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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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 polypeptides 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