02. STRUCTURAL MOLECULAR BIOLOGY

02.1–18 STRUCTURE OF LECINE, ISOLECINE, VALINE-BINDING PROTEIN FROM S. CULI AT 2.6-A RESOLUTION. By Mark A. Saper and Florence M. Saper, Department of Biochemistry, Rice University, Houston, Texas 77251, U.S.A.

Leucine, isoleucine, valine-binding protein (LIV-BP, Mr = 36,770), isolated from the periplasmic space of S. Culi is essential for the transport of branched-chain amino acids. We have aligned the known amino acid sequence of LIV-BP to the polyalanine trace made from a 3.6-A resolution NMR map, and modeled the side chains with the FRODO 3.0 graphics program. CORREL constrained group refinement, NMR phase combination, followed by Hendrickson-Konnert refinement produced a much improved structure (currently R = 24% at 2.8 A 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 B-sheet folding pattern like that in nucleotide-binding coenzymes, 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 crossovers 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 3.0-A resolution. Studies of LIV-BP, consisting of two distinct domains separated by a 3-fold axis. The 3-fold related active sites are approximately spherical, large central cavity of each asymmetric unit. Its x-ray structure has been determined at 2.8-A resolution with the 331,350 (1975). X-RAY STRUCTURE DETERMINATION OF HISTIDINE DECARBOXYLASE FROM LACTOBACILLUS 30a.

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Histidine decarboxylase from Lactobacillus 30a is a pyruvyl dependent enzyme that produces the neurotransmitter histamine. The molecule (Mr = 200K) has an (a8)-subunit composition, crystallizing in space group 14122 (a=212.7, c=107.1A, 8 space group) with half the molecule per asymmetric unit. Its x-ray structure has been determined at 3.0A resolution by the MINPHAS refinement program. The 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 20,905 independent, observed reflections used to calculate the electron density map. The molecule is dumbbell shaped. Each lobe is approximately spherical, ~65A in diameter, with a large central cavity ~30A 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 ~23A. The "upper" half of each (a8) unit has an unusual repeating structure consisting of two overlapping, antiparallel 8-stranded sheets, bordered on one side by a helix. Each (a8) unit contributes two inner edge strands from the 8-sandwich structure and one a-helix to form the walls of the central cavity. The complete amino acid sequence has also been fitted to the electron density map. Studies directed toward understanding interactions of substrate at the active site are underway, as is the structural determination of the corresponding prohistidine decarboxylase (G222; a=97, b=119, c=202A). We wish to acknowledge Drs. K.G. Xuong and R. Hamlin for assistance with collecting the high resolution data set. This work is supported by NIH grant GM30105.

02.1–20 STUDY OF COMPOUND I OF CYTOCHROME PEROXIDASE. By K.G. Xuong, S. Edwards, P. Pouls, J. Kreut, E. Finzel, and Ru. Nielsen. Department of Biochemistry, Rice University, Houston, Texas 77251, U.S.A.

Our laboratory has recently collected the X-ray data on the enzyme-substrate intermediate:Compound I of cytochrome peroxidase. Cytochrome 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 (Ziman and Yonetani, Biochim. Biophys. Acta 313, 350 (1975)).

Due to some significant technical advances on the Multisite Area-Detector Diffractometer (MADD) (Xuong, K.L. et al., Acta Crystal. A43, 289 (1978)), we were able to investigate the structure of Compound I by means of X-ray crystallography. Preliminary experiments showed that our 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 the visible spectrum is very similar to native CCP (Ziman and Yonetani, 1975).

The data were collected on the Mark II MAD 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 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 analysed after data collection, the average amount of Compound I remaining was 80%. The result was 51,700 observations of the 101,500 reflections to 2.5 A resolution. This is the total data from six crystals which merged together with a scaling factor of R = 14.9.

Our original plan was to determine the nature of Compound I by examining a difference Fourier map, (IF(COMPound I)-IF(native)) - (native), with the native phases and structure factors collected at room temperature (Pouls 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 A resolution and about half the data is to 1.5 A. 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, +22°C) difference map.

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