02.1–16 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. Roderick, J. Sacchettini, R. C. Hamlin and N-H. Xoung; Dept. Biol. Chem., Washington University School of Medicine, St. Louis, Mo 63110 and Dept. Physics, University of California at San Diego, La Jolla, Ca. 92093, USA.

L-3-Hydroxyacyl CoA dehydrogenase (BHDH) (E.C.1.1.1.35) is a NAD dependent enzyme that participates in the beta-oxidation of long fatty acids. BHDH contains two identical subunits of known amino acid sequence (Bitar et al, FEBS Lett., 116, 196, (1980)) and has a molecular weight of about 67,000. The enzyme crystallizes in space group C222(1) with unit cell dimensions of A = 227.2 A, B = 82.2 A and C = 124.7. The unit cell contains twelve molecules and the asymmetric unit 1.5 molecule (= 3 subunits).

The initial structure determination of BHDH was performed at 5.25 A resolution (Holden and Banaszak, JBC 258, 2383, (1983)), and has now been extended to 3.5 A resolution. Phase information was initially derived from two heavy atom derivatives (methyl mercury chloride and potassium platinum hexachloride) and in addition the coenzyme NAD was used as a heavy atom. The resulting electron density (e.d.) maps confirmed 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 leveling as well as molecular averaging methods. Both of these methods led to improvements 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 BHDH molecule is located in a general crystallographic position and another BHDH 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 crystallographic independent subunits appears to be fairly similar. However, some minor conformational differences do seem to exist. This is also reflected in the non-equivalence in the binding stoichiometry of the heavy atoms and of the coenzyme NAD.

The description of the BHDH subunit as a pronounced bilobal structure with a large cleft separating the two lobes is confirmed. This cleft is quite shallow and it can be speculated that the binding site for the fatty acyl CoA moiety might be found here. The larger lobe is approximately twice the size of the smaller lobe and contains the binding site for NAD. One end of the bound NAD is in contact with the above mentioned cleft, and the other end is on the molecular surface. The two NAD binding sites located within a molecule are about 35 A apart.

Recently high resolution data extending to 2.8 A have been collected on the UCSD multi-wire detector, MARK-II. These data are currently being processed. The resulting electron density maps and their analysis will be presented and discussed at the meeting.

Supported by NSF grant PCM 7921864.

02.1–17 THE CRYSTAL STRUCTURE OF HUMAN ERYTHROCYTIC PURINE NUCLEOSIDE PHOSPHORYLASE AT 6 Å RESOLUTION. Steven E. Ealick, Trevor J. Greenhough, Y. Sudhakar Babu, William J. Cook, Charles E. Bugg, Department of Biochemistry and Comprehensive Cancer Center, University of Alabama in Birmingham, Birmingham, Alabama 35294 U.S.A., Steven Rule, George Habash and John Helliwell, Science Research Council, Daresbury Laboratory, Warrington WA4 4AD, England.

The three-dimensional structure of purine nucleoside phosphorylase (PNP) from human erythrocytes has been determined at 6-Å resolution by X-ray diffraction methods. PNP catalyzes the reversible phosphorolysis of ribonucleosides and 2'-deoxy-ribonucleosides of guanine and hypoxanthine as well as many related analog nucleosides. The importance of PNP in immunodevelopment and purine nucleoside analog metabolism has led to detailed structural and kinetic studies and generated strong interest in developing inhibitors of the enzyme. PNP was purified using a chromatofocusing column as the final step and crystallized from ammonium sulfate solutions. Crystals of PNP are trigonal, space group R32, diffract beyond 2.8 Å resolution and are moderately stable to X-rays. The unit cell dimensions, using hexagonal axes, are a = b = 142.9 Å and c = 165.2 Å. 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.

Diffractometer data, with d > 6 Å, were collected from native crystals and from several heavy-atom derivative crystals in which the heavy-atom compound was either a sulfhydryl reagent or a substrate analog. Additional data, with d > 3.2 Å, were collected for native and two derivatives using the Synchrotron Radiation Source (SRS), Daresbury, England and photographic methods. The positions of the heavy atoms were located from three-dimensional Patterson syntheses or cross-difference 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 Å 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 Å. Pairs of trimers related by a two-fold axis form loosely bound hexamers. Large solvent cavities are observed which are approximately 100 Å across and 80 Å 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 Å. 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 α-helices. Seven of these have been fit with idealized α-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 α-helices.

At this time, data have been collected to 3.2 Å from crystals of the native enzyme, two derivatives and the enzyme 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 Å resolution.

S. Ealick is a Special Fellow of the Leukemia Society of America. Further support was provided by the American Cancer Society (CH-213A), RCDA #CA-00696 and N.C.I. grants CA-12159 and CA-13148.