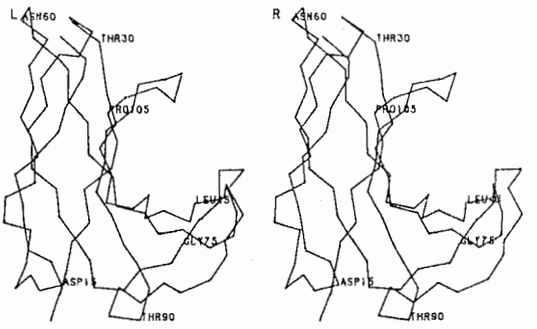
isomorphous with the I-Phe-Tyr-NH_2 complex, the four crystallographically distinct neurophysin molecules were found to associate into an elongated tetramer consisting of two similar neurophysin dimers. However, in contrast to the structure of the I-Phe-Tyr-NH_2 complex, in which the neurophysin tetramer 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 20.1%. Details of the structure determination as well as analysis of the structure will be presented.

Work supported by NIH grant GM-46828 and a grant from the Pittsburgh Supercomputing Center.



PS–03.11.14 REFINEMENT AND INTERPRETATION OF THE X-RAY STRUCTURE OF APO-NEOCARZINOSTATIN AT 1.8Å RESOLUTION. by M.Ramanadham*, Solid State Physics Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India, and Larry C. Sieker, Department of Biological Structure, University of Washington, Seattle, WA 98195, USA

Apo-neocarzinostatin (apo-NCS), the 113 amino-acid long polypeptide component of the antitumour, antibiotic protein neocarzinostatin (NCS), has been refined at 1.8Å resolution by the method of stereochemically restrained least-squares. (Sp.gr. $P2_12_12_1$, a=27.36, b=33.89, c=101.9Å, Z=4). Prior to this, the structure was refined at 2.25Å resolution to an R-value of 0.37 for a model consisting of 751 out of 778 protein atoms. Numerous difficulties encountered in extending this refinement further had taken us back to the MIR 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 2.25Å resolution, commenced only after the conclusion of these steps, had resulted in an R-value of 0.27. The data were then extended to 1.8Å resolution (7,559 observations in the d-spacing range 10-1.79Å), and refinement was continued, eventually leading to a model consisting of all the 778 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 2.5Å resolution. Efforts are also underway to model the chromophore binding to NCS at 2Å resolution.

PS–03.11.15 CRYSTALLISATION AND X-RAY DIFFRACTION STUDIES OF RECOMBINANT HUMAN PLATELET-DERIVED ENDOTHELIAL CELL GROWTH FACTOR

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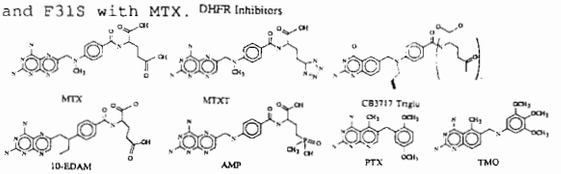
Thymidine phosphorylase (TP) is one of two pyrimidine phosphorylases in the base and nucleoside salvage pathway. A search of the NBRF-PIR sequence database has revealed a striking homology (40% identity over 438 aligned positions) between TP derived from *E. coli* and human platelet-derived endothelial cell growth factor (PD-ECGF) (Barton *et al.* , 1992). 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 pool brought about by thymidine phosphorolysis.

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 (Hampel *et al.*, 1968) One of the forms proved suitable for X-ray analysis. These crystals belong to the space group $P2_12_12_1$ with unit cell dimensions a=63.7 b=70.4 c=219.6 Å $\alpha = \beta = \gamma = 90°$. 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 1σ level to 3.5Å Bragg spacings.

PS–03.11.16 CRYSTAL STRUCTURE ANALYSIS OF HUMAN DIHYDROFOLATE REDUCTASE INHIBITOR COMPLEXES. Vivian Cody*, Joseph R. Luft, Erik Jensen, Walter Pangborn, Andrzej Wojtczak, Nikolai Galitsky, J.H. Freisheim[!] and R. Blakley[+], Medical Foundation of Buffalo, Buffalo, NY 14203, [!]Medical College of Ohio, Toledo, OH 43699, [+]St. Jude Children's Hospital, Memphis, TN 38101.

Data from a methotrexate (MTX)-resistant human cancer cell line reveal a natural F31S mutation in the enzyme dihydrofolate reductase (DHFR). Kinetic data for F31 mutants 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 R3 crystal complexes with anti-

cancer folate and antifolate inhibitors which probe specific regions of the active site. Data were collected to a maximum resolution of 2.0A on a Rigaku Imaging Plate area detector for the following wild type ternary antifolate complexes: methotrexate-γ-tetrazole (MTXT), aminopterin-γ-phosphinothricin (AMP), piretrexim (PTX), and the folate 5,8-dideaza-N10-propargyl-folyltriglutamate (CB3717TG). Complexes with the following F31 mutants are also in progress: F31A with CB3717, TMQ and 10-EDAM, F31G with 10-EDAM, and F31S with MTX. DHFR Inhibitors



Analysis of these data reveals their secondary structures are similar to the isomorphous folate binary complex previously reported, although there is a different fold of the flexible loop near residues 40-47 and a cis-peptide link at P66. Structure determination of the ternary lipophilic antifolate PTX complex reveals an unusal orientation of the methoxybenzyl ring which is positioned near the cofactor rather than binding in the pocket occupied by the p-aminobenzyl group of natural folates. Interpretation of data for the folate inhibitor CB3717 indicate partial occupancy of the cofactor which causes the N10-propargyl group to make short contacts with the side chain of L22 and backbone of S59, in contrast to its orientation in the binary complex of the F31A mutant. The triglutamate side chain in the wild type complex partially occupies two positions across the channel entrance, but is not observed in the binary complex with F31A. In wild type DHFR structures, the F31 phenyl ring makes hydrophobic contacts with the pteridine and p-aminobenzyl rings of the antifolates. Data for F31 mutants indicates a water molecule fills this hydrophobic space. The interactions of MTXT and AMP are similar to those of MTX observed in bacterial ternary complexes in that E30 hydrogen bonds to the antifolate N1 and N2 and to T136 which in turn interacts with a conserved water that also hydrogen bonds to N2; N4 hydrogen bonds to the oxygens of I7 and Y121. While the α-COOH of both MTXT and AMP hydrogen bonds to the conserved R70; there are no enzyme contacts to the γ-tetrazole ring or phosphino group of AMP, both of which hydrogen bond to water. NADPH has an extended conformation, similar to other cofactor complexes and makes a close contact to the antifolate N5 position. These data have provided insight into the mechanisms of antifolate binding and catalysis. Supported in part by NCI CA34714 (VC), CA31922 (RB) and CA41461 (JF).

PS-03.11.17 STRUCTURAL DETERMINATION OF THE NEURAMINIDASE OF INFLUENZA VIRUS A N2 SUBTYPE.
By Lan Zhou* and Ming Luo, Center for Macromolecular Crystallography, Department of Microbiology, University of Alabama at Birmingham, U.S.A

In general the influenza viruses are spherical, enveloped particles with two types of surface glycoproteins spikes, haemagglutinin and neuraminidase. Neuraminidase may facilitate mobility of the virus to and from the site infection, and thus may be an important factor in the spread of the infection. It is a tetramer of M.W. 240,000, reducing to 200,000 when solubilized from the Pronase. The sequences of several strains of N1, N2, N5, N7, N8, N9 and B subtype are known. Influenza A virus is the classic pandemic virus which infects human and affects persons in all area of the world.

The neuraminidase of strain A/Tokyo belongs to the N2 subtype and the subunit contains 469 amino acids. The product of the Pronase from N2 A/Tokyo virus neuraminidase , residue 83 to residue 469, was crystallized by vapor diffusion in hanging drops. Diffraction data at 2.63 Å was collected. Space group is $C222_1$ and cell dimensions are a = 120.36 Å, b=139.60 Å, c = 140.75 Å. The structure was determined by molecular replacement, using one of N9 subtype neruaminidase structure as the starting model. After XPLOR refinement, the crystallographic R factor is 29%.

03.12 – Other Macromolecular Structures

PS-03.12.01 CRYSTALLOGRAPHIC REFINEMENT OF TRICHOSANTHIN AT 1.1Å RESOLUTION. By Xing-qi Ma*, Lei Jin, Hai Yun Gong, Da Cheng Wang, Institute of Biophysic, Academia Sinica, Beijing, China.

Trichosanthin(TCS) is a member of a larger group proteins called ribosome-inactivating proteins(RIP). These proteins all function to catalytically inactivate eukaryotic 60S ribosomal subunits leading to rapid shutdown of protein synthesis.

Interest in RIP is growing due to several recent discoveries. The antiviral activity of the RIPs has focused attention on their use as potential anti-HIV agent,and the abortfacient activity of Tian Hua Fen, a popular Chinese medicine widely used in China prepared from the root tuber of Trichosanthes Kirilowii, has been shown to be due to Trichosanthin which is identified as an RIP also recently.

TCS crystalizes in the space group P212121 with one protein molecule (247 amino-acid residues) in the asymmetric unit. The cell constants are a=38.23 b=76.58 c=79.12. The diffraction data employed in the initial stage of the study were collected from the area detector at 1.7Å resolution, from which an initial TCS structural model was built up. Recently we have obtained a set of data at 1.1Å resolution using synchrotron radiation from KEK of Japan. The reflection with $F>1.5\sigma(F)$ are 62% of possible total reflections. The overall merging R factor was 5.6%. The earlier refinement provided starting parameters for the work here. The TCS model was further refined at this very high resolution by the reciprocal space refinement with energy restraint (EREF), and the 'foreign' solvents were excluded. The conventional R-value is now 0.25 for the 59064 reflections with $F>1.5\sigma(F)$ and 10-1.1Å resolution. The precision of the model is much improved over the earlier refinement. In the best determined regions of the TCS molecule, H atoms are visible in the difference map. Structural heterogeneity is observed for a significant fraction of the amino-acid residues in the protein. Most commen are flexible side chain on the protein surface. Discrete disorder extents into the ordered solvent regions of the crystal as well. Now the crystallographic refinement and the model rebuilding are still in progress.

PS-03.12.02 SOLVENT STRUCTURAL STUDY ON DEUTERATED SPERM WHALE MYOGLOBIN BY BOTH X-RAY AND NEUTRON DIFFRACTIONS. By Fang Shu*[†], V. Ramakrishnan[‡] and Benno P. Schoenborn[§], [†]Physics Department, SUNY at Stony Brook, [‡]Biology Department, Brookhaven National Laboratory, [§]Life Science Division, Los Alamos National Laboratory, USA.

Neutron diffraction has become one of the best ways to study light atoms, such as hydrogens. Hydrogen, however, has a large incoherent scattering factor, leading to high background, while deuterium has virtually no