Acta Cryst. (2002). A**58** (Supplement), C297

STRUCTURAL STUDIES OF COLLAGEN X AND COLLAGEN VIII NC1 DOMAINS

M. Kvansakul[1] O. Bogin[2] E. Rom[2] J. Singer[2] A. Yayon[2] E. Hohenester[1]
[1]Biophysics Section, Blackett Laboratory, Department of Biological Sciences Imperial College Prince Consort Road LONDON SW7 2BW UK [2]ProChon Biotech, Rehovot 76114, Israel

Collagen X is expressed specifically in the growth plate of long bones, where it forms a transient matrix during endochondral ossification. Its C1q–like C–terminal NC1 domain forms a stable homotrimer and is crucial for collagen X assembly. Mutations in the NC1 domain cause Schmid metaphyseal chondrodysplasia (SMCD), an autosomal dominant disorder characterised by a mild dwarfism and skeletal deformities. The crystal structure at 2.0 Å resolution of the human collagen X NC1 domain reveals an intimate trimeric assembly with a central hydrophilic channel. The apical loops of the cone–shaped NC1 trimer bind a cluster of four calcium ions. The structure explains the unusual stability of the collagen X NC1 trimer, which results from extensive subunit contacts and the buried cluster of calcium ions. Furthermore, the structure suggests how NC1 domains direct the assembly of collagen X and VIII into polygonal lattices. Three stripes of exposed aromatic residues are likely to be involved in this process. Most SMCD mutations are unlikely to be tolerated structurally and are predicted to cause disease by haploinsufficiency. Other mutations affect residues at or near the trimer surface and may be tolerated, but could affect the collagen X suprastructure in a dominant-negative manner. Their location thus highlights critical regions for collagen X interactions. Additionally, the collagen X NC1 structure is compared to the recently solved structure of the close homologue collagen VIII NC1.

Keywords: COLLAGEN, C1Q LIKE DOMAIN, CALCIUM BINDING

Acta Cryst. (2002). A**58** (Supplement), C297

CRYSTAL STRUCTURE DETERMINATION OF A SNAKE VENOM PLA2 PROTEIN (MiPLA3).

S. Lok[1] R Gao[2] P Gopalakrishnakone[2] K Swaminathan[1]
[1]Institute of Molecular Agrobiology X-Ray Crystallography 1 Research Link, National University of Singapore, Singapore 117604. SINGAPORE SINGAPORE 117604 SINGAPORE [2]Department of Anatomy, The National University of Singapore, Singapore 119260

Phospholipase A2 (PLA2) hydrolyzes phospholipids at the sn-2 position releasing free fatty acid and 1-acyl lysophospholipid. Snake venom PLA2 belongs to group 1A or group 1B phospholipase. In contrast to Group 1A proteins, Group 1B consist of an additional pancreatic loop between amino acids 62 and 68. Previously, all group 1B PLA2s consist of only nontoxic mammalian PLA2 while those in group 1A are mostly toxic. Therefore, the function of the pancreatic loop in Group 1B PLA2 was proposed to detoxify the whole protein. However, recently snake venom PLA2 (Mipla3) with pancreatic loop has been proven toxic. Previous PLA2 structures containing pancreatic loop showed that the loop possess high degree of conformational flexibility. Its presence on the surface of the molecule suggest that it could involve in protein-protein interaction. The structural difference of the pancreatic loop between Mipla3 and the non-toxic pancreatic PLA2 would thus indicate the importance of this pancreatic loop. Protein crystals of MiPLA3 are produced in 100mM MES, pH6.5, 1.8M ammonium sulphate. The crystal diffracts up to 2.8 Å with a completeness of 96% and Rsym value of 8.8%. Spacegroup is determined to be $P4_122$ with one molecule in an asymmetric unit. Matthews coefficient is 3.5 indicating that the percentage of solvent in the unit cell is 66%. Molecular replacement has been carried out using the program Molrep. Using bovine pancreatic PLA2 as a search model, a peak is detected with R factor of 54% and correlation factor of 28%

Keywords: PHOSPHOLIPASE A2, SNAKE, PANCREATIC LOOP

Acta Cryst. (2002). A**58** (Supplement), C297

STRUCTURE OF SET1, A SUPERANTIGEN LIKE PROTEIN WITHOUT SUPERANTIGENIC ACTIVITY

C.E. Naylor[1] D. Briggs[1] A.K. Basak[1] D.S. Moss[1] B. Henderson[2] S. Nair[2]
[1]Birkbeck College, University of London Department of Crystallography Malet St LONDON WC1E 7HX UK [2]Eastman Dental Institute, University College London, Grays Inn Rd, LONDON, WC1X8LD

The staphylococcal exotoxins (SETs) are a recently discovered cluster of genes in the Staphylococcal aureus genome. Fourteen family members have been identified to date sharing between 38 and 80% sequence identity. It has been noted that the SETs have between 20 and 30% sequence identity to the superantigen protein family: the highest homologies are between SET5 and Streptococcal pyogenes exotoxin G and SET5 and toxic shock syndrome toxin. The superantigens are a group of bacterial toxins that play a pivotal role in the pathogenesis of a number of diseases. They do this by non-specifically crosslinking MHC class II and T-Cell Receptor molecules, thereby causing massive, inappropriate stimulation of the immune system. I some cases as much as 20% of the T-cell population can be activated (compared to 0.0001% for a normal antigen. This stimulation results in acute toxic shock and scarlet fever. Interestingly, extensive investigation of the SET proteins has failed to identify any superantigenic activity, the first superantigenic-like molecule not to show such behaviour. We have solved the structure of SET1 to 2.7Å by molecular replacement. The crystals contain a dimer in the asymmetric unit. We will compare the structure of SET1 with other superantigen-type structures, and relate differences to the SET proteins apparent lack of activity.

Keywords: STAPHYLOCOCCAL EXOTOXINS,SUPERANTIGENS,S AUREUS

Acta Cryst. (2002). A**58** (Supplement), C297

STRUCTURAL STUDIES OF A COLLAGEN BINDING FRAGMENT OF YERSINIA ADHESIN YadA

H. Nummelin[1] M. Merckel[1] Y. El Tahir[2] P. Ollikka[2] M. Skurnik[2] A. Goldman[1]
[1]Institute of Biotechnology Srtuctural Biology and Biophysics Biocenter 3, Viikinkaari 1, P.O.Box 65 UNIVERSITY OF HELSINKI FIN-00014 FINLAND [2]Department of Medical Biochemistry and Molecular Biology, University of Turku, FIN-20520 Turku, Finland

Yersinia adhesin, YadA, is a trimeric outer membrane protein encoded by a Yersinia virulence plasmid. YadA is one of the main virulence factors as it is largely responsible for the adhesion of Y. enterocolitica and Y. pseudotuberculosis to the intestinal tissues *in vitro*, through binding to the extra cellular matrix proteins on the surface of the host cells [1]. We have produced, purified and crystallized a collagen-binding fragment of YadA to determine its structure. For the crystallographic studies the shortest fragment of yadA of Y. enterocolitica, which was still binding collagen, was cloned and transformed into *E. coli*. The N-terminally His_6-tagged protein was purified from cytoplasm using a metal chelating affinity column. Soluble protein was separated from the aggregate by a gel filtration and concentrated for the crystallization. Diamond shaped crystals that belong to space group *R*3 grew within a week. A full data set was collected to 1.55 Å at synchrotron radiation source. For the selenomethionyl-labelled protein, two I-->M mutations were constructed to collagen-binding epitopes [1]. Expression of labelled protein in minimal medium, and purification as native protein, yielded to hexagonal crystals. Crystals belonged to space group *R*32 and the MAD data with three wavelength was collected to 2.0 Å resolution at synchrotron radiation source. The structure was solved using two selenium sites and the refinement is on the way.

References
[1] Straley, S.C. et al. (1993) Infect. Immun. 61: 3105-3110.
[2] El Tahir, Y., Kuusela, P. And Skurnik, M. (2000). Mol. Microbiol. 37(1), 192-206.

Keywords: YERSINIA ADHESIN COLLAGEN