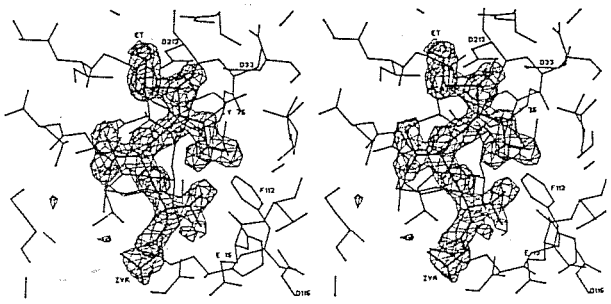
02.10-5 THE USE OF NORMAL MODES IN THERMAL PARAMETER REFINEMENT. By R. Diamond, M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge, England.

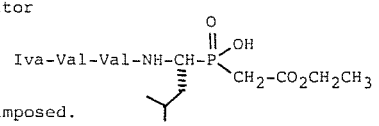
It is now possible to estimate the displacement vectors for each atom of a macromolecule for each normal mode of vibration, especially for the low frequency high amplitude modes. (Levitt M., Sander C. and Stern P.S. (1985) J. Mol. Biol., 181, 423-447). In the present work amplitude coefficients of each of a number of such modes are treated as independent variables in a refinement against X-ray data. This enables anisotropic thermal parameters to be estimated for each atom with a degree of detail dependent on the number of modes employed. Comparisons between results obtained by this means and by conventional isotropic temperature factor refinement will be shown.

02 11-1 REACTION PATHWAY OF ASPARTIC PROTEINASES SUPPORTED BY SUBSTRATE ANALOGUE BINDING TO PENICILLOPEPSIN. By Anita R. Sielecki, Natalie C.J. Strynadka and Michael N.G. James, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Penicillopepsin belongs to the aspartic proteinase class of hydrolytic enzymes. It has been proposed that these enzymes catalyze the hydrolysis of peptide bonds via a non-covalently bound tetrahedral intermediate (James & Sielecki, Biochemistry 24, 3701 [1985]). This pathway was deduced from the 1.8 Å resolution structure of native penicillopepsin and the 1.8 Å resolution structure of the inhibitor Iva-Val-Val-Sta-ethylester bound to penicillopepsin [Sta = statine or (4S,3S)-4-amino-3-hydroxyl-6-methylheptanoic acid]. Both structures were crystallographically refined to relatively low R-factors (0.136 and 0.130). The proposed tetrahedral intermediate of a peptide substrate results from the general base assisted attack of a water molecule on the carbonyl-carbon atom of the scissile bond. Stabilization of this intermediate results from hydrogen-bonding interactions of the gem-diol with the carboxylate of Asp33 in the active site of penicillopepsin. In collaboration with Michael Gelb and Robert Abeles of Brandeis University we have extended these studies to inhibitors that have difluorostatine and difluorostatone in the P_1 sites. Both inhibitors bind in an analogous fashion as the one described in the initial work with Iva-Val-Val-Sta ethyl-ester. The difluorostatone analogue is catalytically hydrated by penicillopepsin. The resulting gem-diol is an excellent mimic of the tetrahedral intermediate. The refined structure at 1.8 Å resolution (R = 0.140) convincingly confirms the previously proposed model-built tetrahedral intermediate of a good substrate. Recent work in collaboration with Paul Bartlett, University of California at Berkeley, shows the mode of binding of a phosphonate inhibitor (see Figure) to aspartyl proteinases. The tetrahedral phosphonyl group does not bind similarly to the gem-diol of the difluorostatone, presumably due to the larger size of the phosphorous atom.



Difference electron density map with model of the inhibitor



superimposed.

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02.12-1 WATER NETWORKS IN THE CRAMBIN CRYSTAL STRUCTURE. Martha M. Teeter, Dept. of Chem., Boston College, Chestnut Hill, MA 02167 USA.

Crystals of the hydrophobic protein crambin (5000 MW) diffract to better than 1.0 Å resolution and have more than 90% of the solvent in the crystal ordered. This protein provides an excellent opportunity to study the distribution of water molecules at a protein surface in atomic detail which is not generally available for other protein crystal structures. The function of the plant protein crambin is not yet known, however it is homologous to the membrane-active plant toxins purothionin (from wheat germ) and viscotoxin (from mistletoe), which are lytic to plant pathogenic bacteria.

Crambin's X-ray diffraction data to 0.945 Å interplanar d spacing at 300 K and to 0.83 Å at 130 K have been collected and the model of the structure has been refined. Two types of water networks are formed: chains at the hydrophilic protein surface and rings, primarily pentagons, at the hydrophobic protein surface.

Analysis of the 140 K model has revealed alternate chains of water molecules which completely traverse the largest channel in the structure. The details of these networks will be described. Water molecues are distributed in four primary regions of the crystal. The variation in the disorder and vibration of water in each of these regions will be noted. Correlations will be made with the neutron diffraction data collected on crambin at 300K to 1.1Å.

Insight into role of water in stabilizing the protein is gained which applies to stabllization of proteins in general and to modelling the effect of changes on protein conformation on this water structure. The resulting atomic picture of the role of water at a protein surface permits more realistic predictions of hydration at a protein surface to be made.