03-Crystallography of Biological Macromolecules

isomorphous with the 1-Phe-Tyr-NH$_2$ complex, the four crystallographically distinct neurophysin molecules were found to associate into an elongated tetramer consisting of two similar neurophysin dimers. However, in contrast to the structure of the 1-Phe-Tyr-NH$_2$ complex, in which the neurophysin monomer was found to contain five dipeptide molecules, the native neurophysin tetramer contains only four bound dipeptide molecules. A full crystallographic refinement using XPLOR is underway. The current R factor is 30.1%. Details of the structure determination as well as analysis of the structure will be presented.

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PS-03.11.14 REFINEMENT AND INTERPRETATION OF THE X-RAY STRUCTURE OF APC-NEOCARINOSTATIN AT 1.8A RESOLUTION. by M. Ramamadhan, Solid State Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India, and Larry G. Sieker, Department of Biological Structure, University of Washington, Seattle, WA 98195, USA

Apo-neocarinostatin (apo-NCS), the 113 amino-acid long polypeptide component of the antitumour, antibiotic — protein neocarinostatin (NCS), has been refined at 1.8A resolution by the method of stereochemically restrained least-squares. (Sp. gr. P2$_1$2$_1$2, a=27.36, b=31.89, c=101.9A, Z=4). Prior to this, the structure was refined at 2.25A resolution to an R-value of 0.37 for a model consisting of 751 out of 776 protein atoms. Numerous difficulties encountered in extending this refinement further had taken us back to the map. A thorough re-examination of this map resulted in a model, which is consistent with not only the electron density distribution, but also the accepted rules of conformation and inter-atomic interactions. Further model improvements were affected by the use of Fourier maps phased by the atomic positions obtained during the previous step. Refinement and periodic model editing at 2.25A resolution, commenced only after the conclusion of these steps, had resulted in an R-value of 0.27. The data were then extended to 1.8A resolution (7,559 observations in the d-spacing range 10-1.79A), and refinement was continued, eventually leading to a model consisting of all the 776 protein atoms and 248 solvent atoms with an R-value of 0.155. The final model is completely unambiguous in terms of the Fourier map interpretation, and is quite satisfactory from the point of view of structural and stereochemical considerations. A detailed analysis of the structure, conformation, and the chromophore binding site has been carried out. This model is currently in use for interpreting the binding of ethidium bromide to NCS at 2.2A resolution. Efforts are also underway to model the chromophore binding to NCS at 2A resolution.

PS-03.11.15 CRYSTALLISATION AND X-RAY DIFFRACTION STUDIES OF RECOMBINANT HUMAN PLATELET-DERIVED ENDOTHELIAL CB1L GROWTH FACTOR. by Spraggon G., Poole D., James E. Y., Barton, G. J., Stuart D. I., Finn C. and Sleep D.

Laboratory of Molecular Biophysics, University of Oxford, Oxford, UK; Delta Biotechnology Ltd, Castle Court, 39 Castle Boulevard, Nottingham, NG7 3FD, UK

Thymidine phosphorylase (TP) is one of two pyrimidine phosphorylases in the base and nucleoside salvage pathways. A search of the NBRF-PFIR sequence database has revealed a striking homology (40% identity over 438 aligned positions) between TP derived from E. coli and human platelet-derived endothelial cell growth factor (PD-ECGF). This strongly suggests that human PD-ECGF is identical to human TP. PD-ECGF is known to stimulate the growth and chemotaxis of endothelial cells in vitro and possesses angiogenic activity in vivo. It is likely that endothelial cells respond specifically to a modulation in intracellular DNA precursor pools brought about by thymidine phosphorylase.

Five different crystal forms of recombinant human PD-ECGF from yeast were produced from initial screening of crystal growth parameters using the "magic Fifty" set of conditions (Jancarik & Kim, 1991) and the hanging drop vapour diffusion technique (Haupt & et al., 1988). One of the forms proved suitable for X-ray analysis. These crystals belong to the space group P2$_2_1$2, with unit cell dimensions a=3.7, b=70.4, c=29.6 (Angstroms) (Angstroms). Assuming a 47% solvent content the crystals contain a single dimer in the asymmetric unit. A set of diffraction data has been obtained using station X11 of the Hamburg synchrotron source which is 84% complete at the 1r level to 2.8A resolution spacings.

PS-03.11.16 CRYSTAL STRUCTURE ANALYSIS OF HUMAN DIOXYPROPANE REDUCTASE INHIBITOR COMPLEXES. Vivian Cady, Joseph R. Luft, Erik Jeness, Walter Pangborn, Andrej Wojcieszak, Nikolai Galitsky, J. H. Freisheim and R. Plakley, Medical Foundation of Buffalo, Buffalo, NY 14203, Medical College of Ohio, Toledo, OH 43614, and St. Jude Children's Research Hospital, Memphis, TN 38101.

Data from a methotrexate (MTHX)-resistant human cancer cell line reveals a natural F31S mutation in the enzyme dihydrofolate reductase (DHFR). Kinetic data for F31 mutations shows greater effects on binding for folates than antifolates. To understand the structural basis for selectivity and specificity for binding to DHFR, and to determine the fundamental role of F31 in binding and catalysis, we have co-crystallized a series of antifolate inhibitors with both human wild type and F31 mutant DHFR as binary and ternary complexes with the cofactor NADPH. We report structural data for isomorphous 03 crystal complexes with anti-