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FROM PHOTONS TO PROTONS IN THE PHOTOCYCLE OF BACTERIAL REACTION CENTER

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Introduction

The membrane bound RC protein plays central role in energetics of photosynthetic bacteria by converting different forms of free energy: light energy into phosphate potential via redox energy and proton electrochemical gradient [1]. The absorption of photons initiates photochemical electron transfer from the excited dimer (P^*) through a chain of electron acceptor molecules, ultimately reducing the primary and secondary quinones Q_A and Q_B, respectively. The electron transfer is coupled to proton binding by RC to solvate (stabilize) semiquinone anions (Q_A^- and Q_B^-) and later to reduce Q_B to hydroquinol, Q_BH_2 [2]. The doubly reduced quinone dissociates from the RC and is reoxidized by the cytochrome bc_1 complex. The vectorial transport of protons from the cytoplasmic to the periplasmic side of the membrane builds up proton electrochemical gradient which drives ATP synthesis.

Proton transport is a key process in utilization of light energy. Some proton transport proteins, e.g. bacteriorhodopsin pump protons directly through the dielectric core of the biological membrane. On the other hand, photosynthetic organisms use indirect way by decreasing the energetic barrier of the membrane for the protons: electrons will be transported through the membrane via redox chain and form subsequently electrically neutral species with the H^+-ions. Thus the protons can pass the membrane with significantly less penalty. The photosynthetic electron transport can be viewed as accessory step that prepare the RC for protonation. Taking this point of view, it is surprising that much less effort has been placed on the proton transport than on electron transfer reactions in RCs.

In this work special emphasis will be put on energetics and kinetics of proton binding to RC as primary step in bacterial proton transport. The results of site-directed mutagenesis and structure-based electrostatic calculations will help us to understand the electrostatic role of the protein in proton binding and the possible pathways of H^+-ions to Q_B. The pH-dependence of the rate of the photocycle will be used to demonstrate how the limitations of quinone/quinol exchange and protonation become the bottleneck of the photocycle in the neutral and alkaline pH-ranges under intense continuous illumination.

Methods

The details of construction of mutants and measurements of electron and proton transfer characteristics were performed as described in cited references.

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The program DELPHI, a generous gift from Dr. Barry Honig, Columbia University, New York was used to solve the Poisson-Boltzmann equation for the electrostatic potential in and around the RC. The coordinates were taken from the Brookhaven Protein Data Bank (structure 4RCR, date 09-SEP-91, resolution 2.8 Å).

Electrostatic profile of the RC

The electrostatic field of the RC was calculated using classical electrostatics as in [3,4]. Figure 1 demonstrates the electrostatic contour levels of 2.kT/e (≈50 mV) in a two-dimensional slice through the main cofactors of P, Q_A and Q_B at low and high pH values. Negative potential is represented by dashed lines and positive potential by solid lines. The surface of the protein is indicated by a heavy solid line. The electrostatic field drives the H^+-ions from the bulk phase to the interior of the protein upon light excitation.

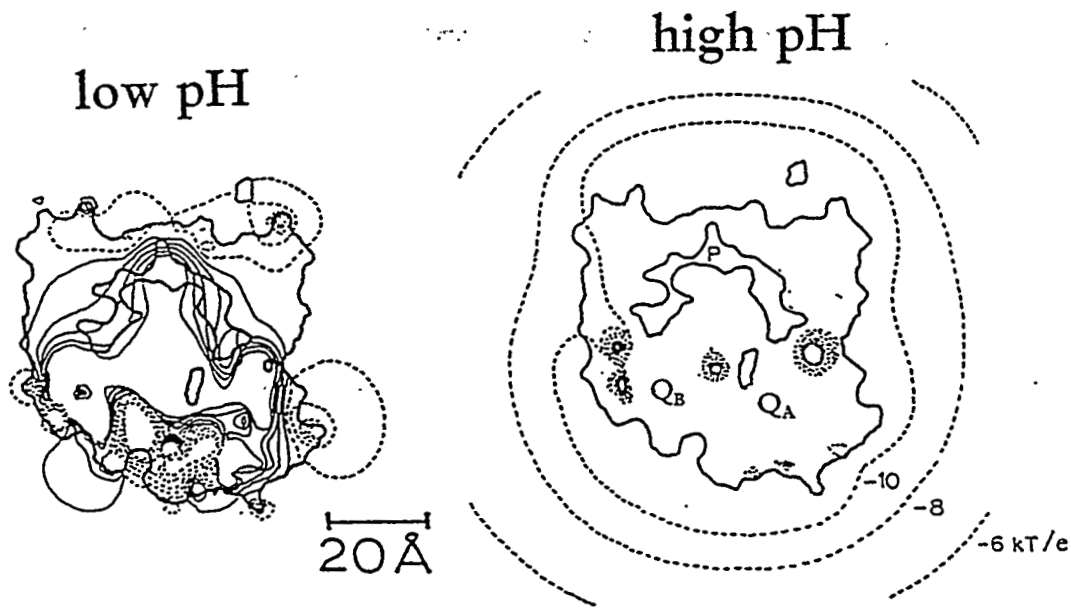

Figure 1 Electrostatic profile of RC at low (Glu and Asp are negatively and His, Lys and Arg positively charged) and high (Glu, Asp, Cys and Tyr are negatively and Arg positively charged) pH values.

The clusters of acidic residues around Q_B binding pocket produce increasing negative surface potential by alkalization of the solution and in some places, the focusing of the electric field in the Q_B pocket can be observed. These effects will facilitate the uptake H^+-ions by the RC in the alkaline pH range where the concentration of H^+-ions and thus the rate of bimolecular collision with the protein dramatically decreases.

Proton uptake due to solvation of semiquinone anions

The light-generated anion radical species, Q_A^- and Q_B^-, are effectively solvated by the protein. The anionic charges induce pK_a shifts in ionizable side-chain groups of various amino acid residues (see above for calculation). The differential compensation of the two semiquinone charges, which includes proton binding by the protein, provides much of the driving force for transfer of the first electron from Q_A^- to Q_B. Studies of site-specific mutants have identified Glu

the charged forms of the quinones and also appear to interact electrostatically with each other: the ionization of Asp L213 suppresses the ionization of Glu L212, which has an unusually high $pK_a \approx 9.6$. Electron and proton transfer in the quinone acceptor complex could be partially accounted for by a simple electrostatic model of interacting Glu L212 and Asp L213, and Q_B^- [12,13]. Protonation of the two amino acids under acidic (Asp L213) and alkaline (Glu L212) conditions determine the pH dependence of the one-electron equilibrium constant of electron transfer from Q_A^- to Q_B [2,4,14,15].

Recent studies on flash-induced proton binding stoichiometry of RCs with substituted quinones at the primary quinone binding site indicated the possible contribution of Tyr H40 in the stabilization of the primary semiquinone [16]. Glu L212 has been identified by time resolved and steady state infrared spectroscopy and significant protonation of Glu L212 has been shown at pH region well below its proposed pK_a value (e.g. ≈ 0.5 H^+/RC at pH 7) [17]. It was concluded that the pH behaviour of Glu L212 could not be explained by the titration of a single isolated residue, but the titration must be unusually broad due the strong interaction with other charged groups in the Q_B site [3,4].

These observations call for re-examination of measurements on pH dependence of electron and proton transfer in RCs of wild type and mutants and revision of the interpretation in terms of the key residues. The responsibility of Asp L213 is also unclear probably due to the location of this residue as part of the acidic charge-cluster involving Asp L210 and Arg L217, amongst others, providing the potential for strong interactions between these groups [6]. The charge cluster can be studied by mutation of protonatable residues for non-protonatable groups. The effect of substituting lysine for Glu L212 is exceptionally interesting as the expected large effects of a net positive charge were not seen and the quinone function was not greatly impaired [18]. Lys L212 may form charge pair with a residue other than Asp L213.

The results from IR measurements require a better understanding of the electrochromic shift of bacteriopheophytins that have been used for tracking interquinone electron transfer. The measured absorption change contains components both from electron transfer and from ionic (proton) relaxation around the quinones [19-21]. Separation of these events is of great importance. One possible way is the observation of dimer fluorescence. The yield of fluorescence of the dimer in the RC is highly dependent on the redox state of the RC: it is high in the closed ($PQ_A^-Q_B$) and low in the open ($PQ_AQ_B^-$) state. The yield of fluorescence is much more sensitive to the position of the electron in the quinone complex than to the ionic configuration in the surroundings, thus the kinetics of the electron transfer will control the yield of dimer fluorescence [see 22].

The study of kinetics of proton binding accompanying semiquinone formation has revealed that much of the H^+-ion uptake appeared to be directed at residues that were not immediately accessible, and the kinetics of proton binding were not simply limited by diffusion of proton donor species [23,24]. In D_2O, little or no solvent isotope effect was observed at pH/D=7, but the ratio of rate constants of proton binding (k_H/k_D) increased to about 3 at higher pH. The kinetic behaviour implied rate-limiting intraprotein proton transfer to specific buried residues in the protein matrix.

Proton delivery pathways to Q_B

After the second light-driven electron transfer Q_BH_2 is formed, and net proton uptake from the aqueous phase must occur. The proton delivery pathway to the quinone head group involves several ionizable amino acid residues in and around the Q_B pocket. Analysis of site-directed mutants indicated the activity of two terminal pathways for proton delivery to Q_B.

One of them involves Ser L223 through Asp L213, whereas the other involves Glu L212 via possibly Asp L213 [2]. Recent Monte Carlo calculations [25] and high resolution X-ray data [26] reveal water molecules located around the Q_B site; their involvement in proton delivery is also likely. Proton uptake measurements of RC films with variable water content would certainly reveal the importance of bound and mobile water molecule in proton transfer.

Not only internal water molecules but subtle structural changes can contribute to proton delivery. Voids which can also be created by mutations, can be filled by water molecules facilitating the transport of H^+-ions in the interior of the protein. Their effect (together with the possible modification of the H-bond network of internal water molecules and protonatable amino acid residues) was studied by double mutants and revertants. It was shown that the compensation mutations (second site revertants) can occur far away from Q_B [11,27] indicating the central role of long-range electrostatic interaction in function of the protein [15,28].

Limitation of proton uptake under continuous illumination

The vast majority of kinetic and energetic data have been obtained from flash kinetic measurements. From physiological point of view, it could be interesting to see how these results will predict the electron and proton transfer characteristics observed under continuous

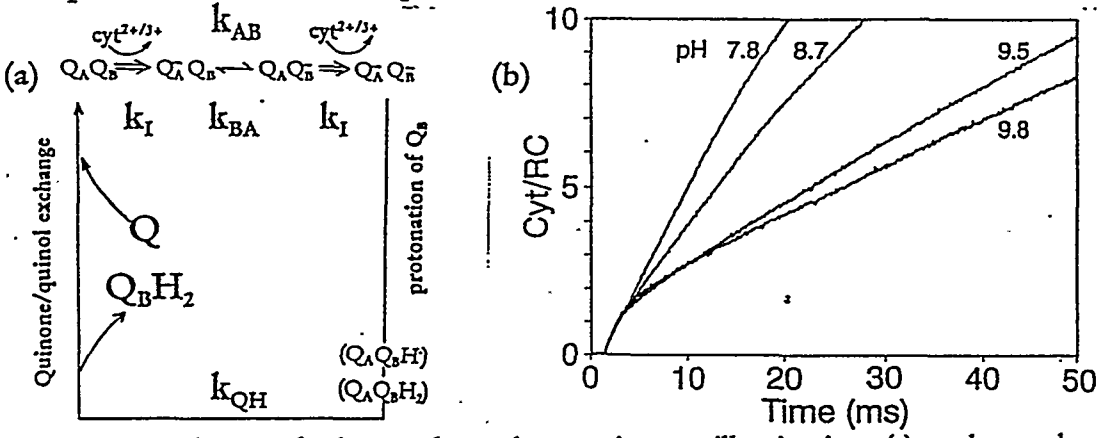

Figure 2 Scheme of photocycle under continuous illumination (a) and cytochrome photooxidation at different pH values (k_I=2.10^3 s^{-1}).

illumination.

The photocycle under stacionary conditions can be modelled by two light and dark reactions alternatively connected in series (Fig. 2a). The rates of the two light reactions (k_I) are equal, k_{AB} and k_{BA} represent the rates of forward and back reactions in semiquinone equilibration, respectively, and k_{QH} denotes the apparent rate of protonation of Q_B and quinone/quinol exchange which depends on the nature of the quinone and detergent. The photocycle can be tracked either by observation of cytochrome oxidation or proton binding. The initial rate is k_I which decreases to a stacionary value of

$$k_{obs} = \frac{k_I}{1 + \frac{k_I}{2} \cdot \left(\frac{1}{k_{QH}} + \frac{1}{k_{AB}}\right) + \frac{k_{BA}}{2 \cdot k_{AB}}} \qquad (1)$$

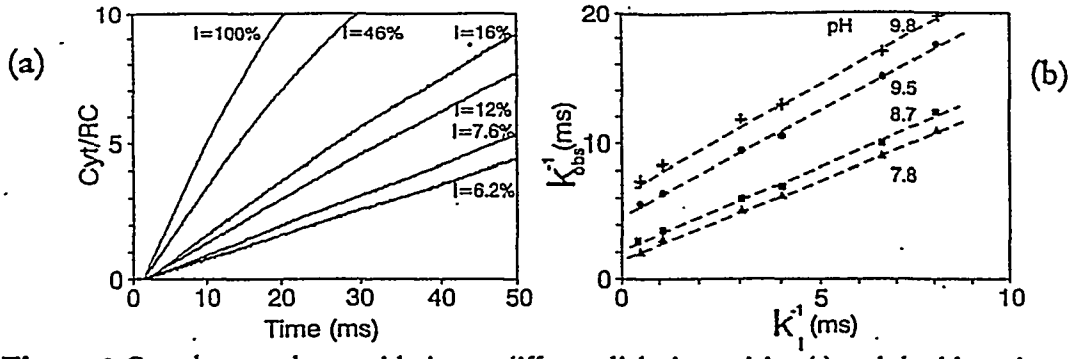

Figure 3 Cytochrome photooxidation at different light intensities (a) and double reciprocal plot to determine k_{QH} according to Eq. (1) (b).

after a transient period much longer than the slowest step in the photocycle (Fig. 2b). If k_{obs} is measured under different light intensities (Fig. 3a), k_{QH} can be derived from interception of a double reciprocal plot (k_{obs}^{-1} vs. k_I^{-1}) (Fig. 3b). According to Fig. 4, k_{QH} is limited by the rate of quinone/quinol exchange below pH 7 and by the rate of protonation in the alkaline pH range.

Inspection of the X-ray structure, the isoprenoid tail of the ubiquinone paves the way for the head group at dissociation from the RC. An aliphatic residue on the L subunit, Leu L232, is positioned close to the tail of Q_B in such a way that it may regulate the diffusion of the quinone/quinol into and out of the RC. The equivalent amino acid on the Q_A site is Trp M266, the indole ring of which interacts with the tail of the primary quinone and could prohibit the release of Q_A under physiological conditions. Measurement of quinone/quinol exchange rate of site-directed mutants at Leu L232 would bring us closer to understanding the export of reducing equivalents from RC at molecular (atomic) level.

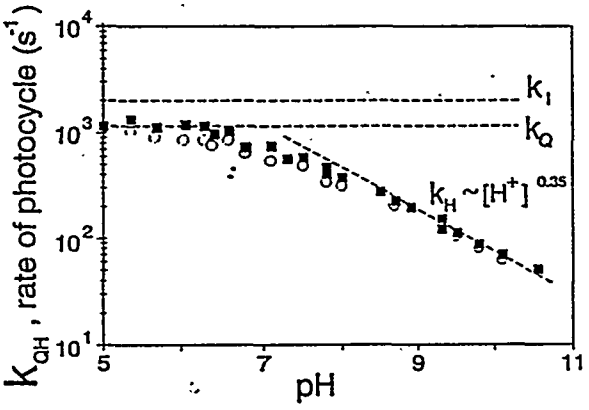

Figure 4 PH-dependence of the rate of photocycle.

CONCLUSIONS

The detailed knowledge of the atomic coordinates of the bacterial RCs has permitted a close scrutiny of structure/function relationships not only of the quinones but of the protein itself with its internal water structure. Protonatable groups were identified as intrinsic part of the redox reactions, providing charge compensation (important for semiquinone formation) and forming channels for the movement of H^+-ions to Q_B^{2-}. The nature and position of these groups give rise to electrostatic profile that determines the kinetics and energetics of proton transport. Fine tuning or dramatic variations of proton delivery pathways can adapt the photocycle to changes in bulk phase pH values, buffering capacities and primary structure (mutations) of the reaction center.

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