

## Minireview

Electrogenic steps during electron transfer via the cytochrome  $bc_1$  complex of *Rhodobacter sphaeroides* chromatophores

Alexey Yu. Semenov

A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation

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The results of the flash-induced electrometrical investigation on the functioning of the photosynthetic bacterial cytochrome  $bc_1$  complex are discussed. The data suggest possible arrangement of redox centers in the  $bc_1$  complex and propose that the total electrogenesis within the  $bc_1$  complex includes: (i) electron transfer between the low- and the high-potential cytochrome  $b$  hemes, (ii) proton binding by doubly reduced  $Q_C^{2-}$  at ubiquinone-reducing center 'C', and (iii) proton release on oxidation of  $QH_2$  at ubiquinol-oxidizing center 'Z'.

Bacterial photosynthesis;  $bc_1$  complex; Electrogenic reaction; *Rhodobacter sphaeroides*

## 1. INTRODUCTION

The cytochrome  $bc_1$  complex is one of the two main pigment-protein complexes which participate in light-energy conversion in photosynthetic bacteria. It is involved in the cyclic electron flow and serves to oxidize the reaction center (RC) ubiquinol and to reduce cytochrome  $c_2$ , an electron donor for the RC (for reviews see [1–3]). This process is accompanied by generation of the transmembrane electric potential difference ( $\Delta\psi$ ). Our knowledge of electrogenic events within the  $bc_1$  complex of purple bacteria is essentially derived from measurements of the slow phase of carotenoid electrochromic absorption changes [4,5]. By means of this method it has been shown that electrogenic reactions in the  $bc_1$  complex are partly suppressed by antimycin A, the inhibitor of the quinone-reducing site ( $Q_C$ ), and completely by myxothiazol, the inhibitor of the quinol-oxidizing site ( $Q_Z$ ) [5,6]. In the presence of myxothiazol under alkaline conditions the negative electrogenic reaction is observed [6–8].

From the results obtained by carotenoid bandshift assay a general model of electrogenic reactions in the  $bc_1$  complex was proposed. According to the model pro-

posed by Robertson and Dutton [6], there are two main electrogenic reactions, namely: (i) electron transfer between the low- ( $b_l$ ) and the high-potential ( $b_h$ ) cytochrome  $b$  hemes, and (ii) a subsequent electron transfer between reduced  $b_h$  and the ubiquinone molecule at the quinone-reducing  $Q_C$ -site. The antimycin-sensitive negative electrogenic step observed in the presence of myxothiazol was ascribed to the back electron flow between  $Q_C$  and  $b_h$ . Note that in accordance with this scheme both the  $Q_Z$  and  $Q_C$  sites should be located at or close to the borders of the membrane dielectric. Consequently both protonation of the doubly reduced  $Q_C^{2-}$  and deprotonation of  $QH_2$  at the  $Q_Z$  site should be non-electrogenic reactions. This model is consistent with the spatial organization of the redox active centers in the mitochondrial  $bc_1$  complex estimated by Ohnishi et al. [9] by the paramagnetic probe method.

The model recently proposed by Konstantinov [10] predicts a different arrangement of redox-centers in the  $bc_1$  complex. According to this model, the total electrogenesis within the  $bc_1$  complex is due to (i) electron transfer between  $b_l$  and  $b_h$ , and (ii) antimycin-sensitive deprotonation of  $QH_2$  at the  $Q_Z$  site. Correspondingly,  $Q_C$  and  $b_h$  should be located at the outer surface of chromatophore membrane, whereas  $Q_Z$  and  $b_l$  should reside approximately in the middle of the membrane dielectric. Actually this inference was mainly based on the analysis of energy-linked responses of cytochrome  $b$  hemes [11] and on the accessibility of  $b$  hemes to membrane-impermeable redox agents [12].

To clarify these points a direct electrometrical assay, developed by our group earlier [13–15], was applied for the investigation of electrogenic reactions in the  $bc_1$

*Correspondence address:* A.Yu. Semenov, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow 119899, Russian Federation. Fax: (7) (095) 939 0338.

*Abbreviations:* RC, reaction center complex;  $\Delta\psi$ , electric potential difference;  $Q_C$  and  $Q_Z$ , the quinone-reducing and quinol-oxidizing sites, respectively;  $b_l$  and  $b_h$ , the low- and the high-potential cytochrome  $b$  hemes, respectively;  $P^+Q_A^-$ , primary dipole in bacterial RC;  $Q_B$ , the secondary quinone acceptor in RC.

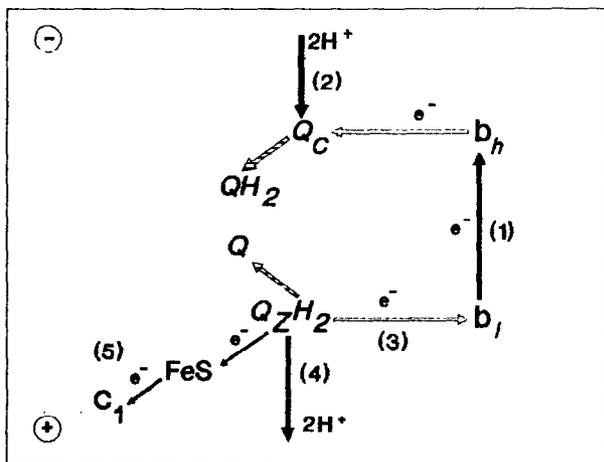


Fig. 1. A general scheme of charge transfer reactions and probable arrangement of redox centers in the chromatophore cytochrome  $bc_1$  complex. The filled arrows indicate main electrogenic reactions. Figures in brackets refer to the numbered reactions (see the text). The pointed lines indicate the conditional borders of the protein (membrane) dielectric.

complex of *Rhodobacter sphaeroides* chromatophores. The high sensitivity of the method means that averaging of experimental data is not necessary and it is possible to register very small electrogenic steps which cannot be detected by other techniques.

## 2. ARRANGEMENT OF REDOX CENTERS

Under oxidizing conditions (at  $E_h = +300$  mV) ubiquinol formation in bacterial RCs takes place only after even numbered flashes [16]. Since at this  $E_h$  value all ubiquinone molecules in the pool are fully oxidized, the reactions in the  $bc_1$  complex could only be triggered by the ubiquinol molecule produced in the RC after its interaction with quinol-oxidizing site,  $Q_Z$ . It was shown [17] after illumination of the dark-adapted chromatophores by the first flash, that only electrogenic phases due to the primary  $P^+Q_A^-$  dipole formation, as well as to the reduction of the oxidized bacteriochlorophyll dimer by cytochrome  $c_2$ , can be observed. After the second flash two new phases appeared in addition: a faster phase associated with protonation of the secondary quinone acceptor,  $Q_B$ , and a slower phase due to ubiquinol oxidation by the  $bc_1$  complex. The difference between the second and the first flashes reflects only electrogenic protonation of  $Q_B$  and subsequent total electrogenesis provided by the  $bc_1$  complex. Antimycin partially inhibited the slower electrogenic phase, while myxothiazol, in the presence of antimycin, caused a total disappearance of the slow phase [17].

This suggests that the electron transfer between the low- and the high-potential cytochrome  $b$  hemes is responsible for the myxothiazol-sensitive electrogenic phase. The antimycin-sensitive phase can be ascribed to

$Q_C^-$  protonation which accompanied the reduction of ubiquinone, and also to the reactions occurring during the additional turnovers of the  $bc_1$  complex. The latter conclusion is mainly based on the fact that the rise time of the antimycin-sensitive electrogenic phase is much slower than the rate of the  $b_h$  oxidation by ubiquinone at center C, and also on the RC- $bc_1$  complex stoichiometry [17]. The possible arrangement of redox centers within the  $bc_1$  complex, and the corresponding charge transfer reactions, are presented in Fig. 1.

## 3. A 'NEGATIVE' ELECTROGENIC PHASE

Addition of myxothiazol without antimycin induces, after the second flash, the appearance of a transient electrogenic phase which has an opposite polarity to all the other phases [17]. This 'negative' or 'reverse' phase is antimycin-sensitive. Its characteristic duration is about 2.5 ms at pH 7.5. It has been shown [18] that the amplitude of the negative phase constitutes about 10% of the amplitude of the electrogenic phase ascribed to the primary charge separation ( $P^+Q_A^-$ ) and does not vary between pH 10.5 and 7.5. With lowering pH the amplitude decreases and disappears approximately at pH 5.0.

The data obtained suggest that the nature of the negative phase, observed by direct electrometry [17,18], differs from that obtained by carotenoid bandshift assay, since the latter was observable only at pH > 8.5, when the electron transfer between  $Q_C$  and  $b_h$  became pronounced [6-8]. Presumably, the negative phase arises as a consequence of proton release out of center C upon ubiquinol binding, and also as a result of the proton transfer from center C to the outer surface of the chromatophore in the reaction:  $QH_2 \rightarrow QH^- + H^+$ . As was suggested by Rich [19] this reaction might precede the ubiquinol oxidation in different quinol:oxidoreductases. The proposed reaction, which leads to the negative electrogenic phase, is presented in Fig. 2. These results suggest that during the normal functioning of the  $bc_1$  complex, at least a part of the antimycin-sensitive electrogenic stage is due to the proton binding by the doubly reduced quinone at the  $Q_C$  site [17,18], and not to the electron transfer between  $b_h$  and  $Q_C$ , as has been originally proposed by Robertson and Dutton [6]. This means that both  $b_h$  and the  $Q_C$ - binding site should be somewhat buried in the protein dielectric (see Figs. 1 and 2). These results showed that the electric distance between center C and the outer aqueous phase is about 4.5-5 Å [18].

## 4. LOCATION OF CENTERS C AND Z

It is interesting to compare this distance with the position of the  $Q_C^-$  semiquinone anion which has recently been determined by Meinhardt and Ohnishi in *Rhodobacter capsulatus* chromatophores [20]. These authors measured the spin relaxation enhancement by an

external probe, and concluded that the distance between  $Q_C^-$  and the cytochrome  $b$  surface outside the chromatophore is between 6 and 10 Å. This estimation of the electrical distance was based on the assumption of a non-variable dielectric constant value in this protein region. Comparison of these two distances makes it possible to evaluate the average enhancement of the dielectric constant between the  $Q_C$  site and the protein surface. The dielectric constant increases almost 2-fold in this protein region in comparison with the central hydrophobic domain.

The obtained data has not allowed a decision as to whether the ubiquinol oxidation at center Z is accompanied by the electrogenic reaction. As mentioned above there are contradictory views to this question (cf. [6] and [10]). Recently site-specific mutagenesis has been used to produce *Rb. sphaeroides* strains lacking the high-potential cytochrome  $b$  heme [21]. Replacement of the axial ligands of cytochrome  $b_h$  (His-111 by either Asp (strain H111D) or Asn (H111N), or His-212 by Asp (H212D)) results in a selective loss of cytochrome  $b_h$  and retention of cytochrome  $b_l$ . Unfortunately, analogous replacements of the cytochrome  $b_l$  axial ligands (His-97 and His-198) is accompanied by a total loss of cytochrome  $b$  hemes. Nevertheless the former mutants could serve as an extremely useful tool for the investigation of redox reactions proceeding under oxidation of ubiquinol at the  $Q_Z$  site. The flash kinetic studies of the H111N mutant have shown that there is no  $b_h$  reduction in the presence of antimycin, while the myxothiazol-sensitive reactions at the  $Q_Z$  site, including reduction of cytochrome  $b_l$ , are still functional [21].

## 5. ELECTROGENIC STEPS

Recently chromatophores of the *Rb. sphaeroides* H111N mutant lacking high-potential  $b$  heme have been investigated by the electrometrical technique [22]. The

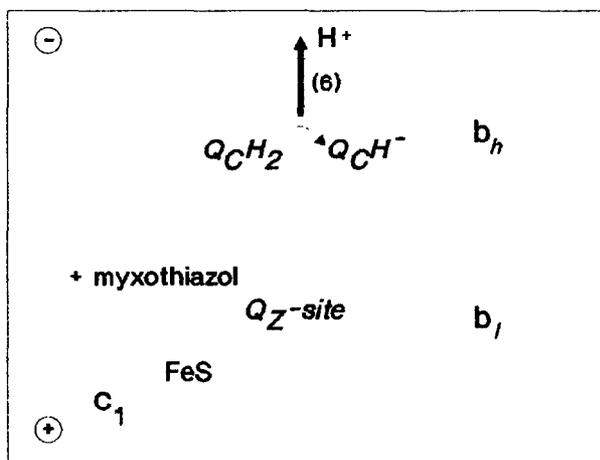


Fig. 2. The proposed scheme of the process leading to the negative electrogenic reaction, which is observed in the presence of myxothiazol.

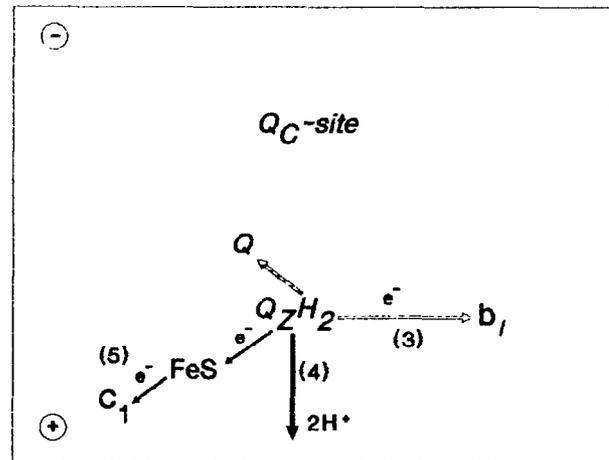


Fig. 3. The scheme of charge transfer reactions and location of redox centers in the cytochrome  $bc_1$  complex of the H111N mutant chromatophores, lacking the high-potential  $b$  heme (see the text for details).

difference between the second and the first flash-induced  $\Delta\psi$  kinetics reveals a fast phase ( $\tau = 90 \mu\text{s}$ ), reflecting protonation of the 'doubly reduced'  $Q_C^{2-}$  in the RC, and a slower phase ( $\tau = 10 \text{ ms}$ ). Myxothiazol has hardly any effect on the faster phase, but it completely inhibits the slower phase and reveals a small additional electrogenic phase of the opposite polarity. Antimycin does not influence the photoelectric responses when it is added either before or after myxothiazol, but stigmatellin partially inhibits the negative phase. The difference between the flashes in the presence and absence of myxothiazol reveals a total myxothiazol-sensitive phase with a rise time of 8 ms and an amplitude which constitutes 10–12% of the primary charge separation stage [22].

The electrogenic steps within the  $bc_1$  complex may include the following reactions (see Figs. 1 and 2).

- (1) Myxothiazol-sensitive but antimycin-insensitive electron transfer between  $b_l$  and  $b_h$  (positive phase).
- (2) Antimycin-sensitive protonation of the  $Q_C^{2-}$  (positive phase).
- (3) Myxothiazol-sensitive electron transfer between  $Q_Z$  and  $b_l$  (positive phase).
- (4) Myxothiazol-sensitive proton release on oxidation of  $QH_2$  at the  $Q_Z$  site (positive phase).
- (5) Electron transfer from  $Q_Z$  to cytochrome  $c_2$  via the Rieske FeS center and cytochrome  $c_1$  (negative phase).
- (6) Antimycin-sensitive partial deprotonation of  $QH_2$  at the  $Q_C$  site in the presence of myxothiazol (negative phase).

Since the chromatophores of the H111N mutant lack heme  $b_h$ , it can be concluded that the slower phase of  $\Delta\psi$  rise ( $\tau = 10 \text{ ms}$ ) is probably related either to reaction (3) or reaction (4) (see discussion below), while the negative phase observed in the presence of myxothiazol is due to reaction (5) rather than reaction (6) (the latter

should not be sensitive to stigmatellin). Redox reactions in the  $bc_1$  complex of the H111N mutant, and the corresponding probable electrogenic steps, are shown in Fig. 3.

Table I presents a comparison of the parameters of different electrogenic reactions in chromatophores from the wild type and mutant (H111N) *Rb. sphaeroides* strains. It can be seen that there is almost no difference either in the amplitudes or in the rise times of the electrogenic phase ascribed to the protonation of  $Q_B$  in RC. In contrast, the amplitude of  $\Delta\psi$  increase ascribed to the functioning of the  $bc_1$  complex is almost 6-fold smaller in the mutant than in the wild-type chromatophores, and it is also about 3-times faster. A more important difference is the lack of the antimycin-sensitive electrogenic step in the mutant strain. The observed negative phase is about 2-fold slower and more than 2-times smaller in the mutant chromatophores. The net amplitude of the myxothiazol-sensitive phase corresponds to a charge transfer through about 5 Å (assuming a P- $Q_A$  distance of 27 Å [23]).

The results need several comments to emphasize the points which remain unresolved.

(i) It should be considered that the negative phase observed in the presence of myxothiazol can partially be due to the passive discharge across chromatophore membrane and represents to some extent the slope of a zero line.

(ii) On the other hand, partial inhibition of this decay by stigmatellin indicates that the observed negative phase at least partially originates from the charge transfer in the  $bc_1$  complex. As mentioned above, both reactions of partial deprotonation of  $Q_C H_2$  and the electron transfer from the FeS center to cytochromes  $c_1$  and  $c_2$  could contribute to this decay. The effect of stigmatellin is consistent with electrogenicity of the proton release, accompanying oxidation of ubiquinol at center Z by cytochrome  $b_1$  (reaction 4) rather than with the electron transfer between  $Q_Z$  and  $b_1$  (reaction 3). If the release of the two protons is actually electrogenic,  $Q_Z$  should be somewhat buried in the protein dielectric, and the electron transfer between FeS and cytochrome  $c_1$ , which is inhibited by stigmatellin, should compensate the electric

potential increase due to the release of one of the two protons. This conclusion is consistent with the lack of accessibility of the FeS Rieske protein for protolytic digestion from the chromatophore interior [24], and the distance of about 20 Å from the protein surface to the FeS cluster, estimated in mitochondria by Ohnishi et al. [25].

In conclusion, the electrogenic reactions in the photosynthetic bacterial cytochrome  $bc_1$  complex involve electron transfer between cytochrome  $b$  hemes, and also two additional steps, which are most probably associated with protonation of the doubly-reduced ubiquinone at the  $Q_C$  site and with deprotonation of ubiquinol at the  $Q_Z$  site.

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Table I

Comparison of the electrogenic reaction parameters in chromatophores of wild-type *Rhodobacter sphaeroides* and GABH111N mutant

Nature of electrogenic phase	$\tau$ (ms)		Magnitude (%) of $P^+Q_A^-$	
	Wild-type	GABH111N	Wild-type	GABH111N
1. Primary dipole $P^+Q_A^-$ formation	$<10^{-4}$	$<10^{-4}$	100	100
2. Protonation of $Q_B^{2-}$	0.1	0.09	16	15
3. $\Delta\psi$ produced by $bc_1$ complex	30	10	45	8
4. Antimycin-sensitive phase	40	0	30	0
5. Myxothiazol-sensitive phase	7	8	15	11
6. Negative phase	2.5	5	9	4

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