information of hydrogen and hydrating water molecules using high resolution x-ray crystallography and neutron crystallography. For higher resolution neutron protein crystallography, it is necessary to exchange hydrogen (H) atoms with deuterium (D) atoms in order to reduce background noise derived from incoherent neutron scattering cross-section of hydrogen. Therefore, we have expressed fully deuterated HIV-1PR using commercially available perdeuterated medium. HIV-1PR was expressed in E. coli as inclusion bodies (50 mg/L of culture medium), refolded by a rapid dilution method and purified by cation exchange chromatography. Total 7.5 mg of perdeuterated HIV-1PR was obtained from 50 mg of inclusion bodies. Then, perdeuterated HIV-1PR with its inhibitor KNI272 was crystallized. X-ray structures of non labeled and perdeuterated HIV-1PR were determined to 1.2 and 1.4Å resolution, respectively, using crystals grown under the same conditions (0.1 M Na acetate buffer, pH 4.6, containing 0.2 M Na formate). Both structures did not show any significant changes. The root mean square distances between the perdeuterated (labeled) and non labeled HIV-1 protease was 0.49Å. Furthermore, we succeeded in growing large perdeuterated HIV-1PR crystal (1.8 × 1.3 × 0.15 mm).

Keywords: HIV-1 protease, deuteration, neutron crystallography

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The effect of deuterium oxide on hydration structure of proteinase K

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Deuterium oxide (D_2O) is the water in which two hydrogen atoms are substituted for deuterium atoms. Because the physicochemical properties of D_2O are similar to normal water (H_2O), D_2O is widely used as solvent in neutron diffraction study and NMR spectroscopy. In particular, all crystals used for neutron crystallographic analysis were obtained from D_2O solution in order to avoid a strong noise from hydrogen atoms. Nevertheless, they have rarely investigated the effect deuterated solution on protein structure and their hydration with the high resolution analysis. The aim of our study is to reveal the effect by high resolution crystallographic analysis of proteinase K (PK). First, we crystallized with several combination of H_2O/D_2O solutions. The crystals have so excellent quality that 1.1Å X-ray diffraction data could be collected using a synchrotron radiation. Their overall B-factors for 100%H_2O, 75%H_2O/25%D_2O, 50%H_2O/50%D_2O and 100%D_2O were 3.69, 3.99, 4.04, 4.86Å, respectively. That suggests D_2O solution had rather small influence on the quality of crystal. Structural refinements of 100%H_2O and 100%D_2O crystals were carried out at 1.1Å. The overall r.m.s.d. between main chains of the two structures is 0.048Å. Moreover, the r.m.s.d. more than 90% waters observed with each structure are within a radius of 0.5Å, except for multi-conformation water molecule, which have the multiplied hydrogen bonding networks via water molecules.

Keywords: deuterium effect, protein crystallography, synchrotron radiation

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Neutron crystallography of 2Zn insulin

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Insulin is one of the most important and wellknown hormones. A monomer of insulin has a mass of about 5700 Da, which is composed of two peptide chains; an A-chain (21 amino acids) and a B-chain (with 30 amino acids). This protein is synthesized and stored in the pancreas as a hexamer with zinc ions and secreted from there in that configuration. A 2Zn insulin crystal used to collect neutron diffraction data was grown by a batch method, and the crystallization condition is as follows: insulin (5 mg/mL), sodium citrate (50 mM), zinc sulfate (6 mM) and acetone (15 %). The obtained crystal is 1.5 × 1.5 × 1.0 mm (volume approximately 2.25 mm^3). The crystal was soaked in the supersaturated D_2O solution for a month. The neutron diffraction experiment was carried out at room temperature using the BIX-4 diffractometer at JRR-3 of JAEA. The neutron wavelength used was 2.6 Å. and the step scan method (with increments of 0.3°) was used for data collection. The HKL software package, DENZO and SCALEPACK were used for data processing and scaling. A total of 5,933 independent reflections were obtained with the overall R-merge of 12 % from 13,038 observed reflections. The completeness of the data set was 81.3 % in the 80 - 2.0 Å resolution range and 23.2 % for the outermost (2.07 - 2.0 Å) resolution shell. The structure refinement and molecular modeling were carried out using programs CNS and XtalView, respectively. The 2Zn insulin structure (PDB ID: 4INS) determined from X-ray data (resolution 1.5 Å) was used as an initial model. As the refinement proceeded, the positions of exchangeable hydrogen atoms could be identified using 2|Fo|-|Fc| and |Fo|-|Fc| nuclear density maps, and the current R-factor is 16.0 %, and free R is 24.3 % at 2.0 Å resolution.

Keywords: 2Zn insulin, neutron single crystal structure determination, crystal growth

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Dehydration-induced phase transition in D-glucose isomerase

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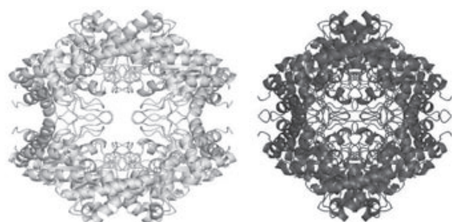
Glucose isomerase, which causes the isomerization of glucose to fructose, has a large market in the food industry because of its application in the production of high-fructose corn syrup. In order to fully understand and control the activity of the protein, a good knowledge of the structural response of the protein to changes in the environmental conditions is necessary. Since proteins function in aqueous media and nearly half of the volume of protein crystals is occupied by water, protein-water interactions are of great interest. We have now identified a dehydration-induced phase transition in D-Glucose isomerase from streptomyces rubiginosus. The transition, characterized using both powder and single-crystal diffraction, occurs at room temperature for relative humidity around eighty percents.

After the transition, the crystal totally recovers its crystalline state and diffraction power. The symmetry is reduced from space-group *I*222 to its subgroup $P2_12_12$ but the effects of this symmetry breaking on the structure are subtle.

The decrease of the unit-cell volume by more that 15 percents produces more intense and interesting structural rearrangements in the crystal (see figure).



Keywords: phase transitions, enzyme structure, polymorphism

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A comparison of hydrated protein models obtained by crystallography, SAXS and other techniques

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Crystallographic or NMR techniques provide information on the precise 3D structure of proteins and reveal the position of some water molecules preferentially bound to certain amino acids. Knowledge of the hydration sites is required for understanding behavior and interactions of hydrated proteins in context with flexibility, dynamics and functionality. A critical comparison of anhydrous and hydrated protein models obtained by crystallography with models from quite different experimental techniques and calculation approaches allows comparing the quality of the models under analysis. Both models and molecular parameters were analyzed: (i) Conventional and ab initio modeling approaches signify satisfactory agreement between crystal- and SAXS-based protein models, provided hydration contributions are taken into account. (ii) Recourse to crystallographic data also allows hydrodynamic modeling; in the case of multibead assemblages efficient bead reductions have to be adopted. (iii) The creation of hydrated models from cryo-electron microscopy data necessitates qualified assumptions regarding hydration. (iv) Combining surface calculation programs and our recent hydration algorithms allows the prediction of individual water molecules; a critical comparison of the water sites on the surface or buried in crevices and channels proves far-reaching identity of crystallographic data and predictions. The good agreement of the results found for hydrated models offers the possibility to complement different techniques and to predict details such as the localization of potential water sites (even in those cases where no crystallographic waters have been identified). Examples presented include proteins ranging from simple proteins to complex, multisubunit, liganded proteins.

Keywords: protein water analysis, small-angle X-ray scattering, modelling

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Flagellar and SOS structural genomics of *Xanthomonas campestris*

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Structural genomics is crucial for understanding the intricate interactions among proteins in a whole organism. We have studied the structural genomics of Xanthomonas campestris (Xcc), a gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. Currently we are working on its flagellar and SOS structural genomics. In the flagellar system, we have solved the first crystal structure of a hook-capping protein FlgD. The core structure reveals a novel hybrid comprising a tudor-like domain interdigitated with a fibronectin type III domain. In the crystal, the monomers form an annular pentamer of dimers of pseudo five-fold symmetry. The resulting asymmetrical star-like decamer complex has a outer dimensions of approximately 110 Å × 90 Å × 65 Å, and a shortest diameter of approximately 20 Å in the center. The outer dimensions of the atomic Xcc hook-capping FlgD complex turn out to be very similar to those of the Salmonella filament cap complex observed by electron microscopy. SOS has been the most intensively studied system induced under DNA damage, and is characterized by the induction of more than 20 genes, which are under the control of LexA. In response to DNA damage, RecA is activated to induce the auto-cleavage of LexA, resulting in de-repression of genes in the SOS regulon. The recX gene is co-transcribed with recA and its product is suggested to regulate RecA function by directly interacting with RecA protein. We have solved the first RecX structure to a resolution of 1.6 Å. It comprise three tandem repeats R1, R2 and R3 of three-helix bundles. Model studies indicate RecX can fit into the helical groove of the RecA filament very well, similar to that reported for the cryoEM image of the RecA/RecX/ATP/ssDNA complex.

Keywords: RecA, RecX, three-helix bundle

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The structural basis of calcium transport by the calcium pump

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The sarcoplasmic reticulum Ca^{2+}-ATPase 1a (SERCA) actively pumps calcium across the SR membrane through formation and break-down of a phosphoenzyme intermediate. The pumping of Ca^{2+} from the muscle cell cytoplasm induces muscle relaxation and the SERCA pump consumes about 25% of the ATP hydrolyzed during muscle activity. The protein has 10 membrane spanning helices, with a cytoplasmic head consisting of three domains (A-actuator, P-phosphorylation and N-nucleotide binding). The vectorial translocation of two Ca^{2+} ions is secured by stringent movements of the cytoplasmic domains coupled to movements of