03-Crystallography of Biological Macromolecules

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 (Sary A. Springer and Stephen G. Sigal,
Proc. Natl. Acad. Sci. USA, 1987, 84, 8684-8685) modified to include part
of the lambda cDNA protein gene (Kosugi Nagai and Hans Christian
Thogersen, Nature, 1984, 309, 610-612) has been cloned into the T7
expression system. The fusion protein has been overexpressed in E. coli
to a very high level both in prokaryotic and eukaryotic cells. Because of
the elution and folding power 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 isomorphous with the native protein crystal, which makes
possible a comparison with previous studies (Xiaodong Cheng and Benno
F. Schoenborn, Acta. Crystal., 1990, 44B, 195-208). We are currently
crystallizing deuterated myoglobin. Results of diffraction experiments
on these samples will be presented.

P5-03.12.03  REFINED CRYSTAL STRUCTURE OF
CHICKEN AXINIX V. A. Walker*, M.C. Bewley &
I.H.Walker, Department of Biochemistry and Molecular Biology,

The annexins are a family of widely distributed calcium
dependent phospholipid binding proteins. Annexins I–XII have
been sequenced. They do not contain the classical F–E
hendecatin-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.

P5-03.12.04  CRYSTAL STRUCTURE OF PEPHORCARPIN B4
A CHYMOTRYPsin INHIBITOR FROM WINGED BEAN SEEDS.
By J.K. Duttagupta, A.Fotton, C. Chakrabarti*, D. Sen,
S.K. Dutta* and M. Singh*. Crystallography and Molecular
Biology Division, Saha Institute of Nuclear Physics, 1/AF
Biharannagar, Calcutta 700 064, India. Indian
Institute of Chemical Biology, Calcutta 32, India.

Pephorcarpin B4, the winged bean (Pelecopedus tetragonolo-
bus) chymotrypsin inhibitor (WCI) is a single-chain
polypeptide (MW 20,600) having 183 amino acid residues.
It belongs to the Kunlun (STLI) family of inhibitors and
has sequence homology with other members of the family
such as Soga bean trypsin inhibitor (STI), Erithrina
trypsin inhibitor (EITI) etc.

The inhibitor, isolated from the seeds and purified
to homogeneity, was crystallized from 3% ammonium
sulphate, 0.01M-Tris HCl, 0.01M NaCl, pH 8.0, using vapor
diffusion method. The crystals are hexagonal, space
group P6221, cell dimensions a=b=6.1 Å, c=210 Å. X-ray
diffraction data (2.9 Å) have been collected on an area
detector and the molecular replacement method has been
used to solve the structure, utilizing the close
homology existing between WCI and STI. The
refinement is underway using restrained least-squares and the current
crystallographic R-factor is 10%.

The three dimensional structure of the inhibitor as
found by us is similar to that of BmI 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
multiple site of binding has been predicted which is
consistent with other serine protease–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 P4 Ser,
the last two acting as spacers. Some structurally
and functionally important residues among Asn 14 and P4
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 in
the reactive site loop and common mode of action of the
leucine inhibitors of this family.

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laboratories at St John's College, Oxford and
University of California during a short visit by her with
Prof. C.R. Low. The X-ray crystallography data were
collected by Prof. D.M. Blow and his colleagues.

P5-03.12.05  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 9QH, England and M.M.
Taiber*. 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 enzymatic activity. Its inclusion in a crystallographic model can be beneficial during refinement or understanding of the mechanism of enzyme action. We have analyzed the patterns in hydration of polar side chains around crombin and 6 other proteins which afforded to better than 1.4 Å 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 acid side chains are more ordered than around carboxyl. This is true especially when both amino and carboxyl 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 crombin and as well as two other protein 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.