IPR BEAMLINE (BL44XU) FOR MACROMOLECULAR ASSEMBLIES AT SPRING-8.

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Biological macromolecular assemblies play significant roles in many biological reaction systems, including energy transfer, protein synthesis, protein analysis, DNA replication and signal transduction. More than 10,000 protein structures are now known since the first crystal structure of hemoglobin and myoglobin were determined. On the other hand, only a few macromolecule assembly structures including viruses have been determined by X-ray crystallography. This is because of the difficulties faced in the preparation, crystallization, X-ray diffraction measurement, and crystal structure determination of large molecular assemblies. A beamline for biological macromolecular assemblies at SPring-8, which is specially designed to collect high resolution and high quality diffraction data of macromolecule assembly crystals with large unit cell, has been operating since September 1999. We have collected X-ray diffraction intensity data from some crystals of macromolecular assemblies. Diffraction data from a macromolecular assembly crystal with two unique axes of over 600 angstrom has been collected at 3.7 Å resolution. The present status of beamline and some diffraction data will be presented.

Keywords: MACROMOLECULAR ASSEMBLIES BEAMLINE DATA COLLECTION

CRYSTAL STRUCTURE OF HEX1 REVEALS ITS MECHANISM OF SELF-ASSEMBLY AND EVOLUTIONARY ORIGIN

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The Woronin body is a membrane bound organelle, which appears to be restricted to the *filamentous Ascomycotina*. It is a specialized peroxisome that functions as a plug for the septal pore, in response to cell lysis. Hex1 is the main component protein involved in the *in vivo* assembly of crystalline Woronin body. The crystal structure of Hex1 at 1.78 Å resolution reveals the intermolecular interactions that promote the Woronin body assembly. The protein contains two mutually perpendicular β-barrels. The N-terminal barrel contains six β-strands and the C-terminal domain contains a five stranded barrel and a flanking α-helix. There are three types of intermolecular interaction. And Groups I and Group II interaction both include the double salt bridges which recruit Hex1 molecular form a coil filament and group III interactions help to crosslink these filaments. Self-assembly is abolished *in vitro* and *in vivo* by mutations in intermolecular contact residues, indicating that the crystal structure is a valid representation of the Woronin body-core. In spite of sharing sequence homology, the tertiary structures of Hex1 and the eukaryotic translation initiation factor 5A (eIF-5A) are very similar, suggesting an ancestral link between them. Since eIF-5A does not self-assemble, comparison of these two protein structures suggests how a crystalline protein complex evolved from a soluble precursor.

Keywords: HEX1 WORONIN BODY SELF ASSEMBLY

CRYSTAL STRUCTURE OF A RIBOSOME INACTIVATING VISCUMIN FROM INDIAN VISCUMIN ALBUM AT 2.8Å RESOLUTION

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Many Plants produce Ribosome Inactivating Proteins (RIPs)-enzymes that act on ribosomes in a highly specific way, thereby inhibiting protein synthesis. Some RIPs can bind to and enter cells, making them among the most-toxic substances known so far. The lectins from mistletoe belong, like ricin, abrin and modeccin to the group of toxic lectins of A and B chains. The A chain is an enzyme whereas B chain is a lectin. The heterodimeric toxic viscumin was isolated from a partial-parasite obtained from Indian western Himalayas. The purified viscumin was crystallised by hanging drop vapour diffusion method against the same buffer containing 55% ammonium sulphate. The crystals belong to hexagonal space group $P6_522$ with a = b = 109Å and c = 309.4Å. The structure has been determined by molecular replacement method and is currently being refined. The present R-factor is 23.2% for all data to 2.8 Å resolution. The overall protein fold is similar to recin. It shows considerable sequence and structural differences with the European viscumin. The root mean-square-deviations (r.m.s) calculated for Ca atoms of European ML-1 and Indian viscumin show higher deviation for the A chain and lower for the B chain. The association of A and B sub units is predominantly hydrophobic in nature.

Keywords: MISTLETOE, CRYSTAL STRUCTURE, RIP ENZYME

STRUCTURE ANALYSIS OF HUMAN DIHYDROFOLATE REDUCTASE COMPLEXES WITH TRIMETHOPRIM AND EPIROPRIM

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As part of a program to understand the role of active site residues on selectivity and specificity of inhibition of dihydrofolate reductase (DHFR), we have determined the crystal structures of human (h) DHFR in complex with trimethoprim (TMP), a highly selective antibacterial, and epiroprim (EPM), its 4-pyrrolyl analogue, and compare these structures with those from other DHFR species. Despite TMPs weaker binding inhibition to human and Pneumocystis carinii (pc) DHFR, it is used to treat pc pneumonia in AIDS patients. EPM is active against TMP-resistant strains, gram-positive bacteria, and enhances the antipneumocystis activity of other drugs. Structural data are reported for wild type hDHFR binary complex with TMP, and the F31G variant of hDHFR ternary complex with NADPH and EPM. Data were collected to 1.9Å for rhombohedral crystals of the TMP complex, and to 2.4Å for tetrahedral crystals of the F31 G variant EPM complex. The TMP bridge conformation in the hDHFR binary complex has torsion angles of -159° and 66°, similar to that observed for the NADPH ternary complexes for pc (176°, 70%) and *Mycobacterium tuberculosis* (mtb) (179°, 71°), as well as for the *E. coli* (-177°, 76°) binary complex, but not for the avian TMP ternary complex (-85°, 102°) or hF31G EPM (-101°,-64°). The major differences among these structures are the orientation (tilt of the pyrimidine ring, δ 30°) within the binding site and the strength of its enzyme interactions.
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Keywords: DIHYDROFOLATE REDUCTASE ANTIBIOTIC DRUG DESIGN