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Enthalpy/entropy driven activation of the first interquinone electron transfer in bacterial photosynthetic reaction centers embedded in vesicles of physiologically important phospholipids

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Abstract

The thermodynamics and kinetics of light-induced electron transfer in bacterial photosynthetic RCs are sensitive to physiologically important lipids (phosphatidylcholine, cardiolipin and phosphatidylglycerol) in the environment. The analysis of the temperature-dependence of the rate of the $P^+Q_AQ_B \rightarrow P^+Q_AQ_B^-$ interquinone electron transfer revealed high enthalpy change of activation in zwitterionic or neutral micelles and vesicles and low enthalpy change of activation in vesicles constituted of negatively charged phospholipids. The entropy change of activation was compensated by the changes of enthalpy, thus the free energy change of activation (≈ 500 meV) did not show large variation in vesicles of different lipids.

Keywords: Reaction centers; Liposomes; Interquinone electron transfer

1. Introduction

The primary processes of photosynthesis take place in a specialized pigment–protein system called photosynthetic reaction center (RC) embedded in the photosynthetic membrane, the thylakoid membranes of chloroplast or intracytoplasmic membrane systems (ICM) of cyanobacteria and photosynthetic bacteria. The capture of light energy in bacterial RC of *Rhodobacter sphaeroides* initiates intraprotein electron transfer (ET) along the active branch from the excited singlet bacteriochlorophyll dimer (P), through a bacteriochlorophyll monomer (B) and bacteriopheophytin monomer (H) to the primary quinone Q_A ≈ 25 Å away in 150 ps. Q_A then reduces the secondary quinone Q_B 15 Å away (edge to edge) in about 100 μs (for reviews, see [1–3]). The transfer of the first electron from Q_A^- to Q_B is reversible and gives rise to equilibrium between the states $Q_A^-Q_B$ and $Q_AQ_B^-$ [4]. The standard free energy level of $P^+Q_B^-$ (ΔG^0_{AB}) is placed about 60 meV below that of $P^+Q_A^-$, therefore the interquinone ET becomes energetically favorable. ΔG^0_{AB} is sensitive to the chemical nature of the quinones, the protein and the environment [5,6]. In RCs of *Rhodopseudomonas viridis* much larger ΔG^0_{AB} is observed [7].

The majority of thermodynamic and kinetic data have been obtained from RCs solubilized in detergent micelles. However, the electron and proton transfer reactions and the light-induced conformation changes could be quite different if the protein is embedded in native membrane environment as consequence of specific interaction between RC and lipids of the membrane.

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Although the lipid composition of photosynthetic bacteria shows large variation according to growth conditions, the major lipid components are phosphatidylcholine (PC), phosphatidylglycerol (PG) and phosphatidylethanolamyne (PE) [8–11]. Recent crystallographic studies have shown that physiologically important phospholipids (negatively charged cardiolipin (CL) [12], and zwitterionic PC [13]) are strictly associated to the RC protein. It was shown that binding of phospholipids as PC, CL and PG modified the standard free energy levels of the quinones, and the free energy gap between the $P^+Q_A^-Q_B$ and $P^+Q_AQ_B^-$ states increased in the order of LDAO, PC, PG and PC+CL [14]. The first [15] and the second [16] electron transfer, as well as the $P^+Q_A^-\rightarrow PQ_A$, $P^+Q_B^-\rightarrow PQ_B$ charge recombination reactions [17] have been found to be sensitive to the membrane environment. Not only the electronic nature of the head group [18,19], but also the length and level of saturation [16,20] of the lipids hydrophobic side chain are important.

The kinetics of the first interquinone ET is determined not by the standard free energy states of the quinones but by the free energy gap of activation between them. It is well known that the interquinone ET requires high enthalpy of activation that is exposed to considerable changes both in the RC and its environment. The ET is accompanied with substantial conformation changes revealed by absorption change [21] and photothermal measurements [22,23] and by theoretical considerations [24]. In chromatophores (membrane fractions), the ET is significantly faster (≈ 30 μs) than in micelles (≈ 100 μs). In contrast to standard free energy states of the quinones, the activation parameters of the interquinone ET are far less revealed for different environments of the RC. In this work, we determined the thermodynamics of activation of the first interquinone ET of RC embedded in vesicles made of different and physiologically important phospholipids. We observed that negatively charged vesicles lowered the enthalpy of activation in expense of significant entropy contribution.

2. Experimental

2.1. Preparation of samples

Rb. sphaeroides R-26 cells were grown photoheterotrophically under anaerobic conditions. RCs were prepared by detergent (LDAO, *N*,*N*-dimethyldodecylamine-*N*-oxide) solubilization followed by ammonium sulphate precipitation and DEAE Sephacell anion exchange chromatography [25]. Unilamellar RC/phospholipids vesicles were made by the micelle-to-vesicle transition method [26]. The photochemical activity of the secondary quinone of the RC was reconstituted by addition of excess ubiquinone-50 (UQ_{10}).

2.2. Kinetic absorption spectrophotometry

Flash induced absorbance change at 771 nm due to the electrochromic response of the absorption of bacteriopheophytins to the $Q_A^-Q_B$ and $Q_AQ_B^-$ states were detected by a single-beam kinetic spectrophotometer of local design supplemented with a temperature controlled sample holder [14,25]. The kinetics of $P^+(Q_AQ_B)^-\rightarrow PQ_AQ_B$ charge recombination was followed at 430 nm.

2.3. Chemicals

1,2-Diacyl-*sn*-glycerol-3-phosphocholine (99%—phosphatidylcholine, PC) from soybean, 1,2-diacyl-*sn*-glycerol-3-phosphoryl glycerol (98%—phosphatidylglycerol, PG) and cardiolipin from bovine heart and UQ_{10} were purchased from Sigma of the highest available purity and used without further purification.

2.4. Data evaluation

The standard free energy difference of the quinones, ΔG^0_{AB} was determined from the apparent one-electron equilibrium constant in the acceptor quinone complex [25,27]: $K_{AB}=[Q_AQ_B^-]/[Q_A^-Q_B]=k_f/k_s-1$, where k_f and k_s are the fast and slow rate constants of the $P^+(Q_AQ_B)^-\rightarrow PQ_AQ_B$ charge recombination, respectively and $\Delta G^0_{AB}=-R\cdot T\cdot \ln K_{AB}$, where R and T are the universal gas constant and the absolute temperature, respectively. The standard enthalpy difference of the quinone states were determined from the slope of the van't Hoff plot of the temperature dependence of the equilibrium constant: $d(\ln K_{AB})/dT=\Delta H^0/RT^2$.

The thermodynamic parameters of activation related to interquinone ET were determined from transition state theory (Eyring's equation):

$$\ln\frac{k}{T}=\ln\frac{\kappa\cdot R}{h}+\frac{\Delta S^{\#}}{R}-\frac{\Delta H^{\#}}{R}\cdot\frac{1}{T} \quad (1)$$

where k is the observed rate constant, κ is the transmission coefficient (usually equals to 1 [28]), h is the Planck constant, R is the universal gas constant and $\Delta H^{\#}$ and $\Delta S^{\#}$ are the enthalpy and entropy changes of activation, respectively. $\Delta H^{\#}$ is calculated from the slope and $\Delta S^{\#}$ is determined from the interception of the best-fit straight lines through the measured data.

3. Results

The thermodynamic parameters of the standard states of the quinones and the activation of the interquinone ET were measured and compared in RCs embedded in environment featured by different phospholipids of physiological importance.

3.1. Thermodynamics of standard states of quinones

The difference of standard free energies of the two quinones was obtained from K_{AB} and the difference of standard enthalpies could be obtained from its temperature-dependence (van't Hoff representation). The data related to different environments are summarized in Table 1. The reaction free energy change ΔG^0_{AB} as well as the enthalpy change ΔH^0_{AB} are definitely larger than $R\cdot T$ (=25 meV/mol at room temperature, the average thermal energy) in all lipids here investigated. The values of ΔG^0_{AB} are in good agreement with those found earlier in LDAO [28,29] or in proteoliposomes formed by different

Table 1
Changes of standard thermodynamic quantities of the $P^+Q_A^-Q_B \leftrightarrow P^+Q_AQ_B^-$ equilibrium in purified photosynthetic reaction centers embedded in micelle (LDAO) and in different phospholipids

	LDAO ($r^2>0.96$)	PC ($r^2>0.98$)	PG ($r^2>0.87$)	PC+CL ($r^2>0.98$)
ΔH^0_{AB}	−105±24 (−150±11)[a] (−230)[b]	−101±7	−102±14	−78±9
$T\Delta S^0_{AB}$	43±21 (78±9)[a] (161)[b]	19±4	26±10	−16±10
$\Delta G^{0,c}_{AB}$	−62±2 (−71±1)[a] (−68)[b]	−81±3	−84±4	−93±4

The values are given in meV/mol. The standard deviation and r^2 values of the fittings are also indicated. Conditions are same as in Fig. 1.
[a] Mancino et al. [28].
[b] Kleinfeld et al. [29].
[c] Note that ΔG^0_{AB} was determined from the rate constants of the charge recombination (see Experimental) and not from the temperature dependence of K_{AB}.

lipids [14]. The entropic contribution for the RCs incorporated in phospholipid vesicle results smaller compared to the detergent case.

3.2. Activation parameters of interquinone ET

The kinetics of interquinone ET of RCs incorporated in different lipid vesicles is shown in Fig. 1. The traces were decomposed into two (fast and slow) components: the rate of the fast phase, $k_{AB}(1)_{fast}$, was above $(100\ \mu s)^{-1}$ and that of the slow phase, $k_{AB}(1)_{slow}$ was 4–10 times smaller in good agreement with our earlier results [14]. The rate constant of the fast phase showed less pronounced temperature-dependence in LDAO (in good agreement with earlier results [15]) and in PC environment in the presence of CL. Interestingly, in single component liposomes, i.e. in PC and PG, the temperature dependence of the two components did not differ considerably (Fig. 2). From the transition state analysis of the temperature-dependence of the rate constants, the thermodynamic parameters of activation listed in Table 2 were obtained. The activation free energy, $\Delta G^{\#}_{AB}$, for both of the fast and the slow phases is not changed considerably in the four

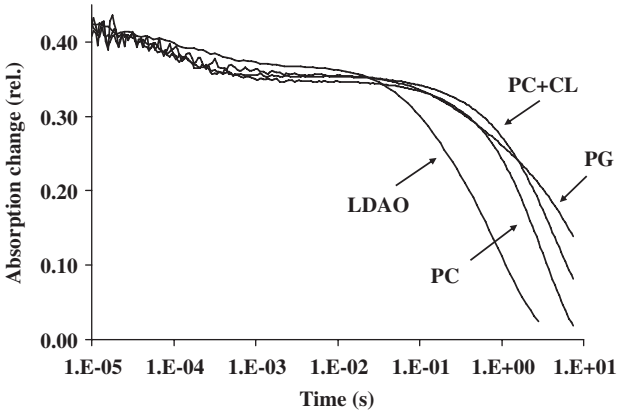

Fig. 1. The absorption change of RCs of *Rb. sphaeroides* R-26 after single saturating laser flash excitation measured at 771 nm. The RCs were incorporated into LDAO detergent, PC, PG or PC+CL liposomes as indicated. The average of 50 recordings was taken. Curves represent the typical traces of 3 (6 for PG) samples. Conditions: excitation at 597 nm; 3 μM RC, 10 mM TRIS, 100 mM NaCl, 0.01% LDAO, pH 8.0 (LDAO) and 5 mM phosphate, 5 mM KCl, pH 6.8 (PC, PG and PC+CL liposomes), temperature 298 K. The molar ratio of lipid/UQ10/RC was 2000:50:1, in the case of PC+CL sample PC/CL/UQ10/RC was 1000:1000:50:1.

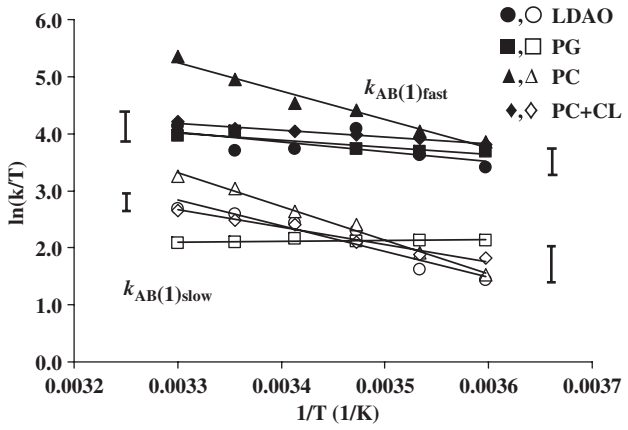

Fig. 2. The Eyring plots of the $k_{AB}(1)_{fast}$ and $k_{AB}(1)_{slow}$ components of the first electron transfer rate measured at 771 nm in the RCs isolated from *Rb. sphaeroides* R-26 and reconstituted in different environments, as indicated. Conditions are same as described in Fig. 1, except the temperature was changed between 5 and 30 °C. Error bars indicate the standard deviations at the lowest (5 °C) and highest (30 °C) temperatures.

Table 2
The thermodynamic parameters of activation of the $P^+Q_A^-Q_B \rightarrow P^+Q_AQ_B^-$ electron transfer in purified photosynthetic reaction centers embedded in micelles (LDAO) and in vesicles made of different phospholipids

	LDAO		PC	PG	PC+CL	DMPC[a]
$\Delta H^{\#}_{AB}(1)_{fast}$	264±81 (255)[b]		384 ±45	173±62	89±25	
$\Delta H^{\#}_{AB}(1)_{slow}$	497±73 (570)[b]	(400±10)[a] (590±41)[c]	500 ±24	−18 ±15	282 ±103	260±10
$T\Delta S^{\#}_{AB}(1)_{fast}$	−270±119		−99 ±49	−366 ±103	−400 ±21	
$T\Delta S^{\#}_{AB}(1)_{slow}$	−7±3	(56±52)[a]	−33 ±17	−567 ±115	−248 ±120	
$\Delta G^{\#}_{AB}(1)_{fast}$	487±200		482 ±94	499±165	488±46	
$\Delta G^{\#}_{AB}(1)_{slow}$	491±76	(520±10)[a] (534±3)[c]	533 ±41	501±130	530 ±223	510±5
$\Delta G^{\#}_{BA}$	608±78 (553)[d]		577 ±46 (614)[d]	600±134 (585)[d]	555 ±227 (623)[d]	

The values are given in meV/mol. r^2 for fitting was 0.78 and 0.84 for the $k_{AB}(1)_{fast}$ in the case of the LDAO and PG samples, respectively, and larger than 0.91 in all the other cases. The values of standard deviation are also indicated. Conditions are same as in Fig. 1.
[a] Tally et al. [16].
[b] Data were calculated by using the connection between the empirical Arrhenius and the absolute rate Eyring model, $E_a = RT + \Delta H^{\#}$, taking the activation energy, E_a, from Tiede et al. [15].
[c] Mancino et al. [28].
[d] Calculated from the rate of the $P^+Q_A^-Q_B \rightarrow P^+Q_AQ_B^-$ electron transfer, $k_{AB}(1)_{slow}$, and the ΔG^0_{AB} free energy difference between the $P^+Q_A^-Q_B$ and $P^+Q_AQ_B^-$ states (c.f. Table 1).

investigated lipid environments. The smaller activation enthalpy change, $\Delta H^{\#}$, is compensated by the larger entropy contribution, $\Delta S^{\#}$, if the negatively charged phospholipids are introduced. The results of the LDAO/RC system are in good agreement with the values obtained by Tiede et al. [15] if the empirical activation energy (E_a) from the Arrhenius plot is converted to $\Delta H^{\#}$ by $E_a = RT + \Delta H^{\#}$. We could calculate the activation free energy, $\Delta G^{\#}_{BA}$, for the reverse $P^+Q_AQ_B^- \rightarrow P^+Q_A^-Q_B$ reaction as the sum of the activation free energy, $\Delta G^{\#}_{AB_{slow}}$, and the equilibrium standard free energy, ΔG^0_{AB}.

4. Discussion

The thermodynamics of the $P^+Q_A^-Q_B \leftrightarrow P^+Q_AQ_B^-$ equilibrium and the $P^+Q_A^-Q_B \rightarrow P^+Q_AQ_B^-$ electron transfer in detergent have long been characterized (e.g. [28]). Recently, this knowledge was extended for RCs embedded in DMPC liposomes and chromatophores [16]. Here, the RCs were reconstituted in PC liposomes where the lipid species resembled those of the in vivo membrane. The standard free energy level of the $P^+Q_AQ_B^-$ state compared to that of $P^+Q_A^-Q_B$ (ΔG^0_{AB}) depended strongly on the lipid environment: The electron transfer was found highly exothermic, -137 meV/mol $< \Delta H^0_{AB} < -84$ meV/mol and the entropic contribution to the free energy remained within the level of the average thermal energy, $R \cdot T$.

The intrinsic rate of interquinone ET per se is very large and the observed value is limited by much slower conformational processes [21]. Proton transfer, charge redistribution, Q_B rearrangement and two-step mechanism of ET are among the best characterized gating reactions. Depending on the rate-limiting process, the observed kinetics of interquinone ET can be decomposed into phases of different rate constants of variable contributions (amplitudes). The slow component refers to the charge compensating relaxation around the redox cofactors, and the fast component is characteristic of the intrinsic electron transfer. When the temperature-dependence of the observed rate constants is exposed to activation analysis, the thermodynamic parameters of the rate limiting processes will be obtained.

The "conformational gating" mechanism is more complicated than simply a limitation of Q_A^- to Q_B electron transfer by the some conformational state of the RC [30–32]. Protonation of specific amino acid side chains accompanied by the change in the hydrogen bonding network and van der Waals contacts between the molecules should also play an important role. As both the slow and the fast components have similar activation parameters, possibly a single gating mechanism of the observed ET dominates.

We found that the presence of negative charges in the proteoliposomes modifies the relative enthalpic and entropic contributions to the free energy change in the interquinone ET reaction, suggesting that the rate-limiting conformational process might be connected with electrostatic charge redistribution.

The predominance of the entropic contribution in negatively charged liposomes may reflect the fact that during the ET reaction, the protein scaffolding has to pass through a more "ordered" conformation if compared to that needed in zwitterionic environments. Furthermore, the decrease of the entropic terms when the effect of negative charges of the CL are diminished by addition of zwitterionic PC, may indicate that the transition state is organized mostly by the electrostatic field generated by the charges surrounding the protein. Additionally, the bound phospholipids may also play role in modulation of the transition state. Further analysis of thermodynamic parameters of the activation may shed more light on the energetic and kinetic details of the interquinone ET and the nature (species, organization, etc.) of the transition state.

5. Summary

Binding of physiologically important phospholipids to the quinone acceptor site affects the stabilization of the $P^+Q_A^-Q_B$/$P^+Q_AQ_B^-$ state. The $P^+Q_A^-Q_B \rightarrow P^+Q_AQ_B^-$ electron transfer is driven mainly by enthalpy change of activation in LDAO and PC, whereas the entropy contribution of activation becomes larger if negatively charged lipids are introduced.

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