PS-03.04.09 X-RAY CRYSTALLOGRAPHIC ANALYSIS OF BOVINE α-LACTALBUMIN. By Kyung Kye Kim, Jeaom Ji Jeong, Jin Ho Moon, Kwang Yeon Hwang, and Se Won Suh, Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea.


In this study bovine α-lactalbumin has been crystallized and its structure has been determined. Space group is P2₁2₁2₁, with unit cell dimensions a = 40.61 Å, b = 48.02 Å, c = 58.94 Å. This crystal form diffracts to about 1.9 Å. A data set was collected to 2.2 Å. The orientation and position of bovine α-lactalbumin were determined using the model structure of bovine α-lactalbumin. Rigid-body refinement followed by conventional positional refinement, simulated annealing refinement, and B-factor refinement reduced the crystallographic R-factor to 0.22 for the 512 unique reflections with an Rبت. The root-mean-square deviation from identity is 0.018 Å for covalent bond distances and 3.9° for bond angles.

PS-03.04.10 CRYSTAL STRUCTURE OF SIX MUTANTS OF AZURIN FROM PSEUDOMENAS AERUGINOSA. By L. C. Tsai*, V. Langer and L. Björn, Department of Inorganic Chemistry, University of Technology and The University of Göteborg, Sweden.

Azurin is a "Type I" blue copper protein that acts as an electron mediator between its presumed physiological redox partners (cyclooxygenase and nitric oxide reductase) of a certain types of bacteria. It consists of a single polypeptide of 128 amino acid residues (which are organized into eight β-strands and a short α-helix) and one copper atom. The Cu atom is coordinated by five ligands (NISCO), three in-plane bonds, His46, Cys12 and His117, and two in axial positions, Gley5 and Met121 and the copper atom is in the interior molecule of the level 7 Å under the surface. The coordination geometry might be described as a distorted trigonal bipyramid.

Site-directed mutagenesis was now carried out to prepare azurin mutants in which certain amino acids have been replaced by others in order to investigate for example azurin's particular role in the electron transport scheme. These specific mutation sites are located close to the copper site or on the periphery of the protein surface as the hydrophobic patch. Most of the prepared mutants have subsequently been characterized by optical absorption spectroscopy and EPR and, in addition, the reduction potential of most of them has been measured.

Crystallographic experiments have been performed on all azurin mutants and so far we have been able to crystallize and collect X-ray data from six of them, five of the mutants have been crystallized from PDG-4000; while (NH₄)₂SO₄ was used in the remaining case.

The crystals of these mutants all exhibit different unit cell parameters. However, they belong to three different crystal systems so that two mutants, Ple144Ala and Met121Glu, crystallize in the monoclinic system (P2₁). The other two, Asp140Ala and Thr48Met and Met131Leu, crystallize in the orthorhombic (with space group P2₁2₁2₁) and finally Glu91Gln crystallizes in the triclinic, space group P1. The X-ray diffraction data extend to a resolution between 2.3–2.7 Å for the six mutants and in each crystal form there are four molecules in the asymmetric unit. They are packed as a dimer of dimers. These mutant structures have all been solved utilizing molecular replacement methods in cooperation with Dr. Herbert Ni, Max Planck Institute for Biochemistry, Munich, Germany.

The extensive β-sheet structure of all these mutants is the same as that of native azurin determined at 1.93 Å resolution (Nar et al., 1991). The dimer contact regions are different in all mutant structures compared to those of native azurin. These differences are significant and lead to a new interpretation of the earlier suggested pathway for the self-exchange electron transport. Finally, there is a significant change in the copper site geometry in the mutants Ple144Ala and Glu91Gln (and the consequences and implications of these changes are also discussed).

Reference

PS-03.04.11 STRUCTURAL STUDY OF HYDROGENASE AT 6 Å RESOLUTION. By Y. Higashino, S. Masaki, AND Y. Yasuda* Faculty of Science, Himeji Institute of Technology, 1783-1, Nachi, Kamigori, Amagasaki, Hyogo, Japan 678-012

Hydrogenase catalyzes the reduction and oxidation of molecular hydrogen on the surface of the bacterial cell. The hydrogenase from sulfur-reducing bacteria, Desulfovibrio vulgaris Miyazaki F comprises two subunits (A+B) with total molecular mass of 90kDalton. This is a membrane protein and was solubilized by Ethanol treatment and crystallized in orthorhombic crystal system by polyethylene glycol (PEG) and 2methyl-2,4-pentanediol (MPD) as precipitating agents. The crystals are in space group P2₁2₁2₁, but differ slightly in their cell dimensions. They are divided into roughly two groups, one (Group A) have cell dimensions of a=102.1, b=126.8, c=69.9 Å, and the other (Group B), a=99.2, b=127.9, c=68.7 Å. The crystals in Group A are generally grown from MPD solution while those from Group B are grown from PEG solution. They do not diffuse X-ray to higher resolution compared to those of Group A, but are suitable for preparing the heavy atom derivatives than the crystals in Group A. Six kinds of heavy atom derivatives (Kg, P, Li) have been found for the crystals from MPD solution. All data sets were collected with Weissenberg camera for microcrystalline crystalllography at Photon Factory, High Energy Physics in Tsukuba. One heavy atom derivative site was initially located from the difference Patterson map for a mercury derivative. The heavy atom sites for the other derivatives were determined from difference fourier maps calculated with the coefficients of F(max) - F(ave) with native phases extended from single isomorphous data of mercury derivative. The heavy atom parameters were refined by phase refinement program using PROTEIN program package (W. Steigemann, Doctoral thesis, Technische Universität, München,1974).

The native fourier map calculated at 6 Å resolution clearly shows a molecular boundary in a unit cell. The size of a molecule is approximately, E4A X 35À X 70À in the direction of each unit cell axis, and c. From the peak heights of this map, three iron-sulfur clusters, which have strongest features in the electron density map, can be located.

The phases were refined by iterative solvent flattening procedure B.G. Wang, Methods Enzymol., 115 (1985), 90-112 I, and extended to the upper resolution. The phase combination with native multilwavelength anomalous data from one crystal is now in progress.

PS-03.04.12 CRYSTALLOGRAPHIC STUDIES ON SULFOLOBUS ACIDOCALDARIUS FERREDOXIN. By Tomomi FUJII, Masato OZKII, Hideaki MORIYAMA, Nobuo TANAKA, Takayoshi WAKAGI, and Taito OSHIMA, Department of Life Science, Faculty of Bio-Science and Biotechnology, Tokyo Institute of Technology, Japan.