]Department of Food and Nutrition, Faculty of Agriculture,Kinki University,Nakamachi, Nara 631-8505, Japan

The pectate lyase (PL) is produced by many plant-associated microorganisms. PL catalyzes the cleavage of α-1,4-glycosidic linkages in the polygalacturonic acid chains, which are a major component of pectic substances of plant cell walls. The thermostable PL is of a considerable interest for a biochemical process such as scouring of cotton fabrics. We have determined the crystal structure of thermostable PL 47 from a thermophilic *Bacillus sp*. TS 47 to clarify structural features participating in the thermostability of PL. The recombinant PL 47 was expressed in B. subtilis MI112 and the purified enzyme was crystallized using PEG 4 K as a precipitant. X-Ray diffraction data were collected to a resolution of 1.8 Å at SPring-8. The crystals belonged to the trigonal space group $P3121$ with unit cell parameters $a = b = 58.8$ and $c = 229.7$ Å. The structure was solved by the molecular-replacement method, using PL from *B. subtilis* SO113 as a starting model. The current model was refined to a crystallographic R-factor of 20.1%. The crystal structure revealed that the PL 47 has a unique motif of right-handed parallel β-helix in common with PL. The PL 47, however, had a number of hydrophobic and acidic amino acids in the loop region at the C terminus side. Ion-pair networks and hydrophobic regions formed in this area seemed to contribute in the thermostability of the PL 47.

Keywords: **PECTATE LYASE CRYSTALLOGRAPHY THERMOSTABLE**

Acta Cryst. (2002). A**58** (Supplement), C109

CRYSTALLOGRAPHIC STUDIES OF THE LARGE SIALIDASE, NAN H, FROM CLOSTRIDIUM PERFRINGENS.

S. Newstead[1] C Chien[2] M Taylor[1] G Taylor[1]
[1]University of St Andrews Centre For Biomolecular Sciences Rm. 4.13 North Haugh ST ANDREWS FIFE KY16 9ST UK [2]Institute of Biochemistry, National Yang-Ming University, Taipei, Taiwan R.O.C

Sialidases (or neuraminidases, ec 3.2.1.18) are widely distributed in nature, where they are responsible for the turnover and regulation of sialoglycoconjugates through hydrolysis of terminal a glycosidically bound sialic acids. Structural studies on several bacterial, viral and parasitic sialidases have revealed a superfamily of multidomian enzymes built around the canonical catalytic b-propeller fold. Additional domains, where present, appear to be involved in carbohydrate recognition, and targeting to specific environments and substrates. The anaerobic gram-positive rod clostridium perfringens, a frequent causative agent of gas gangrene and peritonitis, is known to possess two sialidase isoenzymes, a large (77 kda) and small (43 kda) form, with strikingly different biochemical properties.
We have expressed and purified the native 77 kDa nanH enzyme using a pQE-70 expression vector with a C-terminal poly- Histidine tail in *Escherichia coli*, which readily crystallized using the sitting drop technique. However, mass spectrometry and N-terminal sequencing analysis of a crystal showed that it contained the 50 kDa C-terminal catalytic domain. We will present the 1.62 Å structure of this domain and its comparison with other sialidases. The structure was solved using molecular replacement, employing the catalytic domain of the trans-sialidase from the leech *Marcobdella decora* (1SLL) as a search model. We will also present on progress with crystals of the complete 77 kDa enzyme. The origin and biological function of the microbial sialidases has not been fully resolved. Their importance as therapeutic targets therefore, cannot be fully assessed at present. We expect that the high-resolution crystal structure of a further superfamily member will aid in this assessment.

Keywords: SIALIDASE; CLOSTRIDIUM PERFRINGENS

Acta Cryst. (2002). A**58** (Supplement), C109

CRYSTAL STRUCTURE OF A CALCIUM-FREE α-AMYLASE AmyK38

T. Nonaka[1] M. Fujihashi[1] A. Kita[1] H. Hagihara[2] K. Ozaki[2] S. Ito[3] K. Miki[1, 4]
[1]Graduate School of Science, Kyoto University Department of Chemistry Sakyo-Ku KYOTO 606-8502 JAPAN [2]Biological Science Laboratories, Kao Corporation [3]Japan Marine Science and Technology Center [4]RIKEN Harima Institute/SPring-

All known α-amylases need calcium ions to retain their structure and function. This is a disadvantage when the enzymes are used as a component of detergents, because common detergents contain chelating agents to remove metal ions. A novel α-amylase from *Bacillus sp*. KSM-K38 (AmyK38) is considered to have no calcium ions based on an element analysis and a relation between its activity and a concentration of calcium ion. We have determined the three-dimensional structure of the AmyK38 by X-ray crystallography in order to investigate the mechanism to retain the structure without calcium ions.
AmyK38 was crystallized using polyethyleneglycol 8000 as a precipitant. The crystals, which belong to the space group $P23$ (a = 132.1 Å), diffracted X-rays to 2.13 Å resolution at the BL45XU beamline in SPring-8.
The structure of AmyK38 was solved by the molecular replacement method with a program AMORE using the structure of *Bacillus licheniformis* α-amylase (BLA; PDB code, 1bli) as a search model. The structure of AmyK38 was refined to R=19.9 % at 2.13Å. The overall structure of AmyK38 was almost similar to that of BLA.
Three sodium ions were found in the enzyme molecule, two of which are located at the positions corresponding to those of two calcium ions of BLA. It is suggested that sodium ions, instead of calcium ions, are used to retain the structure and function of AmyK38.

Keywords: **ALKALINE α-AMYLASE, CALCIUM-FREE AMYLASE, CRYSTAL STRUCTURE**