method is generic and can be applied to any system composed of rod-shaped molecules.

The X-ray scattering experiments were performed at ESRF microfocus beamline ID13 on collagen tendon fibers extracted from mouse tails.

[1] J. Doucet, F. Briki, A. Gourrier, C. Pichon, L. Gumez, S. Bensamoun, J.-F. Sadoc *J. Structural Biology* **2011**, *173*, 197–201.

Keywords: collagen, paracrystal, modeling

MS08.P09

Acta Cryst. (2011) A**67,** C265

Structural changes of bovine cytochrome *c* oxidase dependent on the redox states

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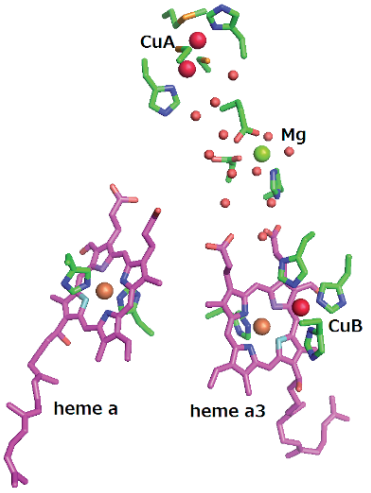
Cytochrome *c* oxidase (CcO) is a terminal oxidase in the respiratory chain. Mitochondrial CcO contains four redox active metal sites, CuA, heme a, heme a3 and CuB. The oxygen molecule is bound to the reduced heme a3-CuB site and reduced to water molecules by electrons from cytochrome c. The oxygen reduction is coupled to the proton translocation across the membrane, generating electrochemical proton gradient. To understand energy transducing mechanism, we have been determined the structural changes of bovine mitochondrial CcO in the resting oxidized and the reduced states. In previous analyses, the conformational changes were found at the peptide segment containing aspartate residue on the CcO surface and the α-helix between hemes a and a3 [1, 2]. It has been suggested that these changes contribute to the proton transfer.

In this study, we prepared bovine CcO crystals in the reduced state which was identified by the measurement of the UV/vis absorption spectrum of the single crystal. We performed X-ray diffraction experiment at BL44XU in SPring-8 and determined the detailed structural changes at 1.4 Å resolution. The FO-FO electron density map calculated by using the data from the resting oxidized and the reduced states indicates that the changes are localized at the inside of CcO, where the redox active metal sites are located. In addition to the previously found conformational changes, it is found that water molecules in the water cluster between subunit I and II are moved dependent on the redox states. These waters are located near the CuA site in the subunit II and the Mg site which is octahedrally coordinated by one aspartate residues in the subunit II, one aspartate and one histidine residues in the subunit I and three water molecules. Furthermore, refined structural changes were found at heme a3 porphyrin plane and the CuB ligand. It is suggests that these changes are involved in the energy transducing reaction.



[1] S. Yoshikawa et al. *Science 280*, 1723-1729. [2] T. Tsukihara et al. *PNAS* **2003**, *100*, 15304-15309.

Keywords: Supramolecular_Protein, X-ray_Structure, Redox_Metal

MS08.P10

Acta Cryst. (2011) A**67,** C265-C266

High resolution analysis and anomalous dispersion analysis of bovine cytochrome *c* oxidase

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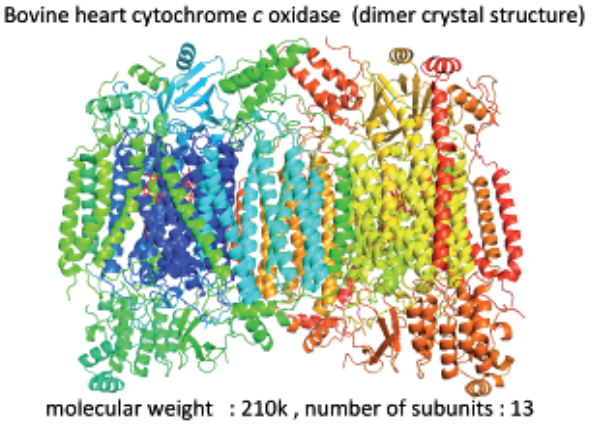
Cytochrome *c* oxidase (CcO) in the respiratory chain is supramolecular membrane protein. CcO functions as a terminal oxidase catalyzing the molecular oxygen reduction coupled to the proton transfer across the membrane. The X-ray structures of mitochondrial and several bacterial CcOs were determined and its resolutions have been improved step by step [1-2].

To understand proton transfer mechanism, we aim to determine orientations and protonation states of amino acid residues (imidazole and carboxyl group etc.) in the proton pathways. In this study, we performed X-ray diffraction experiment at BL44XU in SPring-8 using bovine mitochondrial CcO crystals in the resting oxidized state and improved the resolution to 1.4 Å. To reduce X-ray irradiation damage, diffraction data were collected from many isomorphous crystals. To improve accuracy of the data, the redundancy of data was increased and low quality images (R_{merge} > 0.3 or mosaicity > 0.3) were removed. Resultant data shows 1.6 of I/ σ (I) at 1.42-1.40 Å resolution range. Electron density map shows many multi-conformations of amino acid residues (Met etc.). Structures of phospholipids and ligand at the O_2 reduction site are refined.

CcO crystals give anisotropic diffraction intensities dependent on the crystal axis and the direction of X-ray beam. Processing of the diffraction images at anisotropic resolution ranges improved R and R_{free} values without lowering quality of the electron density.

Anomalous scattering analysis was carried out to determine phosphorus atom positions in phospholipids and to identify the atomic species of the ligand at the O_2 reduction site. The difference anomalous peaks indicate that 7 phospholipids are located on the CcO surface, so we correct previous models [3]. No difference anomalous peak was observed at the O_2 binding site indicating that the ligand is not chloride ion.



[1] K. Muramoto et al. *PNAS* **2010**, *107*, 7740-7745. [2] Liu et al. *PNAS* **2011**, *108*, 1284-1289. [3] K. Shinzawa-Itoh et al. *EMBO J.* **2007**, *26*, 1713-1725.

Keywords: Supramolecular_Protein, X-ray_Structure, Anomalous_Scattering

MS08.P11

Acta Cryst. (2011) A**67,** C266

Structural basis of allosteric regulation of eukaryotic phosphofructokinases

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The enzyme 6-phosphofructokinase (Pfk) catalyses the formation of fructose 1,6-bisphosphate from fructose 6-phosphate and MgATP and contributes to the control of glycolysis in prokaryotic and eukaryotic cells. The catalytic activity is tightly regulated in a wide variety of organisms by diverse positive (e.g. fructose-2,6-bisphosphate, AMP) and negative (e.g. ATP, citrate) effectors.

Eukaryotic Pfk has evolved by a process of tandem gene duplication and fusion to yield a protein with a much more complex structural organization and allosteric regulation. The N-terminal half of a Pfk subunit obviously retained the catalytic function, whereas in the C-terminal half allosteric ligand binding sites have evolved from former catalytic and regulatory sites.

Pichia pastoris Pfk (PpPfk) is with ~1 MDa the most complex and probably largest eukaryotic Pfk [2]. We have determined the crystal structure of full-length *Pp*Pfk to 3.05 Å resolution in the T-state [1]. *Pp*Pfk forms an $(\alpha\beta\gamma)_4$ dodecamer of D_2 symmetry with dimensions of 161 x 157 x 233 Å mainly via interactions of the α-chains. The N-terminal domains of the α- and β-chains have folds that are distantly related to glyoxalase I, but the active sites are no longer functional. Interestingly, these domains located at the two distal ends of this protein along the long two-fold axis form a $(\alpha\beta)_2$ dimer as does the core Pfk domains, however, the domains are swapped across the tetramerization interface. In *Pp*Pfk, the unique γ-subunit participates in oligomerization of the αβ-chains. This modulator protein was acquired from an ancient SAM-dependent methyltransferase. The identification of novel ATP binding sites, which do not correspond to the bacterial catalytic or effector binding sites, point at marked structural and functional differences between bacterial and eukaryotic Pfk.

[1] N. Sträter. *et al*.; *J. FASEB* **2011**; *25*; 89-98. [2] K. Tanneberger. *et al*.; *JBC* **2007**; *282*; 23687-23697

Keywords: 6-phosphofructokinase, allosteric, T-state

MS08.P12

Acta Cryst. (2011) A**67,** C266

Molecular mechanism of metal selective uptake by neutrophil-activating protein

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Helicobacter pylori is a pathogen associated with gastric diseases, including gastric ulcers and stomach cancers. *Helicobacter pylori* neutrophil-activating protein (HP-NAP) is reported to be a major 17 kDa antigen of *H. pylori*. This protein promotes the neutrophil adhesion to endothelial cells and the production of reactive oxygen species which is thought to be a cause of cancer. HP-NAP is a ferritin-like iron storage protein and forms a hollow spherical dodecamer whereas ferritin forms 24mer. Crystal structure of HP-NAP was solved in 2002 at 2.5 Å resolution[1]. The structure contained a Fe atom per subunit at a ferroxidase center. We previously determined the metal-free structure at 2.2 Å resolution[2]. The crystal shows higher symmetry (space group: *F*432, Zanotti's one: $P2_1$) and a high water content of 65.9% (*Vs*). Therefore, the *F*432 crystal is worthwhile for soaking experiments. In comparison with Zanotti's structure, the large conformational changes by the metal coordination were observed for three amino-acid residues (His37, Asp52 and Glu56). In order to estimate the selective mechanism of iron uptake, storage and elimination, we have determined the crystal structures containing various metal ions.

HP-NAP was expressed as a His-tag fused protein and crystallized using the hanging drop vapor diffusion method at 20 °C. To prevent the protein from aggregation, 0.1 M L-arginine was added during purification and crystallization processes. Crystals were grown against 25% ethylene glycol precipitant. By cocrystallization method, crystals including Fe^{2+} or Cd^{2+} ions were obtained using the reservoir solution containing each metal ion. Soaking methods were also performed for Fe^{2+} and Zn^{2+} ions. Anomalous difference data were collected at the Photon Factory in Japan. Ferrous ions incorporated into HP-NAP are probably oxidized to ferric ions instantly by HP-NAP's ferroxidase activity. When Fe^{2+} is added to the droplet, the crystal color is changed to be tinged with yellow. Therefore, Fe atoms in the structure were assigned as ferric ions and refined.

Cd^{2+} and Fe^{3+} structures through cocrystallization were obtained at 2.2 and 2.7 **Å** resolution, respectively. Each subunit contains fourteen cadmium ions and two ferric ions, respectively, and these ions were chelated in a different manner. Fe^{3+} and Zn^{2+} structures obtained through soaking method, were refined at 3.0 and 2.5 **Å** resolution, respectively. Three ferric ions and six Zn^{2+} ions were chelated in ordinary mononucleated manners. Number of metal ions through soaking method is greater than that through cocrystallization. Negative-charged residues are abundant in the inner surface of HP-NAP, which contributes to this additional iron storage mechanism. The most major metal ions were located at the ferroxidase center of the subunit interface. At these metal chelation sites, metal ions adopt the different coordination involved in one or two water ligands. Zn^{2+} and Cd^{2+} ions form binuclear coordination and Fe^{3+} builds mononucleated chelation.

There are two different kinds of pore channels, one of which must be the selective metal ion path from the outer hydrophilic shell to the inner space and vice versa, and are formed at the 3-fold rotational symmetry axes. In Fe^{3+} and Zn^{2+} structures, metal ions are clearly observed at one of the pores.

[1] G. Zanotti, E. Papinutto, W. G. Dundon, *et al*. *Journal of Molecular Biology* **2002**, *323*, 125–130. [2] H. Yokoyama, N. Akao, S. Fujii, *et al*. *in preparation*

Keywords: biomolecule, assembly, structure

MS08.P13

Acta Cryst. (2011) A**67,** C266-C267

Crystal structure of the allosteric-defective chaperonin $GroEL_{E434K}$ mutant

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