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Biochimica et Biophysica Acta 1232 (1995) 180–186

BBA Biochimica et Biophysica Acta

Energetics of the quinone electron acceptor complex in *Rubrivivax gelatinosus*

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Received 19 December 1994; revised 22 May 1995; accepted 28 June 1995

Abstract

The pH and temperature dependences of the free energy stabilization of the Q_A^- and Q_B^- semiquinone anions (Q_A and Q_B are respectively the primary and secondary quinone electron acceptors) were studied in antenna-reaction centre complex from *Rubrivivax (R.) gelatinosus*. This was achieved by measuring the rate constants of the $P^+Q_A^-$ (k_{AP}) and $P^+Q_B^-$ (k_{BP}) (P is the primary electron donor) charge recombination processes by flash-induced absorption spectroscopy. Despite the high primary sequence analogies of the Q_A and Q_B protein pockets between *R. gelatinosus* and the much more studied species as *Rps. viridis*, *Rb. sphaeroides* and *Rb. capsulatus*, the energetic behaviour of the quinone complex of *R. gelatinosus* appears to be somewhat different: (i) above pH 10, k_{AP} decreases, whereas it increases in *Rps. viridis*; this suggests the presence of a protonatable group that stabilizes I^- (I is a bacteriopheophytin electron acceptor) rather than Q_A^-; (ii) the pH dependence of k_{BP} is unusually flat in the range 4–7.5, possibly reflecting that a substantial part of the $P^+Q_B^-$ charge recombination proceeds via the direct route through the protein by an electron tunnelling mechanism, at variance to what is observed in the three species mentioned above; (iii) the very substantial increase of k_{BP} observed above pH 7.5 is reasonably well described by the presence of two apparent protonatable groups: $pK_{1Q_B} = 9.4$, $pK_{1Q_B^-} = 11$ and $pK_{2Q_B} = 8.5$, $pK_{2Q_B^-} = 9.4$. The latter group was not reported in *Rps. viridis*, *Rb. sphaeroides* or *Rb. capsulatus*. We conclude that the apparent pK values measured here in *R. gelatinosus* may reflect the contribution as a whole of several and/or distant groups rather than of well-defined residues.

Keywords: Reaction center; Purple bacterium; Photosynthesis; Proton

1. Introduction

Photosynthetic purple bacteria present a wide variety of phylogenetic, physiological, biochemical and spectroscopic aspects. It is therefore of interest to investigate these properties on different strains to verify the degree of generality of the conclusions derived. *Rubrivivax (R.) gelatinosus* is a non-sulfur purple bacterium that belongs to the β family (*Rhodocyclus*), which has been much less studied than the α family, which contains among others species, *Rhodobacter (Rb). capsulatus*, *Rb. sphaeroides* and *Rhodopseudomonas (Rps.) viridis*. The properties of the photochemical reaction centres (RCs) of these species have been extensively studied. These membrane proteins of the two latter strains have been crystallized and their three-dimensional structures have been resolved [1–6].

The bacterial RCs are composed of three subunits, L, M and H with molecular masses in the range 30–35 kDa. In some species as *R. gelatinosus*, *Chr. minutissimum*, and *Rps. viridis*, a tetrahemic cytochrome *c* ($\approx$ 40 kDa) is tightly bound to the RC at the periplasmic interface. L and M carry all the chromophores present in the complex. Four bacteriochlorophylls (two of which are arranged in a dimer and form the primary electron donor, P), two bacteriopheophytins, two quinone molecules Q_A and Q_B (the first stable electron acceptors), a non-heme Fe (or alternatively Mn) atom and a carotenoid molecule are present in the complex. Many studies have been achieved on the kinetic and energetic properties of the quinone electron acceptor system in RCs from different purple [7] and green bacteria like *Chloroflexus aurantiancus* [8]. At variance, those of the *Rhodocyclus* family have not yet been characterized. In *R. gelatinosus*, Q_A is a menaquinone 8 and Q_B is a ubiquinone 8 [9]. The primary sequences of L and M subunits of this species ([10,11] and Picaud, M., unpub-

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SSDI 0005-2728(95)00110-7

lished results) are very homologous to the respective one of *Rb. sphaeroides*, *Rb. capsulatus* and *Rps. viridis* [12–14], in particular, in the quinone binding sites. However, it is of interest to note that the BChl and BPhe molecules are of the '*a*' type in *R. gelatinosus* and in *Rb. sphaeroides* but of the '*b*' type in *Rps. viridis*. On the other hand, in *R. gelatinosus* Q_A and Q_B are MQ and UQ, respectively, as in *Rps. viridis*, whereas in *Rb. sphaeroides* and *Rb. capsulatus* both quinones are UQ_{10}. Probably due to this different pigment composition, charge recombination between the oxidized primary electron donor, P^+, and $P^+Q_B^-$ occurs by two different pathways in *Rps. viridis* and in *Rb. sphaeroides*. In the latter, this phenomenon occurs directly through the protein by an electron tunnelling effect. In contrast, in *Rps. viridis*, $P^+Q_A^-$ charge recombination proceeds by thermal repopulation of a relaxed state of P^+I^- (where I is the bacteriopheophytin intermediate electron acceptor) [15]. It was therefore interesting to compare the energetics of the quinone system between *R. gelatinosus*, *Rps. viridis* and *Rb. sphaeroides* to track the respective roles of the protein and of the chemical nature of the pigments in these processes.

2. Material and methods

All the experiments described in the present paper have been done on LH1-RC complexes (which keep 85–100% Q_B) instead of purified RCs which have lost the secondary quinone [16] and are unstable especially at high pH (Agalidis, I., unpublished results). The LH1-RC complex from *R. gelatinosus* strain S_1 [17] was prepared as described [9] except that it was purified in the presence of lower detergent concentration (0.04% $C_{12}E_8$ instead of 0.1%). Flash-induced absorbance decays were obtained using a home made spectrophotometer [18]. P^+ absorbance changes decay was monitored at 787 nm. pH buffers used were: Mes, Bistris propane or CAPS. The Arrhenius plots of the rate constants of $P^+Q_A^-$ (k_{AP}) and $P^+Q_B^-$ (k_{BP}) decays between 0 and 40°C were measured as described [19]. The low-temperature experiments were carried out in the presence of 60% glycerol (v/v) in A. Vermeglio's group on a home-made spectrophotometer.

3. Results

3.1. Temperature dependence of k_{AP}, the rate constant of $P^+Q_A^-$ charge recombination

The temperature dependence of k_{AP}, between 310 and 90 K is presented in Fig. 1. This curve can be divided in two parts. The first one, between 310 and 240 K, corresponds to a decrease of k_{AP} from 180 s^{-1} to about 30 s^{-1}. Such room-temperature dependence is similar to what was observed in *Rps. viridis* [15,20]. In this case, $P^+Q_A^-$

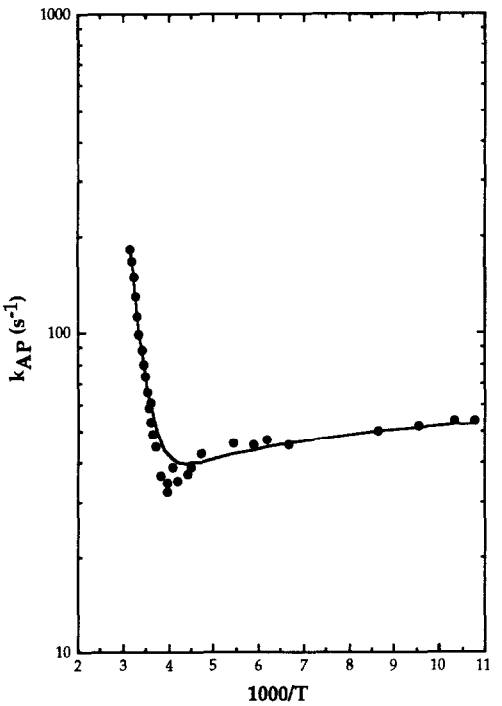

Fig. 1. Temperature dependence of k_{AP}, the rate constant of $P^+Q_A^-$ charge recombination in LH1-RC complexes (ΔO_{875} nm ≈ 2) from *R. gelatinosus*. The line is the result of the fitting of the data with Eq. (2). k_d was taken as $7 \cdot 10^7$ s^{-1}. The following parameter values are derived: $\Delta G^\circ_{AI} = 0.344$ eV, $k(0) = 58$ s^{-1} and $\hbar\omega = 145$ cm^{-1}. Conditions: 10 mM Tris (pH 8), 1 mM EDTA, 0.1% $C_{12}E_8$ 50 μM terbutryn + 60% glycerol (v/v) below 0°C.

recombination was interpreted to occur through a thermal repopulation of a relaxed state of P^+I^- [15,20]. It is likely that this is also the case in *R. gelatinosus*. To describe the k_{AP} variations between 275 and 310 K we used the equation proposed by Shopes and Wraight [15]:

$$k_{AP} = k_d \times e^{-(\Delta G^\circ_{AI}/kT)} + k_T \quad (1)$$

where k_d is the rate constant of charge recombination from the P^+I^- state, k_T is the limiting value of k_{AP} measured at low temperature, ΔG°_{AI} is the free energy gap between the P^+I^- and $P^+Q_A^-$ charge separated states. ΔG°_{AI} is the sum of ΔH°_{AI} and $-T\Delta S^\circ_{AI}$, which are deduced from the slope of the $\ln(k_{AP} - k_T)$ vs. $1000/T$ curve and from the intercept, respectively. Since k_d has not yet been measured in *R. gelatinosus*, we have used the value measured in *Rps. viridis*: $7 \cdot 10^7$ s^{-1} [21]. This is a reasonable assumption, since this parameter is very similar in *Rps. viridis* and *Rb. sphaeroides* [22] which do not possess the same type of BChl and BPhe ('*b*' type vs. '*a*' type). k_T was taken as the lowest value of k_{AP}, at 240 K, i.e., 23 s^{-1}. However, if the choice of k_T does modify the ΔH°_{AI} value, a compensating effect on $T\Delta S^\circ_{AI}$ [23] results in a poor relationship between k_T and the ΔGI°_{AI} value. The activation parameters calculated at pH 8 and 298 K are presented in Table 1. The free energy gap between the P^+I^- and $P^+Q_A^-$ states is thus estimated to be 0.334 ± 0.005 eV.

Table 1
Thermodynamic parameters (eV) associated with the $P^+Q_A^-$ charge recombination process in the antenna-RC complex from *R. gelatinosus*

ΔH°_{AI}	$-T\Delta S^\circ_{AI}$	ΔG°_{AI}
0.314 ± 0.005	0.020 ± 0.005	0.334 ± 0.005

The second part of the curve of Fig. 1 corresponding to the low-temperature range shows a slight acceleration of the $P^+Q_A^-$ back reaction as temperature decreases from 240 K to 90 K, probably reflecting a tunnelling process for the electron transfer from Q_A^- to P^+ as already shown in *Rps. viridis* and *Rps. sphaeroides* [15,24,25]. In *Rb. sphaeroides*, the increase of k_{AP} between 240 K and 90 K was shown to be more pronounced than in *R. gelatinosus*, whereas, in *Rps. viridis*, under the same experimental conditions (i.e., in the presence of 60% glycerol) the temperature dependence of k_{AP} was found to be flat below 160 K [15].

In order to fit the curve of k_{AP} in the whole temperature range, the following equation was used:

$$k_{AP} = k_d \times e^{-(\Delta G^\circ_{AI}/kT)} + k(0) \times \left[\frac{e^{h\omega/kT} - 1}{e^{h\omega/kT} + 1}\right]^{1/2} \quad (2)$$

The second term of the right part of Eq. (2) has been proposed [26] to account for possible coupling of the electron transfer to a single polar mode of the protein. $h\omega$ designates the average frequency of this mode, and $k(0)$, the limiting value of the rate constant at 0 K. The fitting of the data presented in Fig. 1 leads to $k(0) = 58\ s^{-1}$ and $h\omega = 145\ cm^{-1}$.

3.2. pH dependences of k_{AP} and k_{BP}

The pH dependences of k_{AP} and k_{BP} in the pH range 4–12 are presented in Fig. 2. We should point out that the exceptional stability of the antenna-RC complex from *R. gelatinosus* allowed a titration in such a large pH range. These data were obtained on three different preparations. The reversibility of the pH dependences of the rates was systematically checked at low and high pH.

3.2.1. pH dependence of k_{AP}

In the pH range 4–9, k_{AP} smoothly increases, from 45 to 90 s^{-1}, at 21°C. A plateau is observed from pH 9 to pH 10. Above pH 10, k_{AP} decreases, reaching a value of 45 s^{-1} at pH 12. The shape observed below pH 9 is similar to what was measured in RCs from *Rb. sphaeroides* and *Rb. capsulatus*, but with different absolute values of k_{AP}. It is considered that, in these species, at room temperature, the $P^+Q_A^-$ charge recombination proceeds directly to the ground state by an electron tunnelling process, poorly dependent on the free energy level of $P^+Q_A^-$ and therefore on the protonation state of the protein near Q_A. The decrease of k_{AP} above pH 10 (Fig. 2) is unusual in species where $P^+Q_A^-$ recombines by a thermally activated process. Thus, in the case of *Rps. viridis*, where Q_A is an MQ_9, a 2–3-fold increase of k_{AP} was observed in the pH range 9–11 [15,19,20]. The same pattern was observed in *Rb. sphaeroides*, where QA was replaced by quinones of low redox potential [27–29]. This acceleration at high pH has been interpreted as due to deprotonation of a proteic group with stronger electrostatic interaction with Q_A^- than with I^-; the apparent pK of this group in the presence of I^-, pK_I, being smaller than in the presence of Q_A^- (pK_A). If we apply this reasoning for *R. gelatinosus*, it turns out that a protonatable group could interact more strongly with I^- than with Q_A^-. To fit the data of Fig. 2, we have used the equation proposed by Shopes and Wraight [15]:

$$k_{AP} = k_{AP_{H^+}} \times \frac{1 + 10^{(pH - pK_I)}}{1 + 10^{(pH - pK_A)}} + k_T \quad (3)$$

where $k_{AP_{H^+}}$ is the value of k_{AP} at some arbitrary low pH value. The line drawn through the k_{AP} points results from these calculations which indicate a pK_I value of 12.2 ± 0.2 and a pK_A value of 11.6 ± 0.2 (Table 1). This difference is consistent with a stronger interaction energy of a putative protonatable group with I^- than with Q_A^- by about 35 meV, i.e., 58 meV $\times$ (pK_I − pK_A).

3.2.2. pH dependence of k_{BP}

The pH dependence curve of k_{BP} shows two different regions. In the pH range 8–12, k_{BP} increases with an

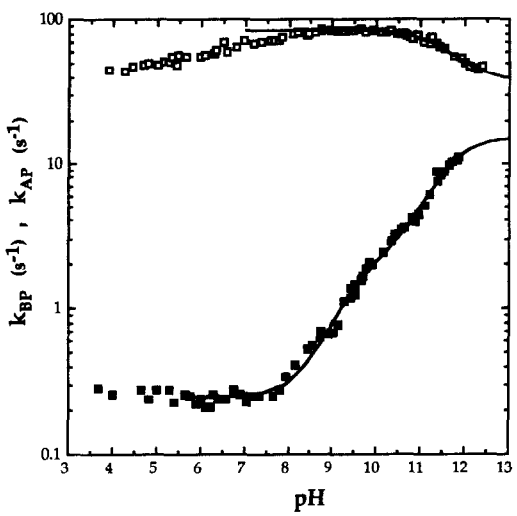

Fig. 2. pH dependence of the rate constants of $P^+Q_A^-$ (open squares, upper data) and $P^+Q_B^-$ (closed squares, lower data) charge recombination in LH1-RC complexes (ΔO_{875} nm = 2) from *R. gelatinosus* at room temperature. The lines are the results of the fitting of the data by Eqs. (3) and (4), respectively. This adjustment leads to the following parameter values: For $P^+Q_A^-$, p$K_I = 12.20 \pm 0.20$, p$K_A = 11.60 \pm 0.20$; $\Delta G^\circ_{AI} = 0.334 \pm 0.005$ eV (deduced from the fitting of Fig. 1). For $P^+Q_B^-$, the existence of two protonatable groups is suggested whose pK values are: p$K_{2Q_B} = 8.45 \pm 0.15$, p$K_{2Q_B^-} = 9.40 \pm 0.15$ and p$K_{1Q_B} = 10.70 \pm 0.15$, p$K_{1Q_B^-} = 11.55 \pm 0.20$. $k_{BP_H^+}$ was adjusted to 0.24 s^{-1}. Other conditions as in Fig. 1 but without glycerol. The pH buffers are indicated in Section 2.

average slope corresponding to about 0.5 H^+/per e^-. This figure is different from those obtained with *Rps. viridis*, *Rb. sphaeroides* and *Rb. capsulatus* RCs, where destabilization of the $P^+Q_B^-$ state (or increase of k_{BP}) starts only above pH 9.7–10. Assuming a simple model where two amino-acid residues modulate the free energy level of Q_B^-, we have fitted the pH dependence of k_{BP} (Fig. 2) by the following equation:

$$k_{BP} = k_{BP_{H^+}} \times \frac{1 + 10^{(pH - pK_{1Q_B})}}{1 + 10^{(pH - pK_{1Q^-_B})}} \times \frac{1 + 10^{(pH - pK_{2Q_B})}}{1 + 10^{(pH - pK_{2Q^-_B})}} \quad (4)$$

where $k_{BP_{H^+}}$ represents the value of k_{BP} at some arbitrary low pH value, and pK_{iQ_B} and $pK_{iQ_B^-}$ ($i = 1,2$) are the pK of the groups i, depending on whether Q_B is in its neutral or semiquinone form, respectively. The analysis of the curve leads to the determination of two groups: ($pK_{1Q_B} = 10.70 \pm 0.15$, $pK_{1Q_B^-} = 11.55 \pm 0.20$) and ($pK_{2Q_B} = 8.45 \pm 0.10$, $pK_{2Q_B^-} = 9.40 \pm 0.10$) respectively (see Table 2). The pK values of the first group are reminescent of those estimated for the group responsible for k_{BP} increase at high pH in *Rb. capsulatus* [30] and slightly higher than those estimated for the same group in *Rb. sphaeroides* [31]. Although in *Rps. viridis* an ionizable group with an apparent $pK_{Q_B} = 9.20$ has been reported by Gao et al. [32], the examination of k_{BP} dependence on pH in this species seems to indicate different protonation properties relative to the group 2 determined here in *R gelatinosus*.

In the pH range 3.5–8, the $P^+Q_B^-$ back reaction is very slow ($k_{BP} = 0.25$–0.30 s^{-1}) and nearly pH-independent. This is still a different behaviour relative to the other species. In *Rps. viridis* [15], *Rb. sphaeroides* [33] and *Rb. capsulatus* [30], k_{BP} substantially decreases at low pH, reflecting a notable stabilization of $P^+Q_B^-$ by some protonations nearby Q_B^-. However, this is not the case in *E. mobilis* [34] and *Chr. minutissimum* RCs [35], which both possess MQ and UQ as first and second electron acceptor, respectively. In *E. mobilis*, k_{BP} does not depend on pH in the range 6–10 and in *Chr. minutissimum* it varies very smoothly below pH 7. The slow back reactions observed in these species (6–10 s) were suggested to reflect a tunnelling process for the return of the electron from Q_B^- to P^+ directly through the protein. Since the primary sequence of Q_B protein pocket is very conserved in *R. gelatinosus* as compared to *Rps. viridis*, *Rb. sphaeroides* and *Rb. capsulatus* (see Table 4) the quasi absence of Q_B^- stabilization observed here is somewhat surprising. We have therefore investigated the possibility that, below pH 8, in *R. gelatinosus*, $P^+Q_B^-$ charge recombination proceeds by a two-step mechanism involving an activationless path through the protein and a thermal reactivation of $P^+Q_A^-$ and P^+I^- states. We have measured the temperature dependence of k_{BP} at different pH. This was done at pH 6.0, 6.8, 9.5 and 10.4. The Arrhenius plots resulting from these measurements are presented in Fig. 3 and the deduced activation energies, ΔH_B°, in Table 3. The ΔH_B° values measured below pH 8, are identical within the experimental error and more than 2-times smaller than

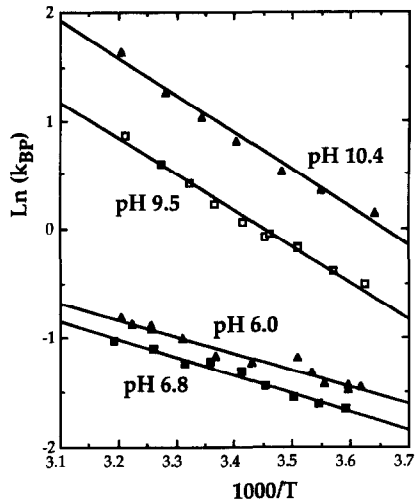

Fig. 3. Arrhenius plots of the rate constants of the $P^+Q_B^-$ charge recombination measured at pH 6.0, 6.8, 9.5 and 10.4. Conditions as in Fig. 2.

Table 2
Apparent pK values involved in the stabilization of Q_A^- and Q_B^- in *R. gelatinosus*

	pK_I	pK_A	pK_{2QB}	$pK_{2Q_B^-}$	pK_{1QB}	$pK_{1Q_B^-}$
R. gelatinosus	12.20 ± 0.2	11.60 ± 0.20	8.45 ± 0.15	9.40 ± 0.15	10.70 ± 0.15	11.55 ± 0.15
Rps. viridis	9.40 [a,b]	9.70 [a,b]		9.2 [b]	10.20 [b] ± 0.10	11.30 [b] ± 0.20
Rb. sphaeroides					9.80 [c]	11.30 [c]
Rb. capsulatus					10.3 [d] ± 0.10	11.60 [d] ± 0.30

[a,b,c] and [d] respectively relate to the work of Shopes and Wraight [15], Gao et al. [32], Kleinfeld et al. [31] and Baciou et al. [30].

Table 3
pH dependence of the enthalpy of the $P^+Q_B^-$ charge recombination process

	pH 6.0	pH 6.8	pH 9.5	pH 10.5
ΔH_B° (eV)	0.129 ± 0.010	0.137 ± 0.010	0.280 ± 0.010	0.289 ± 0.010

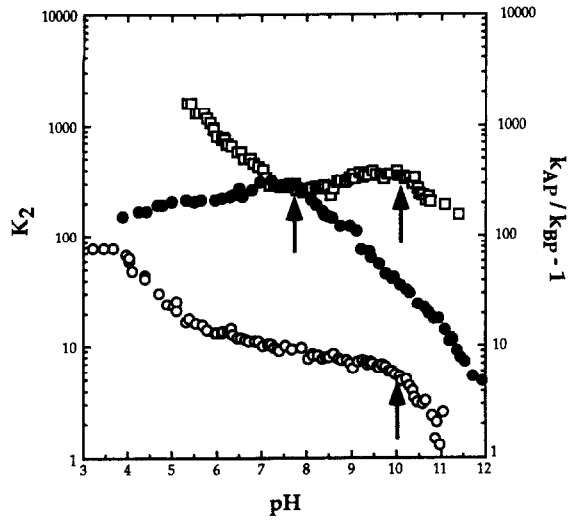

Fig. 4. pH dependence of K_2, the $Q_A^- Q_B \rightleftharpoons Q_A Q_B^-$ equilibrium constant value in *R. gelatinosus* antenna-RC complex determined here (●), in chromatophores from *Rps. viridis* [19] (□) and in RCs from *Rb. capsulatus* [30] (○). Since in *R. gelatinosus*, below pH 8, the direct recombination is likely to be the dominant process of $P^+ Q_B^-$ charge recombination, the K_2 values calculated from the expression derived by Mancino et al. [36], $K_2 = k_{AP}/k_{BP} - 1$ may be underestimated. Therefore, the data points for *R. gelatinosus*, below pH 8, strictly refer to the right ordinate axis, i.e., $k_{AP}/k_{BP} - 1$.

those measured above this pH. This is consistent with the idea of different pathways for the back electron transfer from Q_B^- to P^+, at pH 6.0 and 6.8 on the one hand and at pH 9.5 and 10.4 on the other hand. Indeed, the activationless process at low pH seems to be more important at low pH than at high pH.

3.3. pH dependence of the equilibrium constant of the Q_A^- to Q_B electron transfer process

According to Mancino et al. [36], the apparent equilibrium constant K_2 of the Q_A^- to Q_B electron transfer process may be written as: $K_2 = k_{AP}/k_{BP} - 1$. However, in the case of back electron transfer from Q_B^- to P^+ by an activationless path, the K_2 value calculated with the above equation underestimates its real value. Consequently, in Fig. 4, for *R. gelatinosus*, the points below pH 8 are only relevant to the right-ordinate axis ($k_{AP}/k_{BP} - 1$). In *R. gelatinosus*, due to the concomitant increase of k_{BP} above pH 8 and the decrease of k_{AP} above pH 10, K_2 strongly decreases in the pH range 8–12 from about 300 to about 4. For comparison, we have also presented in Fig. 4 the pH dependence of K_2 in *Rps. viridis* chromatophores [19] and in purified RCs from *Rb. capsulatus* [30]. The arrows show the turning points of the K_2 curves at high pH: 10.2 in *Rps. viridis*, 9.8 in *Rb. capsulatus*. In *R. gelatinosus*, this value is about 7.8 or could even be smaller when considering that, in this species, below this pH value, k_{BP} is not very sensitive to protonation events. These kinetics have been carried out on RCs extracted from three species and having a different degree of purification; nevertheless the patterns of the k_{BP} curves in the three species are sufficiently different to suggest a substantial lowering of the onset of k_{BP} in *R. gelatinosus*.

4. Discussion

The pH dependence of the energetics of the quinone acceptor complex of *R. gelatinosus* appears to be quite different from that of the extensively studied species *Rps. viridis*, *Rb. sphaeroides* and *Rb. capsulatus*. The data presented here show that in *R. gelatinosus* at room temperature $P^+Q_A^-$ recombines by a thermally activated process. The free energy gap between this state and $P^+Q_A^-$ is found to be about 0.33 eV. This is somewhat higher than the values found in *Rps. viridis*, i.e., 0.25–0.29 eV, depending on the kind of preparations (isolated RCs, chromatophores or RCs reconstituted in liposomes) [15,19]. The difference could be accounted for by the different proteic environments of I and Q_A in both species and/or to the different chemical nature of the acceptors (BPhe *a* and MQ_8 in *R. gelatinosus* vs. BPheo *b* and MQ_9 in *Rps. viridis*).

We can estimate the free energy gap between P^* and $P^+Q_A^-$ (ΔG^*) in *R. gelatinosus*. If we take for the free energy difference between P^* and P^+I^- the value of 0.2 eV as measured in *Rb. sphaeroides* by Woodbury and Parson [37] and for the extent of free energy relaxation between P^+I^- and its totally relaxed state M, a value

Table 4
Comparison of amino-acid sequence of the L subunit between L181–L230 in *R. gelatinosus* strain S_1 and in other purple bacteria with identical residues in bold letters

Species	Amino-acid sequence
Vir	**FVNAMALGLH.GGLILSVANP**.GDGDKVKTAE.**HE**NQY**FRDVV.GYSIGALSIH**
Sph.	**FTNALALALH.GALVLSAANP**.EKGKEMRTPD.**HEDTFFRDLV.GYSIGTLGIH**
Caps.	**FTTAWALAMH.GALVLSAANP**.VKGKTMRTPD.**HEDTYFRDLM.GYSVGTLGIH**
Gel.	**FTTTLAMSMH.GGLILSAANP**.KKGEPMK**TTD.HEDTFFRDAV.GYSIGSLGIH**
	181 190 200 210 220 230

Sequences from the following: *Rps. viridis* (*Vir.*) [13]; *Rb. sphaeroides* (*Sph.*) [12]; *Rb. capsulatus* (*Caps.*) [14]; and *R. gelatinosus* (*Gel.*), Picaud, M., personal communication.

≤ 0.2 eV proposed by Sebban [23], the total difference between P^* and $P^+Q_A^-$ states in *R. gelatinosus* may be estimated to be ≤ 0.73 eV. This value is close to the limit (0.8 eV) suggested by Woodbury et al. [38] for the $P^+Q_A^-$ recombination to proceed via a thermal repopulation of P^+I^-. As far as P^+I^- and M energy levels do not change, ΔG^* is essentially modulated by the midpoint redox potential of Q_A as already demonstrated in *Rb. sphaeroides* RCs [23,25,38] The temperature dependence curve indicates that below 240 K the electron transfer occurs by a tunnelling mechanism, whereas above 240 K it occurs by a thermal repopulation of P^+I^- as already determined in *Rps. viridis* [15]. One may note that the low-temperature curve of k_{AP} in *R. gelatinosus* has a quite similar shape (with a negative activation energy) to that determined in *Rb. sphaeroides* where QA was substituted by a menaquinone [15].

Differences of the protein region between Q_A and I in *R. gelatinosus* and in *Rps. viridis* could explain the pH dependences of k_{AP}, especially at high pH. In *Rps. viridis*, a 2–3-fold increase of k_{AP} was previously observed above pH 9, suggesting the presence of a protonatable group between Q_A and I, which interacts by about 30–40 meV more strongly with Q_A^- than with I^- [15,19]. This situation is inverted in *R. gelatinosus*, where a group interacts more strongly with I^- than with Q_A^- (by about 35 meV). This different behaviour is reminiscent of that of the T_3 double mutant from *Rps. viridis* ($Phe^{L216} \rightarrow Ser$ mutation in the Q_B pocket, $Val^{M263} \rightarrow Phe$ mutation in the Q_A pocket) in which k_{AP} decreases above pH 10 [39]. We have previously assumed that, in this mutant, a protein rearrangement has occurred near Q_A, due to the replacement of Val^{M263} by Phe. This proteic modification could result into a displacement towards I of the protonatable group cited above which is in stronger interaction with Q_A^- than with I^- in the WT. Considering the high sequence homology between *R. gelatinosus* and *Rps. viridis* and the small number of protonatable groups in this region of the protein, it is very possible that the same amino acid residue is involved in the relative stabilization of I^- compared to Q_A^- at high pH in *R. gelatinosus*. It has recently been proposed that Tyr^{H40} (the hydroxyl oxygen of which is 13.5 Å apart from the centre of Q_A and 22.5 Å from the centre of I in *Rb. sphaeroides* [6]) may participate in the differential stabilization of I^- and Q_A^- [29]. This ionizable residue is conserved in *Rps. viridis, Rb. sphaeroides* and *Rb. capsulatus*.

The overall shape of k_{BP} dependence on pH in *R. gelatinosus* is also significantly different from that measured in *Rb. sphaeroides* and *Rb. capsulatus*. However, the RCs from these species share very similar primary sequences of the Q_B pocket, including Glu^{L212}, Asp^{L213} and Asp^{L210} [40] (see Table 4). In RCs of the two later species these protonatable residues are considered to stabilize the $P^+Q_B^-$ state, as indicated by a substantial decrease of k_{BP} at low pH. No such behaviour was observed here in *R. gelatinosus*. It is very unlikely that the pK values of Asp^{L213} and Asp^{L210}, could be shifted so much in *R. gelatinosus* that the 'usual' pH dependence of k_{BP} is cancelled. Instead, the much lower activation energy values of k_{BP} measured at pH 6 and 6.8 compared to those at pH 9.5 and pH 10.4, suggest that in *R. gelatinosus*, at least below pH 8, an important part of the $P^+Q_B^-$ recombination occurs by a tunnelling mechanism through the protein directly to the ground state. In *Rb. sphaeroides*, the rate for such mechanism was estimated to be ≤ 0.04–$0.05\ s^{-1}$ [33,41]. The limiting value for this rate would be higher in *R. gelatinosus* RCs ($\approx 0.25\ s^{-1}$).

The pH dependence of k_{BP} measured here in *R. gelatinosus* above pH 8 is different from *Rb. sphaeroides, Rb. capsulatus* and *Rps. viridis*. In these species, a substantial increase of k_{BP} is observed above pH 9.7–10 and is reasonably well described by the apparent deprotonation of one group (group 1) with pK_{QB} in the pH range 9.8–10.5 and pKQ_B^- in the pH range 11.3–11.6. It has been proposed that Glu^{L212} could be involved [42] in this protonation event. We show here that, in *R. gelatinosus*, in addition to this apparent ionizable group, it is necessary to postulate the presence of a second one at lower $pH_{2Q_B^-} = 8.45 \pm 0.15$ and $pK_{2Q_B^-} = 9.40 \pm 0.15$) interacting with Q_B^- with a coulombic energy of about 60 meV. Taking a value of 30–35 for the local dielectric constant leads to a rough estimation of 7–8 Å for the distance between this group and Q_B^-. However, no obvious difference exists in the sequence for protonatable residues in the vicinity of Q_B^- at a distance ≤ 10 Å between *R. gelatinosus* and the other species. The different patterns of the protonation events taking place in *R. gelatinosus, Rps. viridis* and *Rb. capsulatus* is reflected in the pH dependence of K2 (Fig. 4).

It is of interest to note that, in *R. gelatinosus*, a Met is present in position M44 at the interface of the L and M subunit, instead of Asn in *Rb. capsulatus* and *Rb. sphaeroides*. The recent determination of the 3D RC structure from *Rb. sphaeroides* strain Y at 3 Å resolution [6] indicates the existence of an H bond between the backbone oxygen of Asn^{M44} and the hydroxyl group of Tyr^{L222} and another one between Asn^{M44} and the backbone oxygen of Ser^{L223}. These interactions may have an important structural role, at least at the level of the Q_B binding pocket. Replacement of Asn by a hydrophobic amino acid residue like Met in *R. gelatinosus* could modify the local polarity and/or structure of this region of the protein, and lead to the apparition of an apparent new pK group (group 2) which is not necessarily due to a specific residue, but to a bulk contribution of several distant protonatable groups.

5. Conclusion

Our data show that the high primary sequence homologies at the quinone binding sites between *R. gelatinosus* and several purple bacteria do not lead to similar protona-

tion events accompanying the quinone photoreductions. This may arise from different conformations of the protein nearby these cofactors in *R. gelatinosus*, leading to different charge distributions. Alternatively, these observations may underline the influence of distant ionizable residues in the local electrostatic properties in the vicinity of these electron acceptors. This highlights the global influence of the protein in these phenomena and does not support the hypothesis of a direct relationship between a specific residue and its electrostatic influence.

Acknowledgements

We would like to thank Drs F. Reiss-Husson, L. Baciou and C. Vernotte for helpful discussions and careful reading of the manuscript. A. Vermeglio's group is acknowledged for its collaboration in the low-temperature measurements and we are grateful to M. Picaud for communicating unpublished sequences of L and M subunits from *R. gelatinosus* strain S_1.

References

[1] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) J. Mol. Biol. 180, 395–398.
[2] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Nature 318, 618–624.
[3] Allen, J.P., Feher, G., Yeates, T.O., Komiya, H. and Rees, D.C. (1988) Proc. Natl. Acad. Sci. USA 85, 8487–8491.
[4] Chang, C.H., El-Kabbani, O, Tiede, D., Norris, J. and Schiffer, M. (1991) Biochemistry 30, 5352–5360.
[5] Arnoux, B., Ducruix, A., Reiss-Husson, F., Lutz, M., Norris, J., Schiffer, M. and Chang, C.H. (1989) FEBS Lett. 258, 47–50.
[6] Arnoux, B., Gaucher, J.F. and Ducruix, A. and Reiss-Husson, F. (1995) Acta Crist. serie D (in press).
[7] Shinkarev, V.P. and Wraight, C.A. (1993) in The Photosynthetic Reaction Center, Vol. I, pp. 193–255, Academic Press, New York.
[8] Venturoli, G., Trotta, M., Feick, R., Melandri, B.A. and Zannoni, D. (1991) Eur. J. Biochem. 202, 625–634.
[9] Agalidis, I., Rivas, E. and Reiss-Husson, F. (1990) Photosynth. Res. 23, 249–255.
[10] Nagashima, K.V.P., Shimada, K. and Matsuura, K. (1993) Photosynth. Res. 36, 185–191.
[11] Nagashima, K.V.P., Matsuura, K., Ohyama, S. and Shimada, K. (1994) J. Biol. Chem. 269, 2477–2484.
[12] Williams, J.C., Steiner, L.A., Ogden, R.C., Simon, M.I. and Feher, G. (1983) Proc. Natl. Acad. Sci. USA 80, 6505–6509.
[13] Youvan, D.C., Bylina, E.J., Alberti, M., Begusch, H. and Hearst, J.E. (1984) Cell 37, 949–957.
[14] Michel, H., Weyer, K.A., Gruenberg, H., Dunger, I., Oesterhelt, D. and Lottspeich, F. (1986) EMBO J. 6, 1149–1158.
[15] Shopes, R.J. and Wraight, C.A. (1987) Biochim. Biophys. Acta 883, 409–425.
[16] Agalidis, I. and Reiss-Husson, F. (1992) Biochim. Biophys. Acta 1098, 201–208.
[17] Uffen, R.L. (1976) Proc. Natl. Acad. Sci. USA 73, 3298–3202.
[18] Maroti, P., Hanson, D.K., Baciou, L., Schiffer, M. and Sebban, P. (1994) Proc. Natl. Acad. Sci. USA 91, 5617–5621.
[19] Baciou, L., Rivas, E. and Sebban, P. (1990) Biochemistry 29, 2966–2976.
[20] Sebban, P. and Wraight, C.A. (1989) Biochim. Biophys. Acta 974, 54–65.
[21] Kirmaier, C. and Holten, D. (1987) Photosynth. Res. 13, 225–260.
[22] Schenck, C.C., Blankenship, R.E. and Parson, W.W. (1982) Biochim. Biophys Acta 680, 44–59.
[23] Sebban, P. (1988) FEBS. Lett. 233, 331–334.
[24] Gopher, A., Blatt, Y., Schönfeld, M., Okamura, M.Y., Feher, G. and Montal, M. (1985) Biophys. J. 48, 311–320.
[25] Gunner, M.R., Robertson, D.E. and Dutton, P.L. (1986) J. Phys. Chem. 90, 3183–3195.
[26] Bixon, M. and Jortner, J. (1986) FEBS Lett. 200, 303–308.
[27] Kleinfeld, D., Okamura, M.Y. and Feher, G. (1985) Biophys. J. 48, 849–852.
[28] Sebban, P. (1988) Biochim. Biophys. Acta 936, 124–132.
[29] Kalman, L. and Maroti, P. (1994) Biochemistry 33, 9237–9244.
[30] Baciou, L., Bylina, E., J. and Sebban, P. (1993) Biophys. J. 65, 652–660.
[31] Kleinfeld, D., Okamura, M.Y. and Feher, G. (1984) Biochim. Biophys. Acta 766, 126–140.
[32] Gao, J.-L., Shopes, R.J. and Wraight, C.A. (1991) Biochim. Biophys. Acta 1056, 259–272.
[33] Takahashi, E. and Wraight, C.A. (1992) Biochemistry 31, 855–866.
[34] Leguijt, T., Parot, P., Vermeglio, A., Crielaard, W. and Hellingwerf, K.J. (1993) Biochim. Biophys. Acta 1183, 292–300.
[35] Shinkarev, V.P., Verkhovski, M.I., Sabo, J., Zakharova, N.I. and Kononenko, A.A. (1991) Biochim. Biophys. Acta 1098, 117–126.
[36] Mancino, L.J., Dean, D.P. and Blankenship, R.E. (1984) Biochim. Biophys. Acta 764, 46–54.
[37] Woodbury, N.W.T. and Parson, W.W. (1984) Biochim. Biophys. Acta 767, 345–361.
[38] Woodbury, N.W.T., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Biochim. Biophys. Acta 851, 16–22.
[39] Baciou, L., Sinning, I. and Sebban, P. (1991) Biochemistry 30, 9110–9116.
[40] Okamura, M.Y. and Feher, G. (1992) Annu. Rev. Biochem. 61, 861–896.
[41] Labahn, A., Paddock, M.L., McPherson, P.H., Okamura, M.Y. and Feher G. (1994) J. Phys. Chem. 98, 3417–3423.
[42] Paddock, M.L., Rongey, S.H., Feher, G. and Okamura, M.Y. (1989) Proc. Natl. Acad. Sci. USA 86, 6602–6606.