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

structure, act as a filler to match the irregular protein surfaces, or are simply formed by the juxtaposition of the solvent structures present around the isolated protein molecules in solution.

The analysis suggests that the ordered solvent positions are determined largely by short range electrostatic and van der Waal's interactions with the protein surface. The position prediction algorithm has been developed on this basis. The energy of the molecular dipole and van der Waal's sphere of a water molecule interacting with the dipoles, charges and van der Waal's surface of the protein is calculated, assuming the water dipole to be optimally oriented. This energy is determined for points on a grid around the protein, and energy minima in the grid are then identified. Minima positions may then be compared with observed solvent molecule positions. Tests of this method on the refined 1.5 Ångstrom resolution crystal structure of the serine protease SGPA using the 100 most strongly ordered solvent molecules found crystallographically yields 55 of these minima within 1 Ångstrom of an experimental position, using a 0.5 Ångstrom grid. Relaxing the minima criteria to produce about 50% over-prediction increases this score to 77 of the 100. Inspection of the energy surface suggests the undetected molecules lie in minima defined by the detected ones and the protein surface together. This hypothesis is currently being tested.

02.12–5 SOLVENT INTERACTIONS IN B12 COENZYME CRYSTAL HYDRATE

by F. Vovelle[a], J.M. Goodfellow, J.L. Finney, H.F.J. Savage[b], and P. Barnes, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, UK.

The structure of Vitamin B12 coenzyme crystal hydrate has been determined at better than 1.0Å resolution using both X-ray and neutron diffraction data[1]. A highly refined experimental model involving several alternative hydrogen bonding networks, has been obtained for both the ordered pocket and disordered channel regions. For this reason, we have chosen to study these solvent regions using Monte Carlo computer simulation in order to predict both the ordered and disordered regions. The results using several different water models have been compared with experiment using a detailed analysis of hydrogen bonded solvent networks in terms of both average predicted atomic positions and atomic positions estimated from the maxima of probability density maps from all four asymmetric units for this crystal. The solvent molecules are allowed to move independently without crystal symmetry constraints.

The following was found:

(i) Within each asymmetric unit only one hydrogen bonded network was predicted although there were several possible hydrogen atom positions for any one solvent molecule (defined as maxima of probability density).

(ii) Reasonable agreement was obtained between predicted and experimental positions in the ordered solvent region whatever the potential function used. It should be possible to improve this level of agreement as the agreement between experimental and predicted results was always larger than the agreement between the different predicted asymmetric units.

(iii) The positions of the probability density maxima were different in different asymmetric units for the disordered channel region. This led to different hydrogen bonded networks which were not always consistent with the experimentally determined alternative (lower occupancy) sites. This implies that it is essential to simulate more than one asymmetric unit if one wishes to look at disorder in solvent regions.

(iv) Probability density maps were qualitatively very useful for picturing these disordered regions. However, there were no significant differences between quantitative results predicted using either average atomic positions or maxima of the probability density distributions.

Although it is difficult to quantify the best agreement between experimental and predicted disordered solvent networks, the potential which included hydrogen atoms explicitly (EMPWI)[2] seemed to give overall best agreement possibly because it was successful in predicting some of the unusually short hydrogen bonds (less than 2.6Å) which are found in this crystal system.

1) H.F.J. Savage, PhD Thesis, University of London, 1983

2) F. Vovelle and M. Ptak, Int. J. Peptide Protein Res., 435-446 (1979)

a) Centre de Biophysique Moléculaire Orléans, France.

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02.12–6 SOLVENT INTERACTIONS IN NUCLEOTIDE CRYSTAL HYDRATES

by Julia M Goodfellow and P. Lynne Howell, Department of Crystallography, Birkbeck College Malet St., LONDON WC1E 7HX, UK.

Since the earliest fibre diffraction patterns it has been known that solvent plays an important role in the stability and transitions of the different helical forms of nucleic acids. With the accumulation of a number of dinucleotide structures and a few oligonucleotide structures, it is now possible to look theoretically at the molecular nature of the interactions of nucleic acids with solvent (both water and counterions) and with drugs(which may also act as counterions). We have used two techniques to study these interactions at the molecular level. Firstly, computer simulation techniques have been used to predict solvent networks in crystal hydrates. This involves the use of 'realistic' potential energy functions i.e. those which give close agreement with experimental data, if meaningful predictions are to be made. Secondly, we have used accessibility calculations to look at the variation of contact and accessible areas in (a) di- and oligo-nucleotide sequences in the classical helical and crystal forms and (b) dinucleotide and dinucleotide-drug complexes for which there are three-dimensional atomic coordinates. Differences in solvent exposed areas may be important for both the stability of the different helical forms and for sequence specific recognition processes.

In the study of small nucleotide crystal hydrates, computer simulation techniques are being used to predict structural details of the solvent networks using potential energy functions derived for water-amino-acid interactions[1]. A detailed comparison of predicted and experimental results on the structure of the solvent networks has been made and includes an analysis of both