03-Crystallography of Biological Macromolecules

MS-03.03.04 THREE-DIMENSIONAL STRUCTURE OF RECOMBINANT MURINE INTERFERON-β

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Interferons (IFN) are proteins showing antiviral, antitumor and immunomodulator activities with potential therapeutic value. The IFN's have been classified into two categories on the basis of their biological and physical properties. Type I IFN's include fibroblast interferon (IFN-β) and the leukocyte family of interferons (IFN-α) which is composed of at least 10 subspecies. Each member of the Type I IFNs contain ~165 amino acid residues, and competes for the same receptors; furthermore, identical amino acids occupy invariant position in 23 % of their amino acid sequences. In contrast, Type II IFN (IFN-γ) is produced in response to mitogens and antigenic stimuli, contains ~146 amino acid residues and displays no measurable binding to Type I interferon receptors.

We solved the crystal structure of recombinant murine interferon-β (rMuIFN-β) (Senda, 1992) by the multiple isomorphous replacement method using, mersalyl, uranyl acetate, K_2PtCl_4 and $K_3UO_2F_5$ derivatives. The structure was refined to an R-factor of 19.7 % against 2.6 Å X-ray diffraction data using program X-PLOR.

The structure of rMuIFN-β shows a variant of the α-helix bundle with a new chain-folding topology, which seems to represent a basic structural framework of all the IFN-α and IFN-β molecules. Functionally important segments of the polypeptide chain are spatially clustered indicating the binding site(s) to the receptor(s). Comparison of the present structure with those of other α-helical cytokine proteins indicated either a topological similarity in chain folding or a similar spatial arrangement of the α-helices.

Senda,T., Shimazu,T., Matsuda,S., Kawano, G., Shimizu,H., Nakamura,K.T. and Mitsui,Y. (1992) EMBO J., **11**, 3193 - 3201.

MS-03.03.05 CRYSTALLOGRAPHIC STUDIES OF PANCREATIC SPASMOLYTIC POLYPEPTIDE. By Amitabha De*, M. A. Gorman, D. Brown+, M. Sanderson+, M. Carr°, A. N. Lane° & P. S. Freemont. Protein Structure Laboratory, Imperial Cancer Research Fund, +The Randall Institute, Kings College, °National Institute of Medical Research, London, UK.

Porcine pancreatic spasmolytic polypeptide (PSP) belongs to a family of growth factor-like polypeptide and has been shown to inhibit both intestinal motility and gastric acid secretion suggesting involvement in the exocrine function of the pancreas. Recent studies of the human analogue hSP suggest a role in the healing of damaged endodermally-derived tissue, such as in gastrointestinal ulcers. PSP contains 106 amino acid residues in a single chain with two highly homologous domains which are nearly 50% identical. Each domain has three disulphide bonds which gives rise to a novel three looped "trefoil-like" motif which is distinct from the other characterized growth factors. These repeated domains are also seen in human hSP and a single domain is found in rat ITF (intestinal trefoil factor) and human pS2. Understanding the role of pS2 is of particular importance since approximately 50% of oestrogen-dependent human breast tumours secrete pS2 whereas in normal breast tissue no significant expression of pS2 is observed.

We have crystallized PSP from ammonium sulphate solutions at pH 4.2 by the vapour diffusion method. The crystals belong to orthorhombic space group $I2_12_12_1$ with cell dimensions a = 181.68, b = 54.69, c = 72.79 Å and diffract to at least 2.5 Å resolution. The asymmetric unit contains two molecules related by a non-crystallographic two-fold axis. For the native crystal data up to 2.5 Å resolution was collected using Raxis-II image plate. Three heavy-atom derivatives were prepared by soaking, and the X-ray data were collected up to 3 Å resolution for $K_2Pt(NO_2)_4$ and 2.5 Å for $AgNO_3$ and cis-$Pt(NH_3)_2Cl_2$ derivatives. All the three derivatives however have common sites. Phases have been calculated using the MLPHARE program and an initial solvent-flattened electron density map at 2.8 Å resolution showed secondary structure features of PSP.

We have located initial alpha-carbon positions for almost all of the amino-acid residues in the 2.8 Å map with the help of the recently solved NMR structure of one domain of PSP. The complete model building and the refinement is in progress.

MS-03.03.06 STRUCTURE STUDIES OF RECOMBINANT INTERLEUKIN-1 RECEPTOR ANTAGONIST PROTEINS

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The structure of a recombinant version of an endogenous interleukin-1 receptor antagonist protein (IRAP) originally isolated from human U937 monocytes has been determined and is being refined against 2.2Å resolution X-ray diffraction data. Crystals of IRAP belong to space group $P4_32_12$ with a = b = 72.35Å and c = 114.7Å. The structure has been determined with phases calculated from anomalous and isomorphous differences measured from two single-site heavy atom derivatives. The analysis confirms that IRAP is structurally homologous to both IL-1α and IL-1β, sharing the same pseudo-threefold-symmetric β-trefoil structure. We also report the crystallization of recombinant mouse IRAP. An X-ray data set to 2.4Å resolution has been collected from a crystal with space group C2 and unit cell dimensions of a = 79.5Å, b = 76.9Å, c = 58.9Å and β = 116°. Our progress in the determination of the crystal structure of this mouse IL-1 receptor antagonist will also be described.

PS-03.03.07 Crystal structure of the Fv fragment of an anti-dansyllysine antibody at 1.6 Å resolution.

By M.Nakasako, S.Noguchi, Y.Satow, H.Takahashi, I.Shimada and Y.Arata, Faculty of Pharmaceutical Siences, University of Tokyo, JAPAN.

The crystal structure of the Fv fragment of an anti-dansyllysine antibody (IgG2a) is solved by molecular replacement method, using the Fv portion of McPC603 Fab (Satow et al.(1986) J.Mol.Biol.,190,593-604.) as a starting model. The Fv can be prepared by proteolytic digestion of the antibody, which lacks the CH1 domain. The Fv crystal obtained from an ammonium sulfate solution belongs to the space group $P2_12_12_1$ with unit cell dimensions of a=56.51, b=71.67, c=53.93 Å (one Fv / asymmetric unit).

The diffraction intensities for a crystal of 0.1x0.1x 0.3 mm^3 size were collected with an Imaging Plate by means of the oscillation method at BL-14A of Photon Factory (Satow and Iitaka (1989) Rev.Sci.Instrum.,60,

2390-2393.), KEK,Japan. The 1.6 Å data set collected at a shorter wavelength of 0.8200 Å yielded a merging R-factor of 0.046 in intensity. After the phase extension and structure refinement subsequent to the molecular replacement analysis, the R-factor for the final model, which contained 233 residues, 155 water molecules and 1 sulfate ion, was 0.178 in the resolution range of 8.0-1.6 Å. The rms deviation of bond lengths for the model was 0.016 Å and the positional error was estimated to be ca. 0.17 Å.

All the complementarity determining region (CDR) loops are clearly visible in the electron density maps. The H3 loop as well as its side chains are well elucidated and can accomodate the dansyl group of the hapten.

The orientation of the VH and VL domains is significantly different from that of McPC603 Fab; when VL domains are superimposed, the angle between the principal axes of the remaining VH domains is 10.7°.

PS–03.03.08 THE STRUCTURE OF HIV-1 NEUTRALIZING ANTIBODY 50.1 By M.Takimoto-Kamimura*+, R. L. Stanfield, E. A. Stura, A. T. Profy and I. A.Wilson, The Department of Molecular Biology, The Scripps Research Institute, La Jolla, U. S. A.

Much evidence suggests that the principle neutralizing determinant of HIV is located in the third variable region(V3) of HIV envelope glycoprotein, gp120 (Bolognesi et al., AIDS , 1989, 3(suppl. 1)., S111-S118; Steimer et al., Science, 1991, 254, 105-108). Antibody 50.1 was derived from mice immunized with a cyclic forty residue synthetic peptide representing the V3 loop of HIV-1 gp120(MN isolate). 50.1 Fab fragment was produced by papain cleavage. Crystals of the free Fab were obtained from high salt in space groups $P2_12_12_1$ and I222 (Stura et al., Proteins , 1992, 14, 499-508). These crystals have been solved at 2.8 Å resolution by the molecular replacement. Similated annealing with X-PLOR gave a model with R factors of 0.19($P2_12_12_1$), 0.20(I222) for the all data between 2.8 to 10.0Å. Different crystal form but conformation similar as indication CDR loops adapt defined conformations not an assembly that is crystal packing dependent. The CDR H1 loop of 50.1 is two residues longer than previously analyzed H1 loop structures. This is a new H1 loop structure. The insertion occurs at the surface loop formed by residues 30 and 31. In addition, the length and sequence of the 50.1 CDR L1 loop is also placed in a new canonical class.

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