catalyze hydrolysis of the glycosidic linkages in the polygalacturonic acid of pectin. The reaction undergoes the general acid base catalysis in which proton transfer plays the most important role of the mechanism. Thus, we determined the sub-atomic-level structure of endoPG I from *Stereum purpureum* by joint X-ray and neutron diffraction. The enzyme was expressed in *E. coli* Origami and crystallized by a macro seeding method. For the X-ray study, the crystals (*ca* 0.25 mm^3) were soaked into a cryoprotectant solution and flash frozen in liquid nitrogen. X-ray diffraction data (10 - 0.62 Å) was collected at beam line BL41XU in SPring-8 using a helium cryostat (40 K). R_{merge}, $R_{p.i.m.}$. and completeness of the X-ray data are 6.3% (50.0% in the most outer shell), 2.4% (21.6%) and 93.7% (55.0%), respectively. The final model of X-ray (R = 8.8%, R_{free} = 9.8%) contains 2,433 hydrogen. For the neutron study, the crystals (*ca* 4.0 mm^3) were soaked into a D_2O solution to decrease incoherent scattering from hydrogen. Neutron diffraction data (20 - 1.5 Å) was collected using BIX-4 in JRR-3 reactor of JAEA at room temperature. R_{merge} and completeness of the neutron data are 11.7% (24.8%) and 89.1% (68.5%), respectively. The final model of neutron (R = 21.3%, R_{free} = 23.0%) contains 2,227 hydrogen and 301deuterium. Visibility of hydrogen and deuterium atoms on the electron and nuclear density map will be presented.

Keywords: sub-atomic resolution crystallography, neutron crystallography, glycosyl hydrolases

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The high-resolution X-ray crystallography of bovine H-protein of glycine cleavage system

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Recently, high brilliance and small divergence synchrotron beam lines, X-ray data collection at low temperature and technical advances in crystallographic analysis have significantly improved the resolution and quality of X-ray crystal structures. In this study, bovine H-protein of glycine cleavage system was used as a model protein for high-resolution X-ray crystallography. High-resolution crystals were grown by micro-seeding technique. Data collections were performed using synchrotron radiation from Photon Factory beamline BL5A and NW12A. Three data sets were collected for high-, mid- and low-resolution data to avoid the saturation of high intensity diffraction. High-resolution diffraction data of H-protein were observed up to 0.80Å resolution. The data were integrated, scaled and merged using the *DENZO* and *SCALEPACK* programs. H-protein belongs to space group *C*2, with the cell dimensions *a*=84.5Å, *b*=41.3Å, *c*=43.1Å, β =91.2° The overall R_{merge} based on intensities for all data was 5.2% with its completeness of 98.9% against data to 0.88Å resolution. Refinement was carried out by *REFMAC5* and *SHELXL* programs. The refinement of H-protein were proceeding against data to 0.88Å resolution. An R_{factor} and *free*-R_{factor} was 11.6% and 13.4%. Hydrogen atoms were added to the model at predicted positions, lowering the R_{factor} and *free*-R_{factor} by approximately 1.0%. This high-resolution structures provide us more reliable geometric and conformational properties of the protein. We will make improvement to the method of high-resolution X-ray structural analysis, and circumstantially assess the high-resolution structure to obtain the specific information of the protein stereochemistry.

Keywords: high-resolution X-ray crystallography, data collection, refinement

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Crystal structure of an antifreeze protein from snow mold fungi

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Antifreeze proteins (AFPs) are preferentially adsorbed onto the surface of embryonic ice crystal to inhibit further growth of the crystal, leading to reduce the freezing point of the solution below the melting point. Various types of AFPs have been identified from cold-adapted organisms including fishes, insects and plants that can survive subzero temperature. Three-dimensional structures have been determined for several types of AFPs, revealing that they exhibit the distinct structural features. We have identified a novel AFP from snow mold fungi *Typhula ishikariensis*, which has no sequence identity to the known types of AFPs. Recently, proteins that could bind to ice were reported from ice diatom and bacterium in Antarctica, which share high sequence identities with the fungal AFP. Therefore, these AFP homologues are widely distributed in various taxa in microorganisms and considered to be a new type of AFPs. In order to elucidate the antifreeze mechanism underlying in microorganisms, we determined the crystal structure of *T. ishikariensis* AFP. Diffraction data were collected on beamline BL44B2 at SPring-8, Japan. The refined structure of *T. ishikariensis* AFP at 0.95Å resolution revealed that it was composed of a right-handed β-helical domain and a single α-helix aligned parallel to the helical axis of the β-helix. The helical structures have been identified in structures of insect and fish type-I AFPs. While those AFPs have characteristic repeat of residues in the molecular surface considered to contribute to the ice-binding, *T. ishikariensis* AFP exhibits less repetitive residues, suggesting that fungal AFPs bind to the ice by other mechanism. Further experiments including site-directed mutagenesis are necessary for the determination of the ice-binding site.

Keywords: antifreeze proteins, protein structures, protein X-ray crystallography

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The D-pathway mutation N131D decouples the *P. denitrificans* cytochrome c oxidase by influencing E278

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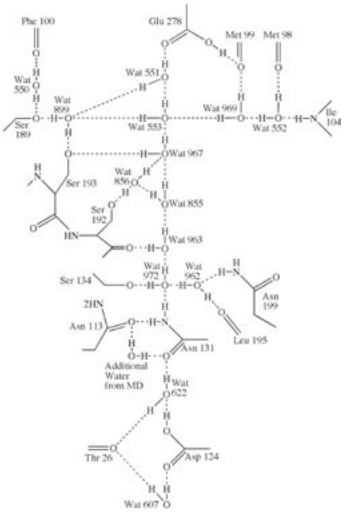
Asparagine 131 near the entrance of the D-pathway of the *P. denitrificans* aa_3 cytochrome c oxidase is important for protein pumping. When replaced by an aspartate, the mutant enzyme is fully decoupled, it retains electron transfer activity, but its proton pumping activity is completely lost. The N131D mutant oxidase was crystallized and its structure solved to 2.32 Å resolution, showing no significant overall changes (rmsd = 0.5 Å) in the protein structure when compared to the wild type structure. However, the variant structure exhibits an alternative orientation of the E278 side chain in addition to the wild type conformation. Moreover, differences in the crystallographically resolved chain of water molecules in the D-pathway are found. Four water molecules are missing, indicating a higher flexibility of these waters, potentially resulting in an decreased rate of Grotthus proton transfer in the D-pathway. Electrochemically induced FTIR difference spectra on several decoupled mutants confirm that the protonation state of E278 is unaltered by these mutations, but indicate a slight pertubation in the hydrogen bonding environment of this residue.



Keywords: membrane protein, Grotthus proton translocation, D-pathway water chain

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High resolution diffraction experiment of bovine cytochrome c oxidase

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Cytochrome c Oxidase (CcO) is an enzyme which is located at the end of the respiratory chain of the mitochondria and reduces an oxygen atom into a water molecule. CcO is a large membrane protein with a molecular weight of 200 kDa which pumps proton coupling with dioxygen reduction. It is essential to directly observe the protonation / deprotonation states of the residues which participate in the proton pumping to make the mechanism clear at atomic resolution. We have determined the three dimensional structures in the oxidized state and the reduced state at 1.8 Å and 1.9 Å, respectively. These structual analysis, however, were not sufficient to observe hydrogen atoms in the electron density. Although contribution of a hydrogen atom to crystal structure factor is small at high resolution range, high resolution diffraction data are required to obtain atomic parameters with high accuracy that are used to calculate (Fo-Fc) difference electron density map. In general, higher than 1.2Å resolution data is needed to observe hydrogen electron density. In order to improve the crystal quality, we have developed a new annealing method and have collected a dataset at 1.6Å resolution on beamline BL44XU at SPring-8. A dataset was collected up to 1.5Å resolution on beamline X06SA at Swiss Light Source. We are trying to determine protonation / deprotonation states at around 1.5Å resolution by using various procedures of structual refinement.

Keywords: membrane proteins, cytochrome oxidase, high-resolution crystal structures

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Water-mediated changes in the quaternary structure of hemoglobin

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Transformations induced by change in solvent content in the crystals of horse methemoglobin were used by Perutz in the early fifties to derive the phase angles of axial reflections in the diffraction data. Protein Crystallography was at its infancy and further structural ramifications of the observation were then not explored. As part of a program involving water mediated transformations, in which protein crystals undergo reversible transformations accompanied by change in solvent content in response to variations in environmental humidity, the crystal structure of high salt horse methemoglobin has been determined at relative humidities (r.h.) of 88%, 79%, 75% and 66%. The molecule is in the R state in the native and the r.h.88% crystals. The water content of the crystal decreases and the molecule moves towards the R2 state when r.h. is reduced to 79%. The crystals undergo a water-mediated transformation with doubling of one of the cell parameters and increase in water content to a level similar to that in the native crystals, when the environmental humidity is further reduced to r.h.75%. The crystal structure at r.h.66% is similar, though not identical, to that at r.h.75%, but the solvent content is substantially reduced and the molecules have a quaternary structure in between those corresponding to the R and R2 states. Thus variation in hydration leads to change in quaternary structure. Furthermore, partial dehydration appears to shift the structure from the R state to the R2 state. We had earlier demonstrated that, in simpler systems, changes in protein structure that accompany partial dehydration tend to be similar to those that occur during protein action. The present work indicates that this is true in multimeric proteins like hemoglobin as well.

Keywords: hemoglobin allostery, quaternary structures, X-ray protein crystallography

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Prediction of hydration structures around polar protein atoms through a database analysis

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