02. 1–3 SUBSTRATE BINDING IN D-GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE - A REVISED MODEL

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The hemithioacetal adduct of D-Glyceraldehyde-3-phosphate Dehydrogenase from Bacillus stearothermophilus has been modeled using the affinity label glycidol phosphate (1,2-epoxypropan-3-phosphate). The structure of the cysteinyl-propan-2-ol-3-phosphate derivative has been determined by difference Fourier methods, model building, and least-squares refinement. The position of the hydroxide and hydrogen at the C1 position of the hemithioacetal have been extrapolated from the geometry of the tetrahedral C1 atom. This model differs from those previously published (D. Moras, K.W. Olsen, M.N. Sabesan, M. Beuhner, G.C. Ford and M.G. Rossmann JBC 250 No. 23 pp.9137-9162 (1975) G. Biesecker, J.I. Harris, J.C. Thierry, J.E. Walker and A.J. Wonacott. NATURE 266 No. 5600, pp.328-333 (1977)) in the position of the terminal phosphate and therefore the assignment of the "substrate" and "inorganic" phosphate sites during the reduction of the co-enzyme.

02. 1–4 XYLOSE ISOMERASE AT 4 Å RESOLUTION. H. L. Carrell, The Institute for Cancer Research, The Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111, U.S.A.

Xylose isomerase catalyzes the conversion of D-xylose to D-xylulose and D-glucose to D-fructose by transposition of a carbon-bound hydrogen atom between two adjacent carbon atoms. The enzyme from Streptomyces rubiginosus has been crystallized and the structure determined at 4 Å resolution using multiple isomorphous phasing techniques. The amino acid sequencing has not yet been done. The polypeptide chain is found to consist of two structural domains one of which contains eight β-strand α-helix (βα) units arranged in a configuration similar to that found for triose phosphate isomerase, 2-keto-3-deoxy-6-phosphogluconate aldolase and pyruvate kinase. The other, smaller domain forms a loop away from the larger domain but overlapping the larger domain of another subunit so that a tightly bound dimer is formed in a "one armed embrace." The tetramer is composed of two such dimers. The location of the active site in the enzyme has been tentatively identified from studies using a crystal grown from a solution containing the inhibitor, xylitol. Data to 3 Å resolution are now being analyzed. A mutant enzyme with a higher specificity for glucose has been crystallized and X-ray data collection has been initiated.

Work supported by National Science Foundation Grants PCM74-21036 and PCM81-04532 and by National Institutes of Health Grant CA-10925.

02. 1–5 STRUCTURE OF APLYSIA MYOGLOBIN AT 2.0 Å RESOLUTION.

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Myoglobin extracted from the buccal muscle of the mediterranean gastropod Aplysia limacina is the prototype molecule for the mollusc globin family. The molecule contains 144 residues per heme, is very rich in hydrophobic/aromatic residues, and lacks the distal histidine (Tentori,L.et al.Int.J.Peptide Prot.Res.(1973),5,187). Aplysia Mb is endowed with peculiar physico-chemical properties,among which a considerable resistance to solvent and/or temperature induced denaturation should be mentioned.(Brunori,M.et al.J.Mol.Biol.(1972),63,139) The molecule, as a simple model for the investigation of heme-globin-ligand interactions, has been subject of extensive ligand binding studies, mainly in its ferric form.(Giacometti,G.M.et al.J.Mol.Biol.(1981),146,363) Aplysia Mb crystallizes in the orthorhombic space group $P2_12_12_1$, with unit cell edges a = 52.9, b = 70.4, c = = 32.5 Å, one molecule per asymmetric unit. The three-dimensional structure has been solved by means of conventional multiple isomorphous replacement techniques, using four heavy-atoms derivatives, to a resolution of 3.6 Å (Bolognesi,M.et al.Int.Congress I.U.Cr. (1978) Warsaw,Commun.04.3-27).Beyond this limit the phasing power of the derivatives falls abruptly, suggesting that an alternative method had to be applied in order to phase native data which extend to 1.6 Å. After inspection of a best Fourier map,calculated at 3.6 Å, it was possible to recognize, with the aid of the sequence, 5 different molecular regions, which comprised approximately 70% of the whole molecule. A skeletal model was built and the corresponding coordinates employed for a number of refinement and model building cycles using the method of Jack and Levitt (Acta Crystallogr.(1978), A34,931) in conjunction with the molecular modeling program FRODO (Jones,T.A.,J.Appl.Crystallogr.(1978),11, 268). Combined (MIR and calculated) phases were used in the first three cycles of refinement together with Sim-weighted double difference Fourier maps, in order to avoid biasing of the developing model. A total of eight refinement cycles have been conducted so far. The molecule is now complete, although some discrepancies with the published sequence have been found; a total of 73 solvent molecules have been located. The R factor at 2.0 Å resolution is 21.2%. Details of the molecular structure, which adopts the typical myoglobin fold, will be displayed and discussed at the meeting.