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Keywords: chitinase, crystal structure, glycoprotein

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Crystal Structures of Two *Bacillus subtilis* Protein YjcG and YflL
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YjcG is a putative protein from *Bacillus Subtilis* with 171 amino acids, it was cloned into the vector pET21-DEST by Gateway system. YjcG was expressed in *E. coli* strain BL21(DE3), purified and crystallized by hanging-drop vapor diffusion method. Since there is no homologous structures, MAD (multi-wavelength anomalous diffraction) method was chosen to solve the YjcG structure. The crystal belongs to spacegroup C2 with unit-cell parameters a=99.30 Å, b=73.77 Å, c=61.58 Å, β=113.541°. The structure is refined to R_{cryst} 0.237 and R_{free} 0.267 at 2.3 Å. Dali search showed that YjcG structure is similar to 2'-5' RNA ligase with Z=14.7, RMSD 2.5 Å with 138 residues.

YflL is a function uncharacterized *B. subtilis* protein with 39.5% identity to acylphosphatase from horse muscle. Crystals suitable for structural studies were obtained from 50 mM potassium phosophate, pH 6.5. The structure was determined by MR (molecular replacement). The crystal diffracted to 1.3 Å and belongs to space group $P2_12_12_1$ with unit-cell parameters of a=26.86 Å, b=48.27 Å, c=59.57 Å. The structure is refined to R_{cryst} 0.181 and R_{free} 0.234. We have identified in vitro that the YflL is not only an acylphosphatase but also an apyrase.
Keywords: *Bacillus subtilis*, MAD method, MR

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Crystal Structures of DAH7PS Synthase from *Pyrococcus furiosus*
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The enzyme 3 deoxy-D-arabino-heptulosate 7-phosphate synthase (DAH7PS) catalyses the condensation reaction between phosphoenol pyruvate (PEP) and the 4 carbon saccharide D-erythrose-4-phosphate (E4P). This reaction is the first step in the shikimate pathway used in plants and microorganisms to synthesize aromatic amino acids and many secondary aromatic metabolites.

DAH7PS from *P furiosus* requires a metal ion for activity and, unlike DAH7PS from other species, is not subject to regulation by aromatic amino acids, It is also able to utilize both 4-carbon and 5-carbon phosphorylated monosaccharides with similar K_{cat} but increased K_m values. This broad substrate specificity and lack of regulation indicates it may be the closest protein to the ancestral enzyme of the type 1 DAH7PS enzymes.

Two crystal structures will be presented. The first – the apo form of the molecule, crystallizes in space group I222 with *a*=87.23Å, *b*=110.02Å, *c*=144.35Å. There are two molecules in the asymmetric unit (the biological unit) which associate with a 2-fold related pair to form distinct tetramers. The structure was solved by molecular replacement using a hybrid model formed from elements of two related structures with low sequence identity (26% and 30%). Maps show the presence of PEP at low occupancy.

The second form, the Cd loaded derivative crystallizes in space group $P2_1$, *a*=48.94Å, *b*=84.33Å, *c*=139.16Å, β=92.60° with two intact tetramers in the unit cell. Current maps show a greatly expanded metal binding site, the presence of PEP at low occupancy, and the presence of some ribose 5-phosphate.
Keywords: enzyme, condensation, synthase

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Crystal Structure of the *Bacillus subtilis* YwlE Protein
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YwlE is a 150 residue protein encoded by the ywlE gene in *Bacillus subtilis*. It is a putative low-molecular-weight protein tyrosine phosphatase (LMW PTP) by sequence comparison.

The full length YwlE was cloned into pET28a and expressed in good amount in soluble form, it was purified by two-step conventional chromatography on a FPLC system. Crystallization trials of the purified protein were preformed by hanging-drop vapor diffusion method, usable crystals appeared in some of the screen drops after a few days. One set of native diffraction data was collected at the MAD beamline of Beijing Synchrotron Radiation Facilities (BSRF), using the oscillation method, and processed to 1.8 Å. The crystal belongs to space group C2 and there is 1 molecule per asymmetric unit. The sequence homology to bovine LMW PTP (Blast score 50.2 bits, 29 %) enabled the structure to be determined through molecular replacement using the CNS program. This gave a model with an R_{free} of 0.48. The program ARP/wARP was used for automated model building and further refinement. A much improved model was obtained after this procedure with R_{free} of 0.27.

The YwlE structure shows significant structural homology to protein-tyrosine-phosphatases, especially in the active site (CX_5R motif). It's hoped that this structure together with enzymology studies will deepen our understanding of protein phosphorylation and signal transduction in general.
Keywords: YwlE protein, tyrosine-phosphotase, bacterial

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Structure of Aldehyde Reductase Complex: Implications for Inhibitor Specificity
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Aldehyde and aldose reductases are members of the aldo-keto reductase superfamily of enzymes that catalyze the NADPH-dependent reduction of a number of different aldehydes to their corresponding alcohols. Both enzymes share significant sequence homology and form a classic α/β TIM-barrel structure with the least conserved residues lining the active site located at the C-terminal loop. Aldose reductase activity has been implicated in glucose over utilization and the aetiology of diabetic complications. Given the abilities of both enzymes to bind common inhibitors, the X-ray structure of porcine aldehyde reductase holoenzyme in complex with NADPH and the potent aldose reductase inhibitor Fidarestat (SNK-860) was determined at an atomic resolution of 1.85Å to elucidate the mechanism of inhibition. The hydrogen bonds between the active site residues Tyr50, His113 and Trp114 are conserved in aldose and aldehyde reductases. In the case of aldehyde reductase residues from the C-terminal loop do not form hydrogen bonds with the inhibitor Fidarestat. Leu300, a residue previously identified as essential in the determination of inhibitor potency for aldose reductase is a Pro in aldehyde reductase and can not form a hydrogen bond. Furthermore, molecular modelling calculations suggest that the conserved Trp220 may play a role in the disparity in IC_{50} values for the two enzymes. These results could provide a structural basis in the design of potent and specific inhibitors for aldose reductase.
Keywords: aldehyde reductase, inhibitor interactions, diabetes