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On the mechanism of proton pumping

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Abstract

The proton pump of purple bacteria is based on the photoinduced trans to cis isomerization of the Schiff base of retinal and the thermal cis–trans reisomerization accompanied by a shift of a proton from the cytoplasm to the extracellular space. Quantum mechanical calculations reveal, in exciting the chromophore, that negative charge is removed from the N atom of the Schiff base, causing a weakening of the HN bond by about 0.5 eV. We assume, in exciting the protonated trans isomer of the Schiff base, that the proton is left behind in the extracellular channel. The Schiff base (after trans to cis isomerization and deexcitation, in which the negative charge is shifted back to the N atom) picks up a proton from the cytoplasmic channel. In the proposed proton pumping mechanism a difficulty of the usually assumed mechanism is avoided, namely the assumption that the cis isomer in the ground state has a pK value increase of ca. 9 pH units only by a change of protein conformation.

1. Introduction

The proton pump in purple bacteria contains retinal attached to a protein by a protonated Schiff base linkage to a lysine side chain (Fig. 1). The complex (bacteriorhodopsin) is incorporated in the cell membrane. Light isomerizes the all-trans chromophore BR_{568} (absorption maximum at 568 nm) into the 13-cis form and a proton is pumped from the inside of the cell to the outside during each cycle leading again to the all-trans form BR by thermal isomerization. The chromophore divides the channel formed by the seven α-helices of the polypeptide into the cytoplasmic part connecting to the inside of the cell and the extracellular part connecting to the outside. The cis form reached after excitation undergoes several changes: from conformation K_{590} to L_{550} to M_{412} (deprotonation of Schiff base) to N_{520} (reprotonation of Schiff base). Then a thermally activated isomerization from cis to trans takes place: the occurrence of intermediate O_{640} and finally back to BR. It is widely accepted that in the step from L to M the Schiff base proton is released into the extracellular channel and in the step from M to N a proton from the cytoplasmic channel is attached to the Schiff base (Fig. 2a), so a proton, effectively, is pumped from the inside to the outside (for reviews see Refs. [1–5]).

However, a number of ingenious experiments with mutants D85N and D85T [6], where the primary proton acceptor in the extracellular channel (amino acid D85) is absent, has led to the conclusion that the trans isomer in these mutants is connected with the extracellular channel and the cis isomer is connected with the cytoplasmic channel, i.e. L releases its proton into the cytoplasmic channel, and M picks up the proton from the cytoplasmic channel (Fig. 2b). It seems reasonable that the connection of the trans and

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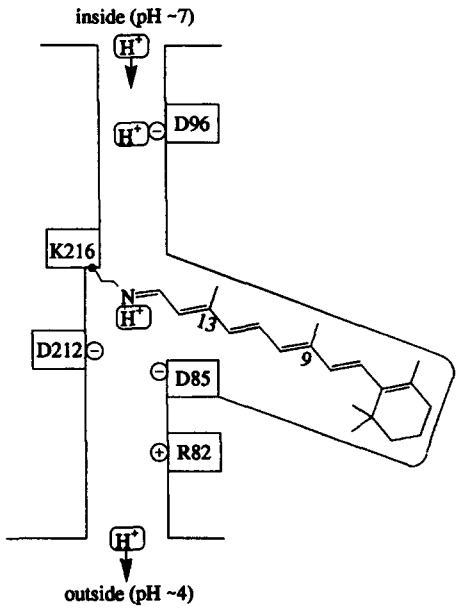


Fig. 1. Proton pump in purple bacteria.

cis isomer with the extracellular and cytoplasmic channel, respectively, is given also in the wild type. But what then is the mechanism of proton pumping?

2. The model

Quantum mechanical considerations on π-electron systems [7–10] [1] indicate a possibility of getting around this difficulty. They lead to the conclusion that the π-electron density at the nitrogen atom of the Schiff base is strongly decreased when exciting the chromophore. The decrease in electron density diminishes the pK of the Schiff base by ca. 9 pH units, i.e. the binding of the proton to the Schiff base is diminished by ca. 0.5 eV. Thus, the Schiff base, after exciting the chromophore, is likely to be deprotonated before being isomerized. Then it can be imagined that after exciting the trans isomer (step 1, Fig. 2c) the proton is released into the extracellular channel to which the trans form is connected when acceptor D85 is available assisting the deprotonation of the Schiff base through hydrogen bridges (step 2).

[1] There are some errata to Ref. [9], i.e. in Eq. (2) $n/2$ should read $1/2n$; in the line before Eq. (7) Δ/a^2 should read $(\Delta/a)^2$; in the chemical formula for butadiene on p. 207 there should appear a single bond in the middle instead of a double bond.

This is followed by the trans to cis isomerization (step 3) and deexcitation (step 4). In deexcitation, the π-electron density at the Schiff base nitrogen is again increased and therefore a sudden binding of a proton from the cytoplasmic channel to which the cis form is connected takes place (step 5). D96 is deprotonated and again protonated by picking up a wandering proton of the cytoplasm (Figs. 3 and 4). During thermal reisomerization (step from N to O, Fig. 3) the π-electron density at the Schiff base

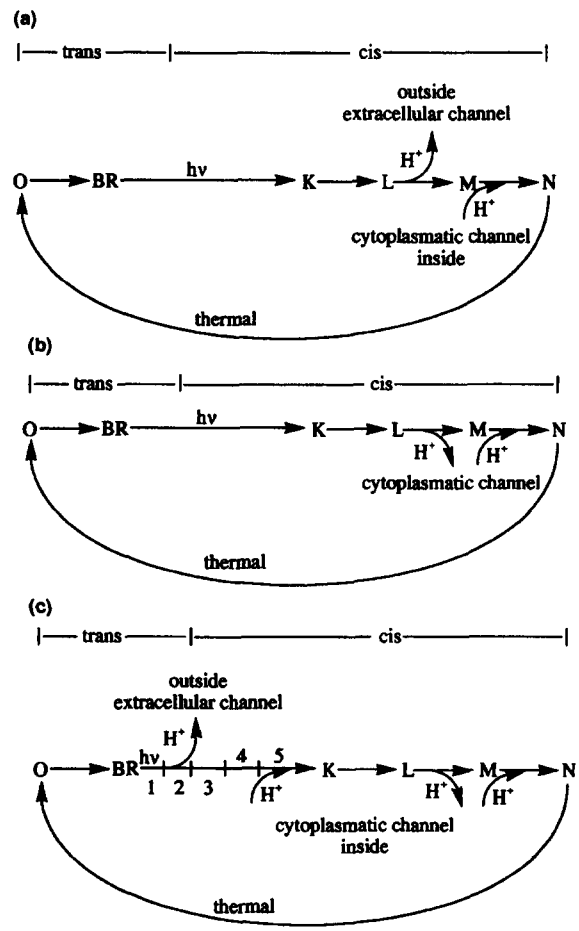


Fig. 2. Processes after photoexcitation of bacteriorhodopsin. (a) Wild type as widely accepted. Proton released into extracellular channel (deprotonation step L to M) and consumed from cytoplasmic channel (protonation step M to N). (b) Mutant deficient in proton acceptor D85. Proton released into cytoplasmic channel (L to M) and consumed from this channel (M to N). (c) Wild type according to the present model. Proton released from chromophore after excitation and before photoexcited trans to cis isomerization

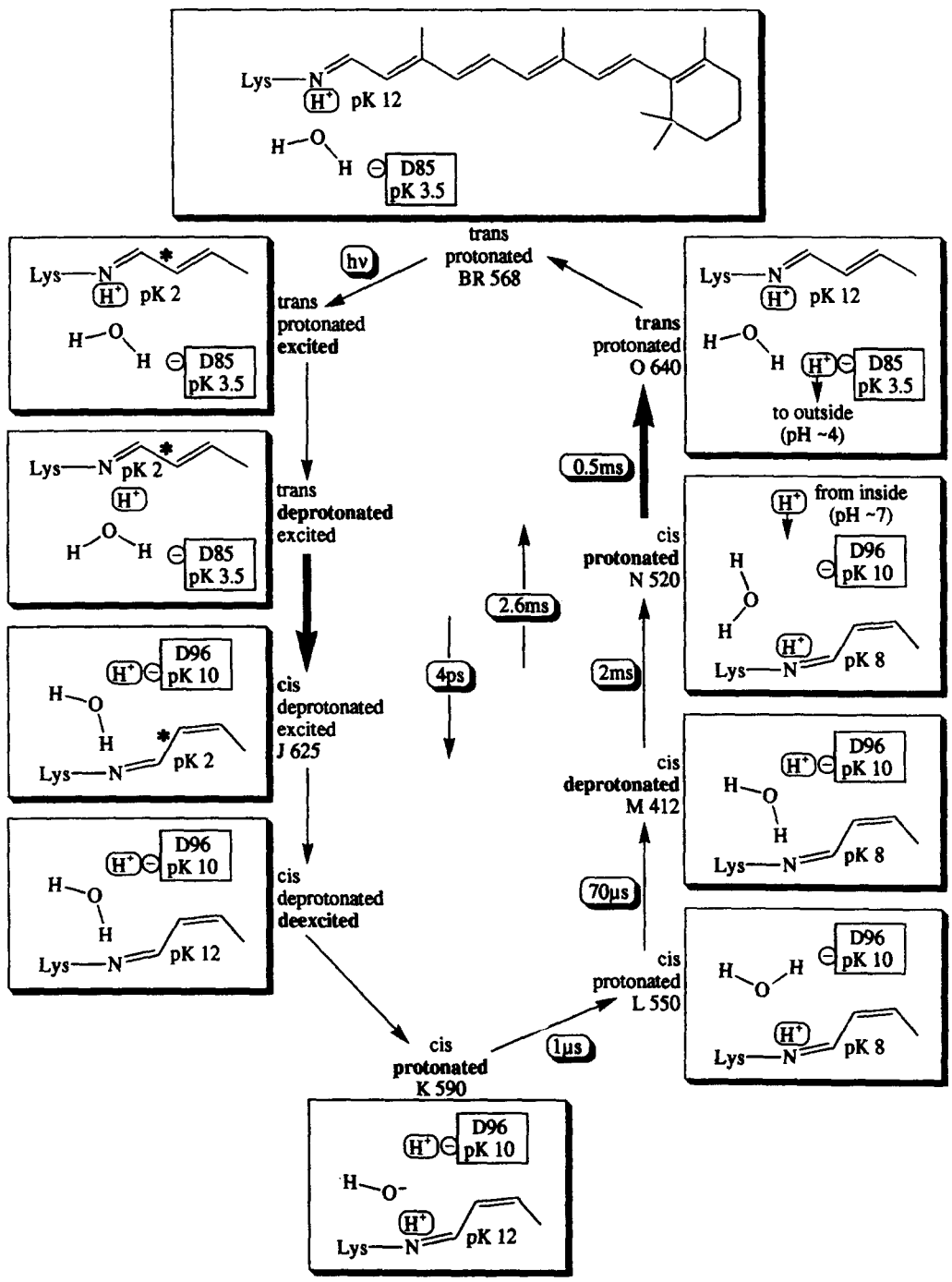


Fig. 3. Sequence of steps in proton pumping cycle according to the present model. The network of water bridges between proton acceptor D85 and Schiff base trans isomer is represented by a single H_2O molecule for simplicity. It is assumed after de-excitation of the cis isomer that the proton is taken from the next H_2O molecule forming a transient HO^- ion which disappears by deprotonating D96.

nitrogen is not diminished, and the proton remains bound to the Schiff base. The proton at D85 is released into the extracellular space. Thus, during each cycle a proton is shifted from the inside to the outside. A difficulty of the model in Fig. 2a is avoided, namely the assumption that the cis isomer in the ground state has a pK value increasing from 3.5 to 12 only by a change of protein conformation. Some specific steps in the proton motion are described in more details below.

– The pK of D85 is about 3.5 [1], the pK of the Schiff base in the ground state about 12 [11]. After excitation the Schiff base must have a pK lower than 3.5, otherwise D85 could not act as a proton acceptor. Therefore, the pK change in exciting the chromophore must be at least 8.5 pH units or the change in hydrogen bond energy must be at least 490 meV (Fig. 3). As mentioned above, the energy change according to the quantum mechanical calculation is about 0.5 eV.

– After deexcitation the Schiff base in the cis form has again a pK of 12. It will accept a proton from the closest H_2O molecule leaving an OH^- ion (occurrence of K) which later accepts a proton; D96 is deprotonated since its pK of about 10 [1,3] is smaller than the pK 12 of the Schiff base.

– A conformation change of the protein is assumed to change the pK of the Schiff base from 12 to, say, 8, so the proton will be shifted back to D96 (pK 10) (occurrence of M).

– The proton is again shifted from D96 to the Schiff base (occurrence of N) and later D96 picks up a proton wandering from the cytoplasm (Figs. 3 and 4).

– The thermal cis to trans isomerization brings the proton of the Schiff base into proximity with D85, the protons repel each other (occurrence of O), thus D85 (pK 3.5) releases its proton into the extracellular space (occurrence of BR).

A number of experimental data is known on proton consumption and release that were found to be in favor of the model in Fig. 2a. Can these data be explained, as well, by the model in Fig. 2c? These questions are discussed in Section 3. Section 4 is concerned with the shifts in the absorption maximum during the cycle. In Section 5 we show that the quantum mechanical model leads to the required pK change in exciting the chromophore.

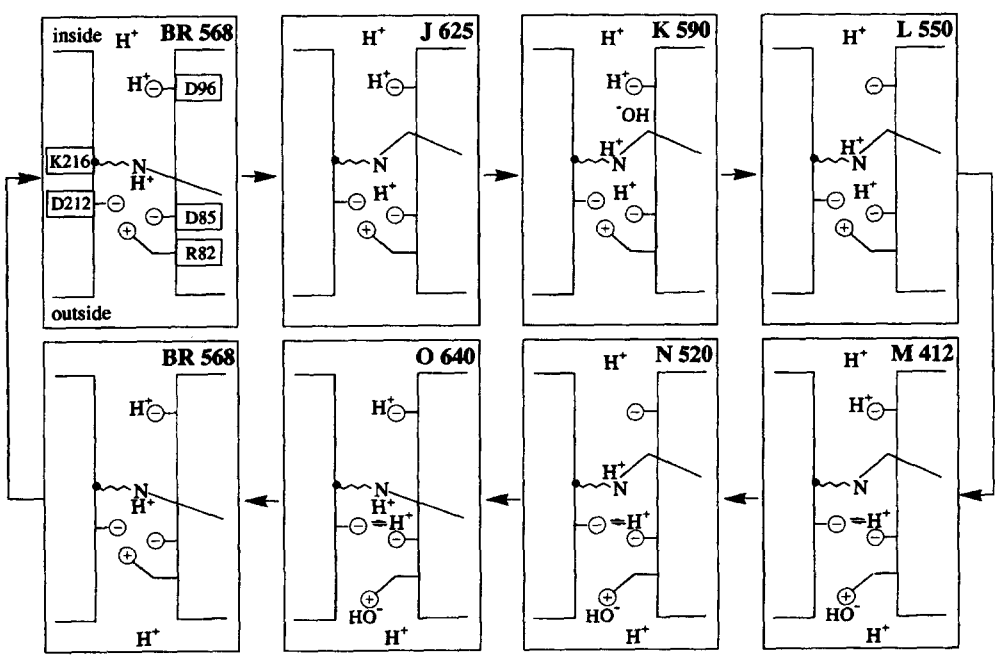


Fig. 4. Shift of protons in the photoinduced cycle of bacteriorhodopsin

3. Proton consumption and release

We summarize the main experimental data used to support the model in Fig. 2a and discuss them in relation to the model in Fig. 2c.

– *D85 protonated (D212 partially protonated) during M formation, deprotonated during O decay.* FT-IR studies (50 μs time resolution) [4] have shown that D85 is protonated in stages M, N and O, and D212 is partially protonated. The protonation of D85 is interpreted according to the model in Fig. 2a as proton transfer from the Schiff base to D85 during M formation [12–15]. The simultaneous protonation of D212 is unclear. In the present model, Fig. 2c, the results are interpreted by assuming that the proton produced in photoexcitation is trapped in the network of hydrogen bridges, liberated by a conformational change in the protein during M formation, and trapped in D85 or D212. The simultaneous protonation of D85 and D212 is reasonable in this view.

– *Proton acceptor D85 missing (mutants D85A, D85N, D85T) [6,16,17].* (i) Proton transport does not occur (Fig. 2b): the proton of the Schiff base is not removed from the N atom during excitation and photoinduced isomerization from trans to cis. (ii) No M intermediate formed [17]: since the proton in step BR to K is shifted into the cytoplasmic channel in this mutant, no proton is removed from D96 after trans to cis isomerization, in contrast to the wild type. Therefore, as protonated D96 is not acting as a proton acceptor, the Schiff base is not deprotonated.

– *D96 deprotonated during L formation, protonated during M formation, deprotonated during N formation established by FT-IR resonance [4].* This is in favor of the assumption that the Schiff base is protonated, deprotonated and reprotonated by proton exchange with D96.

– *Proton donor D96 missing (mutants D96A, D96N) [16,18].* Pumping activity strongly reduced and M decay slowed down at high pH [5]: the proton bonding to the Schiff base must have wandered from the cytoplasm when mediator D96 is absent. This is slow at small proton concentration.

– *The puzzling role of R82.* A proton is released into the extracellular space during M formation [19,20]. This fact does not account for the model in Fig. 2a in favor of the model in Fig. 2c because proton acceptor D85 remains protonated. Possible explanations discussed in the literature [21], including deprotonation of R82, can equally well be applied to the models in Fig. 2a and c. The explanation proposed in Ref. [4] is illustrated in Fig. 4.

– *R82 missing (mutant R82A) [21,17].* Proton release delayed after uptake. The puzzling effect of R82 is omitted. The mutant behaves as expected from the present model in this case.

– *O formation accompanied by uptake of proton from cytoplasm and correlated with reprotonation of D96 [18].* This fact is in accord with the present model (Fig. 4).

– *Rearrangement in stage O.* FT-IR studies [4] indicate protein rearrangement. This may be a reason for the strong bathochromic shift of the absorption band in this case.

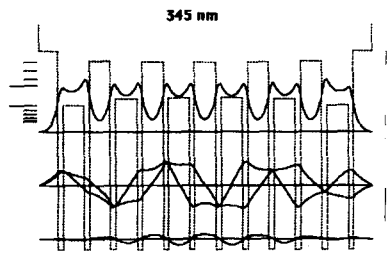
4. Quantum mechanical treatment of chromophore absorption

The chromophore absorption depends on conformational details that have been analysed as summarized in Ref. [2]. We do not go into any detail, our aim is to show that our quantum mechanical model describes the general aspects correctly and is sufficiently accurate to draw the conclusion made in Section 5.

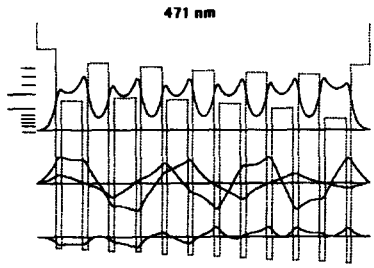
We first consider a polyene with six double bonds. The 12 π-electrons are considered to be in a step potential, C atoms are modeled as potential troughs of -32 eV depth and 0.3 Å width, and the potential in each C–C bond depends on the electron density in the bond determined by self-consistency between bond length and π-electron density [8,10]. The resulting potential corresponding to a chain of alternating double bonds and single bonds is given in Fig. 5a (left). It also shows the π-electron density distribution in the ground state resulting from the model and the wavefunction in the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The absorption maximum given by the energy difference is 345 nm in agreement with the measured value. Fig. 5a (right) corresponds to the same system in an assumed external field $F = 1.9 \times 10^7$ V/cm along the molecular axis with an absorption maximum of 471 nm. The calculated shift $h\Delta\nu = (1/2)\Delta\alpha\, F^2$ with $\Delta\alpha = 760$ Å^3 of the

(a) polyene

$F = 0$

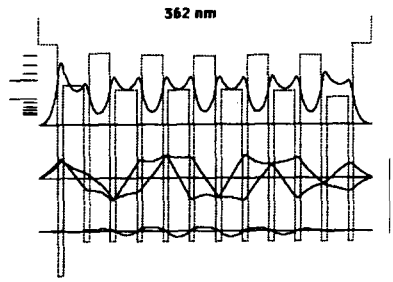


$F = 1.9 \cdot 10^7$ V/cm

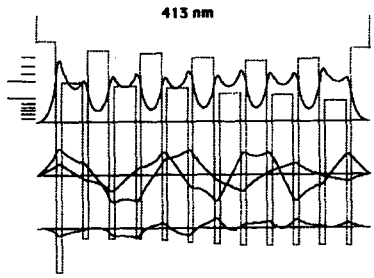


(b) unprotonated Schiff base

$F = 0$

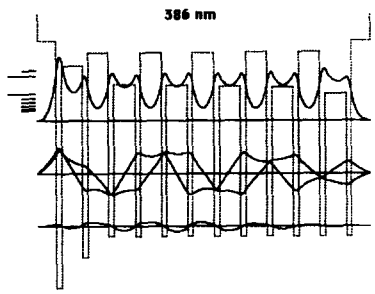


$F = 2.6 \cdot 10^7$ V/cm

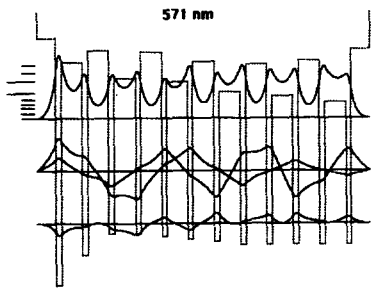


(c) protonated Schiff base

$F = 0$

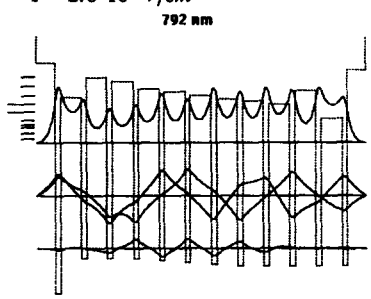


$F = 4.1 \cdot 10^7$ V/cm



(d) unprotonated Schiff base
relaxed excited state

$F = 2.6 \cdot 10^7$ V/cm



absorption band is of the order of the shift ($\Delta\alpha = 220$ Å^3) measured in a similar case [22].

Now we consider the Schiff base. In the unprotonated form the CH group at one end of the polyene is replaced by N. This change is considered in the model by replacing the potential trough of a C atom by a corresponding trough which is lower by a factor given by the ratio of the Slater effective charges of C and N, which are 3.25 and 3.9, respectively. In the protonated Schiff base the N atom and the adjacent C atom are less shielded. The Slater effective charge of N is increased by 0.35 (thus the above ratio becomes 3.25/4.25) and the same increase is estimated for the adjacent C. The self-consistency between bond lengths and π-electron densities is reached for the potentials given in Fig. 5b and c (left). The absorption maxima (362 and 386 nm in the unprotonated and protonated forms, respectively) are in reasonable agreement with experiment (359 and 440 nm, respectively). When applying an external field $F = 1.9 \times 10^7$ V/cm increasing the potential energy of the electron in the direction of the chain toward the N atom (case 1), the calculated absorption maximum of the unprotonated form is at 464 and 512 nm when the field is reversed (case 2); the maxima of the protonated form are at 514 (case 1) and 615 nm (case 2), respectively. The quadratic effect discussed in the polyene is superimposed by a linear effect due to the decreasing bond alternation when applying a field according to case 2, enhancing the bathochromic shift in contrast to case 1.

In bacteriorhodopsin the electric field acting on the chromophore can be approximated by the field of the charged amino acids in proximity (see Fig. 4). This is a rough estimate since this field depends on details of the network of hydrogen bonds. We use a dielectric constant of 1.0 as used in molecular dynamics calculations [23]. Then we find that the sum of the fields of D212, D85 and R82 (Fig. 4, BR 568) is approximately given by a field of 4.1×10^7 V/cm restricted to the section of the chain between C atoms 9 to 13. Similarly, the field of D212, D85, R82 and H^+ in the unprotonated case (Fig. 4, J_{625} and M_{412}) is approximately 2.6×10^7 V/cm. Assuming these fields restricted to the section between C atoms 9 to 13, we obtain the absorption maximum at 571 nm for the protonated Schiff base (see Fig. 5c, right) and at 413 nm for the unprotonated Schiff base (see Fig. 5b, right). The values are in reasonable agreement with experiment (protonated forms BR_{568}, K_{590}, L_{550}, N_{520} and O_{640}, unprotonated form M_{412}). The absorption maximum of J_{625} (which forms within 500 fs and relaxes to K in 3 ps [2,24]) can be compared with the transitions of the unprotonated Schiff base in the relaxed excited state (Fig. 5d, transitions from levels 5 to 6, 6 to 7 and 7 to 8 with maxima at 846, 792 and 777 nm, respectively). It must be considered that these bands couple by forming a strong band at higher energy and weak bands at lower energy [10].

5. Shift of the electron density, pK shift and lowering of the isomerization barrier in the excited chromophore

According to Fig. 5c (right) the LUMO of the protonated Schiff base is smaller at the N atom than the HOMO, thus charge is removed from N when exciting the chromophore: $0.2e$ is removed from N (e = elementary charge). We assume for simplicity that this charge is in the middle of the lobes of the p_z orbital, i.e. at distance $\sqrt{(1\text{ Å})^2 + (0.6\text{ Å})^2} = 1.2$ Å (x, y molecular plane, N–H bond length 1 Å). In moving the H^+ from position NH · · · O to position N · · · HO (by 0.5 Å), the loss in Coulombic energy due to the repulsion of the proton from this charge is

Fig. 5. Electronic structure of (a) polyene, (b–d) Schiff base of retinal in the step potential model. Bond lengths and π-electron densities in the bonds are self-consistent. Course of potential, eigenvalues, absorption maxima (energy difference between LUMO and HOMO), π-electron charge distribution in the ground state, wavefunctions of HOMO and LUMO and π-electron density shift in exciting the ground state (difference of squares of LUMO and HOMO). (a) Polyene, without (left) and with (right) a linear external field of 1.9×10^7 V/cm in the direction of the chain. [b(c)] Unprotonated (protonated) Schiff base of retinal without field (left) and with a field (right) of 2.6×10^7 V/cm (4.1×10^7 V/cm) restricted to the region of C atoms 9–13 simulating the influence of charged amino acids in proximity with the unprotonated (protonated) Schiff base, respectively. (d) Unprotonated Schiff base (case of Fig. 5b, right) in the relaxed excited state, π-electron density shift in de-excitation (difference of squares of HOMO and LUMO).

0.6 eV when assuming an unchanged electronic charge distribution during the process. Using the wavefunctions of the unprotonated Schiff base (Fig. 5b, right) the same consideration gives 0.4 eV [the charge removed from N is 0.15 *e*; a similar value for the change in electronic charge distribution would result when replacing the Schiff base for simplicity by a polyene (Fig. 5a, right)]. The estimated decrease in bonding energy is the average, 0.5 eV (15% of the NH bonding energy) corresponding to a pH shift of 0.5 eV/58 meV ≈ 9.

In relaxing the excited state of the deprotonated Schiff base the π-electron densities of the bonds become roughly equal (Fig. 5d, note that the π-electron density in the 13–14 bond is smaller than in any other double bond) accounting for the photoinduced trans to 13-cis isomerization.

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