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

Koji Shibata, Atsushi Yamaguchi, Ichiro Tanaka, Nobuo Niimura
Ibaraki University, Faculty of Engineering, c/o Prof. Ichiro Tanaka, 4-12-1, Hitachi, Ibaraki, 316-8511, Japan, E-mail：i.tanaka@mx.ibaraki.ac.jp

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

Julian Chen[1], Marc-Michael Blum[1], Paul Langan[2,3], Marat Mustyakimov[2], Heinz Rueterjans[1], Benno Schoenborn[2]
[1]J.W. Goethe University Frankfurt, Institute of Biophysical Chemistry, Max-von-Laue-Str. 9, Frankfurt, Hessen, 60438, Germany, [2]Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87545 USA, [3]Department of Chemistry, University of Toledo, Toledo, OH 53606 USA, E-mail：chen@chemie.uni-frankfurt.de

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)

Eijiro Honjo, Motoyasu Adachi, Taro Tamada, Ryota Kuroki
Japan Atomic Energy Agency, Quantum Beam Science Directorate, 2-4, Shirakata-Shirane, Tokai-mura, Ibaraki, 319-1195, Japan, E-mail：honjo.eijiro@jaea.go.jp

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