02.1-16 02. STRUCTURAL MOLECULAR BIOLOGY

L-3-HYDROXYACYL COENZYME-A DEHYDROGENASE - STRUCTURAL STUDIES AT 3.5 A RESOLUTION: J. J. Birktoft, L. J. Banaszak, H. M. Holden, J. Ross, S. L. Rosierick. J. Sacchettini, R. C. Hamlin and N-H. Young; Dept. Biol. Chem., Washington University School of Medicine, St. Louis Mo 63110 and Dept. Physics, University of California at San Diego, La Jolla, CA 92039, USA.

L-3-Hydroxyacyl CoA dehydrogenase (BHHD) (E.C.1.1.1.35) is a NAD dependent enzyme that participates in the beta-oxidation of long fatty acids. BHHD contains two identical subunits of known amino acid sequence (Birtar et al, FEBS Lett., 116, 196, (1980)) and has a molecular weight of about 67,000. The enzyme crystallizes in space group C2221 with unit cell dimensions of a = 227.2 A, b = 82.2 A and c = 129.7. The unit cell contains twelve molecules and the asymmetric unit 1.5 molecule (= 3 subunits).

The initial structure determination of BHHD was performed at 5.25 A resolution (Holden and Banaszak, JBC 256, 2393, (1981)), and has now been extended to 3.5 A through phase determination. Phase information was initially derived from two heavy atom derivatives (methyl mercury chloride and potassium platinum hexachloride) and in addition the co-crystallization of NAD. The calculated electron density (e.d.) maps refined the conclusions reached from the low resolution studies. The e.d. maps were, however, still not of sufficient quality that the polypeptide chain could easily be followed. Therefore, new e.d. maps were calculated using both solvent level as well as molecular averaging methods. Both of these methods showed some improvement in the overall quality of the e.d. maps. Thus, it is now possible to follow long stretches of polypeptide chain and to identify segments of alpha helix as well as beta sheet structure. In addition the positions of side chains can in numerous instances be identified. However, the connectivity between elements of secondary structure is still ambiguous due to weak and diffuse density. Additional cycles of molecular averaging should improve the quality of the e.d. maps.

The molecular packing in the crystalline lattice is such that one dimeric BHHD molecule is located in a general crystallographic position and another BHHD molecule is located with its molecular dyad superimposed on a crystallographic two fold axis, thus resulting in three subunits per asymmetric unit. The overall folding of the three subunits is such that the overall quality of the e.d. maps. However, some minor conformational differences do seem to exist. This is also reflected in the asymmetry in the binary crystal. The resulting electron density map was calculated using phases determined by analysis of the anomalous scattering data measured at X-rays. The unit cell dimensions, using hexagonal axes, are a = b = 142.9 A and c = 165.2 A. The calculated mass of protein per asymmetric unit is 32,300 daltons, and the crystals are 78% solvent by volume. The molecule exists as a trimer in which subunits are related by a crystallographic three-fold axis. There is one subunit per asymmetric unit.

Diffraction data, with d > 6 A, were collected from native crystals and from several heavy-atom derivative crystals in which the heavy-atom component was ethylenediaminetetraacetic acid (EDTA) reagent or a substrate analog. Additional data, with d > 3.2 A, were collected for native and two derivatives using the Synchrotron Radiation Source (SRS), Daresbury, England and photomac graphic methods. The positions of the heavy atoms were located from three-dimensional Patterson syntheses or cross­difference Fourier maps and refined using least-squares techniques and centric data. A three-dimensional electron density map was calculated using phases determined by multiple-isomorphous-replacement. The figure-of-merit for this phase set was 79% for all data to 6 A resolution. The correct hand was assigned to the data by analysis of the anomalous scattering data measured at SRS.

The boundary of the enzyme is clearly visible in the map. Each subunit is roughly spherical with a diameter of about 40 A. Pairs of trimers related by a two-fold axis form loosely bound hexamers. Large solvent cavities are observed which are approximately 100 A across and 80 A thick. These cavities are joined by solvent channels which run continuously through the crystals and have minimum cross-sectional dimensions of about 40 x 80 A. The active site has been identified from iodinated substrate analogs and from the difference map obtained by subtracting native data from data obtained from crystals soaked in solutions that contain the competitive inhibitor Formycin B. The electron density map contains a number of rod-shaped pieces of electron density which have the appearance of alpha-helices. Seven of these have been fit with idealized alpha-helix models and have an average length of about 15 residues. Analysis of the primary sequence using various secondary structure prediction algorithms shows 7 or 8 possible alpha-helices.

At this time, data have been collected to 3.2 A from crystals of the native enzyme, two derivatives and the entire complex with guanine. Efforts are being made to complete the heavy-atom derivative data collection and to interpret the electron density map at 3.2 A resolution.

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