Proton transfer via a transient linear water-molecule chain in a membrane protein

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High-resolution protein ground-state structures of proton pumps and channels have revealed internal protein-bound water molecules. Their possible active involvement in protein function has recently come into focus. An illustration of the formation of a protonated protein-bound water cluster that is actively involved in proton transfer was described for the membrane protein bacteriorhodopsin (bR) [Garczarek F, Gerwert K (2006) *Nature* 439:109–112]. Here we show through a combination of time-resolved FTIR spectroscopy and molecular dynamics simulations that three protein-bound water molecules are rearranged by a protein conformational change that resulted in a transient Grotthuss-type proton-transfer chain extending through a hydrophobic protein region of bR. This transient linear water chain facilitates proton transfer at an intermediate conformation only, thereby directing proton transfer within the protein. The rearrangement of protein-bound water molecules that we describe, from inactive positions in the ground state to an active chain in an intermediate state, appears to be energetically favored relative to transient incorporation of water molecules from the bulk. Our discovery provides insight into proton-transfer mechanisms through hydrophobic core regions of ubiquitous membrane spanning proteins such as G-protein coupled receptors or cytochrome C oxidases.

FTIR spectroscopy | functional water molecules | proton-transport chain

The membrane protein bacteriorhodopsin (bR) (1) is an established system for the study of the functional role of protein-bound water molecules and is therefore a favorable model relative to other proton pumps and channels that bind and may also rely on active water molecules (2–4). High-resolution X-ray crystal structures resolving protein-bound water molecules are available for bR in addition to functional studies by time-resolved FTIR spectroscopy. Together they provide a detailed molecular reaction mechanism (1, 5–9).

bR performs a light-driven proton-pumping cycle. Proton release to the extracellular solvent and the involvement of protonated water molecules are well characterized (5, 8–10). However, description of a reprotonation pathway from the cytoplasm to the central proton-binding site, the Schiff base (SB), remains incomplete (for more details, see Fig. 1*A*). For the proton transfer from Asp96 to SB in the second cycle-half (12), only two water molecules (Wat501 and 502) appear in ground-state crystal structures. The region between these two residues is primarily hydrophobic (1). Two water molecules alone cannot span a Grotthus-type "proton wire" of hydrogen (H)-bonded water molecules (9, 14) between these positions. However, this hydrophobic barrier blocks proton transfer from the protonated SB (PSB) to the cytoplasmic proton uptake site in the release-cycle step, which would counteract the establishment of a proton gradient.

A bR conformational change during proton uptake is believed to allow water molecules to invade from bulk cytoplasmic water and build a transient proton-transfer chain (15–17). X-ray structures of various photocycle intermediates based on crystallized, mutated bRs at low temperature, show variability in numbers and positions of water molecules in this domain (15–17) (Fig. S1).

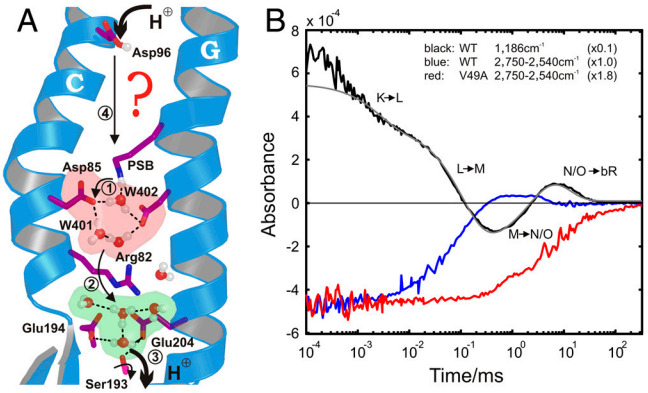

Fig. 1. *Protein-bound water molecules and their IR absorbance changes in bR.* (*A*) Under illumination, bR performs a light-driven proton-pumping cycle including the intermediates J, K, L, M, N, and O (in order of appearance). Following the appearance of M at the end of the first cycle phase, PSB protonates Asp85 (11) (step 1), which cleaves its salt bridge to Arg82. The released Arg82 turns toward Glu194 and Glu204 and pushes the delocalized proton of a protonated water cluster (5) toward these residues (step 2). Transient protonation of Glu194/Glu204 opens the proton diode gate at Ser193, releasing a proton to the extracellular medium (step 3) (9). To reestablish the initial state, SB is reprotonated by Asp96 in N (step 4) (11, 12), which receives a proton from the cytosol in O (12), simultaneous to the isomerization back to all-*trans* retinal. In the last step Asp85 reprotonates the water cluster at the release site (5, 9). (*B*) Time-resolved absorbance changes of strong H-bonded water molecules between 2,750 and 2,540 cm^{-1} for the WT protein are depicted in blue. For comparison, the C_{14}-C_{15} retinal stretching vibration (13) absorbance change at 1,186 cm^{-1} is shown in black, reflecting the (unresolved) appearance of the 13-*cis* isomer, the deprotonation of SB in the L-M transition (step 1), and reprotonation in the M-N transition (step 4). Finally, isomerization back to all-*trans* retinal takes place in the N-O transition and the absorbance change relaxes to the base line. The N-O transition is not resolved individually due to significant back reactions among the O, N, and M intermediates. The infrared absorbance between 2,750 and 2,540 cm^1 in the V49A mutant is depicted in red, which reflects only the change of Wat402. The additional positive signal in M for WT kinetics (blue) reflects the appearance of strong H-bonded water.

To further define how water molecules organize in this space in the WT protein, and to elucidate an active role for protein-bound water molecules in the reprotonation mechanism, we applied time-resolved FTIR spectroscopy to study the WT protein under conditions closer to the physiologically ones.

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