03-Crystallography of Biological Macromolecules

D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is an important enzyme in glycolysis and shows cooperativity in NAD⁺ binding and half-of-the-sites properties toward reaction with some thiol modification reagents. The enzyme was extracted from the tail muscle of South China sea lobster H. americanus. The crystal structure of GAPDH was solved by Rosemann's group and 2.7Å 4-circle diffractometer data. The high resolution data was collected by synchrotron radiation single-crystal imaging plate-Sakabe's Weissenberg camera system at BL6A2 of Photon Factory in KEK. The whole data set contains 173280 reflections (58850 unique reflections) with R-merge of 5.77%. The structure refinement was carried out using the programs XPLOR and PROLSQ and model building techniques on PS 390 based on the 2.7Å model. The current model containing 2 NAD⁺ molecules, 2 sulphate ions and 153 ordered water molecules gives a crystallographic R-factor of 0.216 for 50031 reflections with F>2σ(F) within 5.0-1.8 Å resolution and a stereochemistry with r.m.s. deviations from ideal geometry of 0.018Å for bond lengths and 3.42° for bond angles. The folding of subunit resembles closely the known structure of H. americanus GAPDH and B. stearothermophilus GAPDH. The structure similarity between B. stearothermophilus GAPDH and R. capsulatus GAPDH is higher than that in NAD⁺-binding domain. The conformation of the adenine moiety in the glycosidic bond is anti for the ribose ring not only in the red subunit but also in the green subunit. The r.m.s. differences in atomic positions between the green and red subunits are 0.38Å for the Cα atoms and 1.27Å if other atoms are included, showing the existence of minor side chain asymmetry. Detailed analysis of the structure after further refinement will be presented in the Congress.

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PS-03.09.32 THE CRYSTAL STRUCTURE OF HMG-CoA REDUCTASE FROM PSEUDOMONES MEVALONII. By C. Martin Lawrence; M. V. Rodwell and Cynthia V. Stoffelchen, Department of Biological Sciences, Department of Biochemistry, Purdue University, West Lafayette, IN 47907.

Pseudomonas mevalonii HMG-CoA reductase is a four-electron oxidoreductase that catalyzes the interconversion of HMG-CoA and mevalonate, the first committed step in the biosynthesis of cholesterol. In mammals this reaction is the rate-limiting step in the synthesis of cholesterol and the enzyme is the target of anti-cholesterol drugs. We have crystallized HMG-CoA reductase in the cubic space group P4₃2₁2₁, with two monomers (45 kDa/monomer) per asymmetric unit. Native and derivative data sets have been collected to 2.8Å resolution on a X3-11-Hamilton data detector. Gold and mercury derivatives were used to produce a 3.4Å MIR map in which clear secondary structure was evident. The phases were then