02. 3–1 STRUCTURAL CHANGES IN NATIVE COLLAGEN DURING REPEATED DRYING AND REHYDRATION AS DETECTED BY X-RAY DIFFRACTOMETRY. L.C.Labaki and I.L.Torriani, Instituto de Física, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil, and J.R.Grigera, IFLYSIB, La Plata, Argentina.

It is a well known fact that the conformational state of a biopolymer depends not only on its water content but also on the experimental process followed to take it to the humidity conditions in which it is being studied. Biopolymer hydration processes have been studied by different techniques but its overall features are well described by the water sorption-desorption isotherms, which show characteristic hysteresis loops.

Our study consisted in correlating the changes observed in the X-ray diffraction diagrams obtained for a "powdered" native collagen sample and its degree of hydration. In particular, our interest was focused in gradual drying and rehydration cycles versus one-step cycles.

Hysteresis effects, attributed to complete removal of the strongly bound water, were confirmed by monitoring the intensities of the 10.6 Å equatorial reflection and the 2.9 Å meridional reflection for the samples submitted to several-steps dehydration cycles. Repeated cycles show a gradual loss of structured material with a possible increase in the amorphous content. One step drying and rehydration does not produce the same effects, leading to assume that part of the strongly bound water is not removed by this procedure.

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02. 3–2 The Structure Analysis of Copper , Zinc-Superoxide Dismutase from Spinach. By Y.Kitagawa, N.Tanaka, Y.Katsube, M.Kusunoki, G.P.Lee, Y.Morita*, K.Asada* and S.Aihara*, Institute for Protein Research, Osaka Univ.,Suita,Osaka,565,Japan, *Kyoto Univ.,Japan

Cu,Zn-Superoxide Dismutase from spinach crystallized in space group C2 with cell dimensions of a=166.10,b=45.90, c=85.62 A and β=99.36°. The asymmetric unit contains two dimers. Two heavy atom derivatives of $K_3UO_2F_5$ and $K_2Pt(NO_2)_4$ were prepared, and an electron density map at 2.8 A resolution was computed using the method of multi-isomorphous replacement supplemented by anomalous scattering effect of the uranyl derivative; phase angles of 10,027 independent reflections were determined and the average figure of merit was 0.67. Each dimer is formed by two monomers related by an approximate 2-fold axis, and there is no symmetrical relation between the two dimers in the asymmetric unit. Averaging of the electron density maps of the four monomers gave a very clear map, which allowed a tracing of the main chain in spite of no information on the primary structure. Overall folding of the monomer was very similar to that of the SOD from bovine (J.A.Tainer et al. J.Mol.Biol. 160,181 (1982)); the chain containing about 149-residues forms an 8-strand β-barrel with three loops.

02. 4–1 CRYSTALLOGRAPHIC STUDIES OF ST.TRYPSIN AND BOWMAN-BIRK TYPE PROTEASE INHIBITOR. By T. Yamane, Y. Tsunogae, T. Toida, I. Tanaka and T. Ashida, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya 464, Japan

St.trypsin, an extracellular serine protease from *Streptomyces erythraeus*, is stable to autolysis between pH 4 and 11, in contrast to mammalian serine proteases. This protease consists of 227 amino acid residues, including Asp, His and Ser at sites 88, 42 and 179, respectively, which compose a catalytic triad common to the serine proteases. It crystallizes in $P3_121$ with a = 47.1, c = 179.4 A, Z = 6. The structure analysis of 3.5 A resolution is being carried out, and a rough appearance of a molecule is obtained.

A protease inhibitor, AB-I, isolated from adzuki bean (*Phaseolus angularis*) is a Bowman-Birk type inhibitor which inhibits trypsin and chymotrypsin simultaneously through two independent sites. The inhibitor is a small protein consisting of 81 amino acid residues. The composition is unique, that is, it contains as many as 7 disulfide linkages, many hydrophilic residues, only one Gly, and unusually few hydrophobic residues. This composition suggests an existence of a unique feature in the tertiary structure. Although it gives large crystals of a = 68.7, c = 99.7A, $P3_121$, its crystallinity is very poor. A low resolution (5.5 A) analysis showed that the inhibitor exists as a dimer of about 40 x 40 x 30 A.

On the other hand the (1:1) complex, AB-I+trypsin, gives high quality crystals with a = 55.4, c = 181.5 A, $P4_12_12$, Z = 8. The structure analysis of the complex at 3.2 A resolution is being carried out, and the molecular structure will shortly be delineated.

Two other Bowman-Birk type inhibitors from the same source as that of AB-I are also being studied by X-ray.

02. 4–2 STRUCTURE OF λ_I BENCE-JONES DIMER LOC AT 3 Å RESOLUTION. C-H. Chang, M. T. Short, and M. Schiffer, Argonne Nat. Lab., Argonne, IL 60439.

Bence-Jones protein Loc was crystallized from ammonium sulfate and from distilled water with different unit cell dimensions. We have determined the structure of crystals grown from ammonium sulfate at 3.0 Å resolution and started structure determination on the distilled water form. The structure of the λ_I type immunoglobulin light chain Loc complements the high resolution structural information of the λ_V type protein Mcg. The constant (C) domain sequence of the two proteins is identical; the variable (V) domain differs in 34% of the residues, with significant differences in the third hypervariable region.

The electron density of protein Loc was interpreted by finding the best fit of the individual V and C domains of Mcg. After rigid body refinement the R factor was 34%, which refined with restrained least squares refinement to 27%. Segments where the two proteins differed were left out of the calculation. Probably because of the low resolution of the data, the 2Fo-Fc map calculated with the refined phases contained less additional information than before the refinement. In the Loc light chain dimer, the V-V domain interactions are radically different from those observed in the Mcg protein and in other antibody fragments while the C-C domain interactions are very similar. There is no obvious cavity between the V domains in the Loc dimer; the third hypervariable regions are located close to each other and form a protrusion. Tryptophan residues from these regions interact across the local twofold screw axis, leading to the observed dimer of the Loc V domains. By solving the structure of the crystal of the other form grown from distilled water, we will be able to study the effect of different solvents on immunoglobulin domain interactions. (Supported by the U.S. DOE under contract No. W-32-109-ENG-38.)