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Stabilization of charge separation and cardiolipin confinement in antenna–reaction center complexes purified from *Rhodobacter sphaeroides*

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Abstract

The reaction center-light harvesting complex 1 (RC–LH1) purified from the photosynthetic bacterium *Rhodobacter sphaeroides* has been studied with respect to the kinetics of charge recombination and to the phospholipid and ubiquinone (UQ) complements tightly associated with it. In the antenna-RC complexes, at $6.5<pH<9.0$, $P^+Q_B^-$ recombines with a pH independent average rate constant $\langle k \rangle$ more than three times smaller than that measured in LH1-deprived RCs. At increasing pH values, for which $\langle k \rangle$ increases, the deceleration observed in RC–LH1 complexes is reduced, vanishing at pH >11.0. In both systems kinetics are described by a continuous rate distribution, which broadens at pH >9.5, revealing a strong kinetic heterogeneity, more pronounced in the RC–LH1 complex. In the presence of the antenna the $Q_AQ_B^-$ state is stabilized by about 40 meV at $6.5<pH<9.0$, while it is destabilized at pH >11. The phospholipid/RC and UQ/RC ratios have been compared in chromatophore membranes, in RC–LH1 complexes and in the isolated peripheral antenna (LH2). The UQ concentration in the lipid phase of the RC–LH1 complexes is about one order of magnitude larger than the average concentration in chromatophores and in LH2 complexes. Following detergent washing RC–LH1 complexes retain 80–90 phospholipid and 10–15 ubiquinone molecules per monomer. The fractional composition of the lipid domain tightly bound to the RC–LH1 (determined by TLC and ^{31}P-NMR) differs markedly from that of chromatophores and of the peripheral antenna. The content of cardiolipin, close to 10% weight in chromatophores and LH2 complexes, becomes dominant in the RC–LH1 complexes. We propose that the quinone and cardiolipin confinement observed in core complexes reflects the in vivo heterogeneous distributions of these components. Stabilization of the charge separated state in the RC–LH1 complexes is tentatively ascribed to local electrostatic perturbations due to cardiolipin.

Keywords: LH1-reaction center complex; Bacterial photosynthetic apparatus; Cardiolipin; Ubiquinone pool; Electron transfer; *Rhodobacter sphaeroides*

1. Introduction

Rhodobacter (Rb.) sphaeroides, a member of the proteobacteria α-subgroup, is one of the best-characterized photosynthetic bacteria and its photosynthetic apparatus has become a reference model in studying the primary processes of photosynthesis and, more in general, challenging aspects of bioenergetic electron transfer chains. As in other purple non-sulphur bacteria, the intra-cytoplasmic membrane system of *Rb. sphaeroides* is endowed with several highly organized transmembrane pigment protein complexes which catalyze a light-induced electron transfer coupled to the pumping of protons

Abbreviations: BChl, bacteriochlorophyll; CAPS, 3-[Cyclohexylamino]-1-propane sulfonic acid; CHES, 2-[N-cyclohexylamino]ethane sulfonic acid; CL, cardiolipin; ICP-AES, Inductively Coupled Plasma Atomic Emission Spectroscopy; LDAO, lauryldimethylamine-N-oxide; LH, light harvesting complex; MES, 2-[N-Morpholino]ethanesulfonic acid; NMR, nuclear magnetic resonance; OG, n-octyl-β-D-glucopyranoside; P, primary electron donor of the RC; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PIPES, piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]; Q_A, Q_B, primary and secondary quinone acceptor; *Rb.*, *Rhodobacter*; RC, reaction center; TLC, thin layer chromatography; TRIS, tris[hydroxymethil]aminomethane; UQ, ubiquinone

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across the energy transducing membrane. The resulting electrochemical potential of protons drives the synthesis of ATP via a chemiosmotic circuit, enabling the transformation of electromagnetic energy into chemical energy [1].

The primary light-induced electron transfer events occur in a membrane-bound pigment–protein complex called the reaction center (RC) (for reviews see [2–4]). Within the RC, a bacteriochlorophyll special pair (P), facing the periplasmic side of the membrane, acts as the primary electron donor. Upon light excitation it delivers an electron, via a bacteriopheophytin molecule to the primary quinone acceptor, Q_A, placed close to the opposite, cytoplasmic side of the complex. The primary charge separated state ($P^+Q_A^-$), generated in about 200 ps, is then stabilized by electron transfer from Q_A^- to a second ubiquinone molecule, bound at the Q_B site of the RC. In vitro, when no physiological or artificial electron donor is available to re-reduce flash generated P^+, the electron on Q_B^- recombines with the hole on P^+, restoring the initial ground state of the RC. In vivo, the photoxidized donor, P^+, is rapidly re-reduced by a soluble c-type cytochrome, so that a second charge separation can take place across the RC, leading to the double reduction and protonation of Q_B to ubiquinol (QH_2) [4]. The ubiquinol molecule leaves the RC and is replaced at the Q_B site by oxidized ubiquinone (UQ) from a pool present in stoichiometric excess over the RC. UQH_2 and oxidized cyt c_2 generated by the RC are utilized by the cytochrome bc_1 complex as reductant and oxidant, respectively, resulting in a cyclic electron transfer chain which pumps protons from the cytoplasmic to the periplasmic side of the membrane [1,5].

In vivo, the RC is intimately associated with another integral pigment protein complex, called light-harvesting complex 1 (LH1), whose primary function is to collect photons and funnel excitation energy to the RC. A second, peripheral, light-harvesting complex (LH2) is present in the intracytoplasmic membranes, which transfers excitation energy to the RC only through the LH1 complex [6]. Both antenna systems are composed of two small transmembrane polypeptides (α and β), which bind bacteriochlorophyll (BChl) and carotenoids and form oligomeric ring-shaped structures [6]. The *Rb. sphaeroides*LH2 complex is built from nine αβ heterodimers, arranged in a closed ring with an internal diameter of about 40 Å [7]. The LH1 complex is characterized by an increased number of αβ heterodimers, giving rise to a ring-like structure large enough to surround a RC, forming the RC–LH1 complex [8,9]. This core complex includes an additional small polypeptide (PufX) which is strictly required for the photosynthetic growth under physiological conditions [10]. Electron microscopy images of the RC–LH1–PufX complex in tubular membranes [11] and in 2D crystals [8] showed an S-shaped dimer made up of two C-shaped open rings of LH1, surrounding the RC. Biochemical data support the notion that the presence of PufX decreases the number of αβ heterodimers [12], interrupting the LH1 ring, and that this small polypeptide is involved in dimerization of the complex [13].

A large body of structural information is available for the individual complexes involved in the electron transfer chain of *Rb. sphaeroides* or of closely related species. The crystallographic structure of the *Rb. sphaeroides* RC [14–17] and of the bc_1 complex from *Rhodobacter capsulatus* [18] are known at atomic resolution; lower resolution projections maps of the antenna complexes have been determined more recently [7,8,19]. As a consequence, there is now a growing interest in the supramolecular organization of these complexes and in its functional implications. AFM studies of native membrane from *Rb. sphaeroides* confirm the existence in vivo of RC–LH1–PufX dimers [20]. AFM images obtained in vivo in a closely related species (*Rhodobacter blasticus*) also suggest a highly organized architecture of the bacterial photosynthetic apparatus [21]. Functional studies have led to the proposal of a "supercomplex" structure of the electron transfer proteins, associating two RCs, one bc_1 complex and one cytochrome c_2 (see [22] and references therein). A heterogeneous spatial distribution of the quinone pool has also been postulated on the basis of electron transfer studies performed in chromatophores [23,24]. A related question which has gained recently attention is the role of specific lipids not only in the structural stability and activity of individual membrane complexes, but also in the assembly and stability of supermolecular structures [25–27]. Crystallographic data have shown that the RC co-purify with tightly bound lipids which were structurally resolved [28,29].

The definition of a specific supermolecular architecture in the whole photosynthetic apparatus, its level of static (and/or dynamic) organization and its functional relevance are far from being clarified. When focusing on the behaviour of the LH1–RC core complex there is clear evidence, however, that the thermodynamics and kinetics of electron transfer processes within the RC are markedly affected by the degree of integrity of the system. In a previous work [30] we have compared the kinetics of charge recombination of the $P^+Q_A^-$ and $P^+Q_B^-$ states induced by a single turnover photoexcitation in purified RC–LH1 complexes and in RCs deprived of the antenna. We found that the stability of the $P^+Q_B^-$ state is considerably enhanced in the core complex as compared to purified RCs in the absence of LH1. In the former system, the free energy decrease accompanying electron transfer from Q_A to Q_B approaches that measured in the intact membrane. We also found that a large fraction (about 40%) of the endogenous membrane UQ pool is functionally retained in the purified RC–LH1. This observation, confirmed by Comayras et al. [24], suggests a particular affinity of the quinones for the core complex. However, analysis of the charge recombination, based on a kinetic model which considers rapid quinone binding equilibrium at the Q_B site, indicates that the stabilization of the charged separated state in the core complex cannot be explained solely by a quinone concentration effect. LH1 is likely to maintain within its ring structure a lipid domain whose interaction with the RC could also be responsible for stabilization of the charge separated state. To shed light on these points, in the present paper we have: (i) extended the kinetic analysis of charge recombination in the RC–LH1 complexes by examining its pH dependence; (ii) determined in parallel the quinone and lipid complements associated with the core complexes, comparing them with those of chromatophores; (iii) characterized the fractional composition of phospholipids copurifying with core complexes and compared it with that of