The DMPC lipid phase transition influences differently the first and the second electron transfer reactions in bacterial reaction centers

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Abstract Photosynthetic reaction centers (RCs) from *Rhodobacter sphaeroides* were incorporated in dimyristoylphosphatidylcholine (DMPC) liposomes. The first and second electron transfer rates ($k_{AB}(1)$ and $k_{AB}(2)$, respectively) between the first and the second quinone electron acceptors have been measured as a function of temperature, across the phase transition of DMPC (23°C). The Eyring plots of $k_{AB}(1)$ display straight lines. In contrast, the Eyring plots for $k_{AB}(2)$ in proteoliposomes show a break at about 23.5°C. This physical discrimination between the two electron transfer reactions demonstrates that the stiffness of the lipid environment of the RCs and/or the protein–protein interactions influence the parameters governing $k_{AB}(2)$, but not the gating process limiting $k_{AB}(1)$.

Key words: Liposome; Membrane protein; Reaction center; Electron transfer

1. Introduction

In photosynthetic bacteria, reaction center (RC) proteins convert light energy into chemical free energy. This is accomplished through a series of electron transfer reactions which leads to a transmembrane charge separation between a dimer of bacteriochlorophylls ('P') and a system of two quinones (Q_A and Q_B, respectively, both ubiquinone 10), bound to the cytoplasmic side of the protein [1,2]. The three-dimensional structure of this protein is known at 2.2 Å resolution for the native *Rhodobacter* (Rb.) *sphaeroides* [3].

Following the fast (~200 ps) creation of the $P^+Q_A^-$ state [4,5], the first electron transfer from Q_A^- to Q_B occurs with a rate ($k_{AB}(1)$) of about $(80\ \mu s)^{-1}$ in the native RCs [2,6–11]. This process has been proposed to be 'gated' by protein dynamics and/or protonation events [2,12–25]. The protonation of L212Glu has been suggested to be one of these steps [26–30]. In isolated RCs, the formation of either semiquinone is accompanied by substoichiometric proton uptake by residues interacting with Q_A^- and/or Q_B^- [12–16,20,31–33]. In contrast, in the native chromatophore membranes, below pH 6.8, proton uptake results into the direct protonation of Q_B^- [34].

The second electron transfer, from Q_A^- to Q_B^-, is associated with the net transfer of two protons to Q_B^-. This process, which occurs in isolated RCs with a rate ($k_{AB}(2)$) of about $(300\ \mu s)^{-1}$ at neutral pH, was demonstrated to require the direct participation of L213Asp [35,36]. It has recently been shown that this process depends on the free energy gap between Q_A^- and Q_B^-, therefore suggesting that the electron transfer is likely to be the rate-limiting process [21,22]. Indeed, a fast and reversible proton transfer was suggested to precede the slower electron transfer. Accordingly, it has been proposed that $k_{AB}(2)$ depends on both the proportion of protonated Q_B^- ($f(Q_BH)$) and the electron transfer rate (k_e) [22]:

$$k_{AB}(2) = f(Q_BH) \times k_e \quad (1)$$

The first and second electron transfer reactions have mainly been studied in RCs isolated in detergent. However, the first [7] and the second [34,37] electron transfer processes have been shown to be significantly faster in native membranes than in isolated RC.

In order to investigate the influences of the protein–protein interactions and of the rigidity of the lipid environment on both electron transfer reactions, we have measured the temperature dependencies of these processes in RCs reconstituted in dimyristoylphosphatidylcholine (DMPC) vesicles (the fluid to crystalline phase transition temperature of this lipid is about 23°C [38]) and compared them to RCs isolated in detergent or in native membranes.

We show that the phase transition of DMPC modifies the thermodynamic parameters associated with the second electron transfer process but not with the first one.

2. Materials and methods

2.1. Bacterial culture

Rb. sphaeroides (strain 2.4.1) cells were grown in Erlenmeyer flasks filled to 50% of the total volume with malate yeast medium. The cultures were grown in semi-aerobic conditions in the dark, at 30°C on a gyratory shaker (100 rpm). Since neither the spectroscopic results obtained in chromatophores nor in RCs reconstituted in liposomes show any difference between photosynthetic or semi-aerobic growth conditions, we chose the latter conditions because of their convenience.

2.2. Protein purification

Cells from *Rb. sphaeroides* strains were disrupted by sonication in 20 mM Tris (pH 8) buffer in the presence of DNAse and phenylmethylsulfonyl fluoride (1 mM). The intra-cytoplasmic membranes were purified as described in [39]. For the $k_{AB}(2)$ experiments the supernatant was collected in order to keep the cyt_c as endogenous electron donors to P^+. The membrane solubilization was done first by addition

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Abbreviations: RC, reaction center; DMPC, dimyristoylphosphatidylcholine; P, primary electron donor (bacteriochlorophyll dimer); Q_A, primary quinone; Q_B, secondary quinone

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of LDAO (Fluka Chemie) to a final concentration of 0.35% in the presence of 100 mM NaCl. The RCs were extracted by a second addition of LDAO to a final concentration of 0.8% in similar conditions. The solubilized RCs were subsequently purified on a DEAE Sepharose CL-6B (Pharmacia) column and eluted at an ionic strength equivalent to 250 mM NaCl. The ratio of absorbance at 280/802 nm was in the range 1.4–1.5. The RC isolation and purification were achieved according to the procedure described in [39]. The occupancy of the Q_B site was commonly reconstituted (80% ± 10%) by adding 50 μM of ubiquinone$_6$ (Sigma).

2.3. Proteoliposome preparation

DMPC (14:0/14:0; phase transition at 23°C in water) was obtained from Sigma. The liposomes reconstituted RCs were prepared as in [40].

The orientation of the RC proteins in the liposomes was estimated by comparing the total amount of RCs present in the liposomes (estimated from the extent of P^+ formation measured at 430 nm after a saturating laser flash) to the amount of RCs oriented with P outside the vesicles (estimated from cyt c accessibility). 80 ± 10% of the RC population was found to be oriented with P^+ towards the outside of the liposomes.

The proteoliposomes of the same preparation were used to measure $k_{AB}(1)$ and $k_{AB}(2)$.

2.4. Freeze-fracture electron microscopy

Experiments of freeze-fracture electron microscopy were performed as in [40].

2.5. Spectroscopic measurements

2.5.1. Flash induced absorbance changes. The home-made flash (pulsed YAG laser at 532 nm) spectrophotometer has been described in [41].

2.5.2. Charge recombination kinetics. The rate constant of the $P^+Q_A^- \rightarrow PQ_A$ (k_{AP}) and $P^+Q_B^- \rightarrow PQ_B$ (k_{BP}) charge recombinations were measured at 430 nm, at a wavelength reflecting mainly the P^+–P absorbance changes.

2.5.3. Electron transfer kinetics. The kinetics of the first electron transfer from Q_A^- to Q_B were measured at 750 nm, in the electrochromic band shift of the bacteriopheophytins, in the presence of 25 μm UQ_6 and ~1 μM RCs.

The kinetics of the second electron transfer were measured in RCs at 450 nm, at a wavelength where Q_A^- and Q_B^- slightly differ in their respective extinction coefficients. These kinetics were obtained in the presence of 20 μM reduced cyt c, 500 μM sodium ascorbate, 25 μM UQ_6 and ~1 μM RC. For proteoliposomes, cyt c was pre-reduced by 1 mM sodium ascorbate which was removed on a PD10 column (Sigma). For experiments on chromatophores, 10 μM valinomycin was added to remove transmembranous electric field. Temperature was monitored with a precision of 0.5°C.

2.6. Activation parameters for $k_{AB}(1)$ and $k_{AB}(2)$

In order to determine the thermodynamic parameters associated with $k_{AB}(1)$ and $k_{AB}(2)$, the temperature dependencies of the rates were fitted using the Eyring model [42]:

$$k = (k_B T/h)e^{(-\Delta G^{\ddagger}/RT)} \quad (2)$$

where k_B is the Boltzmann constant, T, the absolute temperature, h, Planck's constant and $\Delta G^{\ddagger}$, the activation free energy.

The activation energy ($\Delta H^{\ddagger}$) and the entropy ($\Delta S^{\ddagger}$) contribution were respectively determined from the slope of the lines and from their intercept with the y-axis.

The temperature titration curves of $k_{AB}(1)$ and $k_{AB}(2)$ were fitted using a Newton algorithm.

3. Results

3.1. Free-fracture electron microscopy

The free-fracture electron micrographs obtained from freezing the samples from 30°C and 4°C, respectively, are presented in Fig. 1. At 30°C, the RCs are randomly arranged in the DMPC liposomes. In contrast, at 4°C, strong segregation of the proteins occurs.

3.2. The first electron transfer reaction

The Eyring plots of $k_{AB}(1)$ (pH 7.8) for the RCs isolated in detergent and for DMPC proteoliposomes are presented in Fig. 2. The kinetic values obtained for the RCs embedded in the lipid membranes are notably higher than in RCs in detergent (Table 1). Indeed, $k_{AB}(1)$ at 13°C measured in RCs in detergent and in DMPC vesicles are 2600 ± 10% s^{-1} and 7050 ± 10% s^{-1}, respectively. At 30°C, these rates increase to 4450 ± 10% s^{-1} and 8700 ± 10% s^{-1}, respectively. The $k_{AB}(1)$ increase observed here in the lipid environment compared to the RCs isolated in detergent is consistent with previous works which have shown that the first electron transfer notably accelerates in chromatophores [34,37,43]. For both samples, the data were well fitted with a straight line assuming Eq. 2. The enthalpy contributions resulting from this fit are $\Delta H^{\ddagger} = 0.40 \pm 0.01$ eV and 0.26 ± 0.01 eV in isolated RCs and in DMPC proteoliposomes, respectively (Table 2). The differences in the enthalpy values are compensated by the entropic contributions, leading to very similar $\Delta G^{\ddagger}$ values in both environments

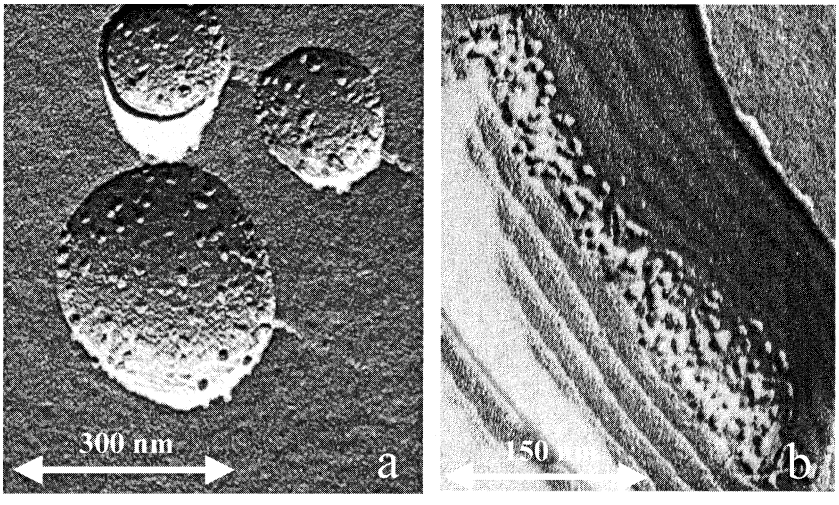

Fig. 1. Freeze-fracture electron micrographs of *Rb. sphaeroides* RC–DMPC vesicles. A: Frozen from $T = 30$°C. B: Frozen from $T = 4$°C.

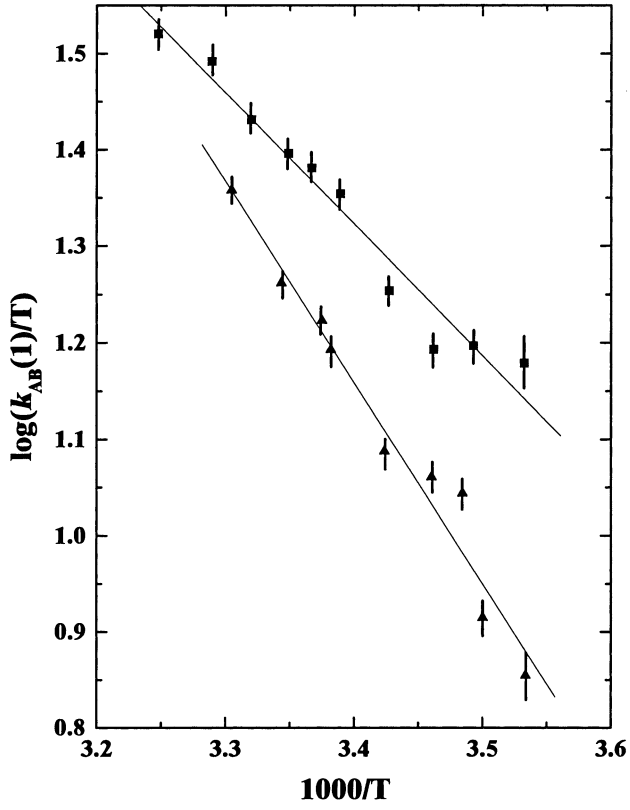

Fig. 2. Eyring plots of the first electron transfer rate measured at 750 nm and pH 7.8 in the RCs (~1 μM) isolated in detergent (triangles) and reconstituted in DMPC vesicles (squares). Conditions: isolated RCs: 0.05% LDAO, 10 mM Tris, 100 mM NaCl, 50 μM UQ_6; in DMPC proteoliposomes: ratio lipid/protein 4, 10 mM Tris, 100 mM NaCl, 50 μM UQ^6. Each data point represents the average of 128 traces. The displayed curve relates to one sample preparation. The titration curves measured in the samples from three different preparations could be well fitted with a straight line. The bars represent the plus and minus standard error on each point.

(0.520 ± 0.005 eV in RCs and 0.510 ± 0.005 eV in proteoliposomes).

In both cases, the kinetics showed bi-exponential decays with rates differing by a factor of 3–4. This heterogeneity has previously been reported [7,10,11,44]. However, in DMPC proteoliposomes, due to a lower signal/noise ratio, the absorption decays were better analyzed with a single exponential. Therefore, for comparison of the data in both environments, we have fitted the decay curves with a single exponential for both samples (Table 1). However, this does not change our conclusions since both rates in a double exponential peeling show a linear Eyring plot for both samples.

3.3. The second electron transfer reaction

Similarly to $k_{AB}(1)$, the DMPC environment induces higher $k_{AB}(2)$ values than the detergent micelle environment (Table 2). Compared to RCs isolated in detergent micelles, the kinetics are approximately three and nine times faster in DMPC and in chromatophores, respectively.

The Eyring plots of $k_{AB}(2)$ (pH 7.8) in the RCs isolated in detergent, in DMPC proteoliposomes and in chromatophores are presented in Fig. 3. The temperature dependence of $k_{AB}(2)$ for isolated RCs is well fitted by a straight line, leading to $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ values of 0.36 ± 0.01 eV and 0.555 ± 0.005 eV, respectively. For chromatophores a straight line is also found and leads to $\Delta H^{\ddagger}$ and $\Delta G^{\ddagger}$ values of 0.29 ± 0.01 eV and 0.550 ± 0.005 eV, respectively.

Interestingly, the figure is different in DMPC proteoliposomes. Indeed, a break is observed in the Eyring plot for $k_{AB}(2)$. The experiments presented were done in three different samples and yielded the same results (the same three have shown straight lines in the Eyring plots of $k_{AB}(1)$). For temperatures above the break point, $\Delta H^{\ddagger}$ for $k_{AB}(2)$ equals 0.27 ± 0.01 eV and $\Delta G^{\ddagger}$ 0.555 ± 0.005 eV. For temperatures below the break point, $\Delta H^{\ddagger} = 0.64 \pm 0.01$ eV, but $\Delta G^{\ddagger}$ remains the same (0.550 ± 0.005 eV) due to a substantial increase of the entropic term.

3.4. Determination of the temperature of the break point of the Eyring plot of $k_{AB}(2)$ in DMPC proteoliposomes

In order to determine the temperature corresponding to the break point, we have split the points into two parts, those below 15°C and those above 27°C. The lines corresponding to the best fit for each series of points cross at a temperature of 23.5°C. To evaluate the error bar on this value, we have drawn for each series of points (below 15°C and above 27°C) the lines corresponding to the envelopes allowed by the maximum error bars on the points. Taking the lowest and the highest possible cross points of the envelope lines leads to 1000/T extreme values of 3.395 and 3.345 (i.e. 21.5°C and 26°C). Therefore, the break point can be estimated to be $T = 23.5 \pm 2.5$°C. This is close to the transition temperature of DMPC in solution.

4. Discussion

The present study shows a discrimination between the effect of the DMPC phase transition on the first and on the second electron transfers between the two quinones in RCs from *Rb. sphaeroides*.

The main structural effect of the fluid to crystalline transition of the DMPC proteoliposomes is to segregate the proteins. This is shown in Fig. 1 and has previously been reported

Table 1
Kinetics of the first and second electron transfers at 13°C and 30°C as a function of the surrounding environment

Electron transfer step	Condition	Kinetics at 13°C (s^{-1}) ± 10%[a]	Kinetics at 30°C (s^{-1}) ± 10%[a]
First	Isolated RC	2600	7050
	DMPC	4450	8700
Second	Isolated RC	213	1180
	DMPC	455	2280
	Chromatophores	620	2860

[a]Experimental error.

for bacteriorhodopsin [45,46], rhodopsin [47] and for RCs from *Rhodopseudomonas viridis* [40]. In these studies it was proposed that below the phase transition temperature, the protein–protein contacts become the dominant process. However, the rigidity of the lipid environment below this temperature may also play an important role. Since both effects are likely to be present at the same time, we analyze below their respective possible consequences on the first and second electron transfer processes.

In the case of increased protein–protein interactions due to the protein segregation below the phase transition, it is likely that the electrostatic environment of the RCs may be modified because of the change from a low dielectric constant value in the DMPC liquid state environment to a higher value due to the close contacts between RCs. In contrast, the stiffness of the environment is expected to affect mainly protein rearrangements associated with the electron transfer process.

4.1. Effect of the DMPC phase transition on the first electron transfer

The first electron transfer is known to be kinetically gated by conformational rearrangements of the protein [2,12–25]. The findings that the Eyring plots of $k_{AB}(1)$ can be fitted with a straight line in DMPC proteoliposomes shows that the increased packing of the RC proteins below the phase transition or/and the more rigid lipid environment do not influence the thermodynamics of the gating process. In addition, the protonation state of L212Glu, which has been proposed to be coupled to this process [26–30], is also not affected by any of these two phenomena. Therefore, neither does the rigidity of the lipids modify the conformational rearrangements associated with the gating process, nor do the possible changes in the electrostatic environment influence the pK_a of L212Glu. In both cases this is rather surprising. This therefore highlights even more the different effect observed on the second electron transfer process.

4.2. Effect of the DMPC phase transition on the second electron transfer

At variance to what is observed for $k_{AB}(1)$, the DMPC lipid phase transition significantly modifies the thermodynamics associated with $k_{AB}(2)$ (Fig. 3, Table 1). Indeed, a break is observed at 23.5°C in the Eyring plots.

The second electron transfer process has been shown to be limited by slow electron transfer preceded by a fast and reversible proton transfer step [21,22]. As described in Eq. 1, $k_{AB}(2)$ depends on both the proportion of protonated Q_B^-, $f(Q_BH)$, and the electron transfer rate, k_e. Both $f(Q_BH)$ and k_e may be affected by the DMPC phase transition.

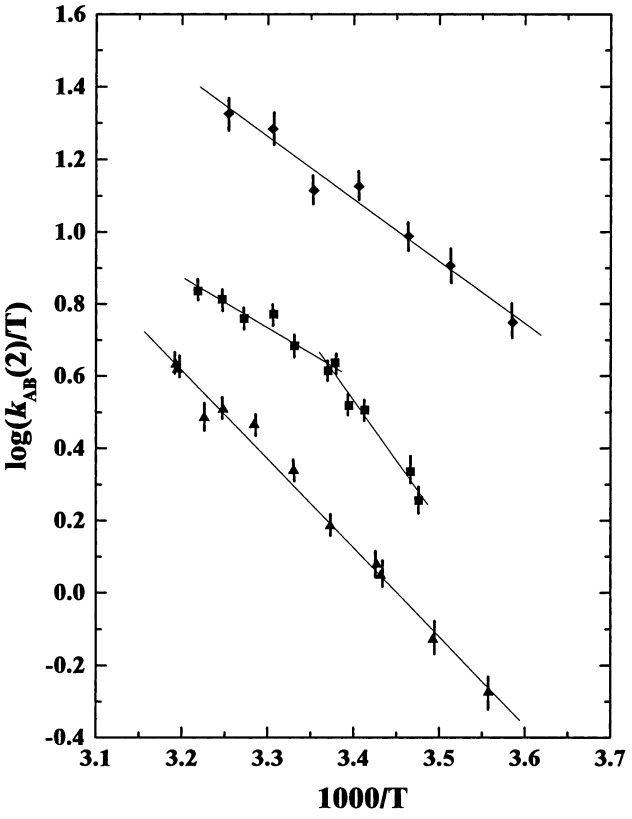

Fig. 3. Eyring plots of the second electron transfer rate measured at 450 nm and pH 7.8 in the RCs isolated in detergent (triangles), reconstituted in DMPC vesicles (squares) and embedded in the native membrane (diamonds). Conditions: isolated RCs: ~1 μM RC, 0.05% LDAO, 10 mM Tris, 100 mM NaCl, 50 μM UQ_6, 20 μM cyt c, 500 μM ascorbate; DMPC proteoliposomes: ~1 μM RC, ratio lipid/protein 4, 10 mM Tris, 100 mM NaCl, 50 μM UQ_6, 20 μM reduced cyt c; native membranes: 100 mM NaCl, 10 μM valinomycin, 20 μM cyt c, 500 μM ascorbate. The displayed curve relates to one sample preparation (the same as that presented in Fig. 2 for the Eyring plot of the first electron transfer rate). The titration curves measured in the samples from three different preparations have shown the same break at ~23.5°C. The bars represent the plus and minus standard error on each point.

4.3. Effect on $f(Q_BH)$

The value of $f(Q_BH)$ depends both on the pK of Q_B^- and on the integrity of the proton transfer pathway to this ultimate proton acceptor.

The pK of Q_B^- when Q_A is oxidized (p$K_{QB^-(QA)}$), has been shown to be notably different in RCs isolated in detergent and in chromatophores [34]. It is likely that the pK of Q_B^-, when

Table 2
Energetics of the first and second electron transfers as a function of the surrounding environment

Electron transfer step	Condition	ΔH (eV) ± 0.01[a]	ΔG (eV) ± 0.005[a]
First	Isolated RC	0.40	0.520
	DMPC	0.26	0.510
Second	Isolated RC	0.36	0.555
	DMPC, $T<23$°C	0.64	0.550
	DMPC, $T>23$°C	0.27	0.555
	Chromatophores	0.29	0.550

The thermodynamic parameters are calculated from the data presented in Figs. 2 and 3.
[a]Experimental error.

Q_A is reduced ($pK_{QB^-(QA^-)}$), also depends on the environment of the RC. According to Eq. 1, a change of $pK_{QB^-(QA^-)}$ due to the modification of the electrostatic environment would affect $k_{AB}(2)$ through the $f(Q_BH)$ factor of Eq. 1. That would explain in part the observed effect. However, no information is available yet on $pK_{QB^-(QA^-)}$ in DMPC liposomes.

Alternatively, if the rigidity of the lipids below the phase transition is involved, it may affect $f(Q_BH)$ through a modification of the proton pathway to L213Asp, which has been demonstrated to be of crucial importance for the delivery of the first proton to Q_B [36,48]. This may happen through a disorganization of the hydrogen bond chains which have been suggested to be involved in this process [49].

4.4. Effect on k_e

The DMPC phase transition may also influence $k_{AB}(2)$ via a modification of the parameters controlling k_e [50]. This may occur via changing the reorganization energy (λ) or/and the free energy barrier (ΔG^0) associated with this electron transfer process. It is likely that a change in λ associated with k_e should also take place in the λ value associated with $k_{AB}(1)$. Since λ and the gating process are related parameters, we should also observe a slope break in the temperature dependence of $k_{AB}(1)$. That is not the case.

We therefore favor the hypothesis that the DMPC phase transition may modify the ΔG^0 value associated with k_e. This is more likely to occur via an electrostatic effect due to the segregation of the RC proteins. Previous measurements of the temperature dependence of the $P^+Q_A^-$ charge recombination in RCs from *R. viridis* reconstituted in DMPC proteoliposomes have shown the same behavior as that observed here for $k_{AB}(2)$ [40]. In that case, the electrostatic influence of the lipid transition was more likely to be involved.

5. Conclusion

The first and second electron transfer processes in RCs reconstituted in DMPC liposomes do not experience similarly the phase transition of the lipids. Indeed, neither the electrostatic changes nor the stiffness of the lipids modifies the thermodynamics associated with $k_{AB}(1)$, at variance to what is observed for $k_{AB}(2)$. This observation confirms the different nature of the two electron transfer processes. Thus, playing with the phase transition of the surrounding lipids may be useful to discriminate between the respective events associated with the transfers of the first and the second electron between both quinones.

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