02. STRUCTURAL MOLECULAR BIOLOGY

02.1-31 PROGRESS IN THE REFINEMENT OF APO M. LACTATE DEHYDROGENASE FROM DOGFISH (SQUALUS ACAUITHUS)." By Celso Adan-Azpirte, Joel L. Sussman and Richard G. Rossmann. Department of Biological Sciences, Purdue University, West Lafayette, Indiana, 47907. USA.

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The chain tracing for the apo M. enzyme subunit of lactate dehydrogenase (LDH) was based on a multiple isomorphous replacement (MIR) electron density map to a resolution of 2.8 A (Adams et al., Nature, 227, 1094-1113, 1970). Minor revisions of the polypeptide tracing were incorporated later and subsequently a model consisting of 329 amino acid residues was built in a Richards optical comparator. A preliminary coordinate list for C atoms has been published (Holbrook et al., In The Enzymes (Boyer, P. D.), ed. 3rd edn. Vol. 11, pp. 191-292,1975) based on a tentative amino acid sequence. This model was revised in a graphics system, using the software developed by Dr. T. A. Jones (Jones, T. A. J. Appl. Crystallogr. 11, 268-272, 1978) and a few xenovus acid residues were substituted (Evansoff et al., Proc. Nat. Acad. Sci. U.S., 74, 2677-2681, 1977). The map used for this interpretation was a 2.8 A resolution, weighted least squares reflection map including the strongest reflections (10%) of the possible in the 2.5 to 2.0 resolution shell.

A total of 31 cycles of constrained-restrained least-squares crystallographic refinement (Sussman et al., Acta Cryst. A33, 800-804, 1977) were performed. Three series of refinement iterations were separated, completed by revisions of the model view in (P - F. J. maps; the R-factor was 25%. A subsequent (P - F. J. map indicated the sites of six sulfate ions and 125 water molecules. Restained least-squares crystallographic refinement of this model using the Hendrickson-Konnoit algorithm and the Purdue University Cyber 205 computer is underway. After 20 cycles of refinement, the R-factor is now 21.3% for 12,183 reflections between 5.0 and 2.5 A resolution limits.

02.1-32 THE STRUCTURE ANALYSIS OF PROTEIN-S; A CALMODULIN LIKE BACTERIAL DEVELOPMENTAL PROTEIN. By Manju Rajamani, G. Jay and R. Sanna, Biochemistry Dept. Scare Univ. of New York, Stony Brook, N.Y. 11790, U.S.

Protein-S is a calcium binding developmental protein produced by the bacterial species, myxococcus xanthus. This bacteria which ordinarily goes through a vegetative cycle, enters a developmental cycle upon starvation and aggregates to form fruiting bodies filled with myxospores. During this stage Protein-S is produced in large quantities. The protein has been crystallized in an orthorhombic space group P212121 with cell dimensions a=27.50 A, b=46.10 A, c=102.16 A. Each asymmetric unit consists of 2 monomers of Protein each having a molecular weight of 23,000. The structure is being determined at a resolution of 2.9 A, using a combination of molecular replacement and multiple isomorph replacement. Platinum chloride and mercapto acetic acid have been used to determine the phase angles.

The amino acid sequence of Protein-S, determined from the sequence of its gene (Inouye, S., Franceschini, T., and Inouye, M. (1983). Proc. Natl. Acad. Sci. U.S.A. Vol. 80, 6259-6283) shows four internally homologous domains. The first and third domains consist of 38 residues and show a homology of 79%, the second and fourth domains consist of 40 residues and show a homology of 65%. There is a sequence of 9 amino acids in the first and third domains that is homologous to the proposed calcium binding sequence of bovine brain calmodulin.

02.1-33 REFINEMENT OF THE CRYSTAL STRUCTURE OF SOYBEAN FERRIC LEMCHEMOLOBIN A NICOTINATE AT 2.4A RESOLUTION. By W. N. Huntebo, C. A. Appleby, J. M. Guise, D. L. Ollis and H. C. Freeman, 1

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The crystal structure was solved at 3.3 A by multiple isomorphos replacement (Ollis et al., Aust. J. Chem. 1983) 36, 451-68. The model obtained from the m.i.r. phases has been improved by a series of real-space (difference maps, computer graphics, PRODO) and reciprocal-space (least-squares refinement, PROLSQ) calculations. Improvement of the model has been accompanied by an extension of the phases from 3.3 A to 2.4 A in three 0.3 A stages.

The asymmetric unit of the crystals comprises two molecules, each with Mr = 17,000. The molecules have been refined independently. The results of the refinement, including a comparison between the two molecules in the asymmetric unit, will be presented.

02.1-34 THE CRYSTAL STRUCTURE OF MPF OF THE PHOSPHOENOLPYRUVATE SUGAR PHOSPHOTRANSFERASE SYSTEM OF ESCHERICHIA COLI AT 2.5A RESOLUTION. By O.A.L. El-Kabbani, R.B. Waygood, G.D. Brayer, and L.T.J. Delbaere. Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0N0.

The phosphoenolpyruvate (PEP) sugar phosphotransferase system (PTS) of E. coli carries out the phosphorylation and concomitant translocation of many hexoses and hexitols across cell membranes. Four proteins are mainly involved in the PTS. These proteins are enzyme I, which is used by all PTS sugars, Hfr which is a histidine-containing phosphocarrier protein for non-fructose PTS sugars, factor III which is a sugar-specific phosphocarrier protein and enzyme II which is both the sugar-specific and membrane-bound protein that phosphorylates and translocates the PTS sugars.

Hfr from E. coli (N90017) has been crystallized from 68% saturated Li2SO4 at pH 3.7 and 14°C. The monochlomic crystals have space group P21 with a=47.50 A, b=46.10 A, c=25.75 A, g=90° and x=92.0°. Quantitative diffraction data to 2.5A resolution were collected for the native and six heavy-atom derivatives. Successful multiple isomorphous replacement phase determination has been carried out for the six derivatives: mercury chloroaldate cis-diamino, dichloro-platinum, gold tetrachloride, sodium mersalil, uranyl nitrate and a double derivative of sodium mersalil + cis-diamino, dichloro-platinum. The centric R values for these derivatives are 0.50, 0.36, 0.47, 0.37, 0.41 and 0.34, respectively, and the over-all figure of merit is 0.80. A 2.3 A electron density map has been interpreted in terms of the amino acid sequence of the protein to produce the three dimensional structure of Hfr. (Supported by the Medical Research Council of Canada. O.A.L. El-Kabbani is the recipient of a Saskatchewan Health Research Board Training Fellowship.)