02. STRUCTURAL MOLECULAR BIOLOGY

02.1-18 STRUCTURE OF LEUCINE, ISOLEUCINE, VALINE-BINDING PROTEIN FROM E. COLI AT 2.4-Å RESOLUTION. By Mark A. Szep and Flore Bau, Department of Biochemistry, Rice University, Houston, Texas 77251, U.S.A.

Leucine, isoleucine, valve-binding protein (LIV-BP, H = 36,770), isolated from the periplasmic space of E. coli is essential for the transport of branched-chain amino acids. We have aligned the known amino acid sequence of LIV-BP to the polypeptide trace made from a 3.0 Å-resolution X-ray map, and modeled the side chains with the FEI NO. 300 graphics program. COREX constrained group refinement, MLK phase combination, followed by Hendrickson-Konnert refinement produced a much improved structure (currently R = 2.4 at 2.8 Å for 7680 reflections). Essentially all of the side chains and carbonyls are unambiguously resolved in the latest map. LIV-BP consists of two distinct domains separated by a wide cleft. The secondary structure elements of each domain are arranged in a ββββ folding pattern like that in nucleotide-binding coasystems, except that in one domain there is an insertion in one of the sheets to helix turns which traverses the cleft to fold the other structurally-similar domain. The bilobate structure as well as the rare multiple crossover between the domains are features found in all binding proteins. We have compared the sequences of the structurally-equivalent regions in all of the known binding protein structures and found no significant homology. The location of the amino acid-binding site in LIV-BP has been confirmed at 2.5 Å resolution by difference Fourier analysis of data collected from crystals soaked in 50 mM leucine. The site abuts one wall of the wide open cleft formed between the two domains.

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02.1-19 X-RAY STRUCTURE DETERMINATION OF HISTIDINE DECARBOXYLASE FROM LACTOBACILLUS 30n. M.H. Haskett, K. Clinger, S.R. Ernst, and E.H. Parks, Dept. of Chemistry, Univ. of Texas, Austin, TX 78712.

Histidine decarboxylase from Lactobacillus 30n is a pyruvyl dependent enzyme that produces the neurotransmitter histamine. The molecule (Mr = 200k) has an (αβ)6-subunit composition, crystallizing in space group P21, (a = 52.1, c = 107.1Å) with half the molecule per asymmetric unit. Its x-ray structure has been determined at 3.0 Å resolution by the MLK phases and structure factors collected at room temperature structure as a starting model (Finzel, to be published 1984). We have increased the resolution by improving the accuracy of the model, and found no significant homology. The location of the pyruvyl acid-binding site in LIV-BP has been confirmed at 2.5 Å resolution by difference Fourier analysis of data collected from crystals soaked in 50 mM leucine. The site abuts one wall of the wide open cleft formed between the two domains.

02.1-20 STUDY OF COMPOUND I OF CYTOCHROME P450 REDUCTASE. By M. h. Xuong, Z. Edwards, T. Poulos, J. Keat, C. Finzel, and F. Quiocho, Dept. of Biochemistry, Rice University, Houston, Texas 77251, U.S.A.

Our laboratory has recently collected the X-ray data on the enyme-substrate intermediate:Compound I of cytochrome c peroxidase. Cytochrome c peroxidase (CCP) catalyzes the breakdown of peroxides to water and alcohol, with cytochrome c serving as the electron source. As per our experiment, if crystals of CCP are soaked in a peroxide solution with no reducing agent, the CCP enzyme will take up the peroxide to begin the reaction and be converted to the semi- stable intermediate, Compound I (Izmaylova and Yonetani, Bioch. Biophys. Acta 209, 350 (1975)).

Due to some significant technical advances on the Multiwire Area Detector Diffractometer (MADD) (Xuong, et al., Acta Cryst. A34, 289 (1978)), we were able to investigate the structure of Compound I by means of X-ray crystallography. Preliminary experiments showed that, unlike CCP, Compound I has no visible spectrum. The decay product is not well defined. In the case of Compound I, the crystal before actual data collection is begun. In the case of a decaying enzyme intermediate, this would have wasted the highest portion of Compound I lifetime on alignment and thereby compromise the quality of the data. We therefore devised a method which allowed us to collect data almost immediately on a crudely aligned crystal by saving complete raw picture images. Using these images, we could determine the exact alignment angles after the data were collected and then post-process them to extract the intensity measurements. This procedure worked extremely well. On six crystals that were discarded and analyzed after data collection, the average amount of Compound I remaining was 80%. The result was 51,700 observations of the 15,000 Miller reflections at 2.5 Å resolution. This is the total data from six crystals which merged together with a scaling factor of R = 0.17.

Our original plan was to determine the nature of Compound I by examining a difference Fourier map, $F_\text{g}(\text{Compound I}) - F_\text{n}(\text{native/native})$, with the native phases and structure factors collected at room temperature (Poulos et al., J. Biol. Chem. 255, 575 (1980)). However, the resulting difference map showed many more changes than expected, indicating that the temperature difference, -15°C versus +22°C, exerts an influence also. To understand the effect of lowering the temperature, we collected native CCP data at -15°C. We collected 264,500 observations of native data which included all of the possible reflections to 2.0 Å resolution and about half the data to 1.8 Å. These data were collected on six crystals which scaled together with an R = 4.92.

We are currently using the low temperature native data to refine the structure using the refined room temperature structure as a starting model (Finzel, to be published 1984). Once this is done, we can calculate the proper Compound I minus CCP (native, -15°C) difference map.

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