Water is an indispensable medium for life. Proteins fold and display their biological functions only in aqueous environments. Thus, to understand why water is necessary for life at nano-scale, the interaction modes between proteins and water molecules, so-called hydration structures, should be investigated. One of the techniques to study the hydration structures is X-ray crystallography. Crystal structure analyses make it possible to identify hydration water molecules adsorbed on protein surfaces. Now, about 37,000 crystal structure models of proteins have been registered in the Protein Data Bank, and the models include a numerous number of hydration water molecules. When analyzing the database as to the hydration structures of proteins, we can obtain statistically reliable information on protein hydration. In the present study, we have developed a suite of programs subject to hydration structure analysis of the database. The analysis provides the probability densities on the distribution of hydration water molecules around polar protein atoms. In addition, it is found that water molecules in the vicinity of protein surfaces interact with hydrogen bond partners in the tetrahedral geometry as observed in bulk water. In the next step, we have developed a novel program suite for predicting the hydration structures around polar protein atoms using the statistically reliable distribution probabilities deduced from the database analysis. We have applied the suite to a structure model of human lysozyme solved at 100 K and compared predicted and crystallographically found water molecules. As a result, the predicted hydration sites are well consistent with crystal water sites particularly in the grooves of the protein surface.

Keywords: protein hydration, hydration structure, bioinformatics

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Methyl group configuration and hydrogen bonds in proteins determined by neutron crystallography

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The authors have created a Hydrogen and Hydration in Proteins Data Base (HHDB) that catalogs all H atom positions in biological macromolecules and in hydration water molecules that have been determined thus far by neutron macromolecular crystallography. In gaseous ethane, C-C bond rotates freely but a staggered conformation is most stable and an eclipsed conformation is most unstable. Our high-resolution neutron diffraction analyses of myoglobin and other proteins have provided this information. All the CH_3 group configurations in amino acid residues are extracted and discussed as follows: In the myoglobin case, 92 CH_3 groups have been identified, It is found that most of the CH_3 groups belong to the stable staggered conformations, but several percents of them belong to eclipsed conformations. According to hydrogen positions determined by high-resolution neutron diffraction of myoglobin, geometrical consideration has been done for hydrogen bonds (H.B.) involved in α-helix. 125 H.B. were identified as donors for acceptor C=O on the main chain α-helix. For these typical alpha-helix hydrogen bonds, It is found that co-linear H.B. were rare and a tendency that H.B. become co-linear as the distance of H.B. becomes shorter. Finally it is found that hydrogen atom positions seen from acceptors C=O can be localized, and that α-helix H.B. occurs with the two features; H.B. is not parallel to the helix axis but rather inclined to C-terminal direction, and hydrogen atoms are located inside, not outside of α -helix.

Keywords: hydrogen, hydration, neutron protein crystallography

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Mechanistic insights from a joint neutron and X-ray structure of diisopropyl fluorophosphatase

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Diisopropyl fluorophosphatase from Loligo vulgaris (DFPase) is a calcium-dependent phosphotriesterase capable of detoxifying a wide range of organophosphorus nerve agents. We have determined the complete room temperature crystal structure of the DFPase holoenzyme inclusive of hydrogen atom positions and protonation states through the application of joint X-ray (1.8 Å) and neutron (2.2 Å) structure refinement. The resulting structure directly reveals a number of features about the active site including the hydrogen bond coordination of water molecules and the protonation states of amino acid side chains. Omit maps unambiguously identify solvent molecule W33, involved in coordinating the catalytic calcium ion in the active site cleft, as a water molecule in a strained, highly unusual orientation, and not a hydroxide, thus excluding water activation by the catalytic calcium. The smallest Ca - O - H angle is 53 degrees, well beyond the angles observed in small molecule hydrated calcium complexes. Residue Asp229, previously identified as the nucleophile, is deprotonated, consistent with our proposed mechanism. The complete network of hydrogen bonding interactions in the water tunnel is revealed, which together with the central calcium ion, stabilize the beta-propeller structure. An analysis of the exchange of labile hydrogen atoms by deuterium shows a number of surface residues resistant to exchange, and directly visualizes the distribution of time scales of H/D exchange in proteins. Furthermore, insights from this joint X-ray and neutron structure may have direct bearing on the phosphotriesterase mechanism of the structurally related enzyme paraoxonase.

Keywords: neutron crystallography, water structure, metalloenzymes

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Structure determination of perdeuterated human immunodeficiency virus type 1 protease (HIV-1PR)

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Human immunodeficiency virus type 1 protease (HIV-1PR, 99 amino acids) is, a member of the aspartic protease family, promotes the specific processing of large viral polyproteins into individual structural proteins and enzymes. Because HIV-1PR is involved in the maturation of HIV-1, it is a prime target for antiviral therapy of AIDS. In order to investigate precise structure-function relationship, we are planning to determine the structure of HIV-1PR including the

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.