pumping a Rhodamin-6G dye laser with a repetition rate of 3 sec^{-1}. The laser wavelength was 590 nm, the energy per pulse 800 μJ, the pulse length 10 nsec. Data were accumulated during about 1000 repetitions. An exponential time dependence corresponding to a lifetime of 5 msec has been observed for certain reflections. The figure shows an example for the time course of an integrated reflection intensity. Such a time behaviour would be in agreement with optical results obtained for CO-Myoglobin in solution at room temperature (Austin, Beeson, Eisenstein, Frauenfelder and Gunsalus, Biochem. (1975)14, 5355). It is, however, at present not yet clear whether the observed intensity changes entirely reflect structural changes accompanying debinding of the ligand, or whether lattice effects may contribute.

Even higher time resolution on a subnanosecond time scale may eventually be achieved by using the pulsed time structure of S.R. and by varying the time lag between the stimulating laser pulse and the S.R. pulse. A test application of this technique is in progress.

02.8-01 CIRCULAR HYDROGEN BONDS. By Wolfram Saenger,

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α-Cyclodextrin (α-CD) is a circular molecule composed of six glucoses and exhibiting six primary as well as 12 secondary hydroxyl groups. It crystallizes as hexahydrate or, in the form of its methanol complex, as pentahydrate. X-ray and neutron diffraction structure analyses allowed to locate water and hydroxyl hydrogens and to derive the hydrogen bonding scheme. In essence, circular structures are formed with four, five or six O-H groups arranged such as to point all in the same direction (homodromic; Saenger, Nature (1979) 279, 343) or to emanate from one H_2O molecule, and the two branches ending at one oxygen (antidromic). It is proposed that such circular hydrogen bonds play a role in water clusters and, in general, in hydration of biological macromolecules.

02.8-02 WATER MOLECULE-β-CYCLODEXTRIN INTERACTIONS.

By Kurt H. Jogun, John M. Maclennan and John J. Stezowski, Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart, Pfaffenwaldring 55, Stuttgart, Federal Republic of Germany.

β-Cyclodextrin (β-CD), cycloheptaamylose, is a medium molecular weight (1135 daltons) cyclic oligosaccharide that mimics biologically important macromolecules in several ways. Among them are its ability to form host:substrate complexes (in some cases catalytic activity is observed) and its ability to associate to form dimers that display good structural integrity in the crystalline state and very likely exist in aqueous solution, particularly at high concentrations.

Crystalline β-CD and many of its host:substrate complexes are highly hydrated. We have found that, in several cases, the crystals can be cooled to $ca.$ 120 K and that high resolution data sets (typically $2\theta_{max}$ = 60 deg., Mo radiation) can be collected which support extensive refinement of the crystallographic model.

A series of structure determinations of β-CD and host:substrate complexes have been carried out that we feel contribute to an understanding of the interaction of water with biological molecules. Aspects of the interaction of water with hydrophilic functional groups and with hydrophobic regions in the crystals will be examined.

02.8-03 NEUTRON DIFFRACTION OF β-CYCLODEXTRIN WATER INCLUSION COMPLEX: AN ANALYSIS OF H-BONDS AND WATER STRUCTURE IN A HYDROPHOBIC CAVITY.

By B. Hingerty[1], Biol. Div., ORNL, Oak Ridge, TN 37830, USA; G. M. Brown[2], Chem. Div., ORNL, Oak Ridge, TN 37830, USA; K. Lindner and W. Saenger, Max Planck Inst. für Experimentelle Med., Göttingen, West Germany.

Cyclodextrins (CD; cycloamyloses) have been used successfully as model structures in the study of non-covalent intermolecular interactions and enzyme mechanisms. These molecules have catalytic activity toward hydrolysis reactions. In order to better understand this activity it is necessary to know the H-bond interactions which are present. While X-ray structures can give the main features of the structure, it is not possible to sort out disordered H-bond networks. To do this we have collected neutron diffraction data for a D_2O exchanged β-CD crystal at the High Flux Isotope Reactor (HFIR) at Oak Ridge. The unit cell is: a = 21.261(6), b = 10.306 (3), c = 15.124(4)Å, β = 112.47(3)°, Z = 2, space group $P2_1$. Monochromatized radiation at λ = 1.015 Å was used initially to collect 3380 reflections with I/σ > 1 to 2θ = 65° (.94 Å resolution). Longer counting times were used at higher angles. From these data all missing deuteriums were located, including some water deuteriums with temperature factors of up to 60 Å^2. The structure was refined to an R-factor of .053 using bond length and angle constraints to retain proper stereochemistry, as is done in protein structures. A complicated H-bond network was revealed with an ice-like water structure. Chains of H-bonded β-CD hydroxyls have two alternate sets of H positions, so that for a given chain direction, all H bonds are OH···O, or alternatively, O···HO. Either a dynamic equilibrium, spatial disorder, or a combination of the two are indicated. More data have since been collected. By means of a structure factor calculation using the coordinates at R = .062, all the

strongest reflections to $2\theta = 130°$ (or .56 Å resolution) were identified. These were then collected, resulting in a total of 6619 reflections with $I/\sigma > 1$ after background averaging. Further analysis will continue with these data which initially have an R-factor of .094 without further refinement.

02.8-04   COMPUTER SIMULATION OF SOLVENT STRUCTURE AROUND BIOMOLECULES. By J. M. Goodfellow, J. L. Finney and P. Barnes, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, United Kingdom.

Monte Carlo computer simulation techniques have been used to investigate water-protein interactions at the molecular level. By extending the polarisable electropole model for water we have developed a set of interatomic potentials describing water-protein interactions. This model specifically accounts for non-pair-additive (cooperative) effects which are thought to be important in hydrogen-bonded systems. Small amino acid crystals have been used as test systems in which all the solvent molecules are well defined. The following parameters were used to compare the predicted structure with the observed structure: (a) average final positions of water oxygen atoms; (b) root-mean-square deviation about these final positions; (c) number and type of molecules coordinating the solvent molecules; and (d) the length and directionality of each hydrogen bond.

We have found that the final predicted water structure is very sensitive to non-bonded parameters, partial charges on the atoms and also to the polarisability of both the water molecules and the amino acid atoms. The magnitude of the increase in dipole moment of the water molecules depended on the system under investigation. We emphasise the need for: (1) adequately testing potential functions before extending the simulations to larger systems, and (2) the importance of including non-pair-additive effects in water-protein systems. Using these optimised interatomic parameters, we are extending this method to look at solvent-related effects in larger systems of biological interest including Vitamin B_{12} coenzyme, aPP, cyclodextrins, calcium-containing drugs and nucleic acids.

02.8-05   WATER STRUCTURE AROUND VITAMIN B_{12} COENZYME : A CHARACTERISATION BY NEUTRON/X-RAY DIFFRACTION AND COMPUTER SIMULATION. By H. F. J. Savage, J. L. Finney, P. F. Lindley, P. Barnes, Department of Crystallography, Birkbeck College, Malet Street, London, WC1E 7HX, UK.

This paper describes the results of neutron and X-ray refinements of the solvent organisation around the coenzyme of vitamin B_{12} and compares them with the hydration structure derived from computer simulation methods. The coenzyme is being used as a test system for further work aimed at understanding the structure and dynamics of larger aqueous macromolecular systems.

The diffraction data were collected at low temperature ($\sim 5°C$) from crystals grown in D_2O. Both the neutron and X-ray D_2O structures have been refined by least-squares methods to the crystallographic residuals $R \sim 13\%$ and $R \sim 14\%$, respectively, at 0.9Å resolution. There are several significant differences between the present D_2O model and the early H_2O model (P. G. Lenhert, Proc. Roy. Soc. A. 303, 45 (1968)). During the initial refinement it was recognised that the coenzyme molecule differed in its position within the unit cell by a small rotation (from Lenhert's model) and one of the sidechains was rotated by $\sim 70°$, causing the water structure in some parts of the unit cell to be noticeably different.

The refined neutron model consists of 21 water oxygen sites per asymmetric unit (several with unit occupancy) and the X-ray model includes 36 oxygen sites : the $\overline{U^2}$ values range from 0.07 to 0.15Å^2. The solvent structure is divided into at least 2 distinct regions :

(1) An ordered monolayer connecting adjacent coenzyme molecules.

(2) A partially ordered region comprising of a solvent channel and an interconnecting region. The waters in the first region appear to be generally coordinated with polar groups while those of the second region are associated with groups which are mainly apolar in character.

A Monte Carlo computer simulation (N. Metropolis et al, J. Chem. Phys., 21 1087 (1953)) in being undertaken using a whole unit cell of 4 coenzyme molecules and 68 initial water positions, selected from the X-ray and neutron sites. The interatomic potentials are based on the polarisable electropole model of water (P. Barnes et al, Nature, 282, 459 (1979)). Each water molecule-coenzyme atom interaction is defined by two non-bonded interaction coefficients and partial atomic charges (J. Goodfellow, see abstract).

The results of a fully equilibrated simulation will be presented. From the initial stages we have found that the water movements were orientational, but in the proceeding stages the following observations were made : (a) the movements of the waters in the ordered region are consistent with the crystallographic temperature factors and (b) there are significantly greater movements of the waters in the partially ordered regions. As in the bulk water case, the dipole distribution shows a relatively wide dispersion, indicating the need to take into account the non-pair additive effects within the system.