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Pathways of proton transfer in the light-driven pump bacteriorhodopsin

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Abstract. The mechanism of proton transport in the light-driven pump bacteriorhodopsin is beginning to be understood. Light causes the all-*trans* to 13-*cis* isomerization of the retinal chromophore. This sets off a sequential and directed series of transient decreases in the pK_a's of a) the retinal Schiff base, b) an extracellular proton release complex which includes asp-85, and c) a cytoplasmic proton uptake complex which includes asp-96. The timing of these pK_a changes during the photoreaction cycle causes sequential proton transfers which result in the net movement of a proton across the protein, from the cytoplasmic to the extracellular surface.
Key words. Bacteriorhodopsin; retinal; proton pump; ion transport.

The cytoplasmic membrane of the extremely halophilic archae contains a unique retinal-protein which functions as a light-driven pump for protons (for reviews see refs 21, 26, 32 and 35). Illumination of this purple protein named bacteriorhodopsin causes the outward flow of protons from the cells and the development of a negative inside membrane potential. The electrochemical gradient for protons across the membrane, so created, will energize ATP synthesis[15, 19, 31, 43] and drive secondary transport systems such as a sodium/proton antiporter[9, 22, 29] and a large number of sodium/amino acid symporters[20, 24]. Together with oxidative phosphorylation which also occurs under aerobic conditions, this system for light-energy capture will contribute to the ability of the cells to exclude sodium ions and accumulate potassium and thus survive at high salt concentrations[27].

How membrane pumps transport ions against a gradient is an important and still largely unsolved question. The simplicity and technical advantages of bacteriorhodopsin have allowed significant advances in the last twenty years in understanding its proton transport mechanism. This short review attempts to give a birds-eye view of recent progress in studies on how such a pump functions.

Bacteriorhodopsin contains seven transmembrane helical segments A through G[17] connected by short loop regions, and a long C-terminal tail with no apparent function for transport. The helices enclose space in the membrane bilayer which is occupied by an all-*trans* retinal lying slightly inclined from the plane of the membrane. It is attached to the ε-amino group of lys-216 near the middle of helix G via the protonated Schiff base linkage $-CH{=}NH^{+}-CH_2-$. Two essential aspartate residues, asp-85 on the extracellular and asp-96 on the cytoplasmic side, together with the Schiff base, form the proton translocation trajectory. This trajectory is roughly normal to the membrane, and defines an 'extracellular proton channel' to one side of the Schiff base and a 'cytoplasmic proton channel' to the other. A rough sketch of this structure is given in figure 1.

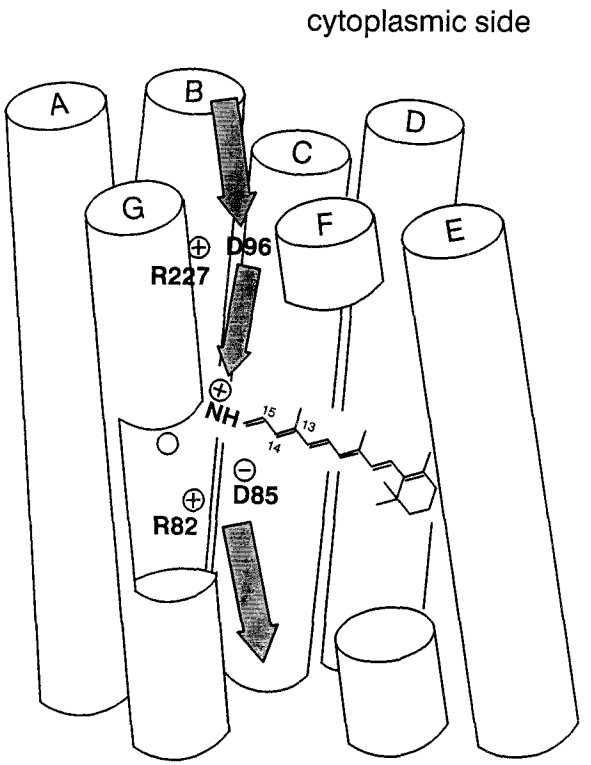

Figure 1. Approximate structure of bacteriorhodopsin and the pathway of the transported proton. The arrangement of helices A through G and the location of the retinal and important residues are drawn after the structural model in ref. 17.

Illumination initiates a reaction cycle for the chromophore with a turnover time of tens of msec. Its intermediate states are distinguished by their changed absorption spectra, for example, in the visible region[2, 23, 41]. The spectral changes identify states termed J, K, L, M, N, and O. The time-courses of their rise and decay are most conveniently determined in single-

turnover experiments where the photo-excitation is by laser flashes and the absorbance changes are followed in real time. The initial even which produces K is isomerization of the retinal from all-*trans* to 13-*cis*. By the time the L state is reached the change in the retinal geometry is communicated to the protein, and this allows transfer of the Schiff base proton to the anionic asp-85. The M intermediate with the deprotonated Schiff base undergoes an important reaction not obviously reflected in spectral changes, except under special conditions[42,44]. In this step, referred to as the reprotonation switch[11,17,26,30,36,40,44] access to the Schiff base changes from the extracellular to the cytoplasmic side. It is designated as the $M_1 \rightarrow M_2$ reaction. The consequence of this switch is that when the Schiff base is reprotonated in the M to N step which follows, it is by the COOH of asp-96 located on the cytoplasmic side. In the N to O reaction the retinal is reisomerized to all-*trans*. The initial protein state is regained in the final O to BR reaction. The result of these reactions is therefore the gain of a proton in the extracellular region of the protein and loss from the cytoplasmic region, i.e. shift in the internal protonation state of the protein in the direction of the transport.

The internal proton transfers, which thus redistribute the protonation state of the two asparate residues mentioned, are coupled to proton release and uptake at the two membrane surfaces. On the extracellular side this comes about because asp-85 is part of a complex of residues which also includes arg-82, tyr-57, and probably a bound water molecule (refs 1, 3, 34, and Brown, Cao, Needleman and Lanyi, in preparation). After asp-85 becomes protonated in the L to M step a proton is released[46], probably from the water molecule, while asp-85 remains protonated until the last step in the cycle. On the cytoplasmic side asp-96 is part of a complex which includes also arg-227 and thr-46, and probably bound water (refs 5, 25, 37, Brown, Cao, Needleman, and Lanyi, in preparation, and Zimányi, Cao, Needleman, Ottolenghi and Lanyi, in preparation). After asp-96 is deprotonated in the M to N step, a proton is taken up, probably here also first by bound water as asp-96 is reprotonated.

The groups which accomplish these proton exchange reactions at the surface must have suitable pK_a's. The proton release complex has initially a high pK_a in order to be protonated as the source of the released proton, but during the photocycle its pK_a will decrease in order to lose the proton. Similarly, the proton uptake complex must have an initially high pK_a so as to have it begin protonated, a transiently lowered pK_a to allow it to transfer its proton to the Schiff base, and it must regain its high pK_a once again in the cycle so as to induce the proton uptake. What are these pK_a's? The pH dependencies of chromophore reaction steps and the proton release and uptake provide some answers to this question.

The photocycle of the asp-96 → asn mutant is simplified by the greatly slowed decay of the M intermediate[4,5,18,28]. Since N and O do not accumulate it is described approximately by the sequence $BR \xrightarrow{hv} K \leftrightarrow L \leftrightarrow M_1 \rightarrow M_2 \rightarrow BR$[45]. The Schiff base deprotonation leads to an equilibrium between L and M at about 10 μs. This equilibrium contains a considerable amount of L. However, at pH 7 or higher the unidirectionality of the $M_1 \rightarrow M_2$ reaction causes the disappearance of L at about 100 μs. At lower pH the L intermediate does not tend to zero but to a second equilibrium with M which persists until the end of the photocycle[46]. The results thus argue for an $M_2 \rightarrow M_1$ back-reaction of increasing rate as the pH is decreased. Analysis of the kinetics in these terms identifies a pH dependent equilibrium during the life-time of M, i.e. argues for the existence of a group whose pK_a determines the pathway of further reactions[46]. There are two alternatives for the photocycle: either it proceeds with deprotonation of this group and a unidirectional $M_1 \rightarrow M_2$ reaction (at $pH > 6$) or it proceeds without it and a reversible $M_1 \leftrightarrow M_2$ reaction (at $pH < 6$). This group must be the proton release complex. Its pK_a, as calculated from the data, is about 6. The unidirectionality of the $M_1 \rightarrow M_2$ reaction above pH 6 is thus explained by the unidirectionality of the proton release at a pH above this pK_a. Strong support for this model is provided by the pH dependency of the proton release itself. The transient release of the proton on the extracellular side can be measured by following the absorbance changes of pH-indicator dyes in the bulk, such as pyranine, during the photocycle. At $pH > 6$ the proton is released approximately during the formation of M[8,14,16,46]. Uptake of another proton on the cytoplasmic side follows approximately during the lifetime of the N intermediate. However, at lower pH the proton is not released in this way. While proton uptake occurs at the usual time, the release follows it rather than precedes it[7,46]. The reversal of the two protonation reactions is detected as the transient net loss of a proton from the protein at the higher pH but a transient net gain of a proton at the lower pH. This is confirmed also by the results of steady illumination experiments. Illumination of purple membrane sheets produces photostationary states[10,13,39,46] in which protons are found to be either released to the bulk (at $pH > 6$) or taken up from the bulk (at $pH < 6$). The pH dependency of the proton release thus also identifies the pK_a of the proton release complex as about 6.

The pK_a associated with proton uptake was explored in a similar way (Zimányi, Cao, Needleman, Ottolenghi and Lanyi, in preparation). At high pH the O intermediate does not accumulate, and the analysis of transient absorption changes of the chromophore and pyranine becomes simpler. Under these conditions the proton uptake occurs after the rise of the N state but before its

decay. The data thus suggest two N states, one before and the other after the proton uptake. The pH dependency of the interconversion of these N states provides a way to calculate the pK_a of the uptake complex. It is approximately 11, i.e. higher than any pH at which the photocycle is normally studied.

These results indicate that under physiological conditions transport of the proton in this pump occurs, because the release on the extracellular side is from a group with low pK_a relative to the pH (low proton affinity) while the uptake on the cytoplasmic side is to a group with high pK_a (high proton affinity). The essence of the transport process in this pump is that both release and uptake groups have initially high enough proton affinities to be protonated, but their proton affinities are transiently decreased in a timely manner during the photocycle. First, the pK_a of the third, internal group (the Schiff base) decreases and it deprotonates, second, the pK_a of the release group decreases and it releases a proton to the bulk, and third, the pK_a of the uptake group decreases and it reprotonates the Schiff base. The pK_a's recover in a different sequence: first, the pK_a of the Schiff base rises and it reprotonates, second, the pK_a of the uptake group rises and it regains a proton from the bulk, and finally, the pK_a of the release group rises and it regains its proton.

The free energy which drives these changes of the pK_a's resides initially in retinal bond rotations and torsions after absorption of a photon[26]. It affects the protein primarily as the Schiff base of the isomerized retinal changes its relationship to the charged residues which constitute the extracellular release complex. After the reprotonation switch it is a protein conformational change detected by crystallographic methods[6, 38], as well as by the thermodynamics of the chromophore reactions[12, 33, 40], which evidently causes changes in the proton affinity of the cytoplasmic uptake complex.

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