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 involved 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 $P 4_1 22$ 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:** COLLAGEN, C1Q LIKE DOMAIN, CALCIUM BINDING

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**STRUCTURAL STUDIES OF COLLAGEN X AND COLLAGEN VIII NC1 DOMAINS**

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Phospholipase A2 (PLA2) hydrolyzes phospholipids at the sn-2 position releasing free fatty acid and 1-acyl lysophospholipid. Snake venom PLA2 belonging 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 $P 4_1 22$ 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%.

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**CRYSTAL STRUCTURE DETERMINATION OF A SNAKE VENOM PLA2 PROTEIN (MIPLA3),**

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**STRUCTURAL STUDIES OF A COLLAGEN BINDING FRAGMENT OF YERSINIA ADHESIN YadA**

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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-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 R3 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 R32 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


**Keywords:** YERSINIA ADHESIN COLLAGEN