

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, K₂PtCl₄ and K₃UO₂F₅ 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 I2₁2₁2₁ 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 K₂Pt(NO₂)₄ and 2.5 Å for AgNO₃ and cis-Pt(NH₃)₂Cl₂ 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

By H. M. Einspahr*, L. L. Clancy, M. J. Bienkowski, T. E. Eessalu & B. C. Finzel, Upjohn Laboratories, Kalamazoo, Michigan 49001, U. S. A.

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 2.2 Å resolution X-ray diffraction data. Crystals of IRAP belong to space group P4₃2₁2 with a = b = 72.35 Å and c = 114.7 Å. 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-1 α and IL-1 β , sharing the same pseudo-threefold-symmetric β -trefoil structure. We also report the crystallization of recombinant mouse IRAP. An X-ray data set to 2.4 Å resolution has been collected from a crystal with space group C2 and unit cell dimensions of a = 79.5 Å, b = 76.9 Å, c = 58.9 Å 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 Sciences, 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 P2₁2₁2₁ 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 0.1x0.1x0.3 mm³ 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,