

Ubiquinone reduction in the photosynthetic reaction centre of *Rhodobacter sphaeroides*: interplay between electron transfer, proton binding and flips of the quinone ring

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Abstract

This review is focused on reactions that gate (control) the electron transfer between the primary quinone Q_A and secondary quinone Q_B in the photosynthetic reaction centre of *Rhodobacter sphaeroides*. The results on electron and proton transfer are discussed in relation to structural information and to the steered molecular dynamics simulations of the Q_B ring flip in its binding pocket. Depending on the initial position of Q_B in the pocket and on certain conditions, the rate of electron transfer is suggested to be limited either by the quinone ring flip or by the charge-compensating proton equilibration between the surface and the buried Q_B site.

Introduction

For 30 years, the bacterial photosynthetic RCs (reaction centres), the first membrane proteins for which the crystal structures were solved [1], served as models for addressing diverse biophysical phenomena (see e.g. [2–8]). The core of a bacterial RC is formed by two membrane subunits L and M that are ‘capped’ by the H subunit. In response to a flash of light, an electron is transferred across the membrane from the bacteriochlorophyll dimer P to two quinone molecules, Q_A and Q_B , which are located, with a non-haem iron atom in between, at the cytoplasmic side of the RC. Q_A functions as a one-electron carrier that transfers electrons to Q_B . The secondary quinone Q_B is reduced first to a semiquinone anion $Q_B^{\bullet-}$, and then, after receiving the second electron, to a quinol Q_BH_2 . The formed quinol molecule can diffuse out of the RC (see [1,9–12] for reviews).

In the 1970s, it was established, by following the charge recombination between the reduced quinone acceptors and P^+ , that electrons could pass beyond Q_A to Q_B only in those samples that were frozen in the light, but not in those frozen in the dark [13]. To explain these observations, Kononenko and co-workers [14] considered a temperature-activated transition between two conformations of the enzyme, namely a ‘non-contact’, dark state and a ‘contact’, light state. Further studies revealed that diverse treatments, which decreased the protein mobility, such as freezing, dehydration, application

of cross-linking agents, embedment in trehalose amorphous matrices etc. could suppress the ET (electron transfer) between Q_A and Q_B (see [3,15,16] and references cited therein). This review is focused on protein dynamics modes that might govern the ET to Q_B in the RC of a purple α -proteobacterium *Rhodobacter sphaeroides*.

ET from $Q_A^{\bullet-}$ to Q_B

The transfer of the first electron in the *Rb. sphaeroides* RC ($Q_A^{\bullet-}Q_B \rightarrow Q_AQ_B^{\bullet-}$ transition) could be observed (i) at the absorption maximum of semiquinone anion (450 nm), (ii) via the electrochromic changes of nearby pigments at 757–770 nm and (iii) by a functional assay involving cytochrome *c* to measure the rate of $Q_A^{\bullet-}$ oxidation [17–23]. The ET kinetics was found to be heterogeneous, being contributed by at least three components with different time constants (τ) and properties. A ‘very fast’ component with τ_{vf} of 3–4 μ s and activation energy (E_a) of approx. 15 kJ/mol [20,21] reflects, most probably, the genuine non-adiabatic ET from $Q_A^{\bullet-}$ to Q_B over an edge-to-edge distance of 13 Å (1 Å = 0.1 nm). The rate of this component, in agreement with the predictions from the ET theory of Marcus and Sutin [4], accelerated with the increase in the driving force, which, in the case of isolated RC preparations, could be varied by substituting low-potential quinones for Q_A [23]. The ‘fast’ component of Q_B reduction was characterized by a τ_f of 50–100 μ s and an E_a of approx. 15–20 kJ/mol [19–22]. In addition, a ‘slow’ component with τ_s of approx. 500 μ s and a higher E_a of approx. 40 kJ/mol was reported [19,21]. The rates of the fast and slow components were independent of the free energy change (ΔG) of ET; therefore these components were

Key words: conformational gating, protein dynamics, proton transfer, *Rhodobacter sphaeroides*, semiquinone, ubiquinol.

Abbreviations used: ET, electron transfer; MD, molecular dynamics; PT, proton transfer; RC, reaction centre.

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suggested to reflect the 'gating' of this ET reaction by protein [21–23].

PT (proton transfer) reactions and the mechanistic scheme of Q_B turnover

The reduction of Q_B to Q_BH_2 is accompanied by binding of two protons. By using pH indicators, Wraight [24] has shown that formation of $Q_B^{\bullet-}$ leads to proton binding; however, since $Q_B^{\bullet-}$ itself was not protonated, it was concluded that protons were accepted by acid group(s), the pK values of which increased in response to a negative charge at $Q_B^{\bullet-}$ [24]. More protons were bound after the second flash, after the formation of Q_BH_2 [24]. The response of pH-dyes roughly correlated with the rate of ET, but only when measured with the preparations of isolated RCs [18,25–27]. When native membrane vesicles (chromatophores) of *Rb. sphaeroides* were studied, the response of pH-dyes was slower than the rate of ET [28,29]. As argued elsewhere [29–32], the retardation was due to a kinetic barrier of approx. 120 meV, which prevented the fast proton equilibration between the membrane surface and the bulk water phase. The kinetic correspondence with ET was better when the proton displacements towards Q_B were traced by the accompanying changes in the transmembrane voltage, either optically, by following the electrochromic changes of native carotenoid pigments [29,33] or by capacitive electrometry [34–37]. The formation of $Q_B^{\bullet-}$ was accompanied by electrogenic PT towards the acid cluster next to $Q_B^{\bullet-}$ [34–36]. At $<15^\circ\text{C}$, it was possible to discriminate between the two components in the kinetics of this PT, namely the fast component with τ_f of approx. 80 μs and an E_a of approx. 10 kJ/mol and a slow component with τ_s of approx. 500 μs at an 10°C and an E_a of approx. 60–70 kJ/mol ([35], compare with the fast and slow components of ET described above). The E_a of the slow component was too high for PT proper, therefore it was hypothesized that in a fraction of RCs, Q_B is initially in a non-functional position that is separated by a high activation barrier from the functional one [35]. And indeed, the low-temperature X-ray structures of the *Rb. sphaeroides* RC [6,38] have revealed that Q_B is distributed between two binding sites in the dark-adapted RC crystals, namely the 'distal' one (see Figure 1A), where quinone is bound to the protein by a single H-bond (hydrogen-bond), and the 'proximal' one (see Figure 1B), where Q_B is 5 Å closer to the Q_A , flipped by 180° around the isoprenoid chain and forms several H-bonds. The kinetics of PT in response to the second flash ($Q_B^{\bullet-} \rightarrow Q_BH_2$ transition) could also be separated into two components, with E_a values of approx. 10 kJ/mol and of approx. 60 kJ/mol at $<15^\circ\text{C}$ ([37], see also Figure 3 below). These components were respectively attributed to the transfer of the first and second protons upon the Q_BH_2 formation [37].

The results on PT could be rationalized by a mechanistic scheme of Q_B turnover that is shown in Figure 1. Since a detailed description of the scheme can be found elsewhere [10,39,40], we focus here only on its main features. (1) In the ground state, the Q_B ring is distributed between the distal

and proximal sites. The two ubiquinone positions seem to be isoenergetic [10], so that their relative occupation might differ depending on the preparation studied, conditions and the length of the ubiquinone tail. In the isolated RCs of *Rb. sphaeroides* and at room temperature, ubiquinone is predominantly in the proximal site [41], although functional evidence of Q_B heterogeneity under compatible conditions can also be found in the literature [42–44]. On decreasing the temperature, quinone partly relocates into the distal site, as it could be inferred from earlier functional studies [45] and from the low-temperature crystal structures of the RC [6,38]. In the recent low-temperature X-ray structure of dark-adapted RCs, as obtained with 1.87 Å resolution, the relative occupation of the distal and proximal positions was 55 and 45% respectively [38]. (2) In the 'light-frozen' RCs, the semi-quinone anion $Q_B^{\bullet-}$ is seen in the proximal site (see Figure 1C), being stabilized by H-bonds with His-L190 (His-190 of the L-polypeptide), Ser-L223, Ile-L224, Gly-L225 and, perhaps, Thr-L226 [6,38]. (3) The transfers of the second electron and of the first proton to $Q_B^{\bullet-}$ are tightly coupled [11]. The formed ubiquinone anion Q_BH^- remains bound by the RC [46], supposedly in the proximal site [9], as shown in Figure 1(D). A high activation barrier of approx. 60 kJ/mol for the transfer of the second proton upon the $Q_BH^- \rightarrow Q_BH_2$ transition [37,39] was explained by its coupling to the relocation of the quinone ring into the distal site (see Figure 1E and [37,39]), where Q_BH_2 is seen in the respective crystal structure (see Figure 1F and [47]).

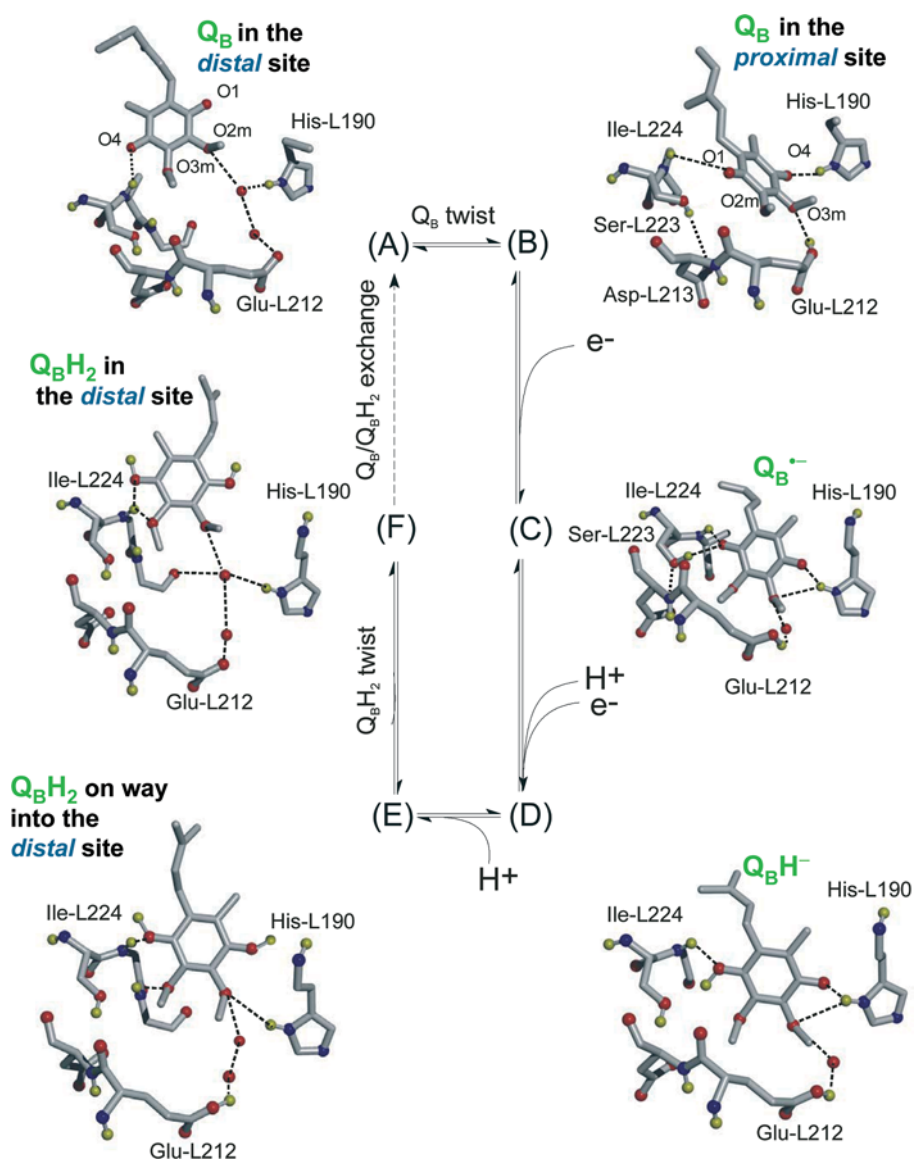
Hence, the proximal Q_B -binding site serves as a catalytic centre where the reduction and protonation of quinone take place. The distal site serves as a 'standby' position for quinone/quinol molecules. The relative occupation of the sites depends on the redox state of quinone. While Q_B can be distributed between the sites, the negatively charged species, namely $Q_B^{\bullet-}$ and Q_BH^- , are stabilized in the proximal position by their electrostatic interaction with the non-haem iron [48]. The ubiquinol Q_BH_2 is seen in the distal site [47].

Tentative gating mechanisms

As noted above, the rate of Q_B reduction was independent of ΔG in a subpopulation of RCs, so that the reaction was suggested to be gated, i.e. limited by some preceding event that made the ET to Q_B energetically favourable [11]. Initially, it has been proposed that the gating event is a transition of the ubiquinone between its 'inactive' distal and 'active' proximal positions [6]. Based on the similarities between the above described fast and slow components of ET and PT respectively, we have speculated that the ET reaction is controlled not by one, but by two relaxation/gating modes [8]. In those RCs, where Q_B was initially in the proximal position, the electron spillover from $Q_A^{\bullet-}$ to Q_B could be driven by proton redistribution that selectively stabilized the $Q_B^{\bullet-}$ state ($\tau \sim 100 \mu\text{s}$ and $E_a \sim 10\text{--}20 \text{ kJ/mol}$). If Q_B was initially in the distal position, it had to be brought into the proximal position ($\tau \sim 500 \mu\text{s}$ and $E_a \sim 60 \text{ kJ/mol}$)

Figure 1 | Mechanistic scheme of ubiquinone reduction in the RC of *Rb. sphaeroides* [as modified from Figure 7 in D.A. Cherepanov, S.I. Bibikov, M.V. Bibikova, D.A. Bloch, L.A. Drachev, O.A. Gupta, D. Oesterhelt, A.Y. Semenov and A.Y. Mulikidjanian (2000) Reduction and protonation of the secondary quinone acceptor of *Rhodobacter sphaeroides* photosynthetic reaction center: kinetic model based on a comparison of wild-type chromatophores with mutants carrying Arg → Ile substitution at sites 207 and 217 in the L-subunit. *Biochim. Biophys. Acta* 1459, pp. 10–34 © Elsevier]

Element colour code: oxygen, red; nitrogen, blue; hydrogen, yellow. H-bonds are depicted by dashed lines. (A) The position of Q_B in the distal site as seen in the low-temperature dark-adapted RC structure (PDB no. 1AJ [6]). (B) Proximal position of Q_B in the catalytic site as seen in the room temperature RC structure (PDB no. 2RCR, [62]). (C) Proximal 'semiquinone' position of $Q_B^{\bullet-}$, as seen in the low-temperature light-adapted RC structure with a trapped $Q_B^{\bullet-}$ (PDB no. 1AG [6]). (D) Proximal position of $Q_B H^-$ as modelled after Lancaster and Michel [9] from the crystal structure of the stigmatellin-containing RC of *Rhodopseudomonas viridis*. (E) The 'detached' $Q_B H_2$ is tentatively shown on its way towards the standby 'quinol' position, before the propeller twist. (F) $Q_B H_2$ position in the distal site as modelled along the X-ray structure of the *Rb. sphaeroides* RCs, which were crystallized in the presence of sodium ascorbate (PDB no. 1PCR [47]).



before the ET and PT could take place [8]. In several recent papers, the role of Ser-L223 in the $Q_B^{\bullet-}$ stabilization has been discussed [12,49,50]. In the $Q_B^{\bullet-}$ -containing crystal structures, Ser-L223 seems to form a H-bond with the C-1-

carbonyl of the quinone ring (see Figure 1C and [6,38]). The electrostatic calculations indicate that a H-bond with Ser-L223 might essentially stabilize the $Q_B^{\bullet-}$ state [50]. As well, the involvement of Ser-L223 in stabilization of $Q_B^{\bullet-}$

was inferred from a comparison of electron nuclear double resonance spectra of differently treated RC preparations [51].

Steered MD (molecular dynamics) simulations of the Q_B rotation

The two quinone-binding sites seem to be ‘linked’ to each other [12]. There is experimental evidence that the formation of $Q_A^{\bullet-}$ might induce conformational changes at the Q_B site [52] and, in particular, the distal to proximal transition of Q_B [53,54]. It has been noted already that protons can be trapped by the amino acid residues of the Q_B pocket in response to the formation of $Q_A^{\bullet-}$ [53,55]. This phenomenon cannot be attributed to the electrostatic interactions alone because charge–neutral replacements of either the Q_A ubiquinone by naphthoquinones [56] or Pro-L209 by Tyr, Phe and Trp [57] suppressed the proton binding in response to the $Q_A^{\bullet-}$ formation. The importance of the proline residue indicates that the linkage between two quinone pockets might be of structural nature.

Recently, we have addressed this topic by steered MD simulations of the *Rb. sphaeroides* RC [58]. We applied the program NAMD2 [59] using an all-atom empirical force field CHARMM22 [60]. For simulations, we used the wild-type (Protein Data Bank no. 1AIJ [6]) and L209PE mutant (PDB no. 1FNQ [61]) crystal structures with Q_B in the distal position. After 400 ps of equilibration, an increasing torque was applied to the four carbon atoms of the Q_B -ring to enforce a rotation around the isoprenoid chain. The free energy ΔG as a function of the flip angle was calculated using the probability distribution function as described in [58]. When the critical force value was reached, the ring flipped. The ΔG value corresponding to the critical force was approx. 60 kJ/mol for the wild-type and approx. 40 kJ/mol for the L209PE mutant. After the Q_B -ring completed the 180° flip, the torque was removed. During the subsequent 1.2 ns equilibration, Q_B spontaneously migrated towards the proximal position and formed H-bonds with His-L190 in both structures. In the L209PE structure, the H-bond with Ser-L223 was also formed (see Figure 2). The smaller ΔG value of the quinone flip in the L209PE RCs might indicate the loosening of the quinone-binding pocket(s) in the absence of the ‘rigid’ Pro-L209. This assumption is consistent with (i) the gained ability of stigmatellin to bind to the Q_A site in the L209PE and L209PY mutants and (ii) the smaller E_a of PT in these mutants [58]. The loosening of the Q_B -pocket might weaken the linkage between the two quinone-binding sites.

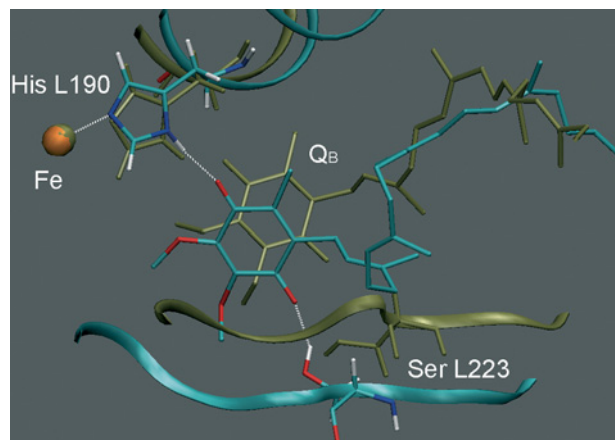
Concluding remarks

Nature of gating reactions

The spontaneous migration of Q_B into the proximal site after the flip, but not before it, indicates that the two positions of the Q_B ring in the *Rb. sphaeroides* RC, as found by X-ray crystallography [6,38], correspond to two energy minima, and that the switching between them requires a flip of the

Figure 2 | The L209PE mutant structure

The structure (coloured by atom type, PDB no. 1FNQ [61]) is equilibrated for 1.2 ns after the forced 180° propeller twist [58] and aligned to the ‘light’ wild-type structure (tan coloured, PDB no. 1AIG [6]).



quinone ring. After Q_B reached the proximal position, the H-bond with Ser-L223 was already formed at approx. 1 ns. Hence, this event by itself is fast and is unlikely to determine the rate of ET between $Q_A^{\bullet-}$ and Q_B at approx. 100 μ s. More plausible is the suggestion that the H-bond between Ser-L223 and $Q_B^{\bullet-}$ becomes stable only after protonation of the acid cluster in the vicinity (see [12] for details). Then, however, the gating rate is determined by proton equilibration between the surface and the acid cluster. As argued elsewhere [8], this reaction takes approx. 50–100 μ s, which is typical for proton equilibration between the protein surface and the buried groups [32] and is compatible with the rate of gated ET between $Q_A^{\bullet-}$ and Q_B [11].

The similarity between the experimentally measured E_a values of the slow PT [35,37,58] and the ΔG values of the quinone ring flip, as obtained from MD simulations [58], support the earlier suggestion on the relation of the slow PT events with an E_a of approx. 60 kJ/mol to the flips of the quinone ring [8,10,35].

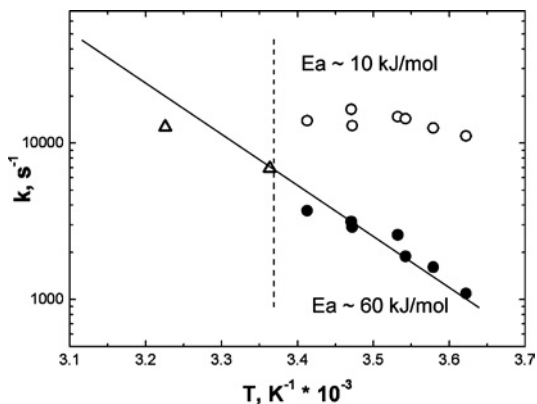
If our attribution of kinetic components with high E_a to the flips of Q_B ring is correct, then, depending on the initial position of Q_B , the rate of the gated ET might be determined either by the quinone ring flip or by the proton equilibration between the surface and the Q_B site [8].

Gating as a function of temperature

The presence of components with an E_a of approx. 60 kJ/mol in the PT kinetics, as described in [35,37], is a rather robust feature: it could be reproduced in a wild-type *Rb. sphaeroides* strain with a plasmid-coded ‘L’ and ‘M’ RC subunits and in a different experimental setup (see Figure 3 and [58]). As seen in Figure 3, two kinetic components of PT were resolvable only at <15–20°C, in agreement with previous results [37]. At room temperature, the components merged because the high E_a components accelerated faster with temperature. The broken line in Figure 3 illustrates that >25°C, an

Figure 3 | Temperature dependence of the fast (○) and slow (●) components of electrometrically measured PT in response to the second flash in *Rb. sphaeroides* chromatophores

The dependence is replotted from data presented in [58] and is similar to the earlier published results [37]. At $>20^{\circ}\text{C}$, the two components merged and the kinetic data were fitted with one exponential (Δ).



event with high E_a does not limit the reaction anymore. Hence, the nature of the rate-limiting reaction in chromatophores of *Rb. sphaeroides* depends on the temperature. Below the 'transition' temperature of approx. 25°C , the Q_B turnover is governed, supposedly, by the Q_B flip ($E_a \sim 60$ kJ/mol), whereas above 25°C the major limiting factor is the proton equilibration ($E_a \sim 10$ kJ/mol). The 'critical' temperature might vary depending on the preparation (e.g. chromatophores versus isolated RCs). In nature, these bacteria live at temperatures $\ll 25^{\circ}\text{C}$, so that the operation of their RC is probably controlled by the quinone flip. It is likely that the affinity of $Q_B\text{H}_2$ to the distal site [47] and a high activation barrier of the flip might prevent eventual oxidation of the membrane ubiquinol via the RC. Such a 'back-reaction' is undesirable, as it leads to energy loss.

We are thankful to Drs L. Baciou and P. Sebban for providing us with a wild-type plasmid-complemented $\text{puf}\Delta\text{LMX21}$ strain of *Rb. sphaeroides*. Very useful discussions with Drs D.A. Bloch, P. Fromme, O. Gopta, L.I. Krishtalik, C.R.D. Lancaster, M.Y. Okamura, M.L. Paddock and C.A. Wraight are appreciated. We are grateful for generous support from Professors E. Bamberg, W. Junge, H. Michel, V. Skulachev and L.S. Yaguzhinsky. The work was funded by grants from the Deutsche Forschungsgemeinschaft (Mu-1285/1, 436-RUS-113/210), Volkswagen Foundation and INTAS (2001-736).

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Received 8 April 2005