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 est imated 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

local environment and hydrogen bonding networks of each water molecule. A correlation between the environment of each unique water molecule and its energetic properties such as dipole moment and binding energy was seen. Moreover, non-pair additive effects were found to be non-negligible. However, agreement between predicted and experimental results was not as good as the agreement between the different predicted asymmetric units (which were all simulated independently of the crystal symmetry constraints). This implies that there is room for improvement in the potential energy functions in order to obtain closer agreement with experimental data. Therefore, we have been studying modifications to these potentials which include either all hydrogen atoms or polar hydrogen atoms explicitly rather than the united atom approach used initially. Further studies involve a more specific solute potential combined with our PE water model.

In the second part of this study of hydration of nucleotides, both accessible and contact areas have been calculated for di-, tetra- and dodeca- nucleotides in the classical A, B and Z helical forms. Changes in the solvent exposure of some residues have been found e.g. the free phosphate oxygens have increased contact and accessible areas as one goes from the A to the B to the Z form, and the 5' sugars have less solvent exposed areas than the 3' sugars but this is due to a different balance of atomic accessibilities in the A and B forms. Sequence specific changes to these solvent exposures have also been investigated as a function of the number and type of neighbouring bases. Similar calculations are being undertaken for (a) dinucleotide drug crystal structures in order to compare the changes in solvent exposure on binding intercalating drugs and (b) oligonucleotide structures to look at the effects of local changes in structure compared with the classical forms.

1. Goodfellow, J.M., J. Theor. Biol., In press (1984).

02.12–7 STRUCTURAL ANALYSIS OF THE PROTEIN CRAMBIN FROM X-RAY DIFFRACTION STUDIES AT 0.945 A. Martha M. Teeter, Department of Chemistry, Boston, University, Boston, MA 02215 USA.

Crambin is a small, hydrophobic plant protein (MW = 4700) with no known function. However, crystals of it diffract to 0.88 A (Teeter and Hendrickson, J. Mol. Biol. (1979) 127, 219-224). The model obtained from the structure solution and refinement at 1.5 A (Hendrickson and Teeter (1981) Nature 290, 107-113) has now been refined against the 0.945 A data by restrained least squares techniques (Hendrickson and Teeter, unpublished).

Crambin crystals contain 32% solvent. More than 80% of the solvent is ordered. Despite the fact that crystals were grown from 60% ethanol, very little ethanol (about 7%) has been located in the crystals. Most of the water found at the surface of crambin is bound to polar groups at the surface. These molecules connect donors and acceptors on the protein in lines. However, a cluster of waters at a hydrophobic intermolecular interface, sandwiched between charged protein side chains, form pentagon arrays around a methyl group on the surface. This is the first time such a cluster has been described at a protein surface and is reminiscent of the water clathrate structures of quaternary amines, aliphatic amines and alcohols. The significance of crambin's pentagonal water array for solvent ordering in general at hydrophobic protein surfaces is unclear and will be discussed.

The detailed geometry of crambin (bond lengths and angles, dihedral and planarity) will be described and compared with peptide geometry information available from analysis of the Cambridge Data File. Features of the secondary structure (hydrogen bonding patterns and distortions from ideal geometry) will be discussed.