

02.1-21 CRYSTALLOGRAPHIC STUDIES ON HUMAN PLASMA holo- AND apo-RETINOL BINDING PROTEIN by H. L. Monaco, G. Zanotti and P. Spadon, Centro Studi Biopolimeri, Istituto Chimica Organica, Padova, Italy, and S. Ottonello, Istituto Biologia Molecolare, Parma, Italy.

Retinol binding protein (RBP) is the specific carrier of vitamin A in plasma. This well characterized molecule is the object of considerable interest because it participates in a protein-protein interaction with specific membrane receptors and prealbumin, in addition to its interaction with retinol and other retinol-analogues. Human-plasma RBP is a monomer of molecular weight 20,600 (182 amino acids) which contains a single binding site for the vitamin. We have crystallized both holo- and apo-RBP in isomorphous crystal forms (space group R3, unit cell parameters  $a=b=104.2 \text{ \AA}$ ,  $c=74.5 \text{ \AA}$ , two molecules per asymmetric unit). Using the data of the native holoprotein and two heavy-atom derivatives, electron density maps at  $3.0 \text{ \AA}$  resolution have been calculated. The two molecules in the crystallographic asymmetric unit are disposed as dimers approximately parallel to the two-fold axis and are essentially composed of beta structure. A low-resolution Fourier-difference map between the holo- and apo-molecule shows clearly the position of the retinol. High resolution data collection of both forms is in progress, in order to allow detailed studies of the changes induced by retinol binding to the protein.

02.1-22 STRUCTURE DETERMINATION OF THE ALLOSTERIC L-LACTATE DEHYDROGENASE FROM LACTOBACILLUS CASEI AT  $3 \text{ \AA}$  RESOLUTION. By M. Buehner and H.J. Hecht, Forschergruppe Röntgenstrukturanalyse, Universität Würzburg, D-8700 Würzburg, Fed. Rep. of Germany.

The allosteric LDH from L.casei has been crystallized as a complex with its activators Fru-1,6-P<sub>2</sub> and Co<sup>2+</sup>. The tetrameric enzyme crystallizes in space group C 2 with 6 tetramers in the unit cell. The overall arrangement is close to the supergroup P 3<sub>1</sub>21, all tetramers have good local 2 2 2-symmetry.

The structure was solved by Molecular Replacement using dogfish muscle apo-LDH and the ternary complex of pig heart LDH as models. In the first stage, 2F<sub>0</sub>-F<sub>0</sub> electron density of all 6 crystallographically independent subunits was averaged. "Chopped 2F<sub>0</sub>-F<sub>0</sub>" maps were used for critical parts of the molecule.

The fold of the polypeptide chain is very similar to the standard LDH fold, with some notable exceptions: The first 12 amino acids are lacking, L.casei LDH does not have an N-terminal arm; the fold of the active site loop is different from all known (apo or ternary) conformations; amino acids 209 - 224 appear not well ordered in an averaged chopped map; the C-terminus is extended by 2 turns of helix.

In the active center, an extended relay system of hydrogen bonds is clearly visible. It connects a sulfate ion (which occupies the substrate position) with His 195, Asp 168 and Glu 199 linearly, finally reaching the solvent.

The activator Fru-1,6-P<sub>2</sub> binds to an extended site not far from the molecular P-axis. One phosphate subsite is formed by arginines 173 and 185 and His 188 (this subsite is an unspecific anion binding site in other LDHes) and the other phosphate subsite involves Arg 256 and, possibly, Lys 259. Hydrogen bonds from the sugar extend to Tyr 190 and to the helix which carries the essential residues Thr 165, Asp 168 and Arg 171.

02.1-23 CRYSTAL STRUCTURE OF THE CALF EYE LENS PROTEIN GAMMA-CRYSTALLIN III. By Yu.N. Chirgadze, N.A. Nevskaya, Yu.V. Sergeev, V.Yu. Lunin<sup>+</sup>, A.G. Urzhumtzev<sup>+</sup>, Institute of Protein Research and <sup>+</sup>Research Computer Center, USSR Academy of Sciences, 142292 Pushchino, Moscow Region, USSR.

A main calf lens protein, the gamma-crystallin fraction IIIb with a molecular mass  $\sim 20.0 \text{ kDa}$  was crystallized in space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with two molecules in the asymmetric part of the unit cell. The phases were obtained at  $3.0 \text{ \AA}$  resolution with  $m=0.72$  by isomorphous replacement and anomalous dispersion with five derivatives. These phases were expanded and refined by an improved version of the method of Agarwall & Isaacs (Proc. Natl. Acad. Sci. USA (1977), 74, 2835) up to  $2.7 \text{ \AA}$  resolution, a limit of crystal diffraction. The preliminary atomic model of gamma-crystallin IIIb consists of about 75% side chains which have been localized during the refinement process. The polypeptide chain forms four-repeated  $\beta$ -structural motifs packed in two similar domains. The majority of functionally important cysteine residues are in the N-terminal domain. Most of them are located inside the domain, though very close to its surface. The main chain folding of gamma-crystallin IIIb is very similar to that of gamma-crystallin II (Wistow et al., J. Mol. Biol. (1983), 170, 175) which has a homology in the amino acid sequence of about 80%. The main difference between the two crystal structures pertains to the crystal packing and intermolecular surface-to-surface interactions.

02.1-24 THE STRUCTURE OF M-TYPE PYRUVATE KINASE. By H. Muirhead and D. Clayden, Department of Biochemistry, University of Bristol, Bristol BS8 1TD, U.K.

Pyruvate Kinase is a tetrameric enzyme which catalyses the transfer of a phosphoryl group from phosphoenol pyruvate (PEP) to ADP in the presence of both bivalent and monovalent cations. The regulation of its activity is important in the control of glycolysis, especially in tissues capable of gluconeogenesis, and several classes of isoenzymes have been identified. The L-type enzyme shows a sigmoidal dependence of activity on PEP concentration and its activity is controlled by various allosteric effectors and inhibitors. In contrast the M-type enzyme does not normally possess allosteric properties. The available evidence suggests that all isoenzymes of pyruvate kinase have essentially the same tertiary structure, which can take up one of several closely related conformations. Normally in vivo the M-type enzyme is in the fully active conformation, while the exact conformation of the other isoenzymes will depend upon the concentration of substrates and the various allosteric activators and inhibitors. The tetrameric quaternary structure may well vary.

The M-type enzyme from cat muscle crystallizes in the orthorhombic space group I222 with unit cell dimensions of  $a = 88.4 \text{ \AA}$ ,  $b = 115.3 \text{ \AA}$  and  $c = 131.0 \text{ \AA}$  and a single subunit in the crystallographic asymmetric unit. The amino-acid sequence is known for the chicken muscle, cat