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

Akifumi Higashiura[1], Takeshi Kurakane[1], Makoto Matsuda[1], Mamoru Suzuki[1], Kazuko Fujiwara[2], Koji Inaka[3], Masaru Sato[4], Tomoyuki Kobayashi[4], Tetsuo Tanaka[4], Hiroaki Tanaka[5], Atsushi Nakagawa[1]

[1]Institute for Protein Research, Osaka University, Yamadaoka 3-2, Suita, Osaka, 5650871, Japan, [2]Institute for Enzyme Research, The University of Tokushima, 3-18-15, Kuramoto, Tokushima, Tokushima, 770-8503, Japan, [3]Maruwa Foods and Biosciences, Inc., 170-1, Tsutsui, Koriyama, Nara, 639-1123, Japan, [4]Japan Aerospace Exploration Agency, 2-1-1 Sengen, Tsukuba,, [5]Confocal Science Inc, 3-3-6, Nihonbash-honcho, Chuo-ku, Tokyo, 103-0023, Japan, E-mail：hgsur-a@protein.osaka-u.ac.jp

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

Hidemasa Kondo[1], Hiroshi Sugimoto[2], Natsuko Noro[1], Nan Xiao[1], Yuichi Hanada[1], Tamotsu Hoshino[1], Sakae Tsuda[1]

[1]National Institute of Advanced Industrial Science and Technology (AIST), Research Institute of Genome-based Biofactory, 2-17-2-1 Tsukisamu-Higashi, Toyohira, Sapporo, Hokkaido, 062-8517, Japan, [2]RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan, E-mail：h.kondo@aist.go.jp

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

Juergen Koepke[1], Katharina Duerr[2], Petra Hellwig[3], Hannelore Mueller[1], Guohong Peng[1], Oliver-Matthias Richter[4], Bernd Ludwig[4], Hartmut Michel[1]

[1]Max-Planck Institute of Biophysics, Molecular Membrane Biology, Max-von-Laue Str. 3, Frankfurt/Main, D-60438, Germany, [2]Technische