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.

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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