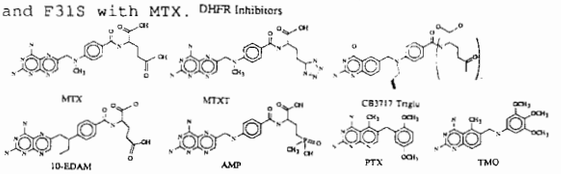
cancer folate and antifolate inhibitors which probe specific regions of the active site. Data were collected to a maximum resolution of 2.0A on a Rigaku Imaging Plate area detector for the following wild type ternary antifolate complexes: methotrexate-γ-tetrazole (MTXT), aminopterin-γ-phosphinothricin (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

incoherent scattering. A fully deuterated sample will thus yield better diffraction data with stronger density in the hydrogen position. On this basis, a sperm whale myoglobin gene (Barry A. Springer and Stephen G. Sligar, Proc. Natl. Acad. Sci. USA, 1987, 84,8961-8965) modified to include part of the lambda cII protein gene (Kiyoshi Nagai and Hans Christian Thogersen, Nature, 1984, 309, 810-812) has been cloned into the T7 expression system. The fusion protein has been overexpressed in *E. coli* to a very high level both in protonated and deuterated media. Because of the solubility and folding problems during purification, different bacterial strains and induction conditions have been searched to work out an optimal procedure. After reconstitution with heme and cleavage with trypsin, milligram amounts of holo-myoglobin have been obtained. Crystallization trials have been successful. The crystals are large enough for both x-ray and neutron studies. The synthetic sperm whale myoglobin crystallizes in $P2_1$ space group isomorphously with the native protein crystal, which makes possible a comparison with previous studies (Xiaodong Cheng and Benno P. Schoenborn, Acta. Crystal., 1990, B46, 195-208). We are currently crystallizing deuterated myoglobin. Results of diffraction experiments on these samples will be presented.

PS-03.12.03 REFINED CRYSTAL STRUCTURE OF CHICKEN ANNEXIN V. D.A.Waller*, M.C.Bewley & J.H.Walker, Department of Biochemistry and Molecular Biology, University of Leeds, Leeds, England

The annexins are a family of widely distributed calcium dependent phopholipid binding proteins. Annexins I–XI have been sequenced. They do not contain the classical E–F hand clacium binding motif of proteins such as calmodulin or troponin C, hence they are a distinct family of calcium–binding proteins.

The structure of chicken annexin V has been solved by molecular replacement using the full coordinates of human annexin V as a search model. It has been refined by restrained least–squares methods to an R–factor of 19.0% at 2.25Å resolution. The structure includes three calcium ions and 82 water molecules.

The calcium ions are bound in three of the eight loops on the surface of the protein which is thought to bind to the membrane. Studies are underway to locate additional metal ion binding sites analogous to those found in the human protein.

PS-03.12.04 CRYSTAL STRUCTURE OF PSOPHOCARPIN B_1 : A CHYMOTRYPSIN INHIBITOR FROM WINGED BEAN SEEDS. By J.K.Dattagupta, A.Podder, C.Chakrabarti*, U.Sen, S.K.Dutta[1] and M.Singh[1], Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhannagar, Calcutta 700 064, India. [1]Indian Institute of Chemical Biology, Calcutta 32, India.

Psophocarpin B_1, the winged bean (*Psophocarpus tetragonolobus*) chymotrypsin inhibitor (WCI) is a single-chain polypeptide (MW 20 KD) having 183 amino acid residues. It belongs to the Kunitz (STI) family of inhibitors and has sequence homology with other members of the family such as Soya bean trypsin inhibitor (STI), Erythrina trypsin inhibitor (ETI) etc.

The inhibitor, isolated from the seeds and purified to homogeneity, was crystallized from 25% ammonium sulphate, 0.1M-Tris·HCℓ, 0.4M NaCℓ, pH 8.0, using vapour diffusion method. The crystals are hexagonal, space group $P6_122$, cell dimensions a=b=61 Å, c=210 Å. X-ray diffraction data (2.9 Å) has been collected on an area detector and the molecular replacement method has been used to solve the structure, utilising the close homology existing between WCI and ETI. Refinement is underway using restrained least-squares and the current crystallographic R-factor is 30%.

The three dimensional structure of the inhibitor as found by us is similar to that of ETI and STI structures - there is however a difference in the reactive site loop (Leu 65 - Ser 66 is the scissile bond) which appears to be somewhat displaced. From the preliminary results of our crystal structure analysis and using the known structure of chymotrypsin, a possible mode of binding has been predicted which is consistent with other serine proteases-protein inhibitor complexes. It is observed that the rigidity of the reactive site loop in the inhibitor is not due to any `S - S' bond or salt bridge but through hydrogen bonding with the N-terminus loop, Asn 14 and P_4 Ser, the last two acting as spacers. Some structurally and functionally important residues alongwith Asn 14 and P_4 Ser are seen to be conserved in all the members of inhibitor family - this may be considered to be responsible for the same type of structural rigidity of the reactive site loop and common mode of action of the legume inhibitors of this family.

One of the authors (A.P) acknowledges the help of Prof. T.L.Blundell, Prof. D.M.Blow and their colleagues for extending the computational facilities of their laboratories at Birkbeck and Imperial Colleges during a short visit by her with a British Council fellowship. The ETI coordinates were kindly made available to us by Prof. D.M.Blow.

PS-03.12.05 PREDICTION OF WATER AROUND POLAR PROTEIN SIDE CHAINS: AN AID TO STRUCTURE REFINEMENT. By S.M. Roe, The Nitrogen Fixation Lab, The University of Sussex, Brighton, BN1 9RQ, England and M.M. Teeter*, Department of Chemistry, Boston College, Chestnut Hill, MA 02167. USA

Water is important in stabilizing the folded conformation of a protein and also is necessary for enzyme activity. Its inclusion in a crystallographic model can be beneficial during refinement or understanding the mechanism of enzyme action. We have analyzed the patterns in hydration of polar side chains around crambin and 6 other proteins which diffract to bettter than 1.4Å resolution (S.M. Roe and M.M. Teeter, J. Mol. Biol., in press (1993)).

Correspondence between the solvent positions around residue side chains can be found by superimposing identical functional groups and their accompanying hydrogen bonding spheres. Well defined hydrogen-bonding shells can be located. Solvent positions around amino side chains are more ordered than around carboxyl. This is true especially where both amino and carbonyl groups are present in the same residue, i.e. asparagine.

A template has been developed which permits prediction of water positions around polar groups. This was tested on crambin and as well as two protein not in our hydrogen bonding database. Rms deviation from water positions were less than the resolution of the structures. The algorithm was also tested in refinement of two proteins. Inclusion of water lowered the R value by 2-4% after refinement. 86-91% of the waters were judged to be well predicted and could refine within the radius of convergence.