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Photosynthetic electrogenic events in native membranes of *Chloroflexus aurantiacus.* Flash-induced charge displacements in the acceptor quinone complex of the photosynthetic reaction center

Armen Y. Mulkidjanian[1,2], Alejandro Hochkoeppler[3], Davide Zannoni[3], Lel A. Drachev[2], Bruno Andrea Melandri[3] & Giovanni Venturoli[3]

[1]*Division of Biophysics, Faculty of Biology and Chemistry, University of Osnabrück, D-49069, Osnabrück, Germany;* [2]*A.N. Belozersky Institute of Physico-Chemical Biology, Moscow University, Moscow, 119899, Russia;* [3]*Laboratory of Biochemistry and Biophysics, Department of Biology, University of Bologna, Bologna, 40126, Italy*

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Abstract

The thermophilic phototroph *Chloroflexus aurantiacus* possesses a photosynthetic reaction center (RC) containing a pair of menaquinones acting as primary (MQ_A) and secondary (MQ_B) electron acceptors and a tetraheme cytochrome c_{554} as an electron donor. We used native, chlorosome-containing photosynthetic membranes of this bacterium to study the MQ_B turnover. The binary oscillations of the semiquinone form MQ_B^- in response to a train of short light flashes were monitored at 416 nm, in the isosbestic point of the light-induced difference spectrum of cytochrome c_{554}. After the first flash MQ_B^- was formed, after the second one it disappeared due to the $MQ_A^-MQ_B^- \rightarrow MQ_AMQ_BH_2$ transition. The latter reaction was kinetically resolved by means of electrometry. For this purpose chromatophores of *Chl. aurantiacus* were adsorbed onto a phospholipid and menaquinone-impregnated collodion film. We found that after the second excitation flash, but not after the first one, the photoelectric response included, in addition to the fast kinetic components reflecting the charge separation between the tetraheme cytochrome *c* and MQ_A, a slower kinetic component with a rise time of 3 μs (pH = 8.3) and a relative amplitude of about 10% of the charge separation phase in the RC. We attributed this reaction to the electrogenic proton transfer which accompanied the transfer of the second electron during the $MQ_A^-MQ_B^- \rightarrow MQ_AMQ_BH_2$ transition. The rise time of the same reaction was reported to be almost three orders of magnitude slower in the isolated, proteoliposome-incorporated RC from this bacterium. The possible reasons of the faster turnover rates observed in the chlorosome-carrying native membrane preparations from *Chl. aurantiacus* are discussed.

Introduction

The gliding yellow-green thermophilic bacterium *Chloroflexus aurantiacus* combines features which are specific to different groups of photosynthetic prokaryotes. This bacterium uses chlorosomes (bacteriochlorophyll *c*-containing flat lipid vesicles attached to the cytoplasmic side of the inner membrane of the cell (Staehelin et al. 1978)) to gather light energy as the green sulfur bacteria do, whereas its photosynthetic reaction center (RC) resembles the pheophytin-quinone type RC of purple bacteria. Still the following features of the *Chl. aurantiacus* RC are unique as compared to the latter (see Blankenship and Fuller (1986), Blankenship et al. (1988), Feick et al. (1995) for reviews): (1) The isolated RC is composed of only two protein subunits, the amino acid sequences of which (determined by (Ovchinnikov et al. (1988a,b) and

Shiozawa et al. (1989)) show homology with the membrane subunits L and M of the purple bacterial RC. The H-subunit that caps the L and M subunits of the purple bacterial RC from the cytoplasmic side of the membrane does not seem to be present. (2) Menaquinone (MQ) is the only quinone moiety found in this bacterium (Hale et al. 1983), thus menaquinone operates not only as a primary (MQ_A) but also as a secondary electron acceptor (MQ_B).

A short flash of light causes oxidation of the bacteriochlorophyll dimer P and initiates the transfer of an electron across the membrane first to MQ_A and then to MQ_B, whereas P^+ is reduced by a membrane-bound tetraheme cytochrome c_{554} (Blankenship et al. 1988; Zannoni and Venturoli 1988; Mulkidjanian et al. 1994). As in purple bacteria (see Okamura and Feher (1995) for a survey on the purple bacterial RC), the secondary quinone acceptor operates as a two-electron gate: a semiquinone $MQ_B{}^-$ is formed after the odd-numbered flashes whereas $MQ_B{}^{2-}$is formed and protonated to menaquinol MQ_BH_2 after the even-numbered ones (Blankenship et al. 1988; Kutuzov et al. 1991). Also, as in the case of purple bacteria, the latter reaction can be monitored both via spectral changes of $MQ_B{}^-$ around 430 nm (Blankenship et al. 1988; Kutuzov et al. 1991) and via the electrogenic reaction which is tightly coupled with the proton transfer from the bulk to $MQ_B{}^{2-}$(Kutuzov et al. 1991).

The application of spectrophotometric techniques is very troublesome with whole membranes from *Chl. aurantiacus*, as this bacterium contains about 1000 molecules of bacteriochlorophyll per RC (Blankenship and Fuller 1986). To overcome this problem, chlorosome-depleted membrane preparations of *Chl. aurantiacus* have been used in the past to improve the signal/noise ratio (Bruce et al. 1982). With this type of preparation it has been shown that the first electron is transferred between MQ_A and MQ_B in 1.3 ms (Blankenship et al. 1988). The electrogenic protonation of $MQ_B{}^{2-}$ after the second flash (which is kinetically coupled to the transfer of the second electron from $MQ_A{}^-$ to $MQ_B{}^-$) has been shown to occur in about 1 ms in the liposome-incorporated isolated RC of *Chl. aurantiacus* (Kutuzov et al. 1991). Both rates are, however, at least one order of magnitude slower than those in the purple bacteria under the same conditions. Moreover, in the chlorosome-depleted preparations the midpoint potential (E_m) of the $MQ_A/MQ_A{}^-$ redox pair has been estimated as -50 mV at pH 8.2 (Bruce et al. 1982), much higher than the respective value of -210 mV which has been determined at this pH value with the native, chlorosome carrying membranes (Venturoli and Zannoni 1988). All this could suggest some structural and/or functional alteration due to the detachment of chlorosome. With the native, chlorosome-carrying membranes of *Chl. aurantiacus* a two-fold increase in the amount of cytochrome c_{554} oxidized by a single Xenon flash (15 μs FWHM) has been observed upon the reduction of the second heme of the tetraheme complex (Zannoni and Venturoli 1988). This may indicate the occurrence of double turnover of RC within 15 μs in this preparation (as discussed by (van Vliet et al. (1991)). In this case, however, the rate-limiting $MQ_A{}^- \rightarrow MQ_B$ electron transfer has to be complete in less than 10 μs, i.e. has to be orders of magnitude faster in the native, chlorosome carrying membranes than in the chlorosome-depleted ones.

In this work, we elucidated directly the kinetic features of the forward reactions within the MQ acceptor pair in native membranes of *Chl. aurantiacus*. We combined differential spectrophotometry with electrometric measurements of the flash-induced transmembrane charge displacements. The onset of the electrogenic protonation of $MQ_B{}^-$ after the second flash was shown to develop with τ of 3 μs at pH 8.3, i.e. almost three orders of magnitude faster than in the absence of chlorosomes in the proteoliposome-incorporated RC of the same bacterium (Kutuzov et al. 1991).

Materials and methods

Cells of *Chloroflexus aurantiacus* were grown and chlorosome-carrying membrane vesicles (chromatophores) were isolated by a French-press treatment of the bacterial cells as described previously (Venturoli and Zannoni 1988; Zannoni and Venturoli 1988).

The spectrophotometric measurements were conducted with a home-built set-up described by Venturoli et al. (1991). A xenon flash (FWHM 4 μs, 3.25 J discharge energy) was used as actinic source in this case.

Photoelectric responses of chromatophores were measured electrometrically with the use of a phospholipid-impregnated collodion film placed between two compartments as described by Drachev et al. (1979, 1989) and Mulkidjanian et al. (1994). The solution used to impregnate the collodion membrane contained 100 mg of azolectin and 30 mg of menaquinone-9 per one ml of *n*-decane. Menaquinone-9 was used to restore the MQ_B function,

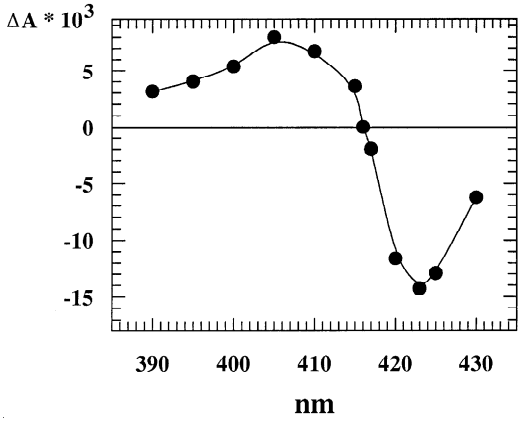

Figure 1. Spectrum of the flash-induced oxidation of cytochrome c_{554} in the Soret region. The absorbance change immediately after the last excitation in a train of 7 flashes, fired 5 ms apart is plotted. Incubation medium: 50 mM MOPS, pH 7.0, 50 mM KCl, 0.5 mM Na ascorbate, 7 μm TMPD. Concentration of membranes equivalent to 1.67 mg protein in 3 ml volume.

as in the electrometric set-up the membrane quinones are washed out from the chromatophore membrane into the measuring one (Kaminskaya et al. 1986). The chromatophore suspension was added into one of the compartments (the final concentration of bacteriochlorophyll was approximately 10 μm). To induce adsorption of chromatophores on the film surface 20 mM $MgCl_2$ was added. Contrary to the usual protocol (Drachev et al. 1979, 1989) the washing step with a low ionic strength buffer removing the non-adsorbed chromatophores was omitted (otherwise the photoelectric signal decreased dramatically). Chromatophores were pre-adapted in the complete darkness for 5 min before applying series of light pulses, to achieve full re-reduction of P^+, and full re-oxidation of the menaquinone acceptor complex by redox mediators presented in the solution. Then, a series of 2 flashes were applied with a 2 s dark interval between the flashes. Light-screened Ag/AgCl electrodes used to measure the electric potential difference between the two bathing solutions separated by the collodion membrane were coupled via an operational amplifier (Analog Devices AD 3554 BM) to a LeCroy digital oscilloscope interfaced to a personal computer. Saturating flashes (λ = 532 nm, FWHM 5 ns, energy 150 mJ) were fired by Nd-YAG laser (Surelite 10, Continuum, Santa Clara, CA). The polarity of the photoelectric response was consistent with the adsorption onto the measuring film of membrane vesicles with the acceptor side of the RC facing predominantly the outside.

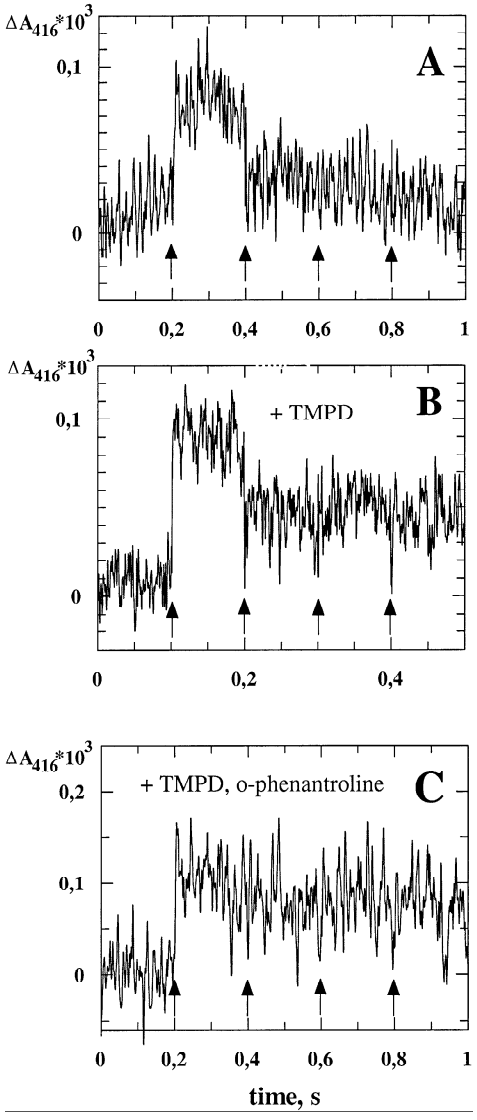

Figure 2. Binary oscillations in MQ_B^- formation measured at 416,1 nm with 64 averages. Incubation medium: 50 mM MOPS, pH 7.0, 50 mM KCl. Concentration of membranes equivalent to 0.45 mg protein in 3 ml volume. Panel A, no redox dyes, 30 μM Na ascorbate, 1.5 min. dark adaptation, train of 4 flashes spaced 200 ms apart. Panel B, 7 μM TMPD , 0.5 mM Na ascorbate, 5 s of dark adaptation, train of 4 flashes spaced 100 ms apart. Panel C, as B except that *o*-phenantroline was added up to 5 mM; the signal is the average of only 16 events.

Results

Binary oscillations of MQ_B^- in whole membranes of Chloroflexus aurantiacus

The conditions under which the binary oscillations of MQ_B^- occur in the native, chlorosome-containing

membranes were tested spectrophotometrically. To avoid the overlap of the flash-induced redox-changes of cytochrome c_{554} we have determined the spectrum of the flash-induced absorption transients of this redox component (Figure 1) and tried to detect the binary oscillations of MQ_B^- at 416 nm corresponding to the isosbestic point. Damped oscillations of MQ_B^- were observed already in the absence of any redox mediators (Figure 2A). It is noteworthy that the binary oscillations in Figure 2A were observed with only 1.5 min of dark adaptation between trains of flashes. This indicates that the MQ_B^- state relaxed faster than in 1 min. The fast electron donation from the reduced cytochrome c_{554} (Venturoli and Zannoni 1988; Mulkidjanian 1994) excluded the oxidation of MQ_B^- via the back reaction with P^+. Then these data suggest the ability of MQ_B^- to interact with some unidentified intrinsic oxidant(s) in the absence of added oxidized redox mediators (contrary to situation in purple bacteria where the latter are generally used to accelerate the oxidation of Q_B^- which otherwise is very slow (see Mulkidjanyan et al. (1986)).

Figure 2B shows the binary oscillations in the presence of 7 μM TMPD (N,N,N,N- tetramethyl-*p*-phenylenediamine) at E_h about 300 mV. Addition of this redox dye under conditions when it was mostly oxidized (midpoint potential (E_m) of TMPD = 270 mV at pH 7.0 (Prince et al. 1981)) accelerated further the oxidation of MQ_B^- state. In the presence of oxidized TMPD a dark adaptation time between trains of flashes of 1–2 s was already long enough to observe the oscillating behavior of MQ_B^-. Moreover when the time between flashes in a train was increased up to 500 ms, the binary oscillations disappeared. Both observations indicate that MQ_B^- was oxidized by TMPD (7 μM) in less than 1 s. Addition of sodium ascorbate caused the reduction of TMPD and the decrease of E_h value up to 150 mV. Under these conditions the lifetime of MQ_B^- increased and the system behaved as the one in the absence of redox-dyes (see the description in Figure 2A). As with purple bacteria, after addition of 5 mM o-phenantroline, a competitive inhibitor of the quinone binding, an absorbance transient was observed only after the first flash, corresponding to the formation of MQ_A^- (Figure 2C); no binary pattern was detected.

Electrogenic reactions in the acceptor part of the RC

Figure 3 shows photoelectric responses measured with the *Chl. aurantiacus* chromatophores as described in 'Materials and methods'. Following a first flash, a fast

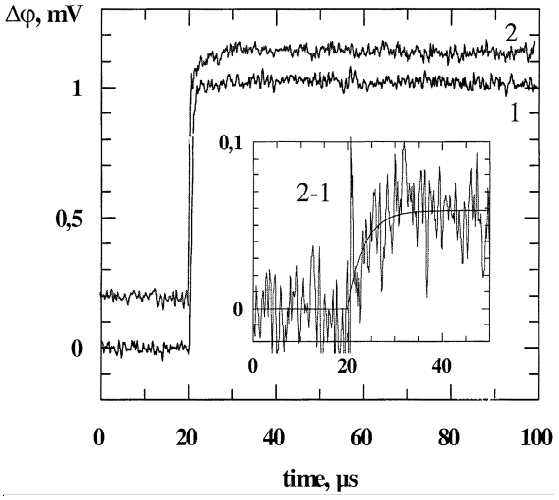

Figure 3. Photoelectric response induced by the first (trace 1) and by the second (trace 2) excitation in a sequence of two subsequent laser flashes fired 2 s apart. Inset, the difference between traces 2 and 1. Each trace is an average of 2 events. The curves were normalized relative to the magnitude of phase A+C. Dark adaptation time between the measurements $\sim$ 5 min. Incubation medium: 50 mM Tricine, pH 8.3, 15 mM $MgCl_2$, 125 μM potassium ferrocyanide, 200 μM Na ascorbate, 25 μM TMPD.

photoelectric response was seen (Figure 3, trace 1). It was due to the charge separation in the RC and consisted of a fast (τ < 10 ns) unresolvable phase *A* due to the electron transfer from P to MQ_A and a slower phase *C* due to the P^+ re-reduction by *c*ytochrome c_{554}. The latter was kinetically resolved by Mulkidjanian et al. (1994) where both phases are discussed in more detail. At the redox poise of the experiment phase C was completed within 1 μs and was almost unresolved on the time scale used in Figure 3. (In the presence of TMPD and Na ascorbate and at E_h about 140 mV the rise time of phase C was about 700 ns and its relative amplitude was about 30% of that of phase A (Mulkidjanian et al. 1994)).

Following a second flash fired after a 2 s interval (Figure 3, trace 2), an additional kinetic component appeared with a rise time of 3 μs and with an amplitude equal to 12% of phase A alone or about 8% of phases A+C. From previous experience (Kaminskaya et al. 1986; Drachev et al. 1990; Kutuzov et al. 1991) this component may be identified as phase *BII* of the electrogenic proton transfer from the bulk which is coupled to the transfer of the *second* electron to MQ_B^- in the course of the $MQ_A^-MQ_B^- \rightarrow MQ_AMQ_BH_2$ transition. Noteworthy, the extent of the fast unresolvable phase (A+C) after the second flash was about 90% of that after the first one. This was

due, to all likelihood, to the presence of an electron on MQ_A^- after the first flash in approximately 10% of RC because of a relatively low equilibrium constant between $MQ_A^-MQ_B$ and $MQ_AMQ_B^-$ states (its value has been estimated as $\sim$ 10 at pH 8 (Venturoli and Zannoni 1988)).

The addition of 4 mM o-phenanthroline was essentially without effect on the second flash-induced electrometric response (not shown). This, however, was in line with our expectations. Indeed, what happened when o-phenanthroline was present in the medium? After the first flash the electron reached Q_A. In the fraction of reaction centers, where the Q_B site was occupied by o-phenanthroline, the electron stayed on Q_A^-. (The back reaction was prevented by very fast reduction of P^+ by cytochrome c_{554}, see Mulkidjanian et al. (1994)). However, the binding of *o*-phenanthroline is not tight (see Shinkarev and Wraight (1993) for a review); the exchange constant for the membranes of *Chl. aurantiacus* could be estimated as $< 10\ s^{-1}$ from the data by Venturoli and Zannoni (1988). Therefore during the time between two flashes (2 s in our set-up) almost in all RC an exchange of an *o*-phenanthroline molecule for a menaquinone took place. This exchange was followed by a fast electron transfer from MQ_A^- to MQ_B and by the trapping of MQ_B^- (the binding constant for a semiquinone is orders of magnitudes higher than the one for neutral quinone or neutral *o*-phenanthroline (reviewed in (Shinkarev and Wraight 1993)). As a result, before the second flash almost all the centers contained MQ_B^- and not *o*-phenanthroline at the MQ_B site. In these centers an electrogenic reaction coupled with the $MQ_A^-MQ_B^- \rightarrow MQ_AMQ_BH_2$ transition could be expected after the second flash, in line with our observations.

In the case of the *Rhodobacter sphaeroides* chromatophores, 3 mM *o*-phenanthroline caused an observable (about 30%) decrease in the relative amplitude of the photoelectric response after the second flash ((Drachev et al. 1988), noteworthy, the *relative* amplitude of phase BII does not change in this case). However in these experiments the chromatophore solution was replaced by buffer before the addition of inhibitor, and the only RC present were those adsorbed onto the measuring film. The high inhibitor/RC ratio compensated the difference in the binding constants for Q_B^- and *o*-phenanthroline, correspondingly. Unfortunately, in the case of *Chl. aurantiacus* we had to use unwashed samples, otherwise the electrometric signal was almost lost (see 'Materials and methods'). Moreover, a 3–4 times higher concentration of photosynthetic membranes had to be used to get acceptable photoelectric responses (Figure 3) than in the case of spectrophotometric measurements (see Figure 2)). According to rough estimates, the inhibitor/RC ratio in our unwashed samples of *Chl. aurantiacus* chromatophores was about two orders of magnitude lower than in the case of washed samples of *Rb. sphaeroides* chromatophores studied by Drachev et al. (1988).

One more way to demonstrate the inhibitor action on the binary oscillations is to decrease the time between the subsequent flashes (as we have done in the case of spectrophotometric measurements, see Figure 2C). This also was not possible in the case of electrometry, because the relaxation time for the slowest components in our electrometric response was in the range of several hundreds of milliseconds.

As the data on the *o*-phenanthroline action were not conclusive in the case of *Chl. aurantiacus* chromatophores, we attributed the 3 μs phase to the electrogenesis of the $MQ_A^-MQ_B^- \rightarrow MQ_AMQ_BH_2$ transition mainly basing on its observation only after the second flash. A further support to this attribution was provided by the observation that by increasing the redox potential of the medium to about 250 mV it was possible to cause the disappearance of the 3 μs phase after the second flash and to equalize the magnitudes of photoelectric responses induced by the first and second flashes (not shown). This was due, to all likelihood, to the oxidation of the $MQ_AMQ_B^-$ state by the oxidized TMPD in the 2 s time between the first and the second flash. The 3 μs phase after the second flash was restored following the reduction of TMPD by Na ascorbate (cf. previous paragraph).

As was shown by Zannoni and Venturoli (1988), the phosphorylating ability of the *Chl. aurantiacus* chromatophores shows a maximum at pH about 8.3 and diminishes at pH values that are higher or lower. This was explained by the decrease of the fraction of coupled vesicles. In correspondence with this observation, the magnitude of the slowly decaying photoelectric response (from the coupled chromatophores) was maximal in a narrow pH range of 8–8.5. At more acidic and more alkaline pH values faster components appeared in the decay (indicating an increase in the fraction of de-coupled chromatophores). These decay components overlapped with the rise time of phase BII and complicated the determination of its pH-dependence. The amplitudes of the fast phases of the photoelectric responses (A+C) induced by the first and second flash were, however, measurable in a rather

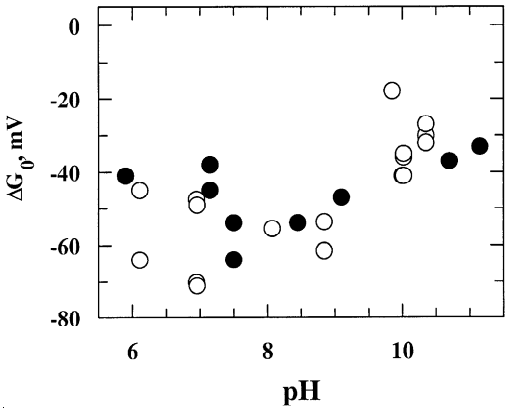

Figure 4. The pH-dependence of the free energy (ΔG_o) difference between the $MQ_A^-MQ_B$ and $MQ_AMQ_B^-$ states. Solid symbols: values determined from the electrometric data as described in the text. ΔG_o was calculated from the equilibrium constant *K* using the equation: $\Delta G_o = -kT*\ln K$. Open symbols: ΔG_o values determined from the comparison of the rates of $MQ_A^- \rightarrow P^+$ and $MQ_B^- \rightarrow P^+$ back reactions (data from Venturoli and Zannoni (1988). Incubation medium during the electrometric measurements: 25 mM Tricine + 25 mM MOPS as pH-buffers, 20 mM $MgCl_2$, 5 μM TMPD, concentration of Na ascorbate in different samples was varied in the range 100 μM – 4 mM.

wide pH range. Their ratio is coupled with the equilibrium constant, and, correspondingly, free energy for the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ reaction by the equation:

$$K = \frac{[Q_AQ_B^-]}{[Q_A^-Q_B]} = \frac{r}{1-r}$$

where $r = \frac{a_2}{a_1}$ (ratio between the fast phases of photoelectric response (A+C) measured after the second and first flashes, correspondingly). Figure 4 shows the pH dependence of the free energy of $MQ_A^-MQ_B \rightarrow MQ_AMQ_B^-$ reaction as determined from the electrometric data (solid symbols). In the same figure we depicted the pH-dependence of the equilibrium constant determined from the comparison of the rates of $MQ_A^- \rightarrow P^+$ and $MQ_B^- \rightarrow P^+$ back reactions (open symbols, data from Venturoli and Zannoni (1988)). The two dependences correspond with each other indicating that the thermodynamics of the $MQ_A^-MQ_B \rightarrow MQ_AMQ_B^-$ reaction in the adsorbed chromatophores was not different from those determined with the chromatophores in solution.

Discussion

As shown above, the electrogenic protonation of MQ_B^- which is kinetically coupled with the transfer of the second electron occurred with τ of 3 μs at pH 8.3 in chlorosome-carrying intact membranes from *Chl. aurantiacus*. Hence, the reaction was almost three orders of magnitude faster than in the case of the proteoliposome-incorporated purified RCs from this bacterium. When the latter were reconstituted with ubiquinone as a secondary acceptor, the characteristic time of the second flash-induced protonation was about 1 ms (pH 8.0) (see (Kutuzov et al. 1991); similar slow rate was observed also when menaquinone-9 was used to restore the MQ_B function in purified RC (A.M., unpublished observation)). The observation that the second flash-induced electrogenic reaction is orders of magnitudes faster in native membranes than in chlorosome-lacking preparations, adds credit to the earlier suggestion of van Vliet et al. (1991) that the first electron is transferred to MQ_B within 10 μs in native membranes (compared to 1.3 ms in the chlorosome-depleted ones (Blankenship et al. 1988)). It is noteworthy that the faster damping pattern in Figure 2 as compared to those measured with the isolated RC (Blankenship et al. 1988, Kutuzov et al. 1991) might be due to the double hits in the part of the RCs. The duration of the 4 μs Xenon flash with which the kinetic traces in Figure 2 were obtained was comparable with the rate of electron transfer both to MQ_B (< 10 μs, estimate from van Vliet et al. (1991)) and to MQ_B^- (about 3 μs, this work).

The differences between thermodynamic and kinetic properties of *Chl. aurantiacus* RCs in chlorosome-carrying and chlorosome-depleted membrane preparations (see also (Venturoli and Zannoni 1988; Venturoli et al. 1991)) suggest that the RC is somehow capped from the cytoplasmic side in the native chlorosome-carrying membrane. At least two possibilities may be discussed: 1) the H subunit of the purple bacterial RC is functionally substituted by the polypetide(s) of the chlorosome base plate; 2) some analogue of the H subunit exists in *Chloroflexus*, but is coded by another operon and has not yet been found because of low homology with the corresponding protein of purple bacteria. At the present experimental stage there is no way to distinguish these two possibilities.

The characteristic time of 3 μs observed in the native membranes of *Chl. aurantiacus* is much shorter than the time of the corresponding electrogenic event

in the RC of purple bacteria.[1] In the case of well studied system represented by chromatophores from *Rhodobacter sphaeroides* the secondary quinone is protonated with τ of about 100 μs at pH 8 (Drachev et al. 1990). It has been recently demonstrated by Graige et al. (1996), that in this RC the rate constant of the second electron transfer ($k_{AB}^{(2)}$) may be determined at neutral pH as $k_{AB}^{(2)} = f(Q_BH)^* k_e$, where $f(Q_BH)$ is the fraction of the protonated semiquinone and k_e is the rate of electron delivery from Q_A^-. According to the current views (Moser et al. 1992), the rate of electron transfer is expected to depend on the distance between MQ_A and MQ_B and on the thermodynamics of the reaction. The latter seems not to differ greatly between RC in purple bacteria and in *Chl. aurantiacus* (both chlorosome-depleted and native ones (Venturoli and Zannoni 1988; Venturoli et al. 1991)). Hence to account for the very fast rate of MQ_B^- protonation in chlorosome-carrying RC of *Chl. aurantiacus* we advance two possible explanations. A first possibility is that the distance between MQ_A and MQ_B may be shorter than in the purple bacterial RC and may increase after the depletion of the chlorosomes; an increase of 2–4 Å will be enough to explain the observed kinetic differences (see Moser et al. (1992)). As discussed above, the transfer of the first electron to MQ_B is, to all likelihood, also much faster in native membranes than in chlorosome-depleted ones (see Zannoni and Venturoli (1988) and van Vliet et al. (1991)), a result supporting this possibility. In line with this is also the observation that the chlorosomes somehow stabilize the RC of *Chl. aurantiacus* and protect the quinone binding sites (Venturoli and Zannoni 1988; Venturoli et al. 1991). A second possibility is that the presence of chlorosome decreases the local pH in the vicinity of MQ_B compared to the chlorosome-depleted RC and the RCs of purple bacteria. A combination of these two effects can also be conceived.

Interestingly, the RC of *Rb. sphaeroides*, when depleted of its H subunit, retained the primary photochemistry, while the electron transfer reaction between $Q_A^- \rightarrow Q_B$ was not detectable (Debus et al. 1985). As the absence of the *observable* electron transfer is equivalent in this case to the drastic slowing of the reaction, it is attractive to speculate that the depletion of the chlorosomes in *Chl. aurantiacus* is, to some extent, equivalent to the removal of the H subunit in *Rb. sphaeroides*.

[1] As a matter of a fact, the electrogenic phase of MQ_B^- protonation shown in Figure 3 is, to our knowledge, the fastest physiological reaction among intra-protein proton transfers experimentally resolved so far.

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