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Effects of low temperature and lipid rigidity on the charge recombination process in *Rps. viridis* and *Rb. sphaeroides* reaction centers

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The pH dependence of the decay rate of the $P^+Q_A^-$ state (P is the primary electron donor, and Q_A, the first stable electron acceptor) was studied at cryogenic temperatures by the absorbance change technique. This study was done in aqueous solvent, in native reaction centers from *Rhodopseudomonas viridis* and in reaction centers from *Rhodobacter sphaeroides* in which the native Q_A was replaced by the 1-amino-5-chloroanthraquinone. The previously reported biphasicity of the $P^+Q_A^-$ decays in both types of reaction centers (Sebban, P. and Wraight, C.A. (1989) Biochim. Biophys. Acta 974, 54–65; Sebban, P. (1988) Biochim. Biophys. Acta 936, 124–132) is also observed in aqueous buffer at low temperature. At variance to room temperature, where a marked pH dependence was previously observed, the relative distributions of the two components of the decay (A_{fast} and A_{slow}) remain constant at 90 K, in the pH range 5.5–11. A_{fast}/A_{slow} is equal to 20:80 and 40:60, in *Rhodopseudomonas viridis* and in modified *Rhodobacter sphaeroides* reaction centers, respectively. To further study the possible influence of rigidity of the protein environment on the above parameters, we have reconstituted the reaction centers from *Rps. viridis* in dimyristoylphosphatidylcholine and dielaidoylphosphatidylcholine liposomes. Below the phase transition temperatures of those lipids ($T_c = 23°C$ and 9.5 °C, respectively), A_{slow} dominates similarly to what occurs at low temperature. However, as the temperature is increased above T_c, i.e. in the fluid phase of the lipid, A_{fast} becomes greater than 50%. The same viscosity effect was observed in glycerol, where the A_{fast}/A_{slow} ratio drops from 1.22 at 35 °C to 0.43 at −10 °C, decreases slightly to about 0.25 at −30 °C, and stays constant until 80 K. Our data support the idea of two well-defined states of the reaction centers whose relative distribution may vary depending on the physical conditions, such as low temperature or viscosity, imposed by the medium. At 90 K, the rate constants of $P^+Q_A^-$ charge recombination in *Rps. viridis* reaction centers observed in aqueous buffer vary in the pH range 5.5–11.5 in a way that is reminiscent of what has previously been observed at room temperature, but with much lower amplitude of the variations. It is suggested that proton distribution present in the dark before cooling or/and proton motion and uptake by the protein at low temperatures modulate the free energy level of the $P^+Q_A^-$ state.

Introduction

The light energy absorbed by the antenna of the photosynthetic organisms is converted into chemical free energy at the level of the reaction centers. In bacteria, the primary electron transfer process leads, in about 200 ps, to a charge-separated state between the primary electron donor, a dimer of bacteriochlorophyll P, and a quinone molecule Q_A. At temperatures below 200 K or in the presence of an inhibitor of the forward electron transfer to a secondary quinone, Q_B, charge recombination between P^+ and Q_A^- takes place. In *Rps. viridis* this process occurs at room temperature via repopulation of a thermally activated state, probably a relaxed state of P^+I^-, where I, a bacteriopheophytin molecule, is an intermediate electron carrier [1–5]. At temperatures lower than about 200 K, the back electron transfer from P^+ to Q_A^- was suggested to follow an electron tunnelling process [4]. It was recently pointed

Abbreviations: Bistris-propane, ((1,3-bis[tris(hydroxymethyl)methylamino]propane); CAPS, cyclohexylaminopropane; DEPC, dielaidoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; LDAO, lauryl dimethylamine *N*-oxide; Mes, 4-morpholineethanosulfonic acid.

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out that at room temperature, in *Rps. viridis*, the $P^+Q_A^-$ decay kinetics are biphasic with a marked pH dependence of the relative distributions of the two phases (A_{fast} and A_{slow}) [6,7]. These phases were attributed to the existence of two conformational states of the reaction centers existing in the dark [7]. Biphasicity of the charge separation kinetics has also been observed at low temperature in *Rb. sphaeroides, Rs. rubrum* and *Rps. viridis* reaction centers and also in chromatophores [6–9]. On the other hand, the decay from $P^+Q_A^-$ is exponential at room temperature in the reaction centers from *Rb. sphaeroides*, where Q_A is ubiquinone. However, when native ubiquinone is replaced by a low-potential quinone, such as an anthraquinone (in order to produce a similar energy gap between $P^+Q_A^-$ and P^+I^- as in *Rps. viridis*), the same type of biphasicity is observed with similar pH dependence of the relative amplitudes [10]. Since in native reaction centers from *Rb. sphaeroides* the decay from $P^+Q_A^-$ is exponential, the above heterogeneity of the decays is likely to arise from different interactions of Q_A with its environment. In order to further understand the possible relationship between the biphasicity observed in the different conditions described above, we have studied the role of temperature and viscosity on the relative distribution of the two phases.

Materials and Methods

Wild-type *Rps. viridis* cells were grown anaerobically (N_2 and CO_2) in light in the Hutner medium. Reaction centers were prepared as previously described [11]. The native reaction centers from *Rb. sphaeroides* were prepared as described by Rivas et al. [12]. To remove the primary quinone, the method of Okamura et al. [13] was used with the modifications introduction by Woodbury et al. [1]. The 1-amino-5-chloroanthraquinone was obtained from Interchim. A 10-fold excess was used in order to reconstitute the Q_A site. Dimyristoylphosphatidylcholine (DMPC; 14:0/14:0) was obtained from Bachem and dielaidoylphosphatidylcholine (DEPC; $18:1t\Delta^9/18:1t\Delta^9$) was obtained from Avanti. *Rps. viridis* reaction center DMPC and DEPC proteoliposomes were prepared as described in Ref. 7 for egg-yolk phosphatidylcholine proteoliposomes except that all steps were completed at about 25–26 °C and 20 °C, respectively, i.e., in the fluid phase of these lipids. For reconstitution, a lipid/protein ratio of 3 (w/w) was used.

The room-temperature absorbance change measurements were done on the same apparatus as in Refs. 5, 7 and 10. For low-temperature experiments, we used the apparatus described in Ref. 8 and 9.

Results and Discussion

Identity of A_{fast} and A_{slow} at room and low temperature

At 90 K, in *Rps. viridis* reaction centers, the biphasicity of the $P^+Q_A^-$ charge recombination is still observed, as was previously reported for the temperature range 80–200 K [6], and at 10 K [8]. The temperature dependence of the rate constants of the two phases, from cryogenic to room temperature, has already been reported [6]. Similar behavior of the two rates was

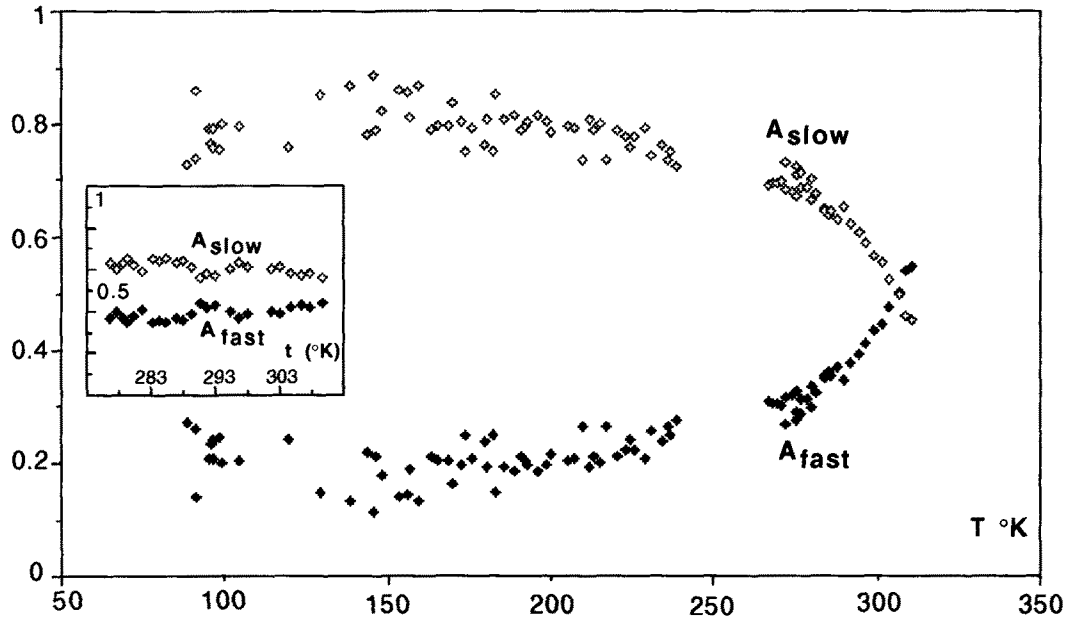

Fig. 1. Temperature dependence of the relative distribution of the two phases present in the $P^+Q_A^-$ recombination decay kinetics in the reaction centers from *Rps. viridis* in 66% glycerol (v/v) (pH 9). Two different sets of data (80–240 K and −10 to 35 °C) are represented on the same plot. Inset: same but without glycerol (pH 9). Buffer: Tris 10 mM, LDAO 0.1%, NaCl 100 mM, 4 mM *o*-phenanthroline.

observed, suggesting the identity of the two phases (or populations) at room and low temperature. To strengthen this hypothesis, we have studied the temperature dependence of the amplitudes of the two phases of $P^+Q_A^-$ charge recombination in reaction centers from *Rps. viridis*, in 66% glycerol (v/v). This is shown in Fig. 1. At 35°C, the A_{fast}/A_{slow} ratio is equal to about 0.55 : 0.45, but readily decreases to about 30 : 70 as the temperature decreases to −10°C. It still decreases slightly until −30°C to reach the value of 20 : 80, which stays constant until cryogenic temperature. Two conclusions can be drawn from these data. (i) The two phases observed at room temperature, where the recombination proceeds via a thermally activated charge-recombination process, are likely to correspond to the phases detected at 90 K, where electron tunnelling process dominates. (ii) The large effect observed between 35°C and −10°C is probably due to the change of the viscosity of the glycerol solution, since no such effect is detected in water, in the temperature range 0–35°C (inset of Fig. 1) (the viscosity change of a 66% glycerol (v/v) solution between 35°C and −10°C is more than 10, but about 2 for water). We shall confirm below this hypothesis, with the data obtained from reconstitution into DMPC and DEPC liposomes.

pH dependence, at low temperature, of A_{fast} and A_{slow} in Rps. viridis and AQ reconstituted Rb. sphaeroides reaction centers

In aqueous medium, at 90K, the lifetimes of the two phases measured in *Rps. viridis* reaction centers are $1/k_{Tfast} = 2.8 \pm 0.5$ ms ($A_{fast} = 20\%$) and $1/k_{Tslow} = 7.5 \pm 0.5$ ms ($A_{slow} = 80\%$), when the sample is cooled at pH 9. These values are temperature-independent from 90 K to 200 K. The samples were frozen in the dark at each pH. It must be noted that these experiments were done in the original buffer, without any cryosolvent. The pH dependencies of A_{fast} and A_{slow} at 90 K and 298 K are reported in Fig. 2. The behavior is obviously very different: the large pH effect observed at room temperature, already reported in Ref. 6, is not found at 90 K. A_{fast} and A_{slow} are constant over the pH range 5.5–11 with relative proportions of 20 : 80, with the possible exception of the values obtained at pH 11 and 11.5. In particular, the marked increase of A_{fast} from pH 7 to pH 9 at room temperature is no longer detected at 90 K. These results suggest that freezing the reaction center protein at low temperature blocks the two conformations and strongly favors the slow component. We have also performed the same titration in the reaction centers from *Rb. sphaeroides*, where the native Q_A has been replaced by the 1-amino-5-chloroanthraquinone (Fig. 3). We have previously demonstrated the biphasicity of the charge recombination in such reaction centers [10]. Similarly to *Rps. viridis*, biphasicity of the $P^+Q_A^-$ charge recombination kinetics was also observed at 90

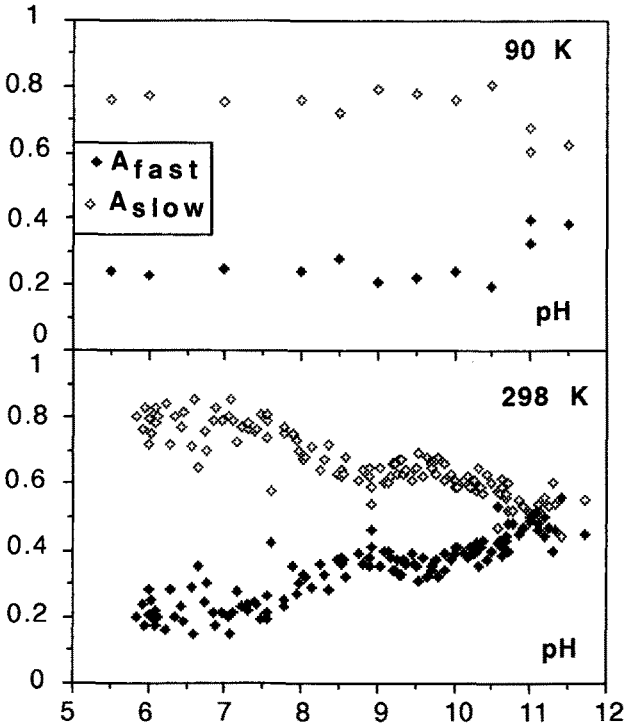

Fig. 2. pH titration of the relative distribution of the two phases of $P^+Q_A^-$ decay kinetics in the reaction centers from *Rps. viridis* at 90 K and 298 K (data from Ref. 5). The low-temperature data are obtained in samples frozen in the dark, in the absence of cryosolvent. Conditions: 20 mM Mes, or Bistris-propane or CAPS depending on the pH, 4 mM *o*-phenanthroline, 100 mM NaCl, 0.1% LDAO.

K. The measured lifetimes are equal to 60 ± 10 ms and 190 ± 20 ms. To minimize the least-square value of the decay curve fitting, the addition of a third component was necessary. Its lifetime is about 18 ± 5 ms and its initial amplitude, 5–10%. These values suggest that this component arises from the reaction center population in which Q_A has not been removed during the biochemical treatment. (i) The lifetime is roughly equal to the average lifetime of the $P^+Q_A^-$ recombination measured at 10 K by Verméglio et al. [8], in native *Rb. sphaeroides* reaction centers from R26 and 2.4.1. strains. (ii) At room temperature, in the same modified reaction center preparation, a similar amplitude (5–10%) was found for the reaction centers which have retained the native Q_A [10]. The difference in the pH titration curves of A_{fast} and A_{slow} measured at 90 K and room temperature [10] (Fig. 2) is even more pronounced than in *Rps. viridis*. At room temperature the strong increase of A_{fast} between pH 7 and pH 8 leads to equal amplitudes of the two phases between pH 8 and pH 10. From pH 10 to pH 11.5 a substantial increase of A_{fast} is also observed leading to a marked inversion of the two populations. This behavior is in contrast to what is measured at low temperature where A_{fast} and A_{slow} remain constant in the pH range 5.5–11.5. These observations support the hypothesis of two conformations of the reaction centers

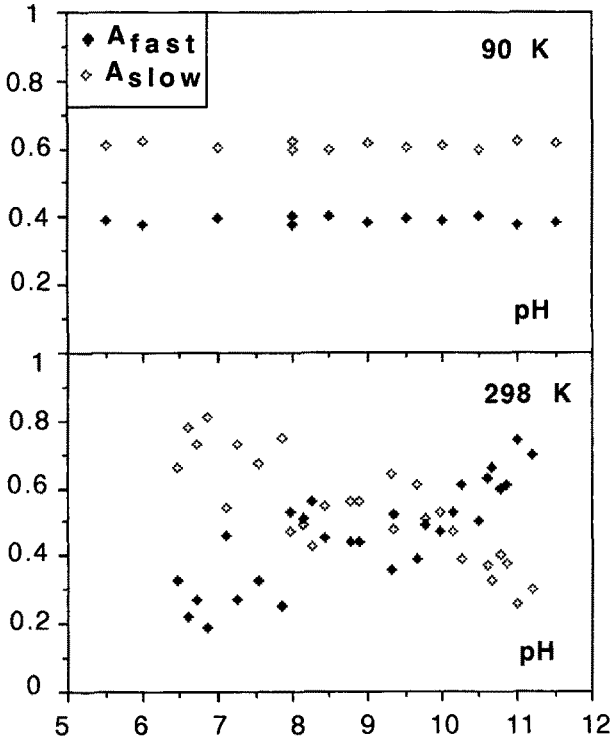

Fig. 3. pH titration of the relative distribution of the two phases of $P^+Q_A^-$ decay kinetics in *Rb. sphaeroides* reaction centers which native Q_A ubiquinone has been replaced by the 1-amino-5-chloroanthraquinone (measurement at 90 K) and with anthraquinone (measurement at 298 K, data from Ref. 6). The low-temperature data are obtained on samples frozen in the dark, in the absence of cryosolvent. Conditions: 20 mM Mes, or Bistris-propane or CAPS depending on the pH, 0.1% LDAO.

stabilized at low temperature whatever the pH or the nature of the quinone acting as primary acceptor. On the other hand, at room temperature it is likely that the equilibration of the two conformational states depends on the interaction of Q_A with its surrounding.

Influence of the lipid rigidity on the relative distribution of A_{fast} and A_{slow}

To confirm the possible influence of the rigidity of the environment (suggested by the glycerol data) on the relative distribution of A_{fast} and A_{slow}, we have reconstituted the reaction centers from *Rps. viridis* in DMPC and in DEPC vesicles. The phase-transition temperatures (T_c) of these phospholipids are 23°C and 9.5°C, respectively [15]. It is very likely that these values are unchanged in the proteoliposomes, since we have used for the reconstitution a very low protein/lipid ratio: i.e., 0.33. At 28°C, i.e., above the T_c values of both lipids, the rate constants of $P^+Q_A^-$ charge recombination were found almost equal, and close to their values in PC liposomes [7]: $1/k_{fast} = 0.64 \pm 0.05$ ms and $1/k_{slow} = 2.1 \pm 0.05$ ms. Fig. 4 shows the A_{fast} and A_{slow} curves over a temperature range that cross over T_c. These experiments were done in the presence of *o*-phenanthroline, at pH 9. Above T_c, A_{fast} represents about 55% of the decay, but its amount decreases when temperature is lowered below T_c, so that at 5°C for DMPC and −2°C for DEPC, A_{fast} represents less than 40% of the $P^+Q_A^-$ decay. We have also measured, at pH 6, 8 and 10, the $P^+Q_A^-$ decay kinetics in the DMPC proteoliposomes below (12°C) and above (35°C) T_c, with an empty Q_B site in the reaction center, or in the presence of terbutryn. The results are presented in Table I. There is no exception to the trend above, observed in the presence of *o*-phenanthroline. For the three samples and the three pH values, A_{slow} is clearly favored by lowering the temperature below T_c. Interestingly, the relative distribution of the two phases depends on the occupancy of the Q_B pocket. In particular, in the pH range 7–9, the highest difference between the amount of A_{fast} and A_{slow} is observed when neither herbicide nor Q_B is present in the pocket. A more detailed analysis of

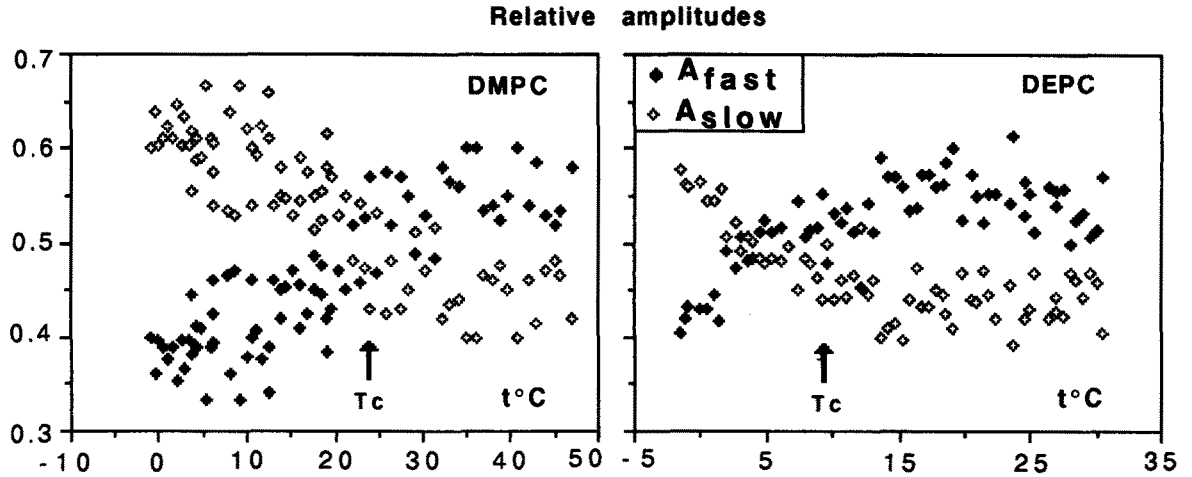

Fig. 4. Lipid phase transition effect on the relative distribution of the two phases of $P^+Q_A^-$ decay kinetics in *Rps. viridis* reaction centers reconstituted in liposomes of DMPC and DEPC. Conditions: 10 mM Tris (pH 9), 4 mM *o*-phenanthroline, 100 mM NaCl.

TABLE I

Effect of lipid phase transition of the relative amplitude of the fast component of $P^+Q_A^-$ charge recombination in Rps. viridis reaction centers reconstituted in DMPC vesicles

The amplitudes are normalized to 1. They are given with a standard error of ±0.05. The measurements below T_c were done at 12 °C, and above T_c at 35 °C.

Conditions	A_{fast}					
	$-Q_B$		+Ophe [a]		+Terb [b]	
	$<T_c$	$>T_c$	$<T_c$	$>T_c$	$<T_c$	$>T_c$
pH 6	0.28	0.45	0.30	0.50	0.35	0.45
pH 8	0.15	0.30	0.40	0.45	0.25	0.40
pH 11	0.45	0.55	0.45	0.63	0.45	0.58

[a] 4 mM *o*-phenanthroline.
[b] 60 μM terbutryn.

the complete pH titration of the A_{fast} and A_{slow} curves, with different contents of the Q_B pocket, will be described elsewhere.

The above data suggest that the relative distribution of the two reaction center conformations is influenced by physical parameter such as freezing by low temperature and lipid rigidification of the close environment of the protein. This supports the view of two well-defined conformations of the reaction centers that can interconvert depending on physical conditions imposed by the medium.

pH dependence of k_{fast} and k_{slow} at low temperature

We have investigated whether, at 90 K, pH has an effect on the rate constants of the kinetics of $P^+Q_A^-$ charge recombination in *Rps. viridis* reaction centers, with no cryosolvent added to the samples. The pH dependence of k_{fast} and k_{slow} at 90 K are shown in Fig. 5. As mentioned above, A_{slow} largely dominates at low temperature. As a consequence the error bars for k_{fast} (≈ 20%) are much larger than for k_{slow} (≈ 6%). The samples were cooled down at each pH in the dark. Shopes and Wraight [4] have suggested that in *Rps. viridis*, pH, and consequently the protonation of some amino-acid groups, modulates the free energy difference between $P^+Q_A^-$ and P^+I^- (ΔG). In addition, proton uptake have been detected in the $P^+Q_A^-$ and PQ_A states of the reaction centers from *Rb. sphaeroides* [16]. As observed in Fig. 5, at low temperature, acceleration of the charge recombination occurs at high pH, reminding the room temperature behavior of the rate constants [6]. Different proton distributions present in the dark in the vicinity of Q_A, may have been frozen when cooling the sample at the different pH. This would modulate the energy level of $P^+Q_A^-$ formed at low temperature. It is also possible, since protons are known to be highly mobile at low temperature (see for example Ref. 17), that some proton uptake by groups nearby Q_A^- still occurs at 90 K after the flash. In the pH titration curve observed in Fig. 5, k_{fast} and k_{slow} variations are small compared to room temperature, where the rate constants accelerate about 2.5-times between pH 8 and pH 11 [6]. Similar effects were observed in 66% glycerol (v/v) at room and low temperature. This suggests that such putative proton uptake at low temperature would arise from water closely associated with the protein or from protonable groups within the protein. It is possible that the variations of the energy level of $P^+Q_A^-$, due to different protonation states of the protein, are still important at low temperature. However, small effects are expected on k_{fast} and k_{slow} since these kinetic parameters are supposed to be directed at these temperatures by an electron tunnelling process. Gunner et al. [18] have demonstrated that the rate of electron transfer from Q_A^- to P^+ is weakly dependent on ΔG at low temperature. Our attempt to detect some slight concomitant increase of k_{fast} and k_{slow} with increasing temperature (which could have revealed some contribution of a thermally activated process) from 80 K to 190 K was unsuccessful (data not shown). The variations of k_{fast} and k_{slow} that we detect in the pH titration curve at 90 K are thus consistent with changes of the energy level of $P^+Q_A^-$ due to protonations established before the flash in the dark or/and to proton motions in the charge-separated state created at low temperature.

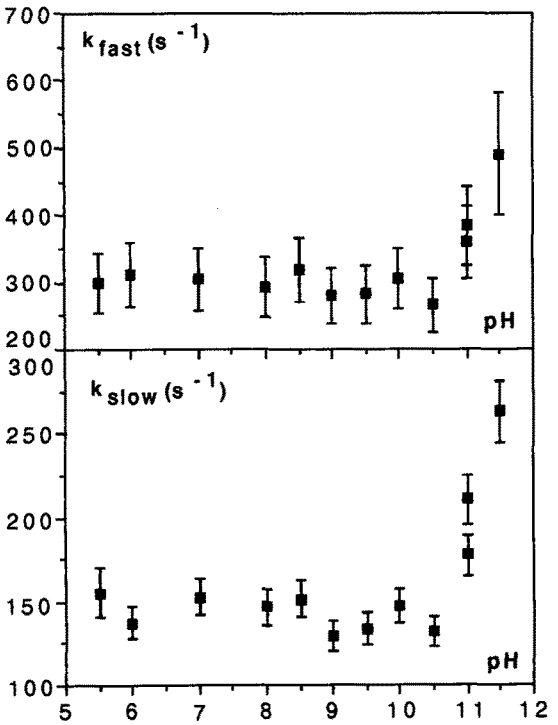

Fig. 5. pH dependence of the kinetic rate constants of $P^+Q_A^-$ charge recombination in the reaction centers from *Rps. viridis* frozen in the dark, measured at 90 K, in the absence of cryosolvent. 4 mM *o*-phenanthroline was present in the samples.

Conclusion

It has previously been reported that two kinetic phases are present in the charge recombinations decays ($P^+Q_A^-$, $P^+Q_B^-$) of native reaction centers from *Rps. viridis* (and of chromatophores) [6,7] and similarly of *Rb. sphaeroides* reaction centers where the native Q_A has been replaced by various low potential quinones such as anthraquinones [5,10]. In addition to the similar pH titration curves of A_{fast} and A_{slow} measured at room temperature for the two types of RC, we have shown that freezing by low temperature cancels in the same way the pH effect on the relative distribution of these phases and favors the slow component. This supports the hypothesis of a similar phenomenon in both kinds of reaction center. In native reaction centers from *Rb. sphaeroides* biphasicity of $P^+Q_A^-$ decays is also induced by lowering the temperature below 200 K [8,9]. Similarities exist between these two kinetic states, and what we have measured in *Rps. viridis* and in *Rb. sphaeroides* modified reaction centers: (i) the lifetimes associated with the two phases have the same ratio, i.e., 3–4; (ii) the two phases have slightly different light-induced absorbance spectra which can be differentiated near the isosbestic point [6,8,9]; (iii) at low temperature, however, the A_{fast}/A_{slow} ratio depends on the nature of Q_A (40 : 60 in *Rb. sphaeroides* reaction centers with anthraquinone acting as Q_A and 60 : 40 in native reaction centers). This suggests that some analogy exists between all these phenomena. The low-temperature-induced biphasicity in *Rb. sphaeroides* reaction centers could be explained by changes, with temperature, of the interactions of Q_A with its surrounding.

We have previously demonstrated that two states of the reaction centers from *Rps. viridis* exist in vivo, probably in the dark, i.e., before any charge separation occurs [7]. We show in this work that, in addition to pH, salt concentration and herbicide effects, the relative distribution of the two populations is sensitive to physical changes such as solvent viscosity, lipid rigidity or low-temperature freezing. These approaches offer simple and effective means to adjust the relative proportions of A_{fast} and A_{slow}, which will be useful in further investigation of the nature of the underlying protein states using 'physical techniques' (FTIR, EPR, ENDOR) to probe changes in the immediate vicinity of the Q_A site.

References

1 Woodbury, N.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Biochim. Biophys. Acta 851, 16–22.
2 Gopher, A., Blatt, Y., Schönfeld, M., Okamura, M.Y. and Feher, G. (1985) Biophys. J. 48, 311–320.
3 Gunner, M.R., Robertson, D.E. and Dutton, P.L. (1986) J. Phys. Chem. 90, 16, 3183–3195.
4 Shopes, R.J. and Wraight, C.A. (1987) Biochim. Biophys. Acta 893, 409–425.
5 Sebban, P. (1988) FEBS Lett. 233, 331–334.
6 Sebban, P. and Wraight, C.A. (1989) Biochim. Biophys. Acta 974, 54–65.
7 Baciou, L., Rivas, E. and Sebban, P. (1990) Biochemistry 29, 2966–2976.
8 Verméglio, A., Thiery, J. and Parot, P. (1988) in The Photosynthetic Bacterial Reaction Center: Structure and Dynamics (Breton, J. and Verméglio, A., ed.s), pp. 251–258, Plenum Press, New York.
9 Parot, P., Thiery, J. and Verméglio, A. (1987) Biochim. Biophys. Acta 893, 534–543.
10 Sebban, P. (1988) Biochim. Biophys. Acta 936, 124–132.
11 Prince, R. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 329–334.
12 Rivas, E., Reiss-Husson, F. and Le Maire, M. (1980) Biochemistry 19, 2943–2950.
13 Okamura, M.V., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. USA 72, 3492–3495.
14 Sinning, I., Michel, H., Mathis, P. and Rutherford, A.W. (1989) Biochemistry 28, 5544–5553.
15 Small, D. (1986) in 'Handbook of Lipid Research': The Physical Chemistry of Lipids (Hanahan, D.J., ed.), Plenum Press, New York.
16 Maròti, P. and Wraight, C.A. (1989) Biochim. Biophys. Acta 934, 329–347.
17 Kunst, M. and Warman, J.M. (1980) Nature 288, 465–467.
18 Gunner, M.R., Robertson, D.E. and Dutton, P.L. (1986) J. Phys. Chem. 90, 3183–3195.