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

P04.24.454

Acta Cryst. (2008). A**64**, C372

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

P04.24.455

Acta Cryst. (2008). A**64**, C372

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

P04.24.456

Acta Cryst. (2008). A**64**, C372−373

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.