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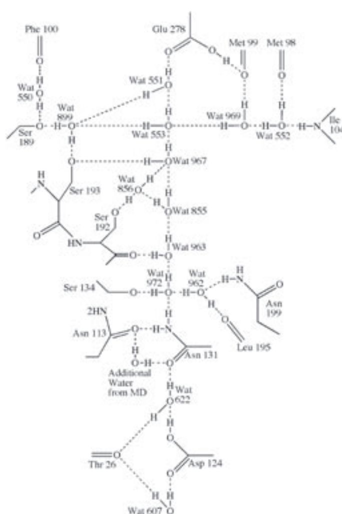
Asparagine 131 near the entrance of the D-pathway of the *P. denitrificans* aa_3 cytochrome c oxidase is important for protein pumping. When replaced by an aspartate, the mutant enzyme is fully decoupled, it retains electron transfer activity, but its proton pumping activity is completely lost. The N131D mutant oxidase was crystallized and its structure solved to 2.32 Å resolution, showing no significant overall changes (rmsd = 0.5 Å) in the protein structure when compared to the wild type structure. However, the variant structure exhibits an alternative orientation of the E278 side chain in addition to the wild type conformation. Moreover, differences in the crystallographically resolved chain of water molecules in the D-pathway are found. Four water molecules are missing, indicating a higher flexibility of these waters, potentially resulting in an decreased rate of Grotthus proton transfer in the D-pathway. Electrochemically induced FTIR difference spectra on several decoupled mutants confirm that the protonation state of E278 is unaltered by these mutations, but indicate a slight pertubation in the hydrogen bonding environment of this residue.



Keywords: membrane protein, Grotthus proton translocation, D-pathway water chain

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High resolution diffraction experiment of bovine cytochrome c oxidase

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Cytochrome c Oxidase (CcO) is an enzyme which is located at the end of the respiratory chain of the mitochondria and reduces an oxygen atom into a water molecule. CcO is a large membrane protein with a molecular weight of 200 kDa which pumps proton coupling with dioxygen reduction. It is essential to directly observe the protonation / deprotonation states of the residues which participate in the proton pumping to make the mechanism clear at atomic resolution. We have determined the three dimensional structures in the oxidized state and the reduced state at 1.8 Å and 1.9 Å, respectively. These structual analysis, however, were not sufficient to observe hydrogen atoms in the electron density. Although contribution of a hydrogen atom to crystal structure factor is small at high resolution range, high resolution diffraction data are required to obtain atomic parameters with high accuracy that are used to calculate (Fo-Fc) difference electron density map. In general, higher than 1.2Å resolution data is needed to observe hydrogen electron density. In order to improve the crystal quality, we have developed a new annealing method and have collected a dataset at 1.6Å resolution on beamline BL44XU at SPring-8. A dataset was collected up to 1.5Å resolution on beamline X06SA at Swiss Light Source. We are trying to determine protonation / deprotonation states at around 1.5Å resolution by using various procedures of structual refinement.

Keywords: membrane proteins, cytochrome oxidase, high-resolution crystal structures

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Water-mediated changes in the quaternary structure of hemoglobin

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Transformations induced by change in solvent content in the crystals of horse methemoglobin were used by Perutz in the early fifties to derive the phase angles of axial reflections in the diffraction data. Protein Crystallography was at its infancy and further structural ramifications of the observation were then not explored. As part of a program involving water mediated transformations, in which protein crystals undergo reversible transformations accompanied by change in solvent content in response to variations in environmental humidity, the crystal structure of high salt horse methemoglobin has been determined at relative humidities (r.h.) of 88%, 79%, 75% and 66%. The molecule is in the R state in the native and the r.h.88% crystals. The water content of the crystal decreases and the molecule moves towards the R2 state when r.h. is reduced to 79%. The crystals undergo a water-mediated transformation with doubling of one of the cell parameters and increase in water content to a level similar to that in the native crystals, when the environmental humidity is further reduced to r.h.75%. The crystal structure at r.h.66% is similar, though not identical, to that at r.h.75%, but the solvent content is substantially reduced and the molecules have a quaternary structure in between those corresponding to the R and R2 states. Thus variation in hydration leads to change in quaternary structure. Furthermore, partial dehydration appears to shift the structure from the R state to the R2 state. We had earlier demonstrated that, in simpler systems, changes in protein structure that accompany partial dehydration tend to be similar to those that occur during protein action. The present work indicates that this is true in multimeric proteins like hemoglobin as well.

Keywords: hemoglobin allostery, quaternary structures, X-ray protein crystallography

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Prediction of hydration structures around polar protein atoms through a database analysis

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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