03-Crystallography of Biological Macromolecules

PS–03.04.09 X-RAY CRYATALLOGRAPHIC ANALYSIS OF BOVINE α-LACTALBUMIN. By Kyeong Kyu Kim, Jeom Gil 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.

α-lactalbumin regulates lactose biosynthesis by modulating the specificity of galactosyltransferase. Crystal structures of baboon and human α-lactalbumin were determined (Acharya, K. R. et al., J. Mol. Biol., 1989, 208, 99-127; Acharya, K. R. et al., J. Mol. Biol., 1991, 221, 571-581). Amino acid sequenecs and three-dimensional structures of these α-lactalbumins and C-type lysozyme are similar to each other.

In this study bovine α-lactalbumin has been crystallized and its structure has been determined. Space group is $P2_12_12_1$ 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 baboon 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 5,112 unique reflections ($F \geq 2\sigma(F)$) between 8.0 Å and 2.2 Å resolution. The root-mean-square deviations from ideality are 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 PSEUDOMONAS AERUGINOSA. By L. C. Tsai*, V. Langer and L. Sjölin, Department of Inorganic Chemistry, Chalmers 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 (cytochrome c_{551} and nitrite reductase) in 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 (N_2S_2O), three in-plane bonds, His46, Cys112 and His117, and two in axial positions, Gly45 and Met121 and the copper atom is in the interior molecule about 7Å under the surface. The coordination geometry might be described as a distorted trigonal bipyramid.
Site-directed mutagenesis has now been utilized to prepare azurin mutants in which certain amino acids have been replaced by others in order to investigate for example azurin's particular role in the electron transport scheme.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 part of the protein surface known 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 for most of them has been measured.
Crystallization 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 PEG 4000, while $(NH_4)_2SO_4$ was used in the remaining case. The crystals of these mutants all exhibit different unit cell parameter. However, they belong to three different crystal systems, so that two mutants, Phe114Ala and Met121Glu, crystallize in the monoclinic system (both with space group $P2_1$). Three of them, Asn47Asp, Trp48Met and Met121Leu, crystallize in the orthorhombic (with space group $P2_12_12_1$) 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 Nar, Max Planck Institute für Biochemie, Munich, Germany.

The extensive β-strand 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 the native dimer. 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 Phe114Ala and Glu91Gln and the consequences and implications of these changes are also discussed.

Reference
Nar, H., Messerschmidt, A., Huber, R., van de Kamp, M. & Canters, G. W. (1991). *J. Mol. Biol.* **221**, 765 - 776.

PS–03.04.11 STRUCTURAL STUDY OF HYDROGENASE AT 6 Å RESOLUTION. By Y. Higuchi, S. Misaki, AND N. Yasuoka* Faculty of Science, Himeji Institute of Technology, 1479-1 Kanaji, Kamigori, Ako, Hyogo, Japan 678-12

Hydrogenase catalyzes the reduction and oxidation of molecular hydrogen on the surface of the bacterial cell. The hydrogenase from sulfate-reducing bacterium, *Desulfovibrio vulgaris* Miyazaki F comprises two subunits (αβ) with total molecular mass of 90k dalton. This is a membrane protein and was solubilized by trypsin digestion, and crystallized in orthorhombic crystal system by polyetheneglycol (PEG) or 2-methyl-2,4-pentanediol (MPD) as precipitating agents. The crystals are in space group of $P2_12_12_1$, but differ slightly in their cell constants. They are divided into roughly two groups, one (Group A) has cell dimensions of a=102.1, b=126.8, c=66.9Å, and the other (Group B), a=99.2, b=127.9, c=66.7Å. The crystals in Group A are generally grown from PEG solution and diffract beyond 1.8Å resolution when synchrotron radiation is used. The crystals in Group B can be obtained from MPD solution. They do not diffract 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 (Hg, Pt, U, Ir) have been found for the crystals from MPD solution. All data sets were collected with Weissenberg Camera for macromolecular crystallography 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 $|F_{deriv} - F_{native}|$ with native phases obtained from single isomorphous data of mercury derivative. The heavy atom parameters were refined by phase refinement procedure using PROTEIN program package (W. Steigemann, Doctoral thesis, Technische Universitat, Munchen,1974).
The native Fourier map calculated at 6Å resolution clearly shows a molecular boundary in a unit cell. The size of a molecule is approximately, 65Å X 75Å X 70Å in the direction of each unit cell axis, *a,b,* 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.C. Wang, Methods Enzymol., 115 (1985), 90-112), and extended to the upper resolution. The phase combination with native multiwavelength anomalous data from one crystal is now in progress.

PS–03.04.12 CRYSTALLOGRAPHIC STUDIES ON *SULFOLOBUS ACIDOCALDARIUS* FERREDOXIN. By Tomomi FUJII*, Masato OOZEKI, Hideaki MORIYAMA, Nobuo TANAKA, Takayoshi WAKAGI, and Tairo OSHIMA, Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan.

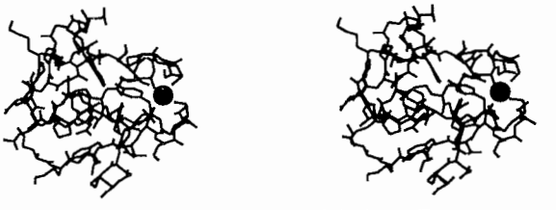
Archaebacteria are classified into a third kingdom of biological world different from both prokaryotes and eukaryotes. Many studies have examined the evolutionary status of archaebacteria from the biochemical and biophysical aspects. We carried out crystallographic studies on the ferredoxin from thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* strain 7 in order to elucidate archaebacterial evolutionary status.
Crystals suitable for X-ray experiments were obtained by a batch method using ammonium sulfate as a precipitant at pH 5.0. The crystals belong to the tetragonal space group $P4_32_12$, with the cell dimensions being a=b=50.12 Å, c=69.52Å. The intensity data of the native crystal was collected by a Rigaku R-AXIS IIC up to 1.8Å resolution. Two derivative data sets within 2.0Å resolution were collected from crystals soaked in $UO_2(NO_3)_2$ and $K_2Pt(CN)_4$. The phase angles were determined by multiple isomorphous replacement method. Bijvoet-difference Fourier using these phases shows two sets of three peaks that may correspond to two [3Fe-4S] clusters. Model building work is underway. The native Fourier map is not so clear, and another derivative is searched to improve the phase angles.

PS-03.04.13 CRYSTAL STRUCTURE OF NEUTRAL PROTEASE FROM *STREPTOMYCES CAESPITOSUS*. By G.Kurisu*, A.Nagara, S.Harada, Y.Kai and N.Kasai, Department of Applied Chemistry, Osaka University, Suita, Osaka 565, Japan.

A neutral protease, i.e., a zinc-containing metalloendoprotease from *Streptomyces caespitosus* (132 residues), is specific for peptide bonds on the amino acid side of aromatic residues. The molecular weight of the enzyme determined by the electrophoresis and ultracentrifugation methods is approximately 15,000. This protease has been crystallized using acetone as a precipitant (orthorhombic, space group $P2_12_12_1$, a=55.21, b=55.27, c=37.60Å V_m=1.9Å/dalton). The crystal diffracts to better than a resolution of 1.5Å with a rotating anode X-ray generator. Protein phase angles were obtained by the multiple isomorphous replacement method using six heavy-atom derivatives (CH_3HgCl, $HgCl_2$, $UO_2(NO_3)_2$, $PbAc_2$, K_3IrCl_6, K_2PtCl_6). The folding pattern of the polypeptide chain could be traced on a electron density map calculated at a resolution of 2.5Å (S.Harada, K.Kitadokoro, T.Kinoshita, Y.Kai & N.Kasai, (1991). *J. Biochem.* **110**,46-49). A large cleft located on the molecular surface was proved to be the active site of the enzyme by structure analyses of inhibitor-complex crystals (N-CBZ-Gly-Phe, N-CBZ-Gly-Tyr) which were prepared by co-crystallization method. A catalytically essential zinc atom was identified in the active site cleft as the highest electron density peak. The zinc ligands of this enzyme is two histidines, which are extrudig from a helix, aspartate and a water molecule. Although the consensus amino acid sequence, (His-Glu-X-X-His, two histidines of which are the zinc ligands in many zinc metalloproteases), was found in the sequence, no structural homology, including amino acid sequence and three-dimensional structure, was not found between this enzyme and other metalloendoprotease. The refinement of the structure is in progress.

PS-03.04.14 CRYSTALLOGRAPHIC STUDY OF RUBREDOXIN FROM DESOLFOVIBRIO VULGARIS MIYAZAKI F. By S.Misaki*, S.Sugiyama, Y.Higuchi and N.Yasuoka, Faculty of Science, Himeji Institute of Technology, Japan. Y.Morimoto, Faculty of Technology, Tokushima University, Japan. T.Yagi, Department of Chemistry, Sizuoka University.

As a part of study to reveal relationship between physical property and structure of proteins from sulfate-reducing bacteria, crystallographic structure determination of rubredoxin from Desulfovibrio vulgaris Miyazaki F (RdDvM) has been carried out.
RdDvM is composed of 52 amino acids. It is highly homologous to rubredoxin (RdDvH) from Desulfovibrio vulgaris Hildenborough. As the physiological role, it is reported that RdDvM, in collaboration with membranous quinone, works as an electron acceptor for intracellular lactate dehydrogenase and the electron extracted from lactate would be transferred to the network of electron carrier proteins to effect electron transfer-coupled phosphorylation (Shimizu F. et al., Biochim., 1989, 71, 1171-1177). Crystal was grown by sitting drop method. Crystal data are as follows ; molecular weight 5574, crystal system trigonal, space group $P3_221$, cell-parameters a=b=43.7 c=50.7 Å γ=120°. Diffraction data (used wavelength 1.0 Å) was collected at beam-line 6A2 in the Photon Factory, KEK in Japan. Total number of measurements is 10630 from 11 films with use of 1 crystal (rotation axis was c*). All measured data were merged by the program PROTEIN. R-merge is 8.9% and 2541 unique reflections are obtained. Completeness of reflection data is 51% within the resolution range of 6~2 Å. Structure was solved by using molecular replacement procedure in the package of the program X_PLOR. Structure of RdDvH was used as the starting model for molecular replacement. R value for correctly searched position of the model through cross rotation search and translation search is 43.4% (resolution range 6~3 Å, number of reflections 941with $|Fo|>3\sigma|Fo|$), which is about 10% lower than those of other possible sets. After rigid body refinement (R=39.4 %), sequence of the model was changed to that of RdDvM. Then structure was refined by simulated annealing method of X_PLOR. With this refinement, R value (initially 47.3 %, resolution range 6~2 Å, number of reflections 2066 with $|Fo|>3\sigma|Fo|$) was reduced to 24.3%. $2|Fo|-|Fc|$ map and omit maps are quite reasonable. From $2|Fo|-|Fc|$ map, possible positions of water molecules were found and added to refinement. With refinement including 14 water molecules, R value is reduced to 21.2% at the present stage.
The molecular structure of RdDvM is illustrated in the figure below. The overall folding pattern is quite similar to that of RdDvH. There are five amino acid substitutions between RdDvM and RdDvH. Four of them are found in the molucular surface, but the rest directs towards the core of molecule which is surrounded with aromatic rings. Detailed structure will be presented.



Molecular structure of RdDvM, ● indicates Fe atom.