# A mechanism for the light-driven proton pump of *Halobacterium halobium*

MITCHELL's hypothesis of chemiosmotic coupling between redox reactions and ATP synthesis in membranes[1] is supported by the finding of a light-driven proton pump in the purple membrane of *Halobacterium halobium*[2–4]. The purple membrane contains the protein bacteriorhodopsin in a crystalline array, with retinal as chromophore[5,6]. We propose here, on the basis of quantumchemical arguments and experimental observations, that the *H. halobium* proton pump may involve proton translocation through photoisomerisation of retinal about its 14–15 single bond.

Most of the information on the light-driven proton pump of *Halobacterium* comes from spectral observations of the pump cycle[7]. Light is absorbed by bacteriorhodopsin with absorption maximum at 568 nm (B_{568}). B_{568} contains all-*trans* retinal bound as a protonated Schiff base to a lysine residue[8–11]. *In vitro* the protonated Schiff base of retinal absorbs around 440 nm, however (ref. 12). It assumes a mesomeric structure between the polyene resonance form
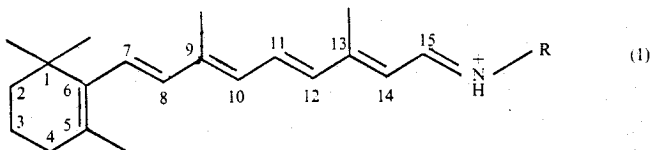

and the polyenylic ion resonance forms, for example
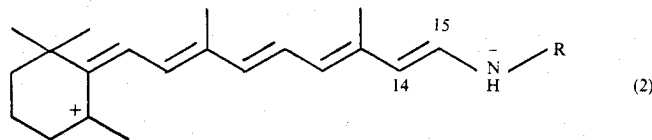
Stabilisation of the latter results in the bathochromic shift[12], for example to 568 nm in bacteriorhodopsin.

The longest lived intermediate of the pump cycle absorbs at 412 nm (M_{412}). In the dark M_{412} reverts to B_{568}; a proton is released to the outer cellular space and another proton absorbed from the cytoplasmic side[7,13]. A knowledge of the structure of the M_{412} intermediate is vital for the elucidation of the molecular mechanism of the proton pump. Lewis *et al.*[9,10] and Aton *et al.*[11] concluded from resonance Raman scattering data that M_{412} represents an unprotonated Schiff base. The blue shift to 412 nm is then to be attributed to the pure polyene type structure
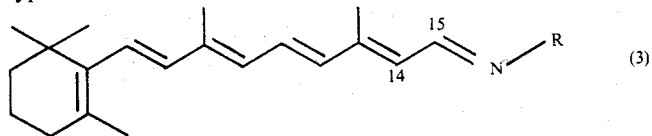
How do deprotonation and reprotonation of B_{568} after optical excitation induce a vectorial proton transfer against a proton gradient? Retinal is known to isomerise around its double bonds on absorption of light. As bacteriorhodopsin exhibits some sterical freedom for bond rotation, proven by the conversion of B_{568} in the dark to a 50:50 mixture of all-*trans* and 13-*cis* retinal which in the light reverts to the all-*trans* chromophore[14], one may expect that the chromophore isomerises during the pump cycle[16] (although thermal back-isomerisation is generally slow—20 min for the *trans* → 13-*cis* isomerisation during dark adaptation[14]). Chemical analysis indicated that during the pump cycle bacteriorhodopsin contains solely all-*trans* retinal[15], but a more recent study by Pettei *et al.*[17] revealed a 13-*cis* M_{412} intermediate, a finding also supported by time-resolved resonance Raman studies[11]. Slifkin and Caplan[18] detected, by time-modulation spectroscopy, the occurrence of two different M_{412} intermediates exhibiting different polarisation behaviour. This finding has been corroborated by Hess and Kuschmitz[19] and Kalisky, Lachish and Ottolenghi (in prepara-

tion). A rapid decay of light-induced linear dichroism synchronous with the M_{412} intermediate indicating a conformational transition of the chromophore, has also been observed at 620 nm (ref. 20).

Thus if conformational transitions of the chromophore play a part in the pump cycle, we must explain how such an isomerisation process could induce changes of the acid-base properties of the chromophore and, thereby, a vectorial transposition of a proton.

The 14–15 single bond adjacent to the C = N group of the Schiff base of retinal exhibits some remarkable properties which makes photoisomerisation about this bond suitable for a vectorial proton translocation. According to the structures (1), (2) and (3) this bond represents a partial double bond in the protonated chromophore B_{568} but a single bond in the unprotonated intermediate M_{412}. To test this supposition we calculated potential surfaces for rotation about the corresponding 6–7 bond of the octatetraene analogue of the retinal Schiff base (Fig. 1). The protonated Schiff base indeed exhibits a high barrier for thermal isomerisation in the ground state. The unprotonated Schiff base, as expected, has a low barrier in the ground state. The energy barrier for photoisomerisation in the lowest excited state, however, is low for the protonated form and high for the unprotonated form. This behaviour of ground and excited state isomerisation potentials suggests a mechanism for the proton pump in *Halobacterium*: photoisomerisation of B_{568} around its 14–15 bond faciliated by a low energy barrier transports the nitrogen proton from an environment E_i in contact with the cytoplasmic side of the purple membrane to an environment E_f in contact with the outer cellular space. Thermal back-isomerisation is prevented for the protonated chromophore by a high barrier for thermal isomerisation, but is possible after the proton has been released at E_f because of the low isomerisation barrier of the unprotonated chromophore in the ground state. Proton diffusion between E_i and E_f without the chromophore carrier is hindered. This mechanism is supported by studies[22] of *trans* → *cis* photoisomerisation of a stilbazolium betaine in the protonated form that thermally reverses only after deprotonation in a basic medium.

The 14*S*-*trans* → 14*S*-*cis* isomerisation suggested probably occurs in connection with a 13–14 double bond rotation. The sterical hindrance imposed on the chromophore by the tight protein cavity of its binding site is likely to enforce an all-*trans* → 13-*cis* photoisomerisation to be accompanied by a 14*S*-*trans* → 14*S*-*cis* bond rotation (D. Oesterhelt & A. Warshel, personal communication). The reversion of the latter motion at the M_{412} stage of the cycle can be accommodated sterically by a complementary bond rotation in the lysine moiety to which the chromophore is bound.

The increase of the barrier of isomerisation on protonation implies a strong dependence of the pK value of the Schiff base nitrogen on the rotation about the 14–15 bond. This feature would greatly increase the efficiency of a proton pump based on the proposed mechanism. This is demonstrated in Fig. 1c which combines Fig. 1a and 1b to model the behaviour of the chromophore in bacteriorhodopsin. The potential curves in Fig. 1c corresponding to the ground and excited states of the unprotonated Schiff base R are labelled $S_0(R)$ and $S_1(R)$, respectively, representing a state $XH^+ + R$, where X represents some proton acceptor on the cytoplasmic side. The potential curves corresponding to the ground and excited state of the protonated Schiff base are labelled $S_0(RH^+)$ and $S_1(RH^+)$, respectively, representing the state $X + RH^+$. The energy difference between $X + RH^+$ and $XH^+ + R$ in the untwisted ground state has been assigned the value 0.17 eV corresponding to a difference of 3 between the pK values of R and X ($\Delta pK = pK(R) - pK(X) > 0$ sets $X + RH^+$ below $XH^+ + R$ by an energy $\Delta E = 2.3kT \Delta pK$). The crossing of the ground state potential curves $S_0(R)$ and $S_0(RH^+)$ implies that on bond rotation the ΔpK value
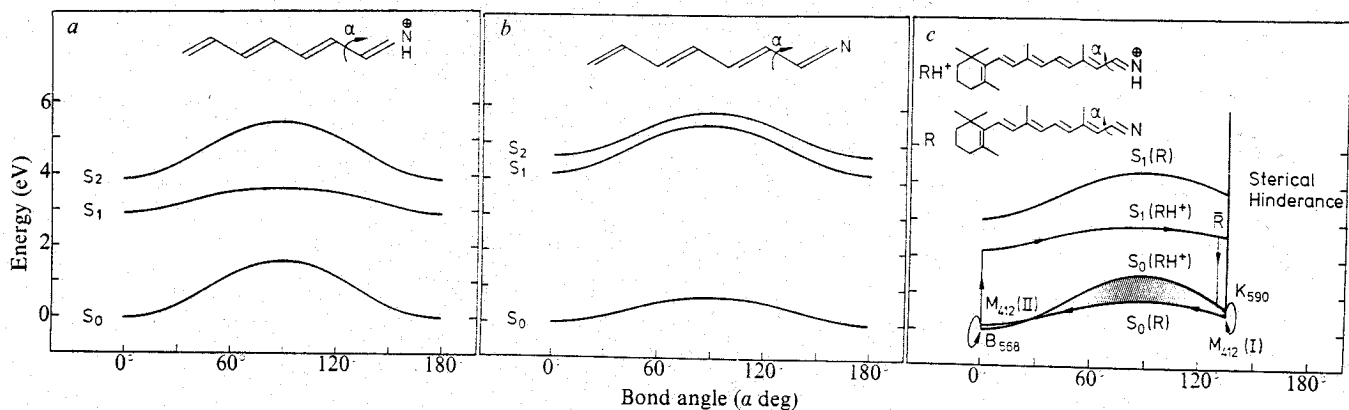
Fig. 1 Isomerisation potentials of polyene Schiff bases contributed by the π electrons and evaluated by a Pariser–Parr–Pople SCF-CI calculation including all single, double, triple and quadruple excitations from the SCF ground state; interaction parameters [ionisation energies, repulsion matrix elements (Ohno approximation) and charges] were taken from ref. 21 describing C=N like pyridine and C=NH$^+$ like pyrrole; for the resonance integrals (in eV) we used the formula $\beta = -2.6 + 3.21\ (r - 1.397)$ where the bond distances r (in Å) were chosen to be 1.46 for single bonds and 1.35 for double bonds. *a*, Protonated Schiff base of octatetraene. *b*, Unprotonated Schiff base of octatetraene. *c*, Combination of the potentials from (*a*) and (*b*) to describe the Schiff base of retinal; the ground state energy ordering is described in the text, the excitation energies for R and RH$^+$ were chosen to conform with the observed absorption maxima in bacteriorhodopsin of 412 nm and 568 nm, respectively.

reverses its sign, that is, the retinal Schiff base becomes more acid than X. Steric hindrance of the 14–15 photoisomerisation by the protein environment as indicated in Fig. 1*c* could fix the chromophore in an acid conformation R thereby transforming light excitation into a change of acid–base properties. A suitable group Y near the translocated nitrogen could accept the proton as long as its *p*K value satisfies the condition

$$E(RH^+ + X) - E(\bar{R} + XH^+) > 2.3kT\,[pK(X) - pK(Y)]$$

If, for example, the $S_0(\bar{R}H^+)$ state lies 0.3 eV above the $S_0(\bar{R})$ state the proton transfer occurs effectively even if the acceptor Y has a *p*K value 5 units below that of X. Thus, the light-driven proton pump could easily overcome a strong *p*H gradient.

After the sterically induced proton release described above the unprotonated chromophore has to transcend the low energy barrier in the $S_0(R)$ state. We suggest that this process (which can be accelerated by optical excitation) has been observed in the experiments described in refs 18–20 and is perhaps to be attributed with the activation energy of 13 kcal mol^{-1} measured for the $B_{412} \to B_{568}$ dark transition[23].

The strong bathochromic shift of the Schiff base of retinal on protonation[12] reflected by the energy difference between the $S_1(R)$ and the $S_1(RH^+)$ states (Fig. 1*c*) implies that the light-excited chromophore is a strong base[24]. This means that during photoisomerisation the chromophore binds the proton tightly at the terminal nitrogen. This proton in turn stabilises during photoisomerisation the chromophore binds the proton tightly at the terminal nitrogen. This proton in turn stabilises the transfer of π-electron charge from the β-ionone ring to

the nitrogen in the excited state $S_1(RH^+)$ (ref. 25) and, thereby, reduces the barrier of photoisomerisation at the $\alpha = 90°$ position of $S_1(RH^+)$.

Figure 2 summarises the suggested mechanism for the light-driven proton pump of *Halobacterium* B_{568} photoisomerises about the 14–15 bond to form the first intermediate K_{590} which exists in a sterically hindered 14*S*-*cis* form. K_{590} releases (probably indirectly) a proton to the outer cellular space and goes over to the unprotonated 14*S*-*cis* M_{412} (I) intermediate. This intermediate isomerises back to all-*trans* M_{412} (II) which accepts a proton from the cytoplasmic side to form B_{568}.

We pointed out above that the 14–15 bond rotation may be induced in the first place by an all-*trans* → 13-*cis* photoisomerisation (included in Fig. 2). It is possible that thermal reversion of this double bond rotation is facilitated by reprotonation of the Schiff base in the same way as thermal rotation around the single bond is being hindered.

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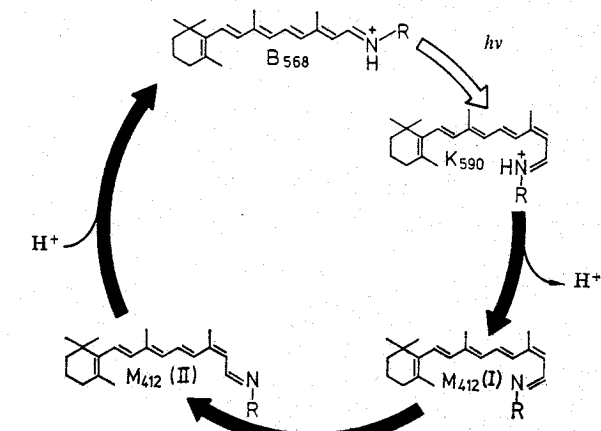25. Mathies, R. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* 73, 2169–2173 (1976).

Fig. 2 Model of the proton pump cycle of *Halobacterium halobium.*