A native electrostatic environment near Q_B is not sufficient to ensure rapid proton delivery in photosynthetic reaction centers

Marie Valerio-Lepiniec[a,*], Jean-Dominique Delcroix[a], Marianne Schiffer[b], Deborah K. Hanson[b], Pierre Sebban[a]

[a]*Centre de Génétique Moléculaire, CNRS, 91198 Gif/Yvette, France*
[b]*Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne, IL 60439, USA*

Received 4 February 1997; revised version received 13 March 1997

Abstract Flash-induced absorption spectroscopy has been used to characterize *Rhodobacter capsulatus* reaction centers mutated in the secondary quinone acceptor site (Q_B). We compared the wild-type, the L212Glu-L213Asp→Ala-Ala photosynthetically incompetent double mutant (DM), and two photocompetent revertants, the DM+L217Arg→Cys and the DM+M5Asn→Asp strains. The electrostatic environment for Q_B^- is different in the two revertant strains. Only the L217Arg→Cys mutation nearly restores the native electrostatic environment of Q_B^-. However, the level of recovery of the reaction center function, measured by the rates of second electron transfer and cytochrome *c* turnover, is quite incomplete in both strains. This shows that a wild-type-like electrostatic environment of Q_B^- cannot ensure on its own, rapid and efficient proton transfer to Q_B^-.

Key words: Photosynthesis; Reaction center; Electrostatic interaction; Proton transfer; Electron transfer; Site-specific mutagenesis

1. Introduction

Bacterial reaction centers convert light energy into chemical free energy by generating a transmembrane charge separation which involves a series of electron and proton carriers. The RC protein is composed of three subunits, L, M and H, and the structure of the complex is known to atomic resolution [1–5]. The cofactors, which consist of bacteriochlorophyll, bacteriopheophytin, and quinone molecules, are bound within the transmembrane portion of the complex. With the absorption of a photon, a radical pair is formed in less than a nanosecond between a dimer of bacteriochlorophyll (P) situated on the periplasmic side of the protein and a quinone molecule, Q_A, situated on the cytoplasmic side. The electron is then transferred in 5–200 μs (depending on the species) to a secondary quinone molecule Q_B. Q_B catalyses the coupling between the transfer of two electrons and two protons by forming the dihydroquinone molecule Q_BH_2. The balance between the various parameters which control the rates of electron and proton transfer to Q_B is an important and current debate. In particular, a critical role for electrostatic interactions between a network of charged residues and the quinones has been pointed out by experimental measurements [6–9] and suggested by theoretical calculations [10,11]. It has been proposed [6–9] that the negative electrostatic potential that is provided by the protein increases the pK_as of proton delivery groups, such as protonatable residues close to Q_B. Independently of its electrostatic influence, a critical role for L213Asp (~6 Å from Q_B) in the delivery of the first proton to Q_B has been established [7,12–14]. For the second proton transfer, the involvement of L212Glu [15] (5 Å from Q_B), perhaps in conjunction with L213Asp [16] has been suggested.

As reported previously, the absence of this pair of acidic residues in the photosynthetically incompetent L212Glu-L213Asp→Ala-Ala double mutant (AA) from *Rhodobacter (Rb.) capsulatus* substantially lowers the free energy level of Q_B^- [6,17]. To further investigate the roles of these residues, we have analyzed reaction centers from two photocompetent mutant strains of *Rb. capsulatus* which also lack both L212Glu and L213Asp. In addition to the AA mutations, each of these strains carries a suppressor mutation, either L217Arg→Cys (AA+L217C) or M5Asn→Asp (AA+M5D). Both suppressor mutations increase the negative electrostatic potential near Q_B and raise the free energy level of Q_B^-. In this paper, data from functional assays of the reaction centers from these two strains, from the previously reported AA double mutant strain, and from the wild-type strain are compared.

Clearly, the greater negative electrostatic potential that is induced in this region by the additional mutations (compared to the AA strain) leads to very different electrostatic environments for Q_B^- in the reaction centers of the two revertants; at pH 8, the energy level of Q_B^- in the AA+L217C reaction center is very similar to that of the wild type. However, the measured rates of second electron transfer and cytochrome *c* turnover clearly show that the level of recovery of the functional capabilities is the same — and is nowhere near complete — in both of the revertant strains. Thus, the extent of the change in electrostatic potential near Q_B does not correlate at all with the rates at which the reaction centers function in these two strains. These results show that although negative electrostatic potential nearby Q_B assists in proton transfer and delivery, it has no effect on its own to ensure rapid delivery of protons to this acceptor.

2. Materials and methods

2.1. Construction and culture of bacterial strains

The construction of the L212Glu-L213Asp→Ala-Ala (AA) double mutant strain has been described [18]. The L212Glu-L213Asp-L217Arg→Ala-Ala-Cys (AA+L217C) and L212Glu-L213Asp-M5Asn→Ala-Ala-Asp (AA+M5D) strains are derived from a selection for phenotypic revertants of a photosynthetically incompetent quadruple mutant, L212Glu-L213Asp-M246Ala-M247Ala→Ala-Ala-Glu-Asp (RQ). Phenotypic revertants of the RQ strain carried mutations in both the L and M genes which restored some functional

*Corresponding author.

PII S0014-5793(97)00328-1

properties of the Q_A and Q_B sites (D.K. Hanson, P.D. Laible, Y.-L. Deng, M. Schiffer, M. Valerio-Lepiniec, and P. Sebban, unpublished observations). In the strains used in this study, AA+L217C and AA+M5D, the Q_A-site mutations that were present in the initially selected RQ revertants were uncoupled from these other mutations by using unique restriction sites present in plasmid pU2922 [19]. For the AA+L217C strain, a *Kpn*I–*Sac*I fragment carrying the wild-type M gene was ligated to the above mutant L gene, replacing the mutant M gene and restoring a wild-type Q_A binding site. For the AA+M5D strain, an *Asc*I–*Sac*I fragment containing the wild-type M gene was ligated to a fragment of pU2922 carrying the L212Ala-L213Ala-M5Asp mutations, thus restoring a wild-type Q_A site. These strains carrying the constructed plasmids retained the photocompetent phenotype. All of the strains described in this study contain the reaction center and light-harvesting I complex, and lack the light-harvesting II complex [20].

Large-scale cultures for RC preparations were grown under chemoheterotrophic conditions (semi-aerobically, dark, 33°C) on RPYE medium [17] containing kanamycin to ensure the presence of the plasmid. RCs of the wild-type, the AA double mutant, and the AA+L217C and AA+M5D phenotypic revertant strains were prepared as previously described [21].

2.2. Flash-induced absorption spectroscopy

The pH dependencies of the $P^+Q_A^-$ charge recombination kinetics were measured in the presence of 50–100 μM terbutryn. To reconstitute the Q_B function of the reaction centers 50–75 μM of UQ_6 was routinely added to the RC preparations. The $P^+Q_A^-$ and $P^+Q_B^-$ decay kinetics were measured at 865 nm. The kinetics of second electron transfer from Q_A^- to Q_B^- were followed at 450 nm in the presence of reduced cytochrome *c*.

Cytochrome photooxidation measurements were made under conditions of strong continuous light. A solution of 4 mM cytochrome *c* was vortexed few seconds with 40 mM sodium ascorbate to reduce it prior to the experiments. This solution was loaded onto a PD-10 column containing Sephadex G-25M gel (Pharmacia). Reduced ascorbate-free cytochrome *c* was collected; free sodium ascorbate was retained by the column. The absence of sodium ascorbate during the measurements prevents from the reduction of part of the exogenous quinone pool (UQ_0), which could artifactually decrease the rates of cytochrome oxidation.

Depending on the pH, the following buffers (5–10 mM) were used in the charge recombination and second electron transfer experiments: succinate, citrate, Mes [2-*N*-morpholino)ethanesulfonic acid], Bis-Tris propane [bis(2-hydroxyethyl)amino] tris(hydroxymethyl) propane], Tris [2-amino-2-(hydroxymethyl)propane-1,3-diol] or CAPS [3-(cyclohexylamino)-1-propanesulfonic acid]. All experiments were carried out on a home-made flash spectrophotometer at 21°C. Photochemical events were triggered by saturating flashes from a YAG laser (Spectra-Physics) (200 mJ per pulse at 532 nm with a 5 ns FWHM).

3. Results

3.1. pH dependencies of $\Delta G°_{AB}$, the free energy gap between the $P^+Q_A^-$ and $P^+Q_B^-$ states

The rate constants of $P^+Q_B^-$ (k_{BP}) and $P^+Q_A^-$ (k_{AP}) charge recombination kinetics in the reaction centers from the AA+L217C and AA+M5D strains were measured at 865 nm (data not shown). Within 5%, k_{AP} was found to be the same for all four strains. The pH dependence curves of K_{AB}, the equilibrium constant relating the $P^+Q_A^-$ and $P^+Q_B^-$ states, can be deduced from the measurements of k_{AP} and k_{BP}, from the following equation [22]: $1+K_{AB}=k_{AP}/k_{BP}$. The free energy gap between the two states can then be calculated: $\Delta G°_{AB}=-k\mathrm{T}\ln K_{AB}$. The pH dependences of $\Delta G°_{AB}$ measured in the AA+L217C and AA+M5D strains, together with the curves from the wild type and from the AA double mutant which were measured previously [6], are shown in Fig. 1. The $\Delta G°_{AB}$ value calculated for the AA+M5D reaction centers may be somewhat underestimated and its pH dependence could be slightly distorted, as suggested previously for

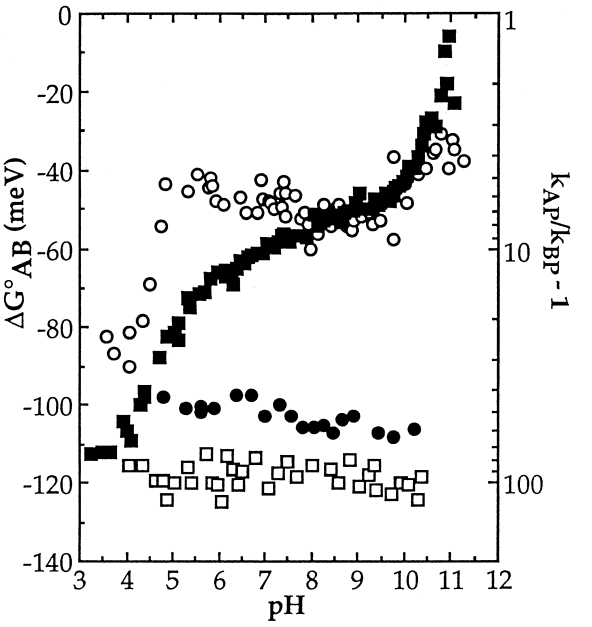

Fig. 1. pH dependences of the free energy gap, $\Delta G°_{AB}$, between the $P^+Q_A^-$ and $P^+Q_B^-$ states in the reaction centers (≈0.5 μM) from the wild type (■) and the L212Glu-L213Asp→Ala-Ala double mutant (□; 'AA'), as compared to those of the AA+L217Arg→Cys (○) and the AA+M5Asn→Asp (●) phenotypic revertant strains. The mutations are described in the text. These $\Delta G°_{AB}$ values were derived from independent measurements of k_{AP} and k_{BP} by $\Delta G°_{AB}=-k\mathrm{T}\ln(k_{AP}/k_{BP})$, as described in the text. Data points from the AA double mutant (previously reported in [6]), and possibly to some extent those of the AA+M5D strain, accurately refer to the right axis but may underestimate the actual free energy gap shown on the left axis (see discussion in the text). Conditions: 50–75 μM UQ_6, 0.03% Triton X-100, 21°C. Buffers were 10 mM succinate, citrate, MES, Bis-Tris propane, Tris, and CAPS.

the AA double mutant [6]. The very slow $P^+Q_B^-$ decay observed for the AA+M5D strain (≈0.10 s^{-1} at pH 7) suggests a direct route for the charge recombination process that does not involve Q_A as an intermediate; the rate constant of $P^+Q_B^-$ decay via the direct pathway was reported [23] to be 0.12 s^{-1} at pH 7.3. Thus, the effective $\Delta G°_{AB}$ in the AA and AA+M5D reaction centers may be even higher than the calculated value that is shown in Fig. 1. While the data points measured for the AA and AA+M5D reaction centers accurately refer to the right-hand axis ($k_{AP}/k_{BP}-1$) of Fig. 1, and may underestimate the actual free energy gap $\Delta G°_{AB}$ (left-hand axis).

Data of Fig. 1 show that most of the $\Delta G°_{AB}$ which is lost in the double mutant at neutral pH, i.e. more than 60 meV, is recovered in the reaction centers from the AA+L217C strain, but not in the AA+M5D strain.

Remarkably, in the AA+L217C reaction centers, the L217Arg→Cys mutation fully compensates for the electrostatic potential increase observed in the AA double mutant including the acceleration of the kinetics detected above pH 9. Moreover, between pH 8 and pH 10.2, the $\Delta G°_{AB}$ curve is superimposeable with that of the wild type. Below pH 7, however, only a slight pH dependence is observed down to pH 5, then a sharp decrease of $\Delta G°_{AB}$ is observed below this pH value, from about −40 meV to about −90 meV at pH 4. Except for the region of the curve between pH 5 and pH 8,

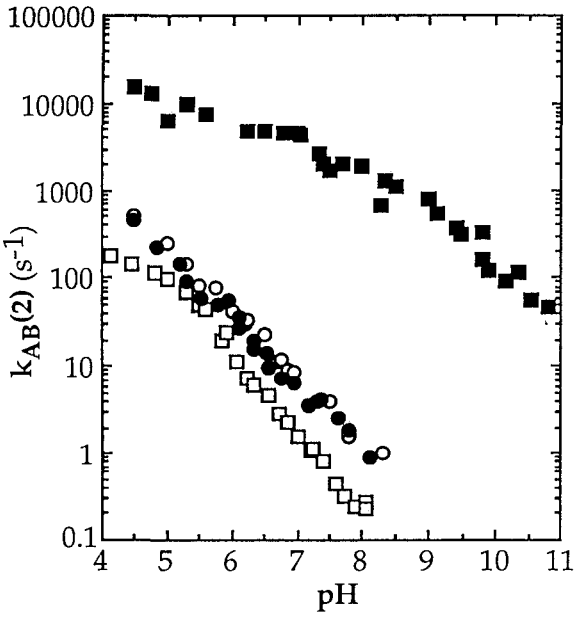

Fig. 2. pH dependences of the rate constants of the second electron transfer reaction [$k_{AB}(2)$] measured at 450 nm, in reaction centers (≈2 μM) from the AA+L217C (○) and the AA+M5D (●) phenotypic revertant strains, compared to those of the wild type (■) and the AA double mutant (□). Conditions: 40 μM cytochrome *c*, 0.15–1 mM sodium ascorbate depending on the pH, 50–75 μM UQ_6 and 0.03% Triton X-100, 21°C. Buffers were 10 mM succinate, citrate, MES, Bis-Tris propane, Tris, and CAPS.

the pH dependence of $\Delta G°_{AB}$ in the AA+L217C strain is very reminiscent to that measured in the wild-type reaction centers.

In reaction centers of the AA+M5D strain, the situation is different. Essentially no pH dependence for $\Delta G°_{AB}$ is observed for the whole pH range studied. Contrary to the AA+L217C strain, only a very small amount (≈10 meV at pH 8) of the $\Delta G°_{AB}$ lost in the AA double mutant strain is recovered. The lifetime of the $P^+Q_B^-$ charge recombination remains about 10 times longer than that of the wild type at the same pH.

3.2. *pH dependence of the rate of second electron transfer from Q_A^- to Q_B^-*

The curves showing the pH dependences of the rates of the second electron transfer [$k_{AB}(2)$] from Q_A^- to Q_B^-, measured at 450 nm in the presence of reduced cytochrome *c* and excess quinone (UQ_6), are presented in Fig. 2. The pH titration curves of $k_{AB}(2)$ in the AA+L217C and AA+M5D strains are superimposeable within the experimental error. A slight acceleration of the second electron transfer process is measured over the whole pH range studied, when compared to the AA double mutant; the increase in $k_{AB}(2)$ is only about 5-fold at pH 7. Therefore, at pH 7, $k_{AB}(2)$ of the AA+L217C and AA+M5D reaction centers is 500-fold smaller than that of the wild type.

3.3. *Rates of cytochrome photooxidation under continuous illumination*

The overall functional capabilities of the reaction centers can be evaluated by measuring, at 550 nm, the rates at which they oxidize external reduced cytochrome *c* under strong continuous illumination in the presence of excess quinone (5 mM UQ_0). The curves measured at pH 7.5 are presented in Fig. 3. The rate measured for the wild-type reaction centers is about 500 $cyt_{ox}RC^{-1}s^{-1}$. This is similar to what was previously observed in the reaction centers from *Rb. sphaeroides* [7,24]. However, after the fast oxidation of 1.5–2 cytochromes, the AA double mutant displays a very slow cytochrome oxidation process leading to a turnover rate of about 5 $cyt_{ox}RC^{-1}s^{-1}$, reflecting the very slow protonation of Q_B^-. Consistent with the data presented in Fig. 2, the reaction centers from the AA+L217C and AA+M5D strains display slightly accelerated turnover rates (≈15 and ≈25 $cyt_{ox}RC^{-1}s^{-1}$, respectively).

4. Discussion

Many experimental studies conducted with *Rb. capsulatus* and *Rb. sphaeroides* reaction centers carrying genetic modifications in the Q_B binding pocket have suggested that electrostatic interactions near Q_B are critical in ensuring rapid, efficient transfer and delivery of protons to Q_B^-. The electrostatic environment of the Q_B pocket has been modified in engineered mutants such as L212Glu→Gln and Ala (*Rb. sphaeroides* [7,15] and *Rb. capsulatus* [25–27]), L213Asp→Asn and Ala (*Rb. sphaeroides* [7,13] and *Rb. capsulatus* [6,17]) and more recently H173Glu→Gln (*Rb. sphaeroides* [6]) substitutions. Phenotypic revertants of photosynthetically incompetent strains have been characterized. Some of these revertants

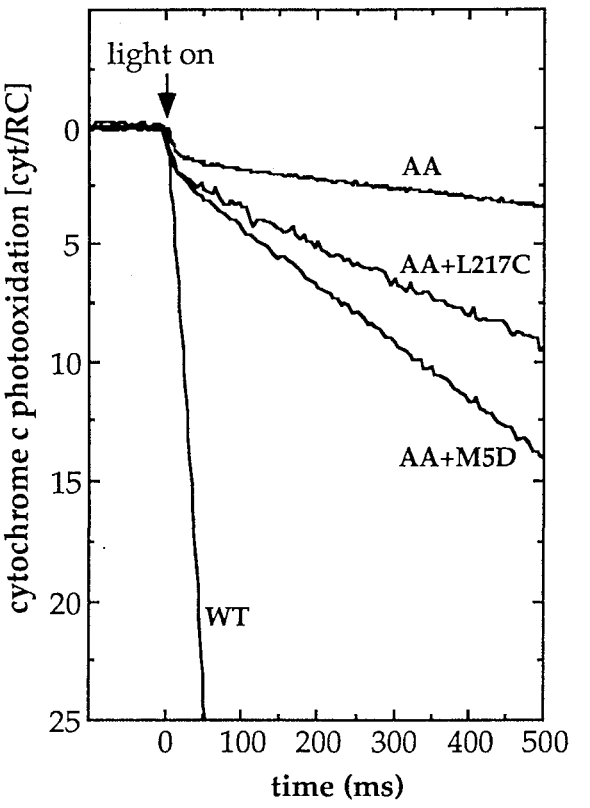

Fig. 3. Kinetics of reaction center turnover as detected by external cytochrome *c* photooxidation under strong continuous illumination (≈1 W/m^2). The rate measured for the wild-type *Rb. capsulatus* is about 500 $cyt_{ox}RC^{-1}s^{-1}$. In the AA double mutant this rate is ≈7 $cyt_{ox}RC^{-1}s^{-1}$ and, ≈15 and ≈25 $cyt_{ox}RC^{-1}s^{-1}$ in the AA+L217C and AA+M5D phenotypic revertant strains, respectively. These rates are corrected for occupancy of the Q_B site which were 100%, 90%, 85% and 90%, respectively. Conditions: 10 mM Bis-Tris propane, pH 7.5, 0.05% sodium cholate, 20 μM reduced cytochrome *c*, 5 mM UQ_0, 21°C.

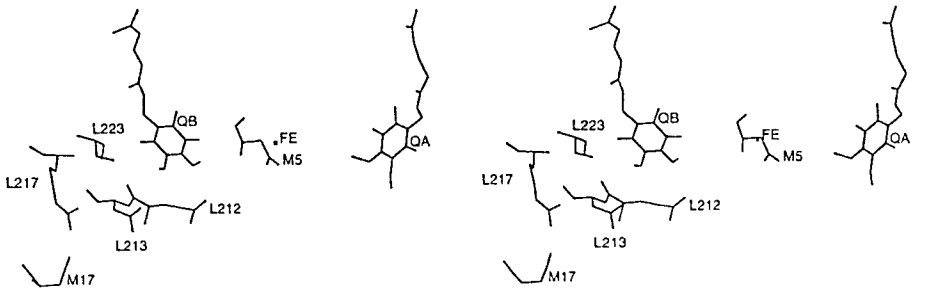

Fig. 4. Stereoview, based on the *Rb. sphaeroides* reaction center structure [2], showing the locations of the quinones and the amino acid residues described in the text. The view shown is perpendicular to the 2-fold symmetry axis of the reaction center. Residue M5Asn, mutated to Asp in the phenotypic revertant strain whose characterization is described in the text, is located 'behind' the non-heme iron atom in this figure, 13 Å distant from Q_B.

have been shown to carry second-site compensatory mutations that act from mid- to long-range to partially restore the functional capabilities of reaction centers that still carry the initial mutation(s) (Fig. 4). Among the compensating mutations are: M231Arg→Leu or Cys (*Rb. capsulatus* [6,17,18]; M233 in *Rb. sphaeroides* [14]), M5Asn→Asp (*Rb. capsulatus* [28]), M43Asn→Asp (*Rb. capsulatus* [6,8,18,29]; M44 in *Rb. sphaeroides* [14]), L231Arg→Cys (*Rb. capsulatus* [25]), and L228Gly→Asp (*Rb. capsulatus* [25]). All of these mutations involve ionizable groups and decrease the free energy gap between the $P^+Q_A^-$ and $P^+Q_B^-$ states relative to that of the original engineered mutant(s). From the results of the mutant/revertant studies, it was suggested that the system requires a negative electrostatic environment near Q_B to ensure the efficient conduction of protons [6,9]. It was proposed that this electrostatic environment tunes the pK_a of ionizable groups that are involved in the proton transport to Q_B, keeping them high enough to facilitate proton transfer. The effect of sodium azide seems to support this hypothesis. Addition of azide at very high concentrations (0.3–1.0 M) to severely impaired mutants, such as the H173Glu→Gln mutant of *Rb. sphaeroides* [9], the L213Asp→Asn mutant of *Rb. sphaeroides* [7,13] and the L212Glu-L213Asp→Ala-Ala double mutant of *Rb. capsulatus* [6], partly restores the native proton transfer rates to Q_B. A direct electrostatic influence on the efficiency of proton transfer to Q_B was suggested as the mode of action of the N_3^- ion, rather than its ability to as act a protonophore [7,13].

Fig. 1 shows that the energy level of Q_B^- in the AA+M5D strain is increased only slightly over that observed for the AA double mutant, and it is pH-independent. In the AA+L217C reaction centers, the L217Arg→Cys mutation restores the free energy gap between the $P^+Q_A^-$ and $P^+Q_B^-$ states to nearly its wild-type value, with a similar pH-dependence. The comparison with the L212Glu-L213Asp→Ala-Ala double mutant suggests that the substitution of L217Arg by a neutral residue is equivalent to a change of more than 60 meV in the energy level of Q_B^- at pH > 5. Surprisingly, above pH 8, the pH dependence curves for $\Delta G°_{AB}$ in the wild-type and the AA+L217C reaction centers are nearly superimposeable. In particular, the L217Arg→Cys mutation restores the sizable acceleration of the $P^+Q_B^-$ charge recombination kinetics which is detected in the wild-type reaction centers of *Rb. capsulatus* above pH 9.5. This behavior has been assigned to L212Glu [6,8,17]. The AA+L217C strain lacks L212Glu, therefore the recovery of an electrostatic potential similar to that observed in the wild type, at high pH, in this mutant reaction center, emphasizes the idea that multiple and complex electrostatic interactions exist between strongly interacting groups which produce apparent pK_a that cannot be necessarily attributed to a specific residue [8–11]. Additionally, in a remarkable way, the L217Arg→Cys mutation mimics the local electrostatic potential experienced by Q_B in the wild-type reaction center at low pH. Indeed, as the pH drops from 5.5 to 3.5, the 50 meV increase in the energy barrier between Q_A and Q_B that is seen in the AA+L217C reaction centers is very reminiscent to that which is observed in the wild-type reaction centers. Again, as for L212Glu at high pH, the low pH behavior of k_{BP} in the wild type has been assigned to L213Asp [7,13], yet this residue is replaced by a non-protonatable Ala in the AA+L217C strain.

The similarities between the electrostatic potential around Q_B in the wild-type and the AA+L217C reaction centers does not, however, correlate at all with any acceleration of the proton delivery to Q_B in the latter strain. Although the relative energy levels of Q_B^- are quite different in the AA+L217C and AA+M5D reaction centers, their rates of second electron transfer and of cytochrome photooxidation under continuous light are essentially the same. The cytochrome photooxidation rates of these two strains are strongly reduced when compared to that of the wild type, to a level that is only slightly higher than that of the photosynthetically incompetent AA double mutant. At pH 7, $k_{AB}(2)$ for these strains is about 5 times greater than that of the AA double mutant, but is still ~500-fold less than that of the wild type.

As has been seen previously [6,8,14,17,18,29], all of the suppressor mutations carried by the phenotypic revertants compensate for the lack of L213Asp by substituting a negatively charged residue for a neutral residue, or by replacing a positively charged residue with a neutral one. It is clear that these suppressor mutations, some of which are distant from L213Asp and/or Q_B [6,8,14,17,18,29], can strongly influence the electrostatic environment of Q_B^-. We have suggested previously [8] that the effects of distant charged groups can be relayed to Q_B via a network of charge-charge interactions that connects the many ionizable groups that are located in this region of the reaction center structure. The AA+M5D strain carries the distant M5Asn→Asp mutation, which can be fitted into the interactive network because it is less than 10 Å from H179Arg, which is itself a part of the network [8]. Similar networks have been predicted by theoretical electrostatic calculations which were based on wild-type structures of the *Rps. viridis* [11] and *Rb. sphaeroides* reaction centers [10]. L217Arg is an integral part of the networks [8,10,11]; in the

wild-type structure, it interacts with several acidic residues — L210Asp, L212Glu, L213Asp, M17Asp and H175Glu. In the AA double mutant and in the AA+L217C revertant, residues L212 and L213 are alanines. The neutral Cys at position L217 in the AA+L217C revertant reaction center can no longer counterbalance the remaining acidic residues. This change would increase the negative electrostatic environment in this region of the reaction center, probably resulting in an increase in the pK_a of nearby H175Glu. Experimentally, when compared to the AA double mutant strain, the loss of L217Arg by mutation to Cys in the revertant strain is reflected in a measurable increase in the negative electrostatic potential surrounding Q_B^- (Fig. 1).

Paddock et al. [7] have suggested that L213Asp serves two functions in the wild-type reaction center, acting indirectly to influence the electrostatic environment of Q_B^- and directly as a proton carrier. From the comparison of the electrostatic and functional capabilities of the AA+L217C and AA+M5D phenotypic revertant strains, it is clear that the amplitude and the pH dependence of the electrostatic properties of the wild-type reaction center protein are, by far, better mimicked by the reaction center from the AA+L217C revertant than the AA+M5D revertant. Thus the L217Arg→Cys substitution is more effective than the M5Asn→Asp substitution in restoring the electrostatic environment of Q_B-, one of the two functions that has been assigned to L213Asp.

However, restoration of the energy gap between $P^+Q_A^-$ and $P^+Q_B^-$ by the L213Arg→Cys mutation does not lead to any improvement in the functional capabilities of the reaction centers from the AA+L217C strain compared to the AA+M5D strain; the rates of second electron transfer and cytochrome photooxidation are the same in these two strains. The L223Ser→Ala mutant [30] is similar to the AA+L217C strain in that both display the wild-type energy gap between $P^+Q_A^-$ and $P^+Q_B^-$ above pH 7.5. However, the rates of proton-coupled second electron transfer are greatly reduced in the L223Ala and AA+L217C strains in comparison to the wild type. These observations suggest that the function of L223Ser and L213Asp, respectively, as proton donors cannot be provided by other residues — or water molecules [31] — in these mutant reaction centers.

The results presented here clearly show that a native electrostatic environment near Q_B is necessary but not sufficient to ensure efficient proton transfer to Q_B^-. A negative potential is obviously of importance in driving protons to the active site, either directly or by maintaining the pK_a of proton-carrying groups at a level that is compatible with their participation in proton transfer. However, the presence of strategically located proton relay groups is required in order to achieve rapid delivery of protons to Q_B^-.

Acknowledgements: The authors thank Drs. L. Baciou for helpful discussions and careful reading of the manuscript and Raj Pokkuluri for the structure figure.

Supported by Human Frontier Science Program Organization Grant RG-329/95 M, by NATO (CRG.920725) and NSF/CNRS (DO/CB 855) grants, by US Public Health Service Grant GM36598 (M.S., D.K. H.) and the US Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38 (M.S., D.K.H.)

References

[1] J.P. Allen, G. Feher, T.O. Yeates, H. Komiya, D.C. Rees, Proc Natl Acad Sci USA 85 (1988) 8487–8491.
[2] C.H. Chang, O. El-Kabbani, D. Tiede, J. Norris, M. Schiffer, Biochemistry 30 (1991) 5352–5360.
[3] U. Ermler, G. Fritzsch, S.K. Buchanan, H. Michel, Structure 2 (1994) 925–936.
[4] B. Arnoux, J.F. Gaucher, A. Ducruix, F. Reiss, Acta Crystallog D51 (1995) 368–379.
[5] J. Deisenhofer, O. Epp, I. Sinning, H. Michel, J Mol Biol 246 (1995) 429–457.
[6] P. Maróti, D.K. Hanson, L. Baciou, M. Schiffer, P. Sebban, Proc Natl Acad Sci USA 91 (1994) 5617–5621.
[7] M.L. Paddock, S.H. Rongey, P.H. McPherson, A. Juth, G. Feher, M.Y. Okamura, Biochemistry 33 (1994) 734–745.
[8] P. Sebban, P. Maróti, M. Schiffer, D.K. Hanson, Biochemistry 34 (1995) 8390–8397.
[9] E. Takahashi, C.A. Wraight, Proc Natl Acad Sci USA 93 (1996) 2640–2645.
[10] P. Beroza, D.R. Fredkin, M.Y. Okamura, G. Feher, Biophys J 68 (1995) 2233–2250.
[11] C.R. Lancaster, H. Michel, B. Honig, M.R. Gunner, Biophys J 70 (1996) 2469–2492.
[12] E. Takahashi, C.A. Wraight, Biochim Biophys Acta 1020 (1990) 107–111.
[13] E. Takahashi, C.A. Wraight, Biochemistry 31 (1992) 855–866.
[14] S.H. Rongey, M.L. Paddock, G. Feher, M.Y. Okamura, Proc Natl Acad Sci USA 90 (1993) 1325–1329.
[15] M.L. Paddock, S.H. Rongey, G. Feher, M.Y. Okamura, Proc Natl Acad Sci USA 86 (1989) 6602–6606.
[16] M.Y. Okamura, G. Feher, Annu Rev Biochem 61 (1992) 861–896.
[17] D.K. Hanson, L. Baciou, D.M. Tiede, S.L. Nance, M. Schiffer, P. Sebban, Biochim Biophys Acta 1102 (1992) 260–265.
[18] D.K. Hanson, D.M. Tiede, S.L. Nance, C.-H. Chang, M. Schiffer, Proc Natl Acad Sci USA 90 (1993) 8929–8933.
[19] E.J. Bylina, R.V.M. Jovine, D.C. Youvan, Bio/Technology 7 (1989) 69–74.
[20] D.C. Youvan, S. Ismail, E.J. Bylina, Gene 38 (1985) 19–30.
[21] L. Baciou, E.J. Bylina, P. Sebban, Biophys J 65 (1993) 652–660.
[22] L.J. Mancino, D.P. Dean, R.E. Blankenship, Biochim Biophys Acta 764 (1984) 46–54.
[23] A. Labahn, J.M. Bruce, M.Y. Okamura, G. Feher, Chem Phys 197 (1995) 355–366.
[24] Maróti P, Osváth S, Tápai C, Hanson DK, Sebban P (1995) in: Photosynthesis: from Light to Biosphere (Mathis P, ed.), Vol. I, pp. 419-424, Kluwer, Dordrecht.
[25] Hanson DK, Deng Y-L, Sebban P, Schiffer M (1995) in: Photosynthesis: from Light to Biosphere (Mathis P, ed.), Vol. I, pp. 859-862, Kluwer, Dordrecht.
[26] P. Maróti, D.K. Hanson, M. Schiffer, P. Sebban, Nature Struct Biol 2 (1995) 1057–1059.
[27] Miksovska J, Maróti P, Tandori J, Schiffer M, Hanson DK, Sebban P (1996) Biochemistry, 3548, 15411–15417.
[28] Delcroix JD, Schiffer M, Hanson DK, Sebban P (1995) in: Photosynthesis: from Light to Biosphere (Mathis P, ed.), Vol. I, pp. 463–466, Kluwer, Dordrecht.
[29] D.K. Hanson, S.L. Nance, M. Schiffer, Photosyn Res 32 (1992) 147–153.
[30] M.L. Paddock, P.H. McPherson, G. Feher, M.Y. Okamura, Proc Natl Acad Sci USA 87 (1990) 6803–6807.
[31] L. Baciou, H. Michel, Biochemistry 34 (1995) 7967–7972.