New Concepts in Biochemistry

General Concept for Ion Translocation by Halobacterial Retinal Proteins: The Isomerization/Switch/Transfer (IST) Model

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ABSTRACT: Bacteriorhodopsin (BR), which transports protons out of the cell in a light-driven process, is one of the best-studied energy-transducing proteins. However, a consensus on the exact molecular mechanism has not been reached. Matters are complicated by two experimental facts. First, recent results using BR mutants (BR-D85T) and the homologous protein sensory rhodopsin I demonstrate that the vectoriality of active proton transport may be reversed under appropriate conditions. Second, in BR-D85T as well as in the homologous halorhodopsin, protons and chloride ions compete for transport; e.g. the same molecule may transport either a positive or a negative ion. To rationalize these results, we propose a general model for ion translocation by bacterial rhodopsins which is mainly based on two assumptions. First, the isomerization state of the retinylidene moiety governs the accessibility of the Schiff base in the protein; e.g. *all-trans*, 15-*anti*, and 13-*cis*-15-*anti* direct the Schiff base to extracellular and cytoplasmic accessibility, respectively, but change in accessibility (called the "switch") is a time-dependent process in the millisecond time range. A light-induced change of the isomerization state induces not only a change in accessibility but also an ion transfer reaction. Second, we propose that these two processes are kinetically independent, e.g. that relative rate constants in a given molecule determine which process occurs first, ultimately defining the vectoriality of active transport.

Active transport of a substance against its chemical potential gradient across a membrane requires input of chemical, electrochemical, or light energy. Understanding the mechanism by which membrane proteins perform this transformation of one form of energy into another is a long sought after goal in bioenergetics. One of the best-studied energy-transducing proteins is the retinal protein bacteriorhodopsin (BR) found in the archaeon *Halobacterium salinarum* which catalyzes light-driven proton extrusion and represents the simplest photosynthetic system known [Oesterhelt & Stoeckenius, 1973; review in Lanyi (1993)]. Three other retinal proteins, related to BR, are found in the same organism. Halorhodopsin (HR) catalyzes the light-driven uptake of chloride ions (Schobert & Lanyi, 1982), while sensory rhodopsin I and II (SRI and SRII) mediate the phototactic behavior of the halobacterium [reviewed in Oesterhelt and Marwan (1993)]. Although it is a sensor, SRI is also able to actively transport protons, as discussed below (Bogomolni *et al.*, 1994; Haupts *et al*., 1995, 1996).

Since determination of membrane protein structure proves notoriously difficult, only a few members of this group are known to (near) atomic resolution, allowing more detailed considerations of their mechanism and function (Mogi *et al.*, 1988). BR and HR were shown to consist of seven membrane-spanning α-helices surrounding a central pore which is divided into two half-channels by the retinal chromophore, which is covalently bound to a lysine residue via a protonated Schiff base (Henderson *et al.*, 1990; Havelka *et al.*, 1995). The half-channels connect the central ion binding site of the protein, e.g. the Schiff base, with the extracellular (EC) and cytoplasmic side (CP) of the membrane, respectively.

It is well established that proton translocation by bacteriorhodopsin is coupled to light-induced *all-trans* to 13-*cis* isomerization of the retinal and its thermal reisomerization to the initial *all-trans* state. This cyclic reaction contains a series of thermally unstable intermediates and is called the "photocycle". The overall reaction thermodynamics of the cycle is governed by light energy absorption and storage of a considerable part of this energy in the protein. Part of the stored energy is used to drive the catalytic cycle, and the other part is transformed into the proton motive force. During the photocycle, the Schiff base proton is released toward the extracellular side of the membrane, while proton uptake occurs from the cytoplasmic side. Clearly, between de- and reprotonation, the Schiff base must have changed its accessibility from the extracellular to the cytoplasmic half-channel, in a process called the "switch" (S). The intermediate with a deprotonated Schiff base has a blue-shifted absorption maximum at 410 nm and is referred to as BR-M. Similar intermediates are formed in the photocycles of SRI (SRI_{380}) and HR (HR^{L}_{410}). HR^{L}_{410} is formed only in a side reaction that inactivates chloride transport by HR but can be accumulated under continuous illumination (Hegemann

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et al., 1985). These blue-shifted intermediates contain 13-*cis*-retinal bound via a deprotonated Schiff base in the *anti* configuration (Stockburger *et al.*, 1979; Diller *et al.*, 1987; Haupts *et al.*, 1994) and can absorb a short wavelength ("blue") photon in a second photoreaction. Although this is not proven for all intermediates, we assume a C═N *anti* configuration for all of them during the following discussion.

The primary reaction of rhodopsins induced by photon absorption is the isomerization of the retinal (abbreviated I* for light-induced and I for thermal reaction). Those molecules that do not isomerize revert to the ground state in a sub-picosecond relaxation process, irrelevant for our discussion of ion transport. In fact, no photoreaction other than isomerization has been reported to be of functional significance for any retinal protein. In halobacterial retinal proteins, the isomerization leads from *all-trans*- to 13-*cis*-retinal when starting from the initial state and in the reverse direction when starting from 13-*cis*-retinal, e.g. in the photoreaction of the blue-shifted intermediates with deprotonated Schiff bases (BR-M, SRI_{380}, and HR^{L}_{410}). On the other hand, thermal isomerization of retinal, on the time scales relevant for proton transport, is considered possible only for protonated Schiff bases (Schulten *et al.*, 1984).

The analysis of BR mutants has identified two aspartate residues critical for high turnover numbers of proton transport. The first is D85, located in the EC half-channel, and is assumed to accept the Schiff base proton after light-induced isomerization (Fahmy *et al.*, 1992; Subramaniam *et al.*, 1992), releasing this proton at a later stage of the photocycle toward the extracellular side (Hessling *et al.*, 1993). The second residue is D96, located in the CP half-channel. D96 is implicated in the reprotonation of the Schiff base (Otto *et al.*, 1989; Gerwert *et al.*, 1990) after it has changed its accessibility from the EC toward the CP half-channel in the switch step (S). Alignments of all known sequences of bacterial rhodopsins from different species revealed that all proton pumps possess aspartes in these two positions (corresponding to Asp85 and Asp96 in BR from *H. salinarum*), while the residues at the corresponding positions are Ala (Asp96) or Thr (Asp85) in all chloride pumps and Tyr or Phe (Asp96) and Asp (Asp85) in all sensory rhodopsins I.

Mutation of residue D85 to a neutral N or T leads to three major effects (Zimányi *et al.*, 1992; Tittor *et al.*, 1994). First, deprotonation of the Schiff base is apparently blocked. However, under continuous illumination, some M intermediate still accumulates. Second, the absorption maximum is red-shifted to ≈600 nm (similar effects are seen when D85 is protonated at low pH), and third, the pK_a of the Schiff base is drastically reduced from >12 in wild type to ≈8 in the mutant (Tittor *et al.*, 1994). Therefore, a significant fraction of molecules has a deprotonated Schiff base (λ_{max} = 410 nm) at neutral pH, even in the dark, and predominantly contains *all-trans*-retinal (Nilsson *et al.*, 1995), referred to as BR-D85T$^{D}_{410}$ (D means formed in the dark; 410 is the absorption maximum). The last step of the photoreaction of this species is the deprotonation of an intermediate absorbing at ≈610 nm. As revealed by electrical measurements (Tittor *et al.*, 1994), this deprotonation occurs through the EC half-channel.

Mutation of the second critical Asp96 to Asn in BR (BR-D96N) leads to a long-lived M intermediate, the decay of which becomes pH-dependent, can be accelerated by azide, and may last up to minutes at high pH (Tittor *et al.*, 1989; Miller & Oesterhelt, 1989). This demonstrates that D96 acts as the proton donor in the reprotonation of the Schiff base. However, there is still a residual transport activity attributable to the slow turnover of photocycling molecules. The residues equivalent to D96 are Y87 and A122 in SRI and HR, respectively. As expected, the thermal decay of HR^{L}_{410} and SRI_{380} is slow and pH-dependent and can be accelerated by azide (Hegemann *et al.*, 1985; Haupts *et al.*, 1996). Most important, the decay is coupled with a proton uptake from the cytoplasmic side (Bamberg *et al.*, 1993; Haupts *et al.*, 1996). These results demonstrate that the deprotonated Schiff bases of the long-lived blue-shifted intermediates containing a 13-*cis*-retinal are accessible from the cytoplasmic side.

The residue corresponding to D85 in BR is D76 in SRI. While the pK_a of this aspartate is <3 in BR, it is 7.4 in SRI (Haupts *et al.*, 1995). Therefore, two species with protonated and deprotonated D76 are in equilibrium at physiological pH, as revealed by their absorption maxima at 590 and 550 nm, respectively (Rath *et al.*, 1996). In contrast to that of BR, protonation of this Asp residue does not slow Schiff base deprotonation (Haupts *et al*., 1996).

Transport Modes of Bacterial Rhodopsins

Ion transport by bacterial rhodopsins can be measured for example as changes in proton concentration in the medium of a cell or vesicle suspension (Oesterhelt, 1982) or as electric currents using the black lipid membrane (BLM) technique [reviewed in Bamberg *et al.* (1993b)]. The use of specific ionophores allows discrimination between the transport of protons or chloride ions. In cases where several different transport modes are active, as discussed below, the measured signal represents the net sum of all transport processes occurring at the same time.

Investigation of the bacterial rhodopsins and some mutants using these and other techniques has revealed different transport modes as summarized in Table 1. In general, the charge movements found are based on one- or two-photon-driven processes which may lead to a net transport in either direction or a transient release and re-uptake of an ion on the same side of the membrane. For example, BR-WT (BR wild type) transports protons out of the cell in a one-photon-driven process. Absorption of a second photon by the M intermediate leads to a shortcut of the transport cycle, and the proton released toward the extracellular side during formation of M is taken up again after the second photoreaction, a process known as "blue light quenching" of proton transport (Ormos *et al.*, 1978). Most importantly, active transport with different vectoriality has been found to be mediated by the same molecule, involving, however, different intermediates. This is demonstrated, for example, by SRI (Haupts *et al.*, 1995, 1996) or BR-D85T which transports protons out of the cell in a one-photon-driven process (Figure 1A, part a) and into the cell in a two-photon-driven process (Tittor *et al.*, 1994). Addition of the protonophore CCCP abolishes the signal, proving that the pH change is induced by a primary proton transport (Figure 1A, part c). It is important to note that this phenomenon of active transport with opposite vectoriality is fundamentally different from a reversible process as found for example for F_0F_1-ATP synthase which is pmf-driven but can also use ATP hydrolysis to extrude protons.

Table 1: Summary of the Transport Modes Found for Bacterial Rhodopsins[a]

reaction sequence		net transport	transported ion	direction	photon requirement	occurrence and active species[m]	ref/figure
I	I*/T/S/T/I/S	yes	H^+	outward	one *hν*	BR-WT	*b*
			H^+			BR-D85T	*c*/Figure 1
			H^+			SRI_{550}	*d, h*
			H^+			HR (*N. pharaonis*)	*e*
			Cl^- [n]	inward		HR_{578}	*f*
			Cl^- [n]			BR-D85T	*c, g*
II	I*/S/T/I*/S/T	yes	H^+	inward	two *hν*	SRI_{590}/SRI_{380}	*h, k*
			H^+			SRI-D76N/SRI_{380}	*h*
			H^+			*Htr*I-SRI/*Htr*I-SRI_{380}	*h*
			H^+			BR-D85T/BR-D85T$^L_{410}$	*i*
			H^+			HR/HR$^L_{410}$ (*H. salinarum*)	*j*
III	I*/S/T/I/S/T		H^+	outward	one *hν*	BR-D85T$^D_{410}$	*i*/Figure 1
IV	I*/T/S/I*/T/S	yes	H^+	outward	two *hν*	SRI_{520}/SRI_{380}	*k*
V	I*/S/I(*)/T/S/T	yes	H^+	inward	one or two *hν*	SRI-D76N	*h*
VI	I*/T/S/I*/S/T	no	H^+	transient outward	two *hν*	BR-WT/BR-M (blue light quenching)	*l*
VII	I*/S/T/I*/T/S	no	H^+	transient inward	two *hν*	not observed	

[a] Grouping is according to the reaction sequence giving rise to the observed charge transport. Sequences I and II are depicted in Figure 2: I*, light-induced isomerization; I, thermal isomerization; S, switch; and T, ion transfer. [b] Oesterhelt and Stoeckenius (1973). [c] Tittor *et al.* (1996). [d] Bogomolni *et al.* (1994). [e] Váró *et al.* (1996). [f] Schobert and Lanyi (1982). [g] Sasaki *et al.* (1995). [h] Haupts *et al.* (1996). [i] Tittor *et al.* (1994). [j] Bamberg *et al.* (1993a). [k] Haupts *et al.* (1995). [l] Ormos *et al.* (1978). [m] Inferred from the wavelength of the required photon(s); action spectra have been obtained for BR-WT, HR, SRI_{550}, and SRI_{520}/SRI_{380}. [n] Other anions are also transported.

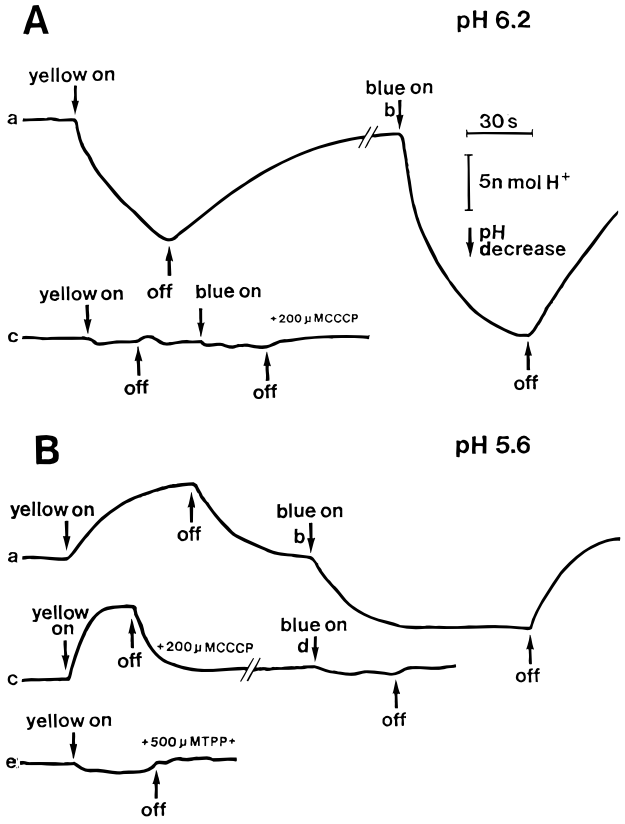

FIGURE 1: Light-induced pH changes in a cell vesicle suspension of mutant BR-D85T (80 mM $MgSO_4$ and 1.2 M NaCl; amount of BR in the sample = 12 nmol). (A) pH 6.2 (a) with a 550 nm cutoff filter, (b) with a 400 ± 25 nm K40 broad band interference filter, and (c) after addition of 200 μM CCCP and the same light conditions as in a and b. (B) pH 5.6 (a) with a 550 nm cutoff filter, (b) with a 400 ± 25 nm K40 broad band interference filter, (c and d) after addition of 200 μM CCCP and the same light conditions as in a and b, and (e) after addition of 500 μM TPP^+.

Interestingly, the same molecule BR-D85T is also capable of blue light-driven proton extrusion on the basis of the BR-D85T$^D_{410}$ starting with a deprotonated Schiff base (part b of Figure 1A and part b of Figure 1B). BR-D85T also provides an example where the same molecule can transport either a proton (Figure 1B, part b) or a chloride ion (Figure 1B, part a; Sasaki *et al.*, 1995). While the yellow light-induced chloride transport is unaffected by addition of CCCP (Figure 1B, part c), the blue light-driven proton tranport is quenched (Figure 1B, part d). Only addition of TTP^+ also quenches the pH change induced by chloride transport (Figure 1B,part e). This demonstrates competition of a negative and a positive ion for transport by the same molecule but different reaction sequences (Table 1, sequences I and III), a situation also found for HR. Both proteins transport protons or chloride ions into the cell in a two- or one-photon-driven process, respectively (Bamberg *et al.*, 1993a; Sasaki *et al.*, 1995; Tittor *et al.*, 1996).

General Concept for the Ion Transport: The IST Model

How can one account for this dizzying variety of activities? It is helpful to consider first some logical requirements to guide any mechanistic explanations. For continuous ion translocation to be observed, each molecule must go through a cyclic process multiple times. This implies that every change introduced at one point must be reversed at a later stage to restore the initial state. Since all proteins start from a thermally relaxed state in the dark, the first step must be a light-induced retinal isomerization (I*) which represents the point of energy input and must be reversed at a later stage of the transport cycle by either a thermal (I) or a second light-induced isomerization (I*). The change in accessibility of the Schiff base between de- and reprotonation, called the switch (S), is another necessary element of the transport cycle and again has to be reversed to re-establish the initial state. De- and reprotonation of the Schiff base may be given a broader definition as ion transfer steps (T) to and from the active center of the molecule, the Schiff base, including chloride or other anions. This leaves us with minimally two isomerization, two switch, and two ion transfer events to compose a transport cycle. A random combination of these six events would yield 720 different sequences. However, taking into account that every transport cycle must begin with a light-induced isomerization and that the same two elements may not succeed one another because they would

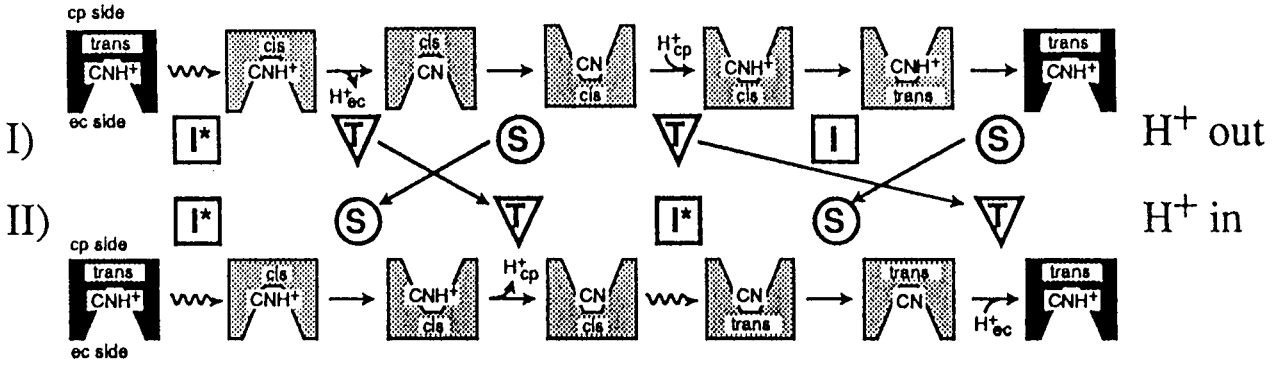

FIGURE 2: Two examples illustrating the IST model of ion translocation by halobacterial retinal proteins. Sequence I represents the BR-WT mode of proton extrusion; sequence II corresponds to the two-photon-driven proton import. The change in vectoriality is reflected by the inversion of the S and T steps in the sequences.

cancel each other, most of the combinations can be excluded. To further reduce the number of sequences to the functionally relevant ones, three basic considerations are introduced.

(1) The accessibility of the Schiff base in the protein is directed by the configuration of the retinal. 13-*cis*-Retinal sets the access to the cytoplasmic and *all-trans*-retinal to the extracellular side (Oesterhelt *et al.*, 1992; but compare with 3). Indeed, Schiff base accessibility from the extracellular side in *all-trans*-retinal containing BR in the dark has been demonstrated (Druckmann *et al.*, 1995), whereas the long-lived blue-shifted intermediates of BR-D96N, SRI, and HR contain 13-*cis*-retinal and exhibit cytoplasmic accessibility. It is very important to note that the change in accessibility is a time-dependent process in the millisecond time range with specific time constants for each molecular species. We make no assumptions whatsoever about the structural changes in the molecules linked to the switch process. These could be as small as a rotation around a single bond in retinal (Schulten *et al.*, 1984) or as large as the experimentally observed conformational change in BR involving helix movement (Dencher *et al.*, 1989; Koch *et al.*, 1992; Subramaniam *et al.*, 1993).

(2) Light-induced retinal isomerization (I*) is assumed to initiate two subsequent processes, one being an ion transfer step (T) to or from the Schiff base and the other being the change of accessibility of the Schiff base, the switch (S) (this is actually a consequence of 1), while thermal isomerization may initiate T and S or only S.

(3) A central point of our proposed concept is the kinetic independence of ion transfer (T) and the switch (S). Thus, isomerization may initiate both processes at the same time, but the ratio of the reaction rates determines the probability of whether ion transfer or switching occurs first. Note that the accessibility of the Schiff base in a distinct intermediate may differ from the strict rule that 13-*cis* corresponds to CP accessiblity and *all-trans* to EC accessibility, when the ion transfer step is faster than the switch event and therefore is first. Particularly, this is the case for the BR-L intermediate.

As a consequence of 3, an isomerization (I or I*) may be followed by T/S as well as S/T. However, since isomerization induces the switch (S), at least one isomerization must occur between two switching events. Surprisingly, only 6 out of the 720 combinatorially allowed sequences fulfill all these requirements, and only 4 of them lead to active transport. All of the different transport activities found for bacterial rhodopsins are attributable to one of these sequences listed in Table 1. Note that the starting point of a transport cycle could in principle be a species containing a deprotonated or a protonated Schiff base in either an *all-trans* or a 13-*cis* retinal configuration (all in the C=N *anti* configuration). Yet the experimentally observed activities all start with *all-trans*-protonated Schiff bases, except that of BR-D85T$^{D}_{410}$ which starts with a deprotonated *all-trans* chromophore.

Some Examples

The sequence describing proton translocation of wild-type BR or SRI$_{550}$ is I*/T/S/T/I/S (Table 1, sequence I). Thus, after light-induced isomerization (I*), the ion transfer step (T) is faster than the switch (S) because a deprotonated aspartate residue in the EC half-channel is ideally positioned to accept the Schiff base proton. This yields an M intermediate with EC accessibility (called MI) which is turned into one with CP accessibility by the switch (called MII). Reisomerization is thought to be blocked as long as the Schiff base is deprotonated. Therefore, completion of the cycle must be in the order T/I/S (Figure 2, sequence I; Table 1, sequence I). Absorption of a second blue photon by the MI or MII intermediate leads to a proton re-uptake from the EC side, implying that the switch has not yet occurred (MI) or is faster than ion transfer (MII); e.g. the sequence describing the blue light-quenched process is I*/T/I*/T or I*/T/S/I*/S/T, with no net transport (Table 1, sequence VI).

In contrast, in the absence of a suitable proton acceptor in the EC half-channel, e.g. in HR or SRI$_{590}$, ion transfer after the initial isomerization (I*) is impaired, allowing the switch to occur before ion transfer, resulting in Schiff base accessibility and ion release toward the cytoplasmic side. Thermal decay of the M intermediate would require reprotonation of the Schiff base from the CP side followed by reisomerization and switch with no net transport. However, the effect of a second blue photon is unaltered, leading to an overall sequence of I*/S/T/I*/S/T. This represents active, two-photon-driven proton transport but with reversed vectoriality compared to BR-WT which has been experimentally demonstrated for BR-D85T, HR, and SRI (Table 1, sequence II). This reversed vectoriality is reflected by the inversion of the sequence of the S and T steps as shown in Figure 2.

BR-D85T is especially interesting because it shows both activities, the one-photon-driven outward as well as the two-photon-driven inward transport (Figure 1A, part b; Tittor *et al.*, 1994). Therefore, after the light-induced isomerization, the switch and the ion transfer step must have similar reaction

rates, highlighting the principle of kinetic competition between switch and ion transfer. This leads to deprotonation toward the EC side followed by a switch in one subset of the molecules, yielding outward transport via the sequence I*/T/S/T/I/S (Table 1, sequence I). In the other subset of the molecules, the sequence is the other way around, leading to no net transport in the absence of blue light (I*/S/T/T/I/S), while in the presence of blue light, the second photoreaction leads to the sequence I*/S/T/I*/S/T, yielding inward transport (Table 1, sequence II).

In the presence of chloride, BR-D85T can show yet another transport activity, the one-photon-driven inward transport of chloride ions (Figure 1B, parts a and c), which is also the physiological function of HR. Although the vectoriality of this transport is opposite to BR-WT proton transport, the sequence describing the activity is the same (I*/T/S/T/I/S; Table 1, sequence I). The different vectoriality is caused by the reversed direction of the ion transfer steps. While I* induces release of the Schiff base proton (T) toward the EC half-channel in BR (and a subpopulation of the molecules of BR-D85T), it is argued that in the other subpopulation of the BR-D85T molecules and in HR a chloride ion moves toward the protonated Schiff base, making closer hydrogen bond contact with it. This was demonstrated for HR through chloride-dependent changes in the C=N stretch frequency during the transport cycle (Braiman *et al.*, 1994). After the switch has occurred, assigned to a $HR_{520}I \leftrightarrow HR_{520}II$ step (Oesterhelt *et al.*, 1992), the chloride ion is released toward the cytoplamic side. In contrast, BR-M picks up a proton from the CP side. BR-D85T and HR are therefore examples of cases where a negative and a positive ion compete for transport under the same conditions (Figure 1B, parts a and b).

Another example of a reaction sequence describing transport of both vectorialities is found when comparing proton transport based on BR-D85T/BR-D85T^{L}_{410} (Table 1, sequence II) and BR-D85T^{D}_{410} (Table 1 sequence III). Again, the different vectoriality is explained by the inversed direction of the ion transfer steps. In the former case, initiation of the transport occurs by absorption of a long wavelength photon, while in the latter case, a blue photon is required, because the starting point is a deprotonated Schiff base. Both absorptions initiate an I*/S/T reaction sequence; however, T represents a de- or a reprotonation in BR-D85T/BR-D85T^{L}_{410} and BR-D85T^{D}_{410}, respectively. While for BR-D85T^{L}_{410} reisomerization must be induced by absorption of a second photon (I*), it may proceed thermally in the case of the BR-D85T^{D}_{410} cycle because it involves a protonated Schiff base. In effect, a proton is transported form the EC to the CP side in a two-photon-driven reaction by BR-D85T/BR-D85T^{L}_{410} but in the reverse direction by BR-D85T^{D}_{410}, driven by one blue photon.

Final Remarks

To conclude, we have shown that the concept of a kinetic independence of proton transfer and the switch yields a consistent mechanistic view of the ion transport activities of halobacterial retinal proteins by combining the three functional elements isomerization (I), switch (S), and ion transfer (T). A cyclic transport process must fulfill logical requirements which reduce the combinatorially allowed possibilities of combining these elements to only four different modes with net transport. For all of these modes, examples have been found, and conversely, all observed transport activities of the halobacterial retinal proteins may be accounted for by one of these modes (Table 1).

The concept of the kinetic independence of the switch and ion transfer has, of course, a tremendous impact on the understanding of how the proton pump bacteriorhodopsin works. According to this, proton translocation is possible because of the optimal balance of reaction rates of different processes that are kinetically independent. The role of the ion binding sites in the molecule (besides the Schiff base, e.g. aspartates in BR and arginines in HR) is to kinetically optimize ion transfer reactions to and from the Schiff base, thereby determining the physiologically transported ion (Miller & Oesterhelt, 1989). This might be called a kinetic model as opposed to a stepwise model in which the switch is only possible after the proton transfer has occurred, as proposed for bacteriorhodopsin (Kataoka *et al.*, 1994; Lanyi, 1995; Spudich & Lanyi, 1996). These authors suggest that a relaxed conformation of the protein provides accessibility of the Schiff base from the cytoplasmic side. Only the electrostatic interaction of the protonated Schiff base with its counterion, mainly Asp85, forces the protein into a strained conformation with accessibility from the extracellular side (Spudich & Lanyi, 1996). This interaction is assumed to be interrupted not only upon mutation of Asp85 in the ground state but also more importantly after light-induced proton transfer of the Schiff base proton to Asp85 in the photocycle. As a result, the protein relaxes to the state with cytoplasmic accessibility. Such a mechanism accounts neither for the observed blue light-driven proton transport of BR-D85T (part b of Figure 1A and part b of Figure 1B; Tittor *et al.*, 1994) nor for two-photon-driven proton transport of either HR (Bamberg *et al.*, 1993a), BR-D85T (Tittor *et al.*, 1994), or SRI_{590} (Haupts *et al.*, 1996). Therefore, we propose a mechanism in which the accessibility of the Schiff base in the protein is directed by the isomerization state of the retinal, *all-trans* being to the extracellular and 13-*cis* to the cytoplasmic side as has been suggested first by Oesterhelt *et al.* (1992). Isomerization of the retinal induces the switch and the ion transfer process which kinetically compete, thus resulting in different vectorialities of the transport depending on the relative reaction rates.

For the future, two questions seem to be a necessary focus. First, what is the structural basis of the switch? Structural changes could be as small as either a 14-*s*-*cis* to 14-*s*-*trans* rotation in retinal or a rearrangement of hydrogen bond networks in the half-channels or as large as movements of transmembrane helices. Second, what are the thermodynamic driving forces of the various transport modes in the different proteins? In other words, how much of the absorbed light energy can be converted into electrochemical energy of an ion gradient? In the case of BR, 280 mV has been determined as the driving force (Michel & Oesterhelt, 1976). A further intriguing question concerns whether the principles outlined for the halobacterial rhodopsins also operate in other energy-coupled transport systems.

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