02.7-03 LOW TEMPERATURE STUDIES OF ELASTASE. By N.-h Xuong, C. Cork, R. Hamlin, A. Howard, B. Katz, Paul Kuttner and C. Nielsen, Depts of Physics, Chemistry and Biology, Univ. of Calif., San Diego, La Jolla, CA 92093 USA.

We have collected x-ray diffraction data on Porcine Pancreas Elastase crystals (Sawyer et al., J. Mol. Biol. (1978) 118, 127) at room temperature, at-55°C with and without the substrate N-carbobenxozy-L-alanyl-p-nitrophenyl ester (ZAP) (Alber et al., Nature (1976) 263, 197) and at -73°C with and without the substrate N-carbobenzoxy-L-alanylamide (ZAM) (Fink & Ahmed, Nature (1976) 263, 294). All data were collected on the multiwire area detector diffractometer (MADD) (Xuong et al., Acta Cryst. (1978) A34, 289). The data collection statistics are given in the following table.

The -55° and -73°C native sets are being refined to 1.8 Å resolution by a restrained parameter least-squares procedure with a bulk solvent correction term incorporated into the calculation. Presently the -55°C data is at a conventional R factor of 18% (all reflections to 1.8 Å) and the -73°C data is at an R factor of 22% (all reflections to 1.8 Å). We have built approximately 240 water molecules into both structures to date. The roles of sulfate and acetate ions and methanol (the cryo-solvent) will be examined as the refinement proceeds.

When the low temperature and room temperature native data sets are all refined to conventional R factors of less than 18%, we will analyze the variation in function of temperature of individual B values (or atomic displacements) of the nonhydrogen atoms in order to get information about the protein flexibility and dynamics.

In addition, there will be an analysis of the difference Fourier maps between the refined enzyme-substrate and enzyme native data sets collected at the lower temperatures. The enzyme-substrate studies are done in collaboration with T. Alber and G. Petsko from MIT and T. Fink from Univ. of Calif., Santa Cruz.

Data Collection Statistics

Data Set	Data Collection conditions	Resolution Limit (Å)	Number of observations
1	Room temp. native	1.6	121727
2	-55 C Native	1.8	55472
3	-55 C + ZAP	1.8	48051
4	-73 C Native	1.6	127767
5	-73 C + ZAM	2.26	42313

Data Set	Number of of reflections collected	R factor (in intensity) (%)	Reflections F>2σ
1	29180	4.61	26655
2	17779	9.60	14054
3	18748	8.2	15786
4	29672	3.77	29423
5	10009	4.4	9883

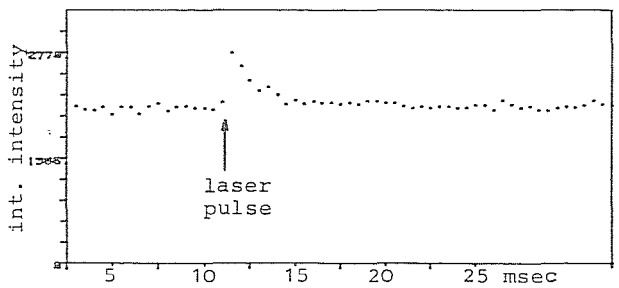
02.7-04 DYNAMICAL PROPERTIES OF PROTEINS BY HYDROGEN EXCHANGE AND NEUTRON DIFFRACTION.

A. A. Kossiakoff and S. A. Spencer, Brookhaven National Laboratory, Upton, N. Y. 11973.

A unique approach to study conformational dynamics of proteins has been developed by coupling the hydrogen exchange technique to neutron diffraction. The direct quantification by neutron diffraction of H-D exchange ratios at specific sites, combined with information about the groups location in the 3-D structure has provided details of the nature and extent of short-lived conformational fluctuations in the proteolytic enzyme, trypsin. The exchange characteristics of trypsin were obtained from a highly refined structure (R=.19 at 1.8 Å) and clearly indicate that rates of D substitution are not a simple function of the distance the labile site is from the solvent interface. 23% of the peptide NH hydrogens are unexchanged; these groups for the most part are incorporated in β- sheet structure. 62% of the NH hydrogens are in a fully deuterated state, but some of these groups are only partially accessible or inaccessible to bulk solvent relative to the static crystallographic structure of trypsin. This indicates that crystallized proteins exhibit similar dynamic behavior to proteins in solution. These observations are being used to determine systematic correlations between conformational fluctuations and specific structural domains. The relative mobilities of the terminal methyl rotor groups of hydrophobic side chains have also been determined. In a majority of cases rotor orientations can be assigned with confidence (±20°). The ratio of staggered to eclipsed conformers was found to be about 20 to 1. This gives important information concerning energy considerations of internal packing of hydrophobic groups. It was also found that D_2O molecules could be unambiguously oriented with respect to their coordinating ligands at the protein-solvent interface. (Supported by the U. S. Dept. of Energy.)

02.7-05 TIME-RESOLVED STUDY OF DYNAMICS IN PROTEINS USING SYNCHROTRON RADIATION AND LASER EXCITATION. By H. D. Bartunik, E. Jerzembek, E.M.B.L. Outstation Hamburg, Notkestrasse 85, Hamburg-52, D. Pruss, G. Huber, Inst. of Appl. Physics, Univ. Hamburg, Jungiusstrasse, Hamburg-36, W-Germany, and H. C. Watson, Dept. Biochemistry, Univ. Bristol, England.

The dynamics of reversible structural changes in protein molecules, which can repeatedly be stimulated by, e.g., laser light, may be investigated in 3 dimensions on a submillisecond time scale by exploiting the high intensity of synchrotron radiation (S.R.). In order to prove the feasibility of such studies, the time course of reflection intensities from CO-Myoglobin (monoclinic $P2_1$) has been measured before and after photodissociation of the ligand by a laser pulse with a time resolution of 500 μsec using S.R. from DORIS at the double-focusing instrument X11. Reflections along layer lines were recorded with a linear position-sensitive gas detector (A. Gabriel). Time frames were generated by a timing unit which also triggered a Xe^*Cl excimer laser



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 figuré 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, Abteilung Chemie, Max-Planck-Institut für Experimentelle Medizin, Hermann-Rein-Straße 3, D-3400-Göttingen, Germany.

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 $I/\sigma > 1$ to $2\theta = 65°$ (.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