a somewhat compact shape inspite of the positive charges on the two bases; the adenine and nicotinamide rings are about 9.6Å apart. The planes through the two bases are parallel and are at 4.08Å apart. The C-NH_2 of the carboxamide group is trans to C(3)-C(4) of the nicotinamide whereas it is cis in the Li^+-complex. Both the nucleotides adopt the preferred conformation usually found for nucleotides, viz: anti, g^+ for C(4')-C(5') and C(2')-endo. Both the sugars exhibit a very pronounced bond-shortening anomeric effect (i.e. C(1')-O(4') < C(4')-O(4') by 0.078Å and 0.039Å respectively for the sugars attached to nicotinamide and adenosine. The pyrophosphate group exhibits what appears to be an inherent asymmetry in the P-O bonds of the P-O-P link; P_N-O greater than P_A-O by 0.04Å. The P_N-O-P_A angle has widened considerably to 133.3(1)°, allowing a variety of possible conformations across the two P-O bonds. Looking along the P...P virtual bond, the phosphate groups are staggered, a conformation quite different from that in the Li^+-complex. There is no intramolecular stacking, but this structure exhibits a novel intermolecular stacking giving rise to sandwiching of nicotinamide half way between adenine and water molecules 6.97Å apart. It is interesting to observe that the binding sites in different enzymes for such a chameleonic coenzyme is so similar.

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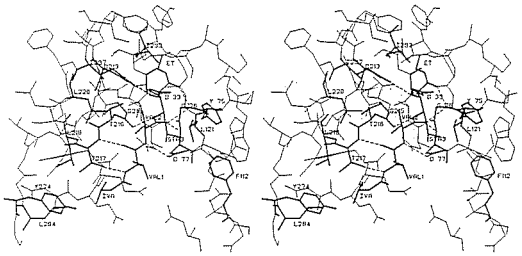
02.11–1 STRUCTURAL TRENDS IN N-ACYLTHIOESTERS OF RELEVANCE TO ACYLPAPAINS. By C. P. Huber and K. I. Varughese, Div. of Biological Sciences, National Research Council of Canada, Ottawa, Canada K1A 0R6.

We are determining the crystal structures of a series of dithio- and thiol-esters which are closely related to the ester groups in the active site of acylpapains. The conformation of eight of these compounds is characterized by small N-C-C-S (ψ') torsional angles, with short N···S(thiol) distances in the range 2.83 - 2.93 Å, and by nearly orthogonal amide and thioester planes, leading to an N···S interaction. The C-N-C-C (ϕ') torsional angles are in the range -75.4 to -97.1° and the ψ' angles are in the range 9.5 to -23.0°. In a Ramachandran-type plot, the values of ϕ', ψ' for four N-acylglycine ethyl dithioesters lie essentially along a straight line which seems to represent the conformational pathway with maximal nitrogen-sulfur orbital interaction. Values of ϕ', ψ' for two N-acylalanine ethyl dithioesters and for two N-acylglycine ethyl thiolesters, while in the same range, show some deviations from the straight line. Resonance Raman spectroscopic studies of transient dithioacylpapains (Ozaki, Pliura, Carey and Storer, (1982), Biochemistry, 21, 3102) indicate that in the major population of the acyl-enzyme the dithioacyl group assumes a comparable conformation with N···S interaction. The fact that the conformations of the thiolesters in our series are very similar to those of the dithioesters suggests that information obtained for the dithioacylenzymes may be transferable to the natural thiol-intermediates.

02.11–2 A STEREOCHEMICAL ANALYSIS OF THE ASPARTYL PROTEINASE HYDROLYTIC MECHANISM. Michael N.G. James and Anita R. Sielecki, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The crystal and molecular structure of penicillopepsin, the aspartyl proteinase from *Penicillium janthinellum*, has been refined at 1.8 Å resolution to an R-factor of 0.136 for the 21,962 reflections with $I \geq 1\sigma(I)$ (James & Sielecki, J. Mol. Biol. 163, 299 [1983]). The close proximity of the two catalytically important carboxyl groups of Asp33(32) and Asp213(215) suggests that they share a proton (d_{O-O} = 2.87 Å) in a tight hydrogen-bonded environment (residue numbers in parentheses refer to those of porcine pepsin). Hydration of this active site region suggests that a specific water molecule hydrogen-bonded to Asp33(32) plays the role of the attacking nucleophile (OH^-) in the hydrolytic mechanism. A plausible substrate binding mode to penicillopepsin has been deduced on the basis of the observed binding of a pepstatin analogue (James *et al.*, Proc. Natl. Acad. Sci. 79, 6137 [1982]). Crystallographic refinement of this molecule, Isovaleryl-valyl-valyl-statyl ethyl ester, in the complex [R = 0.131 for 21,197 reflections, $I \geq 1\sigma(I)$] shows the detailed binding interactions responsible for its inhibitory character ($K_I = 2.4 \times 10^{-8}$M). This inhibitor is taken as a model for the tetrahedral intermediate in the catalytic pathway of a good substrate. The statyl residue has a secondary hydroxyl group on a tetrahedral carbon atom analogous to the C=O group of the scissile bond. The figure below shows the refined structure of the pepstatin analogue bound to penicillopepsin. The hydroxyl group is bound between the two aspartyl carboxyl groups and replaces a strongly bound solvent from the native enzyme.

In spite of the remarkable 2-fold symmetric arrangement of the active site region of the aspartyl proteases, the



interaction with substrates is decidedly asymmetric. Residues in penicillopepsin involved in hydrophobic binding of P_1 residues are tyrosine-75(75), phenylalanine-112(111) and leucine-121(120); those most important for binding P_1' residues of a substrate are: phenylalanine-190(189), isoleucine-211(213), phenylalanine-295(299), isoleucine-297(301) and isoleucine-293(297).

It is proposed that the electrophile is the shared proton between Asp213(215) and Asp33(32); the nucleophile is a water that is activated to an OH^- by the proximity of Asp33(32); the leaving group is protonated by the acidic solution in the cases of those proteases with pH optima in the range 1-4. Analysis of the stereochemistry of this proposed reaction pathway suggests a tetrahedral intermediate with opposite hand to that proposed for the serine proteinases.

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02.12–1 NEUTRON DIFFRACTION STUDY OF THE INTERACTION OF ETHANOL WITH LYSOZYME. By M.S. Lehmann, S.A. Mason and G.J. McIntyre, Institut Laue-Langevin, 156X, 38042 Grenoble Cédex, France.

The study was undertaken to estimate experimentally both how numerous and how specific the hydrophobic interactions can be on a protein surface. An additional aim was to assess the usefulness of neutron diffraction techniques for location of very small deuterated molecules in the protein solvent.

A non-deuterated triclinic crystal of hen egg-white lysozyme (Hodsdon, J.M., Sieker, L.C. & Jensen, L.H. (1975), A.C.A. Abstr. 3, 16) was soaked at pH 4.6 in a solution containing 25% C_2D_5OH. Reflections were measured in 9 days at 280 K on the neutron diffractometer D8, and consisted of all data to 2.4 Å and the 70% strongest to 2.0 Å; in all 6047. The wavelength was 1.675 Å and the cell is 27.332 Å, 32.158 Å, 34.274 Å, 88.34°, 108.63° and 111.80°. Ethanol molecules were located after preliminary refinements, and 16 ordered water molecules from the deuterated structure were included. Each ethanol molecule was described as two hard spheres of radii 1 Å separated by 2 Å. The Hendrickson-Konnert restrained refinement program was used, and the final R value was 0.097. Including the flat solvent did not improve the R factor significantly, but did lead to very good agreement between the thermal parameters for this structure and the previous higher resolution neutron studies of the triclinic lysozyme structure (Mason, S.A., Bentley, G.A. and McIntyre, G.J. (1982) Brookhaven Symposium 32, in press).

In all 15 ethanol sites were found with occupation between 1.0 and 0.2, and in every case there was clear evidence of hydrophobic interactions. Comparison of the experimentally determined local hydrophobicity of the surface with calculation of accessible surface, and with the water structure in non-alcoholic lysozyme will be given.

02.12–2 ON THE CONSERVATION OF PROTEIN-SOLVENT INTERACTIONS IN IMMUNOGLOBULIN VARIABLE DOMAINS. By S. Swaminathan, W. Furey, C.S. Yoo*, B.C. Wang and M. Sax, Biocrystallography Laboratory, P.O. Box 12055, VA Medical Center, Pittsburgh, PA 15240 and the Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260

A detailed study of the water structure of Bence-Jones protein Rhe was made at 1.6Å resolution (Furey, Wang, Yoo and Sax(1983), J. Mol. Biol. 167, 661-692). Several tightly bound water molecules making at least two hydrogen bonds with the protein were found; in some cases the water molecules were located in a cavity and were completely surrounded by residues of non-hypervariable regions. We suggest that these waters may be structurally significant and should be considered an integral part of the protein itself.

As a test of this idea, we have undertaken a study to see whether these structural water molecules are present (or can be accommodated) in other crystal structures of V_L domains. We found that the structural waters in the non-hypervariable regions of Rhe could indeed be accommodated in the corresponding regions of other V_L domains. The method and results will be presented.

* Deceased, August 31, 1983

02.12–3 ENVIRONMENTAL EFFECTS ON WATER-MEDIATED TRANSFORMATIONS IN THE CRYSTALS OF RIBONUCLEASE A. By D.M. Salunke, R. Kodandapani and M. Vijayan, Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560012, India.

We have earlier shown that a new monoclinic form of ribonuclease A, prepared by acetone diffusion into a protein solution in tris buffer, undergoes a reversible transformation, as evidenced by changes in the diffraction pattern and the cell dimensions, when the relative humidity around the crystal is reduced to 93% (Curr. Sci. (1984) in press). Subsequent studies indicate that the well-known crystal form of ribonuclease A grown from 55% 2-methylpentan-2,4-diol (MPD) in phosphate buffer does not transform even when the relative humidity is reduced beyond 75% whereas the same form grown from aqueous ethanol transforms between relative humidities of 93% and 90%. The new crystal form, after being soaked in the phosphate buffer-MPD mixture, transforms only when the relative humidity is reduced to about 75%. It, however, readily transforms at a relative humidity of 90%, when aqueous ethanol is used for soaking. Further experiments on water-mediated transformation of different forms of ribonuclease A and other proteins, under different environmental conditions are in progress. The results obtained so far suggest that environmental factors significantly influence these transformations which could well involve well-defined conformational transitions as a function of hydration, in addition to changes in crystal packing.

02.12–4 ANALYSIS AND PREDICTION OF SOLVENT STRUCTURES IN PROTEIN CRYSTALS. By J. Moult, A.R. Sielecki and M.N.G. James, MRC Group in Protein Structure and Function, Biochemistry Department, University of Alberta, Edmonton, Alberta, Canada.

The refined high resolution structures of eight enzymes and enzyme inhibitor complexes obtained in this laboratory have provided the basis for an analysis of ordered solvent structure in protein crystals. The results of this analysis have been used to develop an algorithm for the prediction of ordered water molecule locations on the surface of globular protein molecules.

In agreement with other workers, we find a monolayer of ordered solvent covering nearly all the accessible surface of these proteins, with approximately 15% of the observed solvent molecules forming part of a second layer. The molecules exhibit a wide range of order and occupancy, with a strong correlation between these properties and the degree of interaction with the protein surface, as assessed in both electrostatic and van der Waal's terms.

Although all these crystals are grown in the presence of high concentrations of salt, we find only three unique ordered ion positions in the entire set. All three are involved in inter-molecular interactions. We conclude that the large disordered solvent regions in these crystals offer an energetically more attractive environment for this type of ion. Thus, binding is only observed in very specific niches, or when the number of ions needed to maintain electrical neutrality is too high for them all to be accommodated in the disordered region.

The ordered solvent structure often forms a bridge between adjacent protein molecule surfaces, and there are rather few direct protein-protein contacts. Possible interpretations of such bridge solvent structures are that they increase the stability of the crystal