03-Crystallography of Biological Macromolecules

We have just determined the three-dimensional structure of procarcinol at 2.8Å resolution by X-ray crystallography. The structure has a rather bizarre elongated shape comprising four domains rich in beta-sheet with one strand longer than 90Å. We have been able to relate various structural features to toxin function (secretion, activation, oligomerization, channel formation and gating) on the basis of the behaviour of a range of single-point mutants. The toxin contains no hydrophobic stretches of sequence long enough to span a membrane, but the dimer interface has a number of hydrophobic patches. Activation to the mature toxin would expose an additional hydrophobic region and this may account for the protein’s ability to penetrate lipid bilayers. Images of a novel oligomer derived from electron microscopy has enabled us to construct a model of the aerolysin channel and suggested a pathway for how the water-soluble toxin penetrates into a membrane to form a voltage-gated channel.

Figure: Cartoon drawing of procarcinol as determined by X-ray crystallography. This figure was produced by the computer program MOLSCRIPT (Kraulis, J. Appl. Cryst. 24, 996-998).

DS-03.08.06 CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN A SINGLE CHAIN ANTIBODY AND NEUROMINIDASE, A BASIS FOR RATIONAL PROTEIN ENGINEERING. By R.L. Murphy 1, M.C. Lawrence 1, W.R. Turp 2, V.R. Harley 2, R.G. Webster 3, P.J. Hudson 1 and P.M. Colman 1 1Biomolecular Research Institute and 2CSIRO Division of Biochemical Engineering, Parkville, Vic. 3052, Australia; 3Department of Neurology, St. Jude Children’s Research Hospital, Memphis, TN 38105, U.S.A.

We have solved the structure of the complex between a recombinant single chain Fv (scFv) fragment of antibody NC10 and its antigen, neuraminidase (NA) from influenza virus. The complex crystals belong to space group P21212 with cell edges a = 114Å, b = 114Å, c = 114Å and contain two complexes per unit asymmetric unit. The single chain Fv consist of the variable heavy (VH) and variable light (VL) chains of the NC10 antibody, joined by a GC(GlyGly) 4(NC10) peptide linker. Single-chain Fv molecules may be useful in therapeutic and diagnostic reagents, since they retain antigen-binding properties and are more stable under physiological conditions than double-chain Fv antibodies. They are also ideal models for protein engineering studies on antibodies (Goldshuhb et al., Biochemistry, 1990, 29, 1362-1367).

DS-03.08.07 THE CRYSTAL STRUCTURE OF 1-1 ISOZYME OF SOYBEAN LIPPOXYGENASE. By Wladek Mincl 1, Jeffrey T. Bollin 1, Janusz Szczekol 2, Bernard Axelrod 2 and Zbybek Owinsowski 3, 1Department of Biological Sciences, 2Department of Biochemistry, Purdue University, W. Lafayette IN 47907, 3Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510

Lipoxigenases are non-heme, non-sulfur, iron-containing dioxygenases catalyzing the hydroperoxidation of polyunsaturated fatty acids. The iron is known to participate in the catalYSIS. All plant and mammalian lipoxigenases that have been so far characterized contain six conserved histidine residues. Five of these occur in a fairly well-conserved region of 40 amino acid residues which contains iron ligands.

The enzyme was crystallized in space group P21 with one 839 amino acid monomer per asymmetric unit, and cell dimensions a = 95Å, b = 124Å, c = 30 Å and 91°. Diffraction data were collected using a SOMA area detector and RAXIS-II imaging plate system to a resolution of 2.6Å. The structure was solved by multiple isomorphous replacement with two heavy atom derivatives. MIR phases were calculated to 3.0Å resolution. Phase improvement and extension to 2.6Å was performed by a solvent flattening technique that incorporates maximum entropy procedures (Z. Owinsowski, to be published). The position of the active site iron atom was located in an anomalous difference electron density map. The iron atom is found in a predominantly alpha helical domain and is attached to two histidines (His 599 and His 504), both of which belong to an eight turn alpha helix. The binding of the iron atom by these two histidines is in agreement with the prediction made earlier by analysis of site specific mutants (Szczekol and Axelrod, Biochem. Biophys. Res. Commun. 1992, 185, 686-690). Detailed interpretation of the electron density map is in progress and will be presented together with procedures used in the structure determination.