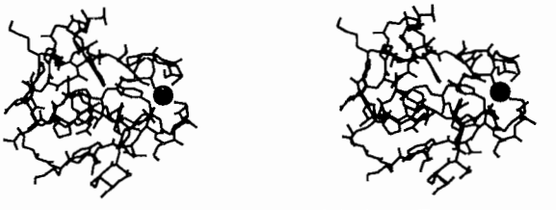
Archaebacteria are classified into a third kingdom of biological world different from both prokaryotes and eukaryotes. Many studies have examined the evolutionary status of archaebacteria from the biochemical and biophysical aspects. We carried out crystallographic studies on the ferredoxin from thermoacidophilic 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.

