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02. STRUCTURAL MOLECULAR BIOLOGY

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 Chimico Organica, Padova, Italy, and S. Ononello, 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 P3, unit cell parameters a=b=104.2 A, c=74.5 A, two molecules per asymmetric unit). Using the data of the native holoprotein and two heavy-atom derivatives, electron density maps at 3.0 A resolution have been calculated. 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 protein-protein interaction with specific molecules.


The allosteric LDH from Lactobacillus casei has been crystallized as a complex with its activator Fru-1,6-P2 and the ternary complex of pig heart LDH as models. The tetrameric enzyme crystallizes in space group C2 with 6 tetramers in the unit cell. The overall arrangement is close to the supergroup P 3 1 2 1, 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, 2Fo-Fc electron density maps were used for critical parts of the molecule. The fold of the polypeptide chain is very similar to that of pig heart LDH as models. The amino acid sequence is known for the chicken muscle, cat heart LDH and a single subunit in the crystal derives.


A main calf lens protein, the gamma-cristallin fraction I1b with a molecular mass 28.0 kdal was crystallized in space group P212121 with two molecules in the asymmetric unit. The phases were obtained at 3.0 A 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 Agarwal & Issacs (Proc. Natl. Acad. Sci. USA (1977), 72, 2615) up to 2.7 A resolution, a limit of crystal diffraction of a complex with its activator framework. The polypeptide chain forms four-repeated structural motifs which have been localized during the refinement process. The polypeptide chain forms an extended site not far from the molecular P-axis.

The activator Fru-1,6-P2 binds to an extended site from the molecular P-axis. One phosphate subite is bound by arginines 173 and 185 and His 190 and Arg 197. The other phosphate subite 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.