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Reaction centers in lipids

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ABSTRACT Specific functional role of physiologically important lipids of the photosynthetic membrane (phosphatidylcholine, cardiolipin and phosphatidylglycerol) was investigated on the thermodynamic and kinetic requirements of the charge movements in bacterial reaction centers. The major effect of these lipids is to increase the stabilization of the separated caharges induced by light excitation during the photosynthetic energy conversion. It can be achieved by (1) changing the redox midpoint potential of the Q_A/Q_A^- and Q_B/Q_B^- redox couples, which results in the increase of the free energy gap that drives the Q_A^- to Q_B electron transfer or (2) by changing the quinone binding/unbinding equilibrium. This study provides evidence that from kinetic point of view the $P^+Q_A^-Q_B \rightarrow P^+Q_AQ_B^-$ charge transfer is mainly driven by the change in the enthalpy in LDAO and PC, whereas the entropy contribution is larger if negatively charged lipids are introduced. **Acta Biol Szeged 49(1-2):195-197 (2005)**

KEY WORDS

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Thanks to the structural crystallography (Nobel prize to Michel, Deisenhofer, Huber) and functional (Nobel prize to Marcus) results together with the works of molecular biology, computer- and electro- techniques, a wealth of information made a relatively clear picture about the kinetics, energetics and stabilisation of electron transport within the photosynthetic reaction center, RC, (see e.g. Sebban et al. 1995, 2003; Wraight 2004). However, several interesting questions can still be addressed.

The physical parameters of the electron transport essentially depend on the type of the RC and environmental factors. From this point of view, the membrane environment is of special interest. The kinetics and thermodynamics of the e^--transfer reactions in RCs are mainly studied in detergents, and the basic processes are already known (Fig. 1). However there are more and more evidences that these parameters have different features in artificial and/or *in vivo* membranes.

The reaction center of *Rhodobacter (Rb.) sphaeroides* consists of three polypeptides, known as L, M and H subunits. The e^--transfer after light excitation is initiated by the primary donor bacteriochlorophyll dimer, (P, $BChl_2$) followed by transient reduction of bacteriochlorophyll monomer (BChl) and bacteriopheophytine (BPheo). Consequently the e^- is trapped by the primary, Q_A, and the secondary quinone, Q_B. Absorbing of one photon in the absence of secondary donor to P^+ the electron is stabilized in the $P^+Q_AQ_B^-$ state. Depending on the free energy of the Q_B/Q_B^- redox couple the charge pair recombines to the ground state PQ_AQ_B with characteristic reaction routes and rates. If secondary electron donor (e.g. cytochrome *in vivo*, or ferrocene, DAD, TMPD etc. *in vitro*) is present, a second electron can be stabilized on the secondary quinone by accepting two protons in the state of $PQ_AQ_BH_2$. The doubly reduced and protonated quinone than leaves the RC and it is replaced by a fully oxidized quinone, Q, from the membrane pool. This cycle can be repeated until one of the reaction components, the donor or the quinone acceptor, is exhausted.

The function of the quinone acceptor complex of the RCs depends both on the redox and bound properties of the quinone molecules (Nagy et al. 2004). If the quinones are bound to the reaction center, their redox properties are determined by the environment and the chemical identity of the molecule.

Here we offer direct evidence for the role of characteristic native phospholipids of the membranes, phosphatidylcholine, PC, phosphatidylglycerol, PG, and cardiolipin, CL, in charge stabilization in RC.

Materials and Methods

Samples

RCs were prepared from *Rb. sphaeroides* R-26 by detergent (LDAO, N,N-dimethyldodecylamine-N-oxide) solubilization followed by ammonium sulphate precipitation and DEAE Sephacell chromatography (Tandori et al. 1995). RC/phospholipid vesicles were made by the micelle-to-vesicle transition method (Trotta et al. 2002).

Kinetic spectrophotometry

Flash induced absorbance change of the P/P^+ redox couple and the electrochromic response of the absorption of bacteriopheophytins to the $Q_A^-Q_B$ and $Q_AQ_B^-$ states (Tiede et

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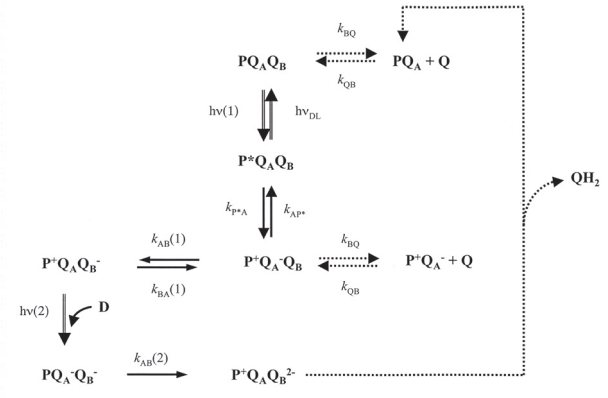

Figure 1. The scheme of the cyclic turnover of the acceptor quinone complex in reaction centers from *Rhodobacter sphaeroides* R-26 arranged according to the level of reduction of the complex and to quinone binding to the Q_B site. Q_A – primary quinone, Q_B – secondary quinone, P – primary donor bacteriochlorophyll dimmer, Q – quinone molecule. Arrows with solid lines: e^--transport reactions; double solid lines: light reactions; dotted lines: quinone exchange reactions. The reaction rates are as indicated.

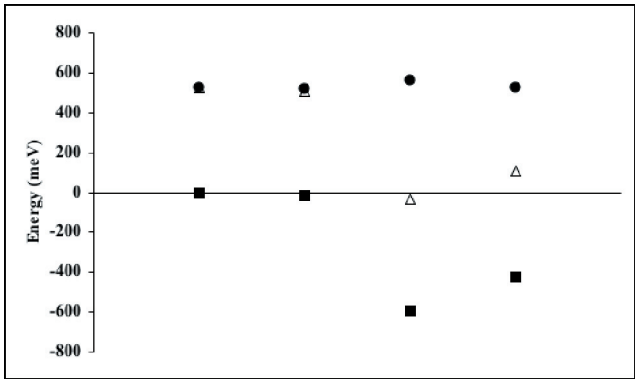

Figure 2. The thermodynamic parameters of the activation processes (the activation free energies, entropy contribution and enthalpy changes) of $P^+Q_A^-Q_B$ to $P^+Q_AQ_B^-$ e^--transfer in different environments of the RCs as indicated.

al. 1996; Nagy et al. 2004) were detected by a single-beam kinetic spectrophotometer of local design supplemented with a temperature controlled sample holder.

Results an Discussion

Kinetics and energetics of the e^--transport

The effect of physiologically important lipids on the kinetics and energetics of the following reactions were investigated (cf. Fig. 1): (1) $P^+Bfeo^-Q_AQ_B \rightarrow P^+BfeoQ_A^-Q_B$, (2) $P^+BfeoQ_A^-Q_B \rightarrow P^+BfeoQ_AQ_B^-$ forward e^--transport; (3) $P^+Bfeo(Q_AQ_B)^- \rightarrow PBfeoQ_AQ_B$ charge recombination and the (4) $P^+BfeoQ_A^-Q_B \leftrightarrow P^+BfeoQ_AQ_B^-$ equilibrium.

The temperature dependence of the reaction rates (Eyring representation) and the K_{AB} equilibrium constant of the $P^+BfeoQ_A^-Q_B \leftrightarrow P^+BfeoQ_AQ_B^-$ reaction (van't Hoff representation) were measured and the thermodynamic parameters of RCs incorporated into detergent (LDAO or TX-100) or different lipids (PC, PG, PC+PG, PC+CL) were calculated. From kinetic pont of view it is interesting to note that the activation free energy, $\Delta G_{AB}^{\#}$, of the $P^+BfeoQ_A^-Q_B \rightarrow P^+BfeoQ_AQ_B^-$ forward e^--transport is not changed considerably by the lipids attached to the RCs. The smaller activation enthalpy, $\Delta H^{\#}$, is compensated by the larger entropy contribution, $T\Delta S^{\#}$, to the free energy change if the negatively charged phospholipids (PG or CL) are introduced (Fig. 2).

From the van't Hoff representation of K_{AB} the change in the free energy, ΔG_{AB}^0, the enthalpy, ΔH_{AB}^0 as well as the entropy $T\Delta S^0$ of the $P^+BfeoQ_A^-Q_B \rightarrow P^+BfeoQ_AQ_B^-$ e^--transport were calculated. The free energy gap between the $Q_A^-Q_B$ and $Q_AQ_B^-$states (the stabilization energy, the absolute value of ΔG_{AB}^0) increased when the RCs were incorporated into physiologically important lipids. ΔG_{AB}^0 was -61 meV, -75 meV, -85 meV, -90 meV in LDAO, PC, PG and PC+CL, respectively. It is important to note that the entropy contribution in the $P^+Q_A^-Q_B \leftrightarrow P^+Q_AQ_B^-$ equilibrium is within the $E=R\cdot T$ (=25 meV/mol at room temperature) in PC, even after addition of CL, however it is larger in LDAO and PG (data not shown).

Charge compensating relaxation in proteins

The light induced difference in the absorption spectra of PQ_A/PQ_A^- and PQ_B/PQ_B^- in the near infrared is due to the shift in the BPheo absorption because of the electric field perturbation around the chromophore. We used 771 nm to measure the electrostatic events due to the Q_A^- to Q_B^- transition. The absorption change of the RCs in LDAO, PC and PG liposomes reflects two main phases in a wide time window of several orders of magnitude. The fast one occurs in submillisecond range, and the slow one is completed on few seconds time scale and there is a plateau between them. Both of them can be divided into a faster and a slower component again.

Both PC and PG lipids modify the size (amplitude) and the rate of the conformation movements. In PC the rate of the overall electron transport, $k_{AB}(1)$, increased by a factor of 1.3 compared to that of measured in the LDAO ($k_{AB}(1) = 3125\ s^{-1}$ increased to $k_{AB}(1) = 4132\ s^{-1}$). In contrast, $k_{AB}(1)$ decreased considerably in PG. We measured $k_{AB}(1) = 714\ s^{-1}$, which value indicates about 4.4 times decrease compared to that of found in LDAO.

The available crystal structures may indicate a dramatic movement of the Q_B prior to its reduction. However, besides this structural evidences there are no direct proves for coupling between the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ e^--transfer and this flip-flop movement. Nevertheless, the change of the electrostatics on the surface of the protein may alter the pK_a's of key amino acids and the redox midpoint potential of Q_A/Q_A^- and/or Q_B/Q_B^- essentially.

Quinone binding equilibrium

In the presence of exogenous donor the periodic appearance and disappearance of the semiquinone, Q^-, occurs after repetitive and saturating flash excitation. It can be followed by monitoring the damped binary oscillation of the absorption change at 450 nm. The oscillation pattern can be controlled by two parameters: the apparent one e^--equilibrium constant, $K_{AB} = [Q_AQ_B^-]/[Q_A^-Q_B]$, and the quinone binding constant, $K_q = [Q_AQ_B]/[Q_A\ldots] = [Q_A^-Q_B]/[Q_A^-\ldots]$.

K_{AB} increased in the order of LDAO < total lipids < PC < PG (9.3, 14.4, 19.0, 28.1). K_q, however, was large in total lipids (40.0), and very small in the PC (8.5), or even smaller in PG (3.2). For comparison, this value is 10.1 in the detergent, LDAO. Since, K_{AB} is large in all samples, it appears that the oscillation pattern is determined by the equilibrium constant of the quinone binding/unbinding. We can assume: 1) the RC may have preferred orientations that make the accessibility to the bulk phase (and thus to the external donor) asymmetric in PG. 2) The availability of quinones is less in PG liposome because of structural and/or kinetic reasons.

Conclusion

Here we provide direct evidence for specific functional role of the photosynthetic membrane probably by binding specific lipid components (like PG, PC and CL) to specific site(s) of the RC protein. The major effect of these lipids is to increase the stabilization of light induced charge(s) in photosynthetic energy conversion. It can be achieved by changing either the redox midpoint potential both of the Q_A/Q_A^- and Q_B/Q_B^- redox couples which results in the increase of the free energy gap that drives the Q_A^- to Q_B electron transfer or the quinone binding/unbinding equilibrium.

Acknowledgements

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