PS04.01.52 PURIFICATION AND CRYSTALLOGRAPHIC STUDIES OF A D-ALANYL-D-ALANINE CARBOXY-PEPTIDASE FROM *B. STEAROTHERMOPHILUS*. Alexandre P. Kuzin[1], Janet L. Frost[2], Steven L. Condron[1], and Judith A. Kelly[1], [1]Department of Molecular and Cell Biology and Institute of Materials Science, University of Connecticut, Storrs, CT 06269-3125, [2]Department of Science, Capital Community Technical College, Hartford, CT 06105

D-alanyl-D-alanine carboxypeptidases (Cpases) are involved in bacterial cell wall biosynthesis and are targets of β-lactam antibiotics. The gene for a Cpase from *B. stearothermolphilus* has been modified by R. Manning and C. Despreaux of Hoffmann-LaRoche to remove a 26-residue C terminal fragment that anchors the enzyme in the bacterial membrane. The engineered gene was inserted in *Pichia pastoris*, and the soluble 43kDa enzyme was excreted into the medium. Ammonium sulfate precipitation and Fast Protein Liquid Chromatography using a Mono-S (cationic) column were used to purify the enzyme. The enzyme can also be solubilized via trypsin or chymotrypsin digestion. Cpase was crystallized from 20mM Tris buffer, pH 7.5, with 0.2M NaCl and 10% PEG 8000 in $P2_12_12$ (a=85.1Å, b=140.3Å, c=167.3Å). There are at least four molecules in the asymmetric unit cell. Native data to 3.2Å and data for a K_2PtCl_4 derivative have been collected. The sequence of Cpase was compared to that of the DD-transpeptidase from *Streptomyces* K15 (Englebert, S. *et al.*, 1994, *J. Mol. Biol.* **241**, 295-297) and aligned using the Multiple Sequence Alignment program (Gupta, S. K. *et al.*, *J. Comput. Biol.*, in press). Seventy of the 262 residues in K15 were found to be identical to the Cpase. Efforts are under way to phase the Cpase with molecular replacement using X-PLOR and AMoRe with K15 as the search model.

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PS04.01.53 CRYSTALLOGRAPHIC STUDIES ON SHIKIMATE KINASE FROM *ERWINIA CHRYSANTHEMI*. A.J. Lapthorn, T. Krell, J.E. Coyle and J.R. Coggins Dept.'s of Chemistry and Biochemistry, University of Glasgow, Glasgow, G12 8QQ, UK

Shikimate kinase (EC 2.7.1.71) catalyses the fifth reaction of the shikimate pathway which involves the conversion of shikimic acid into its phosphorylated derivative, shikimate 3-phosphate, using ATP as a co-substrate. The shikimate pathway is essential to plants and microorganisms for the biosynthesis of aromatic compounds, but importantly is absent from animals. The enzymes are therefore attractive targets for the development of novel antibiotics and herbicides such as glyphosate and fluroshikimate.

Crystals of an ADP-shikimic acid complex of *Erwinia chrysanthemi* shikimate kinase expressed in *E. Coli* have been obtained at room temperature using 0.1M HEPES pH=6.9 and 2.2M sodium chloride as precipitant. The crystals are tetragonal, space group P41212 or enantiomorph, a=108.5Å; c=92.8Å; with 2 molecules in the asymmetric unit, corresponding to a packing density of 3.6Å^{3}Da^{-1}. The crystals diffract to at least 2.6Å at the Daresbury SRS, station 9.5.

We anticipate that shikimate kinase will contain a core structure of 4 β-strands and 4 α-helices as proposed for a number of ATP/GTP-binding proteins [1]. Matsuo and Nishikawa [2] have predicted that shikimate kinase has the same structure as adenylate kinase, but molecular replacement with AmoRe using this structure, and other kinases as search model have been unsuccessful. We are presently using MIR methods to solve the structure.

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PS04.01.54 CRYSTAL STRUCTURE OF PHOSPHOLIPASE A_2 FROM *AGKISTRODON HALYS BLOMHOFFII VENOM*. K. Tomoo[1]*, M. Doi[1], T. Ishida[1], K. Ikeda[2], Y. Samejima[3], [1]Department of Physical Chemistry, [2]Department of Biochemistry, Osaka University of Pharmaceutical Sciences, Japan, [3]Institute for Medical Chemistry, Hoshi University, Japan

PhospholipaseA_2 (PLA_2) are calcium-dependent lipolytic enzymes involved in a number of physiologically important cellular processes, such as the inflammatory response through the release of arachidonic acid from the phospholipids in the plasma membrane. The PLA_2 from the venom of *Agkistrodon halys blomhoffii* (AHB-PLA_2) characteristically reveals its biological activity as a monomer form, whereas many other PLA_2 function as dimmer. In order to understand fully the structure-function relationship in AHB-PLA_2, we studied the X-ray structure analysis of this enzyme.

Crystals of AHB-PLA_2 have been obtained at room temperature using 0.1M TrisHCl buffer(pH=8.0) with 5mM $CaCl_2$ and hexylene glycol as precipitant. Crystal belong to the hexagonal space group $P6_122$ with cell dimensions of a=b=64.4Å,c=172.4Å. The intensity data were collected up to 2.5Å resolution using Rigaku RAXIS IIC. Initial structure was determined by the method of molecular replacement using a start model of PLA_2 from *Crotalus atrox* venom . Refinement was carried out using the program X-PLOR. The analysis of the detailed structure is in progress.

PS04.01.55 THE TRIGONAL AND THE ORTHORHOMBIC FORMS OF THE TRIPLE MUTANT Y52,73F/D99N OF PLA2. K. Sekar, Xin Chen, M.-D. Tsai, M. Sundaralingam, Laboratory of Biological Macromolecular Structure, Departments of Chemistry and Biochemistry, The Ohio State University, 1060 Carmack Road, Columbus, OH 43210, USA

The enzyme PLA2 hydrolyses the sn-2 ester bond of phosphoglycerides in the presence of calcium ion. The catalytic triad Asp - His - water is involved in a complex hydrogen bonding network with other active site residues that lock a structural water and provide structural support. The crystal structure of the double mutant Y52,73F[1] shows the structural water, but it is missing in the single mutant D99N[2]. In order to understand the hydrogen bonding network around the catalytic-active site residues, we have carried out the X-ray studies of the triple mutant Y52,73F/D99N. The crystals are trigonal $P3_121$ with a = b = 47.1Å and c= 102.4Å. 1.9Å data were collected and the refinement of the structure converged to a final R-value of 18.9% for 6809 reflections. It is interesting that the structural water is missing, as in the single mutant, and therefore the triple mutant would be expected to have similar enzymatic activity. The hydrogen bonding network is different but it has many features similar to that of the single mutant. We have also obtained crystals of the orthorhombic form[3] $P2_12_12_1$ with a=46.7Å, b=65.5Å and c=38.0Å. Its 1.8Å structure with the missing water is similar to the trigonal form. The structures of both forms will be compared with the single and double mutants.

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