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Effect of temperature and surface potential on the electrogenic proton uptake in the Q_B site of the *Rhodobacter sphaeroides* photosynthetic reaction center: $Q_A^{-\cdot}Q_B^{-\cdot} \rightarrow Q_AQ_BH_2$ transition

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Abstract

Direct electrometry was used to study the light-induced voltage changes in the *Rhodobacter sphaeroides* chromatophores adsorbed to a phospholipid-impregnated nitrocellulose film. After the second laser flash, a fast increase in the voltage associated with charge separation was followed by a slower increase attributed to the proton uptake in the Q_B site of the photosynthetic reaction centers. Kinetics and relative amplitudes of these voltage changes attributed to the $Q_A^{-\cdot}Q_B^{-\cdot} \rightarrow Q_AQ_BH_2$ transition, were measured as a function of pH and temperature between +4 and +40 °C. The kinetics can be approximated by a single exponent above +23 °C (100 μs at +25 °C, pH 7.2), whereas below this temperature, it was a good fit of two exponential approximation (65 μs and 360 μs with similar contributions at +10 °C, pH 7.2). The faster component diminished with an apparent pK ~8.5, whereas the slower one was maintained at a constant level until pH ~9.5 and then decreased. The calculated activation energy from the temperature dependence of the slower component (55 – 65 kJ/mol) was much higher than that of the faster component (< 10 kJ/mol). The two voltage components can be attributed to the transfer of the first (faster component) and the second (slower component) proton from the reaction center surface to Q_B. We suggested that higher activation energy of the slower component was due to a conformational change in the reaction center kinetically coupled to the second proton transfer to Q_BH^-.

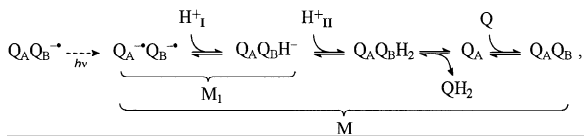
The faster component diminished in the presence of 1 M KCl, with an apparent pK ~7.5. To explain this observation, we assume that: (i) the midpoint potential of the $Q_A/Q_A^{-\cdot}$ redox pair was higher in 1 M KCl because of the reduced surface potential of chromatophores; (ii) the midpoint potential of the $Q_B^{-\cdot}/Q_BH^-$ redox pair was insensitive to the surface potential change; (iii) the equilibrium constant of the reaction $Q_A^{-\cdot}Q_B^{-\cdot} \leftrightarrow Q_AQ_BH^-$ decreased at high ionic strength.

Abbreviations: P – bacteriochlorophyll *a* dimer (P870); Q – coenzyme Q_{10} (ubiquinone-50); Q_A – primary quinone acceptor; Q_B – secondary quinone acceptor; RC – reaction center; Δ_ψ – transmembrane electric potential difference; τ – characteristic time constant

Introduction

The photosynthetic reaction center (RC) of *Rhodobacter sphaeroides* is a membrane pigment–protein complex which catalyzes the conversion of energy of light into chemical energy by stabilizing the products of photochemical separation of electric charges.

Following the flash excitation of RC, an electron is transferred from a bacteriochlorophyll *a* dimer (P, primary donor) to the primary quinone acceptor (Q_A) and then to the secondary quinone acceptor (Q_B) which acts as a two-electron gate. After the first flash, a tightly bound and kinetically stable semiquinone $Q_B^{-\cdot}$ is formed which remains unprotonated at least at pH > 5. The formation of $Q_B^{-\cdot}$ leads to a remarkable proton uptake by certain residues in the vicinity of Q_B within a broad pH range ($\sim$0.5 H^+/RC at pH 7.0) (for reviews, see Shinkarev and Wraight 1993; Okamura and Feher 1995). This proton uptake was shown to be mostly due to protonation of surface groups, and it does not contribute significantly to the observed generation of transmembrane electric potential difference (Δ_ψ) in RC (Drachev et al. 1990; Bibikov et al. 1994; Brzezinski et al. 1997; Gopta et al. 1997). The second flash-induced turnover of the RC leads to transfer of the second electron to $Q_B^{-\cdot}$ (characteristic time constant (τ), $\sim$100 μs in *Rb. sphaeroides* chromatophores at pH 7.0, see Verméglio 1982). The electron transfer is accompanied by the net binding of two protons, and the formation of an ubiquinol (Q_BH_2). The latter is easily exchangeable with the ubiquinone molecules of the membrane quinone pool. The sequential binding of the two protons was proposed (Takahashi and Wraight 1992; McPherson et al. 1993). The following scheme (Equation (1)) illustrates the reactions in the Q_B site induced by the second flash:



(1)

where M_1 is the equilibrium constant of the coupled second electron and first proton transfer reaction, and M is the overall equilibrium constant of Q_BH_2 formation and exchange with Q molecule. Unlike the first electron, the transfer of the second one and accompanying proton transfer reactions (Equation (1)) cause a substantial Δ_ψ generation in RC. The kinetics of these reactions were studied in chromatophores by the electrometric technique (Drachev et al. 1981; Feher and Okamura 1984; Kaminskaya et al. 1986; Drachev et al. 1990; Shinkarev et al. 1993a) and by carotenoid electrochromic bandshift measurements (Drachev et al. 1988). Although these kinetics were shown to be mono-exponential at room temperature, there are some indications on the intrinsic heterogeneity of the proton uptake coupled to the second electron transfer (McPherson et al. 1993; Nauclér and Brzezinski 1993). In this work, we analyzed the kinetics of the voltage changes accompanied the $Q_A^{-\cdot}Q_B^{-\cdot} \rightarrow Q_AQ_BH_2$ reaction in the broad temperature and pH ranges.

Materials and methods

Cells of *Rb. sphaeroides* R2 (purple wild-type strain) were grown photoheterotrophycally at +30 °C, and chromatophores were isolated by French Pressure Cell disruption procedure as in (Drachev et al. 1989; Starkov et al. 1997) and stored frozen in 250 mM sucrose at -20 °C until used. The kinetics of the laser flash-induced $\Delta\psi$ generation in chromatophores were measured by direct electrometry (Drachev et al. 1981, 1990). A 0.2 μm thick nitrocellulose film separating two Teflon compartments was impregnated with a solution of soybean phosphatidylcholine (150 mg/ml, type II-S, Sigma) and coenzyme Q_{10} (20 mg/ml, Sigma) in *n*-decane. Chromatophores ($\sim$80 μm bacteriochlorophyll *a*) were incubated in one of the compartments with 25 mM $CaCl_2$ for 1.5 at 23 °C, then the suspension was replaced by a buffer solution free of $CaCl_2$ and chromatophores. The voltage changes were monitored using Ag|AgCl electrodes, amplified by a high input impedance ($\sim 10^{11}$ Ω) amplifier (3554BM, Burr-Brown, USA), and digitized by a DL1080 oscilloscope (Datalab, UK). Photoexcitation (>98% saturation) of the immobilized chromatophores was provided by a frequency-doubled Q-switched YG-481 Nd:YAG laser (532 nm, FWHM 15 ns, 32 mJ/cm^2 per flash) (Quantel, France). The time resolution of the setup was $\sim$100 ns. The voltage kinetic traces were analyzed without averaging as multi-exponential decays using the DISCRETE algorithm (Provencher 1976) and Microcal Origin 4.1 software package (Microcal Software, Inc., USA). To reveal the voltage kinetic components associated with the charge transfer reactions in the Q_B site, an electron transfer inhibitor terbutryn (5 μM) was added. Then the voltage signal obtained in the presence of terbutryn, was point-by-point subtracted from those following either the first or second successive flashes before addition of the inhibitor (Drachev et al. 1990; Bibikov et al. 1994).

Results

A short pulse of light caused a generation of Δ_ψ across the chromatophore membrane of *Rb. sphaeroides* (Fig-

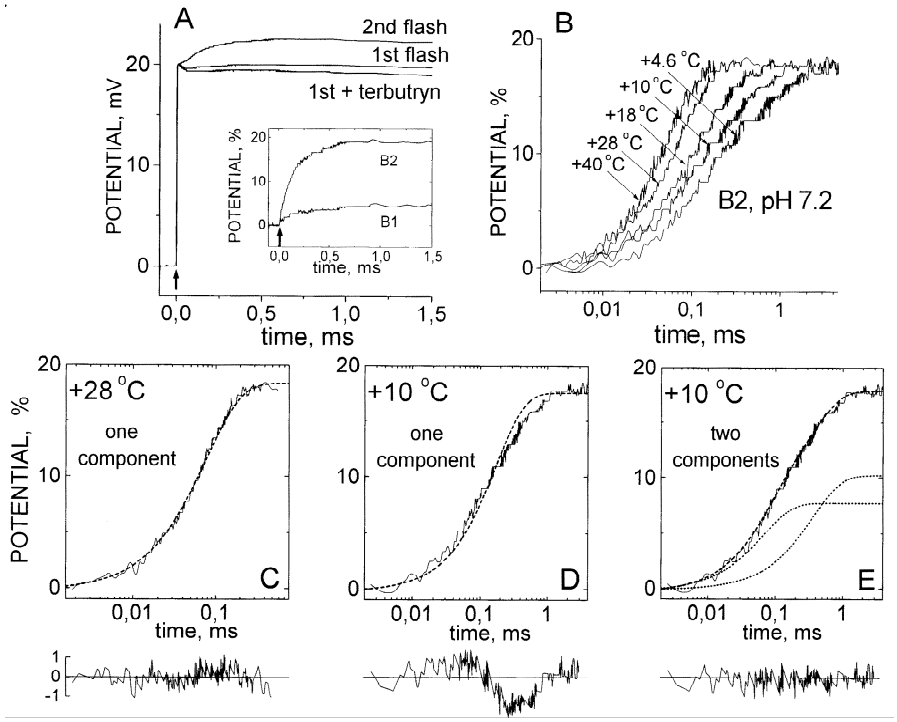


Figure 1. Voltage changes of *Rb. sphaeroides* chromatophores following successive light pulses. (A) Voltage changes following the first and second laser flashes in the absence of inhibitor, and following the first flash in the presence of terbutryn (pH 7.2, +25 °C). (Insert) Kinetic components of the voltage changes associated with charge transfer in the Q_B site induced by the first (B1), and second (B2) flashes. Arrows indicate the laser flashes. (B) The B2 component measured at different temperatures. (C) The B2 component kinetics (+28 °C), the result of the best one-exponential fit (τ = 77 μs, dashed line), and the residuals of the fit (depicted at the bottom of the plot). (D) The B2 component kinetics (+10 °C), the result of the best one-exponential fit (τ = 175 μs, dashed line), and the residuals of the fit. (E) The same kinetic trace as in (D) the result of the best two-exponential fit, the individual exponential components (τ = 65 μs, 43% and τ = 360 μs, 57%, dashed lines), and the residuals of the fit. Note the voltage scale in (A) (mV) is different from that used in other figures (%% of the fast charge-separation amplitude taken as 100%). Conditions: 20 mM MOPS, 50 mM KCl, 2 mM potassium ferrocyanide, 40 μM potassium ferricyanide (E_h +320 mV), 50 μM dimethylferrocene, 4 μM methylene blue, 4 μM myxothiazol, 2 μM antimycin A. Chromatophores were incubated in the dark for 10 – 30 min before series of flashes, to achieve complete re-oxidation of the quinone acceptors (Mulkidjanyan et al. 1986). The dark interval between the first and the second flashes in series, 1 s.

ure 1A). The fast kinetically unresolved component of Δ_ψ generation (τ < 100 ns in our setup) has been assigned previously to the charge separation between the photo-excited P and Q_A (Drachev et al. 1981). Following the first flash, an additional minor voltage component was observed (B1, 3 – 4% of the charge-separation component at neutral pH, see insert to Figure 1A). This component has been attributed to a combination of different processes such as: (a) electron transfer between Q_A and Q_B; (b) proton transfer between the chargeable amino acid residues near Q_B; (c) fast structural changes accompanying Q_B reduction (for details, see Drachev et al. 1990; Bibikov et al. 1994; Brzezinski et al. 1997; Gopta et al. 1997). Following the second flash, an ubiquinol (Q_BH_2) was formed (Equation (1)). The transfer of two protons to Q_B resulted in a substantial voltage component (B2, 18 – 20% of the charge-separation component, see insert to Figure 1A). The possible contribution of the electron transfer between Q_A and Q_B to the B2 component, is thought to be small and could not be considered (Drachev et al. 1990). To discriminate between B1 and B2 components and other possible charge transfer reactions, a triazine herbicide (terbutryn) was used (see 'Materials and methods'). The results obtained with other inhibitors (e.g. atrazin or stigmatellin) were similar to those in the presence of terbutryn (data not shown).

Figure 1B illustrates the voltage kinetics of B2 recorded at different temperatures. The kinetics slowed down and stretched with the temperature decrease. Above +20 °C, the B2 kinetics fits a single exponential component (Figure 1C) further designated as $B2_{single}$. At lower temperature, the kinetics differs

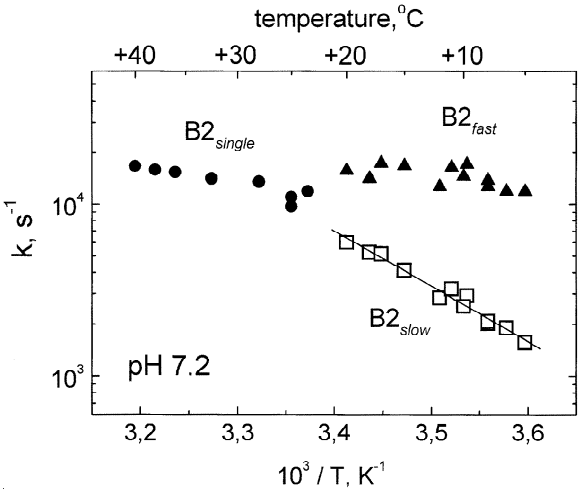


Figure 2. Temperature dependence of the $B2_{single}$ (●), $B2_{fast}$ (▲), and $B2_{slow}$ (□) component rates. The solid line is a least-square fit through the $B2_{slow}$. Conditions: as for Figure 1.

from mono-exponential and can be adequately approximated by a sum of two exponential curves (further designated as $B2_{fast}$ and $B2_{slow}$) (compare Figure 1D and 1E).

The temperature dependence of the B2 kinetics was measured between +4 and +40 °C. Figure 2 shows the Arrhenius plots of the rate constants of $B2_{single}$ (circles) above +23 °C and $B2_{fast}$ (triangles) and $B2_{slow}$ (squares) components below +23 °C (pH 7.2). The slope of the fitted line of $B2_{slow}$ yields activation energy of ∼60 kJ/mol. Similar values (55 – 65 kJ/mol) were obtained at pH between 6.0 and 8.1 (data not shown). The rates of $B2_{single}$ and $B2_{fast}$ components did not significantly depend on temperature; both values can be roughly estimated as ≤10 kJ/mol. Note that within the interval from +20 to +25 °C, the two-exponential kinetics turned to the mono-exponential and in this range, the observed rate constants may deviate from true values.

At +25 °C, the pH-dependencies of the $B2_{single}$ kinetics were in accordance with those published previously (Drachev et al. 1990). The relative amplitude did not depend on pH below pH ∼9.0 and declined at pH above 9.0 (Figure 3A). The rate constant was weakly pH-dependent below pH 7.0 – 7.5 followed by a steeper dependence at higher pH with a slope –0.7 per pH unit (Figure 3B). The dependence of the $B2_{single}$ rate generally resembles that of the second electron transfer in chromatophores (Verméglio 1982). Similar pH dependencies of the second electron transfer and proton uptake rates were reported for solubilized RCs (Shinkarev and Wraight 1993; Okamura and Feher 1995, and references therein) and RC-containing proteoliposomes (Semenov et al. 1990).

At +10 °C, the total amplitude of B2 gradually increased between pH 4.6 and 7.5 – 8.0, and then decreased (Figure 3C, dashed line). The amplitudes of $B2_{fast}$ and $B2_{slow}$ were similar at pH ∼8.0. The amplitude of $B2_{fast}$ increased between pH 4.6 and ∼7.5, and then declined with an apparent pK ∼8.5 (Figure 3C, up triangles). The amplitude of the slower component was pH-independent below pH 9.5 and then decreased (Figure 3C, squares). Thus, only one (slower) component was distinguishable at pH > 9.3. The pH-dependencies of the rate constants of both $B2_{fast}$ and $B2_{slow}$ at +10 °C (Figure 3D, up triangles and squares, respectively) resembled that of $B2_{single}$ at +25 °C (Figure 3D, dotted line); they were characterized by a weak pH-dependence at neutral pH values and a steeper one at pH above 8.0. However, the dependence of $B2_{slow}$ was steeper also at pH < 5.8.

We studied the effect of high ionic strength (1 M KCl added) on the B2 kinetics at +10 °C. Under these conditions, the $B2_{fast}$ component disappeared with an apparent pK 7.5 (Figure 3E, down triangles). The pH-dependencies of the rates of both $B2_{fast}$ and $B2_{slow}$ components (Figure 3F, down triangles and circles, respectively) were stronger than those at 50 mM KCl (Figure 3F, dotted lines).

Discussion

In this work, we observed voltage changes attributed to the charge transfer reactions in the Q_B site in the *Rb. sphaeroides* chromatophores accompanying $Q_B^{-\cdot}$ reduction after the second flash. The observed kinetics were apparently mono-exponential above +23 °C, whereas they differ from a mono-exponential curve at lower temperatures (compare Figure 1C–E). At low temperatures, the non-exponential kinetics turned to the mono-exponential one with an apparent pK ∼8.5; at alkaline pH the kinetics was mono-exponential in the whole temperature range (compare Figure 3B and D).

Generally, the observed deviation of the kinetics from the mono-exponential might be explained by a structural heterogeneity of RC before the second flash. The non-exponential kinetics of the first electron transfer in RC were extensively discussed (see e.g. Tiede et al. 1996; Gopta et al. 1997, and references therein)

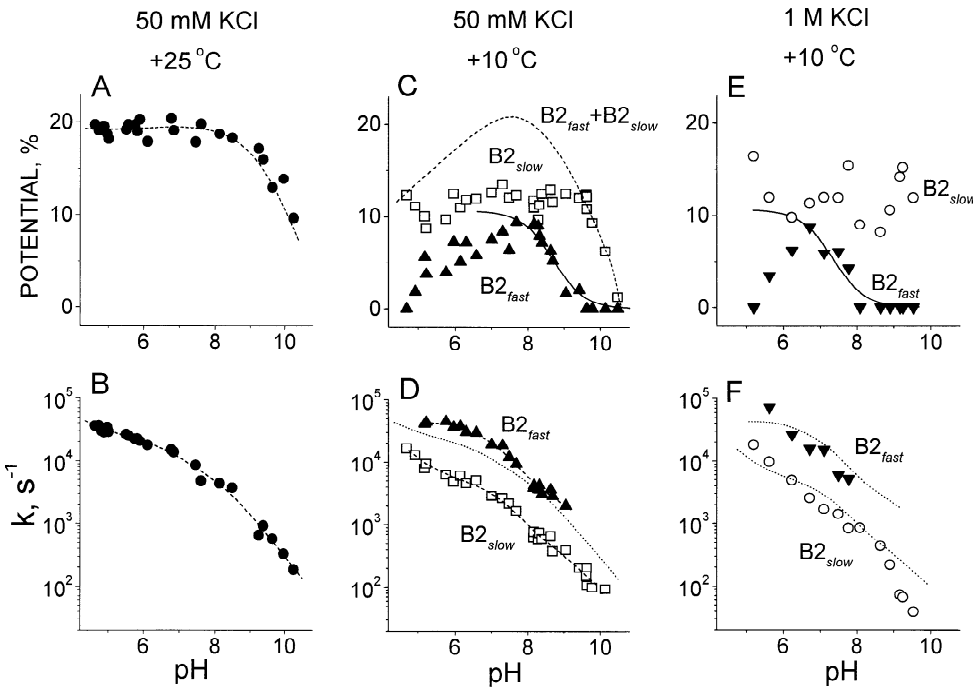


Figure 3. The pH-dependencies of the B2 component amplitudes and rate constants. (A) The amplitude and (B) the rate constant of $B2_{single}$ at +25 °C (●), splines through the data (dashed lines). (C) The amplitudes of $B2_{fast}$ (▲), $B2_{slow}$ (□), and the total B2 (a spline through the data, dashed line) at +10 °C, a titration curve with pK^{app} of 8.5 (solid line). (D) The rate constants of $B2_{fast}$ (▲) and $B2_{slow}$ (□), splines through the data (dashed lines) at +10 °C. Dotted line is the spline of $B2_{single}$ rate constant at +25 °C redrawn from (B) (E) The amplitudes of $B2_{fast}$ (▼) and $B2_{slow}$ (○) at +10 °C, a titration curve with pK^{app} of 7.5 (solid line). (F) The rate constants of $B2_{fast}$ (▼) and $B2_{slow}$ (○) at +10 °C. Dotted lines are the splines of $B2_{fast}$ and $B2_{slow}$ rate constants redrawn from (D). 50 mM KCl was present in (A, B, C, D), 1 M KCl was added in (E, F). Other conditions: as for Figure 1, except: (i) the incubation medium contained 20 mM of one of the following buffers (acetic acid, MES, MOPS, HEPES, Tris-HCl, CHES, CAPS) depending on pH region; (ii) the concentration of methylene blue which was necessary for the complete dark adaptation between series of the flashes (see captions to Figure 1), ranged from 0.1 μM at alkaline pH up to 40 μM at acidic pH. At pH > 9, the observed kinetics of B2 in the presence of 1 M KCl were interfered with another slow pH-independent component of the voltage changes (τ ~30 ms). The contribution of the latter component depended on the concentration of terbutryn; therefore, the real rate of $B2_{slow}$ at 1 M KCl at alkaline pH should be slower than that observed at low salt concentration.

and explained by a distribution over different conformational states of RC and time-dependent structural changes. Unlike Q_B or Q_BH_2 species, $Q_B^{-\cdot}$ is thought to be firmly attached to the site by hydrogen bonding and electrostatic attraction to the non-heme Fe^{2+} atom. The refined atomic displacement factor of $Q_B^{-\cdot}$, as calculated from the high-resolved X-ray structure of *Rb. sphaeroides* RCs frozen to 90 K under illumination, was similar to that of Q_A (Stowell et al., 1997). This fact indicates the relative structural uniformity of RC before the second flash as against the first one.

Alternatively, the observed non-exponential kinetics might be a result of sequential binding of the two protons (Equation (1)) as it was discussed by McPherson et al. (1993). According to the contemporary views, the transfer of the first proton (H^+_I) unlike the second proton (H^+_{II}) to Q_B, is kinetically coupled to the second electron transfer (see e.g. Shinkarev and Wraight 1993; Okamura and Feher 1995). In the Glu-L212⇒Gln mutant, the transfer of H^+_{II} was blocked; therefore, Glu-L212 was considered as a specific donor of H^+_{II} to Q_B (Paddock et al. 1989; Takahashi and Wraight 1992; Shinkarev et al. 1993b; McPherson et al. 1994). The equilibrium constant M_1 of the $Q_A^{-\cdot}Q_B^{-\cdot} + H^+ \leftrightarrow Q_AQ_BH^-$ reaction, decreased with increasing pH and was equal to 1 at pH ~8.5 (Takahashi and Wraight 1992; McPherson et al. 1994). Therefore, at pH > 8.5, the reduction of $Q_B^{-\cdot}$ was thermodynamically driven by the H^+_{II} transfer ($Q_AQ_BH^- + H^+ \leftrightarrow Q_AQ_BH_2$) and/or by the QH_2 exchange with an ubiquinone molecule. The observed amplitude of the $B2_{fast}$ component decreased with an apparent pK ~8.5 (Figure 3C). Therefore, we proposed that at pH < 8.5, this component re-

flected fast transfer of H^+_I, whereas slow component corresponded to the transfer of H^+_{II}. At pH > 8.5, both protons were transferred simultaneously, and two kinetic components were indistinguishable.

A fraction where the observed binding of both protons was determined by one of the subsequent reactions, increased with the same pK ~8.5. However, the decrease of $B2_{fast}$ at pH > 8.5 was not accompanied by a concomitant increase of the amplitude of $B2_{slow}$. This fact can be explained as a consequence of two reasons: (i) the equilibrium constant M of the overall reaction (Equation (1)) decreases at alkaline pH (Shinkarev and Wraight 1993) that leads to the decrease of the total B2 amplitude above pH 9.0 (Figure 3A, C, dashed lines); (ii) at pH > 9.0, Glu-L212 residue is protonated after the first flash, but remains unprotonated before the first, and after the second flash. The protonation of Glu-L212 following the first flash was shown to be electrogenic at high pH (Drachev et al. 1990; Brzezinski et al. 1997); consequently, the total B2 amplitude should decrease.

At pH below 6.5, the total amplitude of B2 at +10 °C was less than that observed at +25 °C (compare Figure 3A and C) while they were similar at other pH values. This effect can be due to a low Q_B occupancy and slow Q binding to the unoccupied site between the first and second flashes at low pH and low temperature (Shinkarev et al. 1985; Gopta et al., unpublished observations).

The effect of ionic strength on the electrogenic proton uptake in *Rb. sphaeroides* chromatophores was previously investigated at room temperature (Shinkarev et al. 1993a). At high salt concentration, the reduction of the electrostatic surface potential of the chromatophore membrane results in a change of the 'local' concentration of protons including that near Q_A or Q_B sites (see Maróti and Wraight 1997 and references therein). The observed B2 kinetics was pH-dependent and, therefore, must be sensitive to the surface potential change. The surface potential near Q_B site is also pH-dependent: its value matches zero at pH ~5.7 (the iso-electric point, pI) and has a negative sign at higher pH (Shinkarev et al. 1993a); consequently, the effect of high ionic strength on the B2 kinetics would be minimal at pH ~5.7 and maximal at high pH. The equilibrium constant M_1 (Equation (1)) should be sensitive to a change of the local electrostatic potential at the site Q_A but not at the site Q_B, because the total charge at Q_B is conserved during the reaction $Q_A^{-\cdot}Q_B^{-\cdot} + H^+ \rightarrow Q_AQ_BH^-$. The pH-dependence of the observed $B2_{fast}$ amplitude was attributed to the reaction $Q_A^{-\cdot}Q_B^{-\cdot} + H^+ \rightarrow Q_AQ_BH^-$ shifted to a more acidic pH in the presence of 1 M KCl (pK^{app} ~7.5) *versus* 50 mM KCl (pK^{app} ~8.5) (compare Figure 3C, up triangles, and 3E, down triangles). This pK shift can be a consequence of the M_1 value decrease at high ionic strength at pH > pI. As the 'local' concentration of protons depends on the surface potential, the transfer of H^+_{II} is expected to be slower at pH > pI under high ionic strength conditions, also in line with the experimental observations (Figure 3F).

The attribution of the $B2_{fast}$ and $B2_{slow}$ components to the H^+_I and H^+_{II} transfer steps, can also be supported by the following arguments: (i) the oxygen atoms of the 'proximal' (O4) and 'distal' (O1) oxo groups of Q_B are located inside the protein dielectric at the same distance from the plane of chromatophore membrane (see e.g. Allen et al. 1987; Chang et al. 1991). Consequently, the individual transfer step of each of the two protons should contribute similarly to the observed total voltage changes after the second flash; (ii) in the Glu-L212⇒Gln mutant, the relative amplitude of the B2 component was approx. two times smaller than in the wild type *Rb. sphaeroides* chromatophores (Shinkarev et al. 1993b); (iii) as it has been shown by McPherson et al. (1993), the second electron transfer rate was less temperature-dependent between +4 °C and +21.5 °C than the proton uptake rate in the isolated RC of *Rb. sphaeroides.* This effect was attributed to the second proton uptake rate being more temperature dependent than that of the first one, although the separate kinetics of H^+_I and H^+_{II} uptake have not been resolved; (iv) the kinetics of the electrogenic protonation of Q_B in the wild type *Rb. sphaeroides* RCs split into two components when measured in D_2O at 6.5 < pD < 8.0; at pD > 8.0 a single kinetic component was observed (Nauclér and Brzezinski 1993).

Higher activation energy of the apparent rate of H^+_{II} transfer does not necessarily imply that the two protons are transferred along different functional pathways. RC was shown to contain voids filled with bound water molecules which protrude from the protein surface almost to Q_B both in *Rb. sphaeroides* (Ermler et al. 1994; Stowell et al. 1997) and *Rhodopseudomonas viridis* (Deisenhofer et al. 1995) that may effectively conduct protons to Q_B. As it was shown by Graige et al. (1996), the H^+_I transfer is not rate-limiting in the reaction $Q_A^{-\cdot}Q_B^{-\cdot} \rightarrow Q_AQ_BH^-$ in *Rb. sphaeroides* RCs. The apparent B2 kinetics did not depend significantly on the concentration of a buffer in a broad concentration range (Gopta

et al., unpublished observations) indicating the proton exchange at the RC surface is not rate-limiting in chromatophores. We further propose that the microscopic H^+_{II} transfer rate is fast and this transfer step is also not rate-limiting. High activation energy of the H^+_{II} transfer could be then attributed to structural changes in the RC accompanying the proton binding.

According to the refined structural data of *Rb. sphaeroides* RC containing $Q_B^{-\cdot}$ when frozen to 90 K under illumination, the semiquinone is in the direct contact with the water chain(s) and the reorientation is not necessary for a proton conduction to the distal O1 atom (Stowell et al. 1997). A network of H-bonded water molecules can in principle provide the fast proton exchange in the protein with low activation energy comparable to that of the proton diffusion in liquid water (8 kJ/mol) (see Prue 1973 and references therein). Stowell et al. (1997) also showed that in the RC crystals containing neutral Q_B when frozen in the dark, the quinone was distributed between the two positions, the 'proximal' one which is close to that of $Q_B^{-\cdot}$ in the 'light-frozen' RC, and the 'distal' one, where quinone is bound ~5 Å apart. These data indicate that in the latter case the reaction $Q_A^{-\cdot}Q_B \rightarrow Q_AQ_B^{-\cdot}$ is accompanied by the Q_B movement and should have a significant activation energy. This result is in agreement with our conclusion that the neutral quinone may be bound in two conformations which are characterized by different activation energies of the first electron transfer (< 10 kJ/mol and 60 – 70 kJ/mol) (Gopta et al. 1997). The activation energy of the first electron transfer and accompanying charge-transfer reactions was found 45 – 50 kJ/mol in solubilized RCs (Kleinfeld et al. 1984; Mancino et al. 1984; Tiede et al. 1996). In the X-ray structure of *Rb. sphaeroides* RC reported by Ermler et al. (1994), Q_B is shifted by ~5 Å relative to its position in other structures (Allen et al. 1987; Chang et al. 1991). These data were explained by *ubiquinol* rather than *ubiquinone* bound to the site (Ermler et al. 1994). This 'quinol' position seems to be close to the 'distal' position in the 'dark-frozen' structure (Stowell et al. 1997). We therefore propose that H^+_{II} binding to O4 atom of Q_BH^- is coupled to the displacement of Q_BH^- or Q_BH_2 from the 'semiquinone' to the 'quinol' binding position. This movement requires breaking of several hydrogen bonds and major conformational changes of the polyisoprenoid side chain (Stowell et al. 1997). Possible temperature-dependent structural changes in the chromatophore lipid membrane may also affect the observed activation energy. These conformational transitions seem to represent a reversion of events accompanying the $Q_A^{-\cdot}Q_B \rightarrow Q_AQ_B^{-\cdot}$ transition in the fraction of RCs with 'distal' Q_B. Thus one can expect similar high activation energies for the conformational changes accompanying both formation of $Q_B^{-\cdot}$ and the protonation of Q_BH^-.

Acknowledgments

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References

Allen JP, Yeats TO, Komiya H and Rees DC (1987) Structure of the reaction center from *Rhodobacter sphaeroides* R-26: The protein subunits. Proc Natl Acad Sci USA 84: 6162–6166

Bibikov SI, Bloch DA, Cherepanov DA, Oesterhelt D and Semenov AY (1994) Flash-induced electrogenic reactions in the SA(L223) reaction center mutant in *Rhodobacter sphaeroides* chromatophores. FEBS Lett 341: 10–14

Brzezinski P, Paddock ML, Okamura MY and Feher G (1997) Light-induced electrogenic events associated with proton uptake upon forming Q_B^- in bacterial wild-type and mutant reaction centers. Biochim Biophys Acta 1321: 149–156

Chang C-H, el-Kabbani O, Tiede D, Norris J and Schiffer M (1991) Structure of the membrane-bound protein photosynthetic reaction center from *Rhodobacter sphaeroides*. Biochemistry 30: 5352–5360

Deisenhofer J, Epp O, Sinning I and Michel H (1995) Crystallografic refinement at 2.3 Å resolution and refined model of the photosynthetic reaction center from *Rhodopseudomonas viridis*. J Mol Biol 246: 429–457

Drachev LA, Semenov AY, Skulachev VP, Smirnova IA, Chamorovsky SK, Kononenko AA, Rubin AB and Uspenskaya NY (1981) Fast stages of photoelectric processes in biological membranes. III Bacterial photosynthetic redox system. Eur J Biochem 117: 483–489

Drachev LA, Mamedov MD, Mulkidjanian AY, Semenov AY, Shinkarev VP and Verkhovsky MI (1988) Phase II of carotenoid bandshift is mainly due to the electrogenic protonation of the secondary quinone acceptor. FEBS Lett 233: 315–318

Drachev LA, Mamedov MD, Mulkidjanian AY, Semenov AY, Shinkarev VP, Verkhovsky MI, Kaurov BS and Skulachev VP (1989) Flash-induced electrogenic events in the photosynthetic reaction center and cytochrome bc_1-complex of *Rhodobacter sphaeroides* chromatophores. Biochim Biophys Acta 973: 189–197

Drachev LA, Mamedov MD, Mulkidjanian AY, Semenov AY, Shinkarev VP and Verkhovsky MI (1990) Electrogenesis associated with proton transfer in the reaction center protein of the purple bacterium *Rhodobacter sphaeroides*. FEBS Lett 259: 324–326

Ermler U, Fritzsch G, Buchanan SK and Michel H (1994) Structure of the photosynthetic reaction center from *Rhodobacter sphaeroides* at 2.65 Å resolution: Cofactors and protein-cofactor interactions. Structure 2: 925–936

Feher G and Okamura MY (1984) Structure and function of the reaction centers from *Rhodopseudomonas sphaeroides*. In: Sybesma C (ed) Advances in Photosynthesis Research, Vol II, pp 155–164. M. Nijhoff/W. Junk Publ., Dordrecht, the Netherlands

Gopta OA, Bloch DA, Cherepanov DA and Mulkidjanian AY (1997) Temperature dependence of the electrogenic reaction in the Q_B site of the *Rhodobacter sphaeroides* photosynthetic reaction center: the $Q_A^- Q_B \rightarrow Q_A Q_B^-$ transition. FEBS Lett 412: 490–494

Graige MS, Paddock ML, Bruce JM, Feher G and Okamura MY (1996) Mechanism of proton-coupled electron transfer for quinone (Q_B) reduction in reaction centers of *Rb. sphaeroides*. J Am Chem Soc 118: 9005–9016

Kaminskaya OP, Drachev AL, Konstantinov AA, Semenov AY and Skulachev VP (1986) Electrogenic reduction of the secondary quinone acceptor in chromatophores of *Rhodospirillum rubrum*. Rapid kinetic measurements. FEBS Lett 202: 224–228

Kleinfeld D, Okamura MY and Feher G (1984) Electron transfer in reaction centers of *Rhodopseudomonas sphaeroides*. I. Determination of the charge recombination pathway of $D^+Q_AQ_B^-$ and free energy and kinetic relations between $Q_A^-Q_B$ and $Q_AQ_B^-$. Biochim Biophys Acta 766: 126–140

Mancino LJ, Dean DP and Blankenship RE (1984) Kinetics and thermodynamics of the $P870^+Q_A^- \rightarrow P870^+Q_B^-$ reaction in isolated reaction centers in the photosynthetic bacterium Rhodopseudomonas sphaeroides. Biochim Biophys Acta 764: 46–54

Maróti P and Wraight C (1997) Kinetics of H^+ ion binding by the $P^+Q_A^-$ state of bacterial photosynthetic reaction centers: Rate limitation within the protein. Biophys J 73: 367–381

McPherson PH, Okamura MY and Feher G (1993) Light induced proton uptake by photosynthetic reaction centers from *Rhodobacter sphaeroides* R-26. II. Protonation of the state $DQ_AQ_B^-$. Biochim Biophys Acta 1144: 309–324

McPherson PH, Schoenfeld M, Paddock ML, Okamura MY and Feher G (1994) Protonation and free energy changes associated with formation of Q_BH_2 in native and Glu-L212→Gln mutant reaction centers from *Rhodobacter sphaeroides*. Biochemistry 33: 1181–1193

Mulkidjanyan AY, Shinkarev VP, Verkhovsky MI and Kaurov BS (1986) A study of the kinetic properties of the stable semiquinone of the reaction-center secondary acceptor in chromatophores of non-sulfur purple bacteria. Biochim Biophys Acta 849: 150–161

Nauclér A and Brzezinski P (1993) Deuterium isotope effect on the rate of proton uptake by Q_B^{2-} in bacterial reaction centers. In: Abstracts of 11th International Biophysics Congress, p 183a. Budapest, Hungary

Okamura MY and Feher G (1995) Proton-coupled electron transfer reactions of Q_B in reaction centers from photosynthetic bacteria. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria, pp 577–594. Kluwer Academic Publishers, Dordrecht, the Netherlands

Paddock ML, Rongey SH, Feher G and Okamura MY (1989) Pathway of proton transfer in bacterial reaction centers: Replacement of Glu 212 in the L subunit inhibits quinone (Q_B) turnover. Proc Natl Acad Sci USA 86: 6602–6606

Provencher SW (1976) A Fourier method for the analysis of exponential decay curves. Biophys J 16: 27–41

Prue JE (1973) Proton, protonic acids and hydrogen bond. In: Bailar JC et al. (eds) Comprehensive Inorganic Chemistry, Vol I, pp 117–138. Pergamon Press, Oxford

Semenov AY, Mamedov MD, Shinkarev VP, Verkhovsky MI and Zakharova NI (1990) The electrogenic event associated with the reduction of the secondary quinone acceptor in *Rhodobacter sphaeroides* reaction centers. In: Drews G and Dawes EA (eds) Molecular Biology of Membrane-Bound Complexes in Phototrophic Bacteria, pp 329–335. Plenum Press, New York

Shinkarev VP, Drachev LA, Mamedov MD, Mulkidjanian AJ, Semenov AY and Verkhovsky MI (1993a) Effect of pH and surface potential on the rate of electric potential generation due to proton uptake by secondary quinone acceptor of reaction centers in *Rhodobacter sphaeroides* chromatophores. Biochim Biophys Acta 1144: 285–294

Shinkarev VP, Takahashi E and Wraight CA (1993b) Flash-induced electric potential generation in wild type and L212EQ mutant chromatophores of *Rhodobacter sphaeroides*: Q_BH_2 is not released from L212EQ mutant reaction centers. Biochim Biophys Acta 1142: 214–216

Shinkarev VP, Kononenko AA and Rubin AB (1985) The molecular mechanism of the electron transfer between the quinone co-factors in the purple bacterial photosynthetic reaction centers. Biologicheskiye Membrany 1: 640–650

Shinkarev VP and Wraight CA (1993) Electron and proton transfer in the acceptor quinone complex of reaction centers of phototrophic bacteria. In: Deisenhofer J and Norris JR (eds) The Photosynthetic Reaction Center, Vol I, pp 193–255. Academic Press, New York

Starkov AA, Bloch DA, Chernyak BV, Dedukhova VI, Mansurova SE, Severina II, Simonyan RA, Vygodina TV and Skulachev VP (1997) 6-Ketocholestanol is a recoupler for mitochondria, chromatophores and cytochrome oxidase proteoliposomes. Biochim Biophys Acta 1318: 159–172

Stowell MHB, McPhillips TM, Rees DC, Soltis SM, Abresch E and Feher G (1997) Light-induced structural changes in photosynthetcis reaction center: implication for mechanism of electron-proton transfer. Science 276: 812–816

Takahashi E and Wraight CA (1992) Proton and electron transfer in the acceptor quinone complex of *Rhodobacter sphaeroides* reaction centers: characterization of site-directed mutants of the two ionizable residues, Glu^{L212} and Asp^{L213}, in the Q_B binding site. Biochemistry 31: 855–866

Tiede DM, Vázquez J, Córdova J and Marone PA (1996) Time-resolved electrochromism associated with the formation of quinone anions in the *Rhodobacter sphaeroides* R26 reaction center. Biochemistry 35: 10763–10775

Verméglio A (1982) Electron transfer between primary and secondary electron acceptors in chromatophores and reaction centers of photosynthetic bacteria. In: Trumpower BL (ed) Function of Quinones in Energy Conserving Systems, pp 169–180. Academic Press, New York