

02.1-18 STRUCTURE OF LEUCINE, ISOLEUCINE, VALINE-BINDING PROTEIN FROM *E. coli* AT 2.8-Å RESOLUTION. By Mark A. Saper and Florante A. Quiocho, Dept. of Biochemistry, Rice University, Houston, Texas 77251, U.S.A.

Leucine, isoleucine, valine-binding protein (LIV-BP, M_r = 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 polyaniline trace made from a 3.0-Å resolution MIR map, and modeled the side chains with the FRODO for PS 300 graphics program. CORELS constrained group refinement, MIR phase combination, followed by Hendrickson-Konnert refinement produced a much improved structure (currently R = 24% 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 ββ folding pattern like that in nucleotide-binding domains, except that in one domain there is an insertion in one of the sheet 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 2.8-Å resolution from 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.

Supported by grants from the NIH, NSF, and the Robert A. Welch Foundation.

02.1-19 X-RAY STRUCTURE DETERMINATION OF HISTIDINE DECARBOXYLASE FROM *LACTOBACILLUS* 30a.

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Histidine decarboxylase from *Lactobacillus* 30a is a pyruvoyl dependent enzyme that produces the neurotransmitter histamine. The molecule (M_r = 204K) has an (αβ)₆ subunit composition, crystallizing in space group I4₁22 (a=221.7 and c=107.1Å) with half the molecule per asymmetric unit. Its x-ray structure has been determined at 3.0Å resolution by the MIR phasing method including anomalous scattering. An area detector was used to collect the high resolution data from a native and two heavy atom derivative crystals. A final mean figure-of-merit of 0.79 was obtained for 26,805 independent, observed reflections used to calculate the electron density map.

The molecule is dumbbell shaped. Each lobe is approximately spherical, ≈65Å in diameter, with a large central cavity ≈30Å deep around the molecular 3-fold axis. The 3-fold related active sites are located around the bottom of this cavity and are separated from each other by ≈23Å. The "upper" half of each (αβ)₆ unit has an unusual repeating structure consisting of two overlapping, antiparallel β-pleated sheets, bordered on each side by an α helix. Each (αβ) unit contributes two inner edge strands from the β-sandwich structure and one α-helix to form the walls of the central cavity. The complete amino acid sequence has been fitted to the electron density map. Studies directed toward understanding interactions of substrate at the active site are under way, as is the structural determination of the corresponding prohistidine decarboxylase (C222₁; a=97, b=119, c=202Å). We wish to acknowledge Drs. Ng.H. Xuong and R. Hamlin for assistance with collecting the high resolution data sets. This work is supported by NIH grant GM30105.

02.1-20 STUDY OF COMPOUND I OF CYTOCHROME c PEROXIDASE. By Ng. h. Xuong, S. Edwards, T. Poulos, J. Kraut, B. Finzel, C. Nielsen and R. Hamlin, Departments of Physics, Chemistry and Biology, University of California, San Diego, LaJolla, California 92093, U.S.A.

Our laboratory has recently collected the X-ray data on the enzyme-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 agents, the CCP enzyme will take up the peroxide to begin the reaction and be converted to the semi-stable intermediate, Compound I (Erman and Yonetani, Biochim. Biophys. Acta 393, 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 small native CCP crystals soaked in peroxide could be X-rayed for four hours at -15°C and still remain about 85% Compound I. The decay product is not well defined, but its visible spectrum is very similar to native CCP (Erman and Yonetani, 1975).

The data were collected on the Mark II MADD system equipped with a rotating anode and low temperature device. The Mark II is capable of collecting data about fifty times faster than a conventional diffractometer. However, normal operation of the Mark II, like other diffractometers, requires alignment of 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 digital 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 dissolved and analyzed after data collection, the average amount of Compound I remaining was 80%. The result was 53,700 observations of the 15,000 unique reflections to 2.5 Å resolution. This is the total data from six crystals which merged together with a scaling factor of R = 5%.

Our original plan was to determine the nature of Compound I by examining a difference Fourier map, F₀(Compound I)-F₀(native)α(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 recollected native CCP data at -15°C. We collected 264,500 observations of 39,700 unique reflections 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 = 4.9%.

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.

Work supported by NSF grant PCM 80-21653 and NIH grant RR01644.