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Review

Coupling of electron transfer to proton uptake at the Q_B site of the bacterial reaction center: A perspective from FTIR difference spectroscopy

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ABSTRACT

FTIR difference spectroscopy provides a unique approach to study directly protonation/deprotonation events of carboxylic acids involved in the photochemical cycle of membrane proteins, such as the bacterial photosynthetic reaction center (RC). In this work, we review the data obtained by light-induced FTIR difference spectroscopy on the first electron transfer to the secondary quinone Q_B in native RCs and a series of mutant RCs. We first examine the approach of isotope-edited FTIR spectroscopy to investigate the binding site of Q_B. This method provides highly specific IR vibrational fingerprints of the bonding interactions of the carbonyls of Q_B and Q_B^- with the protein. The same isotope-edited IR fingerprints for the carbonyls of neutral Q_B have been observed for native *Rhodobacter sphaeroides* RCs and several mutant RCs at the Pro-L209, Ala-M260, or Glu-L212/Asp-L213 sites, for which X-ray crystallography has found the quinone in the proximal position. It is concluded that at room temperature Q_B occupies a single binding site that fits well the description of the proximal site derived from X-ray crystallography and that the conformational gate limiting the rate of the first electron transfer from $Q_A^-Q_B$ to $Q_AQ_B^-$ cannot be the movement of Q_B from its distal to proximal site. Possible alternative gating mechanisms are discussed. In a second part, we review the contribution of the various experimental measurements, theoretical calculations, and molecular dynamics simulations which have been actively conducted to propose which amino acid side chains near Q_B could be proton donors/acceptors. Further, we show how FTIR spectroscopy of mutant RCs has directly allowed several carboxylic acids involved in proton uptake upon first electron transfer to Q_B to be identified. Owing to the importance of a number of residues for high efficiency of coupled electron transfer reactions, the photoreduction of Q_B was studied in a series of single mutant RCs at Asp-L213, Asp-L210, Asp-M17, Glu-L212, Glu-H173, as well as combinations of these mutations in double and triple mutant RCs. The same protonation pattern was observed in the 1760–1700 cm^{-1} region of the Q_B^-/Q_B spectra of native and several mutant (DN-L213, DN-L210, DN-M17, EQ-H173) RCs. However, it was drastically modified in spectra of mutants lacking Glu at L212. The main conclusion of this work is that in native RCs from *Rb. sphaeroides*, Glu-L212 is the only carboxylic acid residue that contributes to proton uptake at all pH values (from pH 4 to pH 11) in response to the formation of Q_B^-. Another important result is that the residues Asp-L213, Asp-L210, Asp-M17, and Glu-H173 are mostly ionized in the Q_B state at neutral pH and do not significantly change their protonation state upon Q_B^- formation. In contrast, interchanging Asp and Glu at L212 and L213 (i.e., in the so-called swap mutant) led to the identification of a novel protonation pattern of carboxylic acids: at least four individual carboxylic acids were affected by Q_B reduction. The pH dependence of IR carboxylic signals in the swap mutant demonstrates that protonation of Glu-L213 occurred at pH > 5 whereas that of Asp-L212 occurred over the entire pH range from 8 to 4. In native RCs from *Rhodobacter sphaeroides*, a broad positive IR continuum around 2600 cm^{-1} in the Q_B^-/Q_B steady-state FTIR spectrum in ^1H_2O was assigned to delocalized proton(s) in a highly polarizable hydrogen-bonded network. The possible relation of the IR continuum band to the carboxylic acid residues and to bound water molecules involved in the proton transfer pathway was investigated by testing the robustness of this band to different mutations of acids. The presence of the band is not correlated with the localization of the proton on Glu-L212. The largest changes of the IR continuum were observed in single and double mutant RCs where Asp-L213 is not present. It is proposed that the changes observed in the mutant RCs with respect to native RCs reflect the specific role of bound protonated water molecule(s) located in the vicinity of Asp-L213 and undergoing hydrogen-bond changes in the network.

Abbreviations: P, primary electron donor; BChl, bacteriochlorophyll; Bphe, bacteriopheophytin; H_A, intermediate electron acceptor; Q_A, (Q_B), primary, (secondary), quinone electron acceptor; Q*n*, ubiquinone-*n*:, 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone; RC, reaction center; *Rb.*, *Rhodobacter*; *B.*, *Blastochloris*; ET, electron transfer; FTIR, Fourier transform infrared; RS, rapid-scan; TR, time-resolved; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance

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1. Introduction

In bioenergetic systems, electron and proton transfer reactions are fundamental processes required for the generation and interconversion of energy. In photosynthetic bacteria, the initial steps of light energy conversion into chemical energy occur in a membrane bound pigment-protein complex called the photochemical reaction center (RC) which couples electron and proton transfer across the bacterial membrane . The RC complex (~100 kDa) is composed of three (L, M, and H) or four (including a cytochrome unit) polypeptide subunits. With the three-dimensional structure determined at ~2 Å resolution, the protein amenable to site-directed mutagenesis, and the possibility to trigger the electron transfer (ET) reactions by a short pulse of light, the RC represents an ideal system for the study of proton-coupled ET reactions.

In the native RC, light-induced ET is exclusively initiated via the active A-branch of cofactors from the primary electron donor P (a dimer of bacteriochlorophyll) through a series of electron acceptors (notably the bacteriopheophytin H_A) to the primary quinone (Q_A), and then to the loosely bound secondary quinone Q_B (Fig. 1A). In RCs from *Rhodobacter* (*Rb.*) *capsulatus* and *Rb. sphaeroides*, Q_A and Q_B are both ubiquinone-10 (Q_{10}). A second ET coupled with the uptake of two protons from the solution results in the formation of the quinol Q_BH_2 that is subsequently released from the Q_B binding site and replaced by another quinone from a pool contained in the membrane. This physiologically important reaction, i.e., the double reduction and protonation of Q_B, together with the oxidation of Q_BH_2 by the cytochrome bc_1 complex, further initiates the formation of the proton gradient required for ATP synthesis [1].

The two-sequential ET reactions between Q_A and Q_B have been subjected to extensive kinetic optical studies [2–4]. Notably, it has been reported that the first ET from Q_A to Q_B is at least biphasic [5–8] with a fast phase (5–10 μs) that is assigned to pure ET and a slow phase (100–200 μs) which appears kinetically gated by a conformational change [9]: following light absorption, the slow phase of ET to Q_B first involves a conformational change followed by an ET step. Several possible structural changes involved in the gating process have been proposed. In addition to the structural changes, proton uptake also plays an important role in the electron transfer. Although the first ET to Q_B in isolated RCs does not involve the direct protonation of the semiquinone itself, substoichiometric proton uptake by the protein following formation of Q_B^- has been experimentally measured [10–13] and also predicted from electrostatic calculations [14–27], based on the X-ray structures. Proton binding by the RC protein reveals a change of the pK_a of amino acid side chains between the states Q_B and Q_B^-.

High resolution structures of the RCs from two purple bacteria, *Rb. sphaeroides* [28–32] and *Blastochloris (B.) viridis* [33–35] show that, unlike Q_A, Q_B is surrounded by many polar and acid residues of the L, M, and H protein subunits. In the *Rb. sphaeroides* RC, the Q_B binding pocket is formed by a cluster of polar and acid residues (and water molecules) including Ser-L223, Asp-L213, Asp-L210, Asp-M17, Glu-H173, and Glu-L212 [36,37]. In particular, Ser-L223, Asp-L213, and Glu-L212, which are all located near Q_B (Fig. 2), were shown to be crucial for rapid coupled electron-proton transfer to reduced Q_B [2–4]. Several other amino acid side chains located between the Q_B site and the surface [2–4] are also potential proton transfer species (such as Asp-L210, Asp-M17, Glu-H173, and Arg-L217). One controversial issue is the binding position of Q_B. While the binding site of Q_A is well-defined, different locations of Q_B have been observed in the various crystallographic structures reported for both *Rb. sphaeroides* and *B. viridis* RCs, and two distinct main binding sites for Q_B have been discussed [30,35,36,38], i.e., a position proximal to the non-heme iron (closest to Q_A) with hydrogen bonds at both carbonyls, and a position termed the distal site displaced by ~4.5 Å (further from the non-heme iron) with only one carbonyl hydrogen bonded to the protein (Fig. 1B). On the other hand, there is a consensus on the location of Q_B^-, which places the semiquinone at the

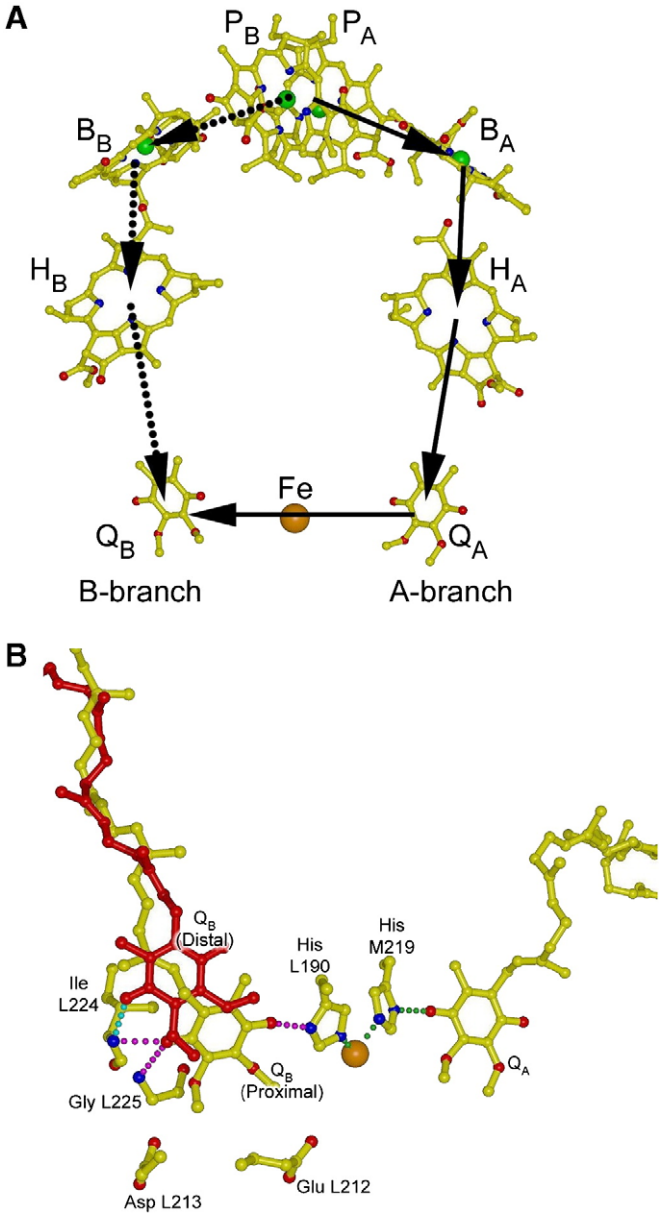

Fig. 1. Structural models of the *Rb. sphaeroides* reaction center. (A) Overall organization of the cofactors showing the bacteriochlorophylls (dimeric primary electron donor P and monomeric (B), bacteriopheophytins (H), and ubiquinones (Q). The cofactors are arranged around the axis of 2-fold symmetry in two branches (A and B) that span the membrane. The route of electron transfer from P to Q_B along the A-branch is shown by the solid arrows. (B) The distal and proximal binding positions of the Q_B ubiquinone in the RC. When in the distal position (red), the C4=O carbonyl accepts a hydrogen bond (green spheres) from the backbone NH group of Ile-L224, while the C_1=O carbonyl is free. When in the proximal position (green yellow), the C_1=O carbonyl is hydrogen-bonded (magenta spheres) to the backbone NH group of Ile-L224 and Gly-L225, while the C4=O carbonyl is hydrogen bonded to the side chain of His-L190. On the symmetrical side, His-M219 is a ligand to Q_A. This figure is courtesy of P. Fyfe and M.R. Jones (University of Bristol, Bristol, U.K.).

location proximal to the non-heme iron and at the approximate symmetry-related position of Q_A. In the proximal position, Q_B and/or Q_B^- form several likely hydrogen bonds to the backbone at L224 and/or L225 at the C_1=O carbonyl and to His-L190 at the C_4=O group [28,36]. Possible hydrogen-bonding interactions between Ser-L223 and Q_B and/or Q_B^- have been discussed [27,34–36,39–41] (see Section 2.4).

With respect to the general stucture-function relationship of Q_B and Q_B^-, the most specific questions that have been addressed and are still