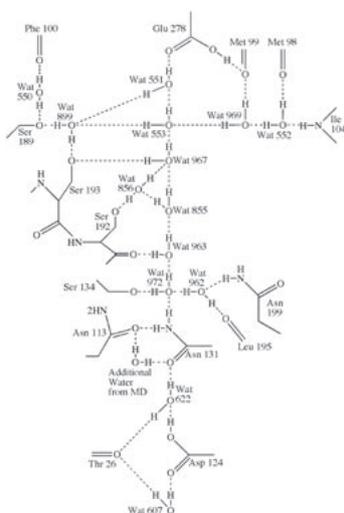


Universitaet Berlin, Strasse des 17. Juni 135, Berlin, D-10623, Germany,
³Universite Louis Pasteur, rue Blaise Pascal 4, Paris, F-67000, France,
⁴Johann Wolfgang Goethe University, Max-von-Laue Str.9, Frankfurt/
 Main, D-60438, Germany, E-mail: juergen.koepke@mpibp-frankfurt.mpg.
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Asparagine 131 near the entrance of the D-pathway of the *P. denitrificans* aa₃ 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 Grothaus 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 perturbation in the hydrogen bonding environment of this residue.



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

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High resolution diffraction experiment of bovine cytochrome c oxidase

Michihiro Suga¹, Kyoko Ito-Sinzawa², Hiroshi Aoyama³, Kazumasa Muramoto², Eiki Yamashita¹, Shinya Yoshikawa²
¹Institute for Protein Research, Yamadaoka3-2, Suita-shi, Osaka-fu, 565-0871, Japan, ²Department of University of Hyogo, Kamigori Akou 3-2-1, Hyogo 678-1297, Japan, ³Graduate School of Pharmaceutical Science, Osaka University, Suita, Osaka, 565-0871, Japan, E-mail : m-suga@protein.osaka-u.ac.jp

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 structural 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 structural refinement.

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

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Water-mediated changes in the quaternary structure of hemoglobin

Prem S Kaushal, R. Sankaranarayanan, M. Vijayan
 Indian Institute of Science, Molecular Biophysics Unit, Molecular Biophysics Unit, Bangalore, Karnataka, 560 012, India, E-mail : ps@mbu.iisc.ernet.in

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

Daisuke Matsuoka, Masayoshi Nakasako
 Keio University, Faculty of Science and Technology, Department of Physics, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa, 223-8522, Japan, E-mail : dmatuoka@phys.keio.ac.jp