02.1-47 THE 2.5 A CRYSTAL STRUCTURE OF A DIMERIC PHOSPHOLIPASE A_2 FROM THE VENOM OF CROTALUS ATROX. By C. Keith, D. Feldman, S. Deganello, J. Glick, K.B. Ward, E.O. Jones, P.B. Sigler, Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL. 60637.

The crystal structure($P2_12_12_1$ 2 protomers/asymmetric unit) of the dimeric (α_2) phospholipase A_2 from Crotalus atrox has been determined to 2.5 Å resolution by MIR augmented by real space direct methods. A skeletal model was fit to the electron density and the stereochemistry of the backbone was idealized. The dimeric molecule forms a smooth oblate ellipsoid composed of two covalently identical subunits related by a local dyad axis which is nearly 'exact'. Much of the architecture of the individual protomers resembles the structure of the homologous monomeric bovine enzyme [Dijkstra, B.W., et al. (1978) J. Mol. Biol. 125, 53-60]. Preferred substrates are phospholipids condensed into membranes or membrane-like aggregates. The patch of residues which stabilizes the enzyme's interaction with lamellar substrates forms the interfacial recognition surface. The putative interfacial recognition surface of each crystalline protomer is exposed to the solvent, but on opposing surfaces of the dimer, suggesting that both surfaces cannot interact with the same membrane surface simultaneously. The extensive intramolecular contact surface between the protomers involves the catalytic and calcium binding sites. Access to an internal cavity formed by enclosed abutting active center regions is restricted. One barrier is the interprotomer salt bridge Asp 49 and Lys 64. Asp 49 is in the Ca^{++}-coordination center; therefore, binding Ca^{2+} to Asp 49 would disrupt this ionically bonded barrier, permitting access of substrate to the internal catalytic centers. /Work supported by grants from the USPHS (GM 22324) and the NSF (PCM 74-15075 and the INT 78-21875).

02.1-48 STRUCTURE OF MITOCHONDRIAL F_1-ATPase TO 9 Å RESOLUTION by L.M. Amzel, P. Narayanan and P.L. Pedersen. Johns Hopkins University School of Medicine, Baltimore, Md., USA.

Crystals of F_1-ATPase from rat liver mitochondria belong to rhombohedral space group R32. Cell data are: a = b = 148, c = 368 Å (hexagonal); M.W. ≃ 380,000 daltons; d_{obs} = 1.216 g.cm^{-3}. With the value of 0.74 for the partial specific volume of the protein, the molecular weight of asymmetric unit is 180,000 daltons. Crystals diffract up to 3.5 Å resolution and we analyzed the structure by X-ray diffraction and electron microscopy and a model of the molecule was obtained at 9 Å resolution. The molecule had been found to be a dimer and each half appears to be composed of three masses. Features of the model can be tentatively correlated with known properties of the molecule.

02.1-49 CRYSTALLOGRAPHIC STUDIES OF NEUROPHYSIN-DIPEPTIDE COMPLEXES. By J. Rose, C.S. Yoo, W. Furey,Jr., B.C. Wang and M. Sax Biocrystallographic Laboratory, VA Medical Center, Pittsburgh, PA 15240 and the Department of Crystallography, University of Pittsburgh, Pittsburgh, PA 15260, and E. Breslow, Department of Biochemistry, Cornell University, New York, NY 10021.

The neurophysins are known to be carriers for the posterior pituitary hormones, oxytocin and vasopressin. Bovine neurophysin-II has been crystallized (Yoo, et al, J. Mol. Biol. 127, 1979) as a binary complex with L-phe-L-tyr amide, a peptide known to bind neurophysin at its active site. The crystals belong to space group $P2_12_12_1$, with cell constants a=121.6, b=67.9, c=62.1 Å. Recently, a modified BNP-II (BNP-II'), in which the three C terminus residues have been deleted, has been crystallized as a binary complex with L-phe-L-tyr amide. $MnCl_2$ or $(NH_4)_2SO_4$ were used as the precipitating agent.

Crystals of both BNP-II and BNP-II' obtained through the $MnCl_2$ precipitation belong to space group $P2_12_12$ with cell constants a=153.4, b=70.1, and c=36.1 Å and appear to be isomorphous with porcine neurophysin-I (Blundell, et al, FEBS Let., 121 No. 1, 41, 1980). Crystals of BNP-II' obtained through $(NH_4)_2SO_4$ precipitation are assumed to be isomorphous with BNP-II reported previously.

Rotation function studies are being carried out on the bovine neurophysin to investigate the state of aggregation in the crystal, which should prove useful in the crystal structure determation.

Isolation of the major ovine neurophysin (ONP-III), which closely resembles BNP(II), has been initiated. The preliminary results of this investigation as well as the results from the BNP-II investigation will be presented.

02.1-50 THE 6 Å STRUCTURE AND BIOLOGICAL ACTIVITY OF CRYSTALS OF Δ^5-3-KETOSTEROID ISOMERASE. By E.W. Westbrook, O.E. Piro and P.B. Sigler, Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL 60637, U.S.A.

Δ^5-3-ketosteroid isomerase (KSI) from P. testosteroni functions as an α_2 dimer having 13,400 dalton protomers. The enzyme catalyzes intramolecular transfer of a proton from C4 to C6 in certain Δ^5-3-ketosteroids thereby isomerizing the Δ^5 double bond to Δ^4. Crystals of KSI grown from high salt have three remarkable properties. First, the unit cells of the two pH-dependent polymorphs are large and the molecular packing is unusually complicated but well ordered, i.e. $P2_1$, (pH>5.5), a = 140, b = 85, c = 95 Å, β = 130.1°, 24 protomers per unit cell; and $P6_122$, (pH<5.5), a = 65, b = 504 Å, 48 protomers per unit cell. Second, the hexagonal form binds specific competitive inhibitors stoichiometrically. Third, molecules in the hexagonal lattice are catalytically active. The crystals give useful intensities to 2.7 Å.

An electron-density map of the hexagonal form was prepared by multiple isomorphous replacement (MIR). The 504 Å c-axis spacing was resolved by Frank's optics. As the complexity of the cell subverted the difference Patterson analysis, the derivatives were interpreted by applying direct methods to the difference amplitudes. The MIR map was improved by real space direct methods (see Schevitz et al. in these abstracts), initially by attenuating the negative density and 'leveling' the solvent and subsequently - after the local symmetry became apparent - by averaging the four protomers of the asymmetric unit and calculating phases from the symmetry-averaged density. The functional dimer is characterized by a local dyad (one of three) that relates protomers in pairs through a substantial contact surface.