Volume 321, number 1, 1–5 FEBS 12315 April 1993

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

Received 1 February 1993; revised version received 4 March 1993

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^{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 proposed 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.

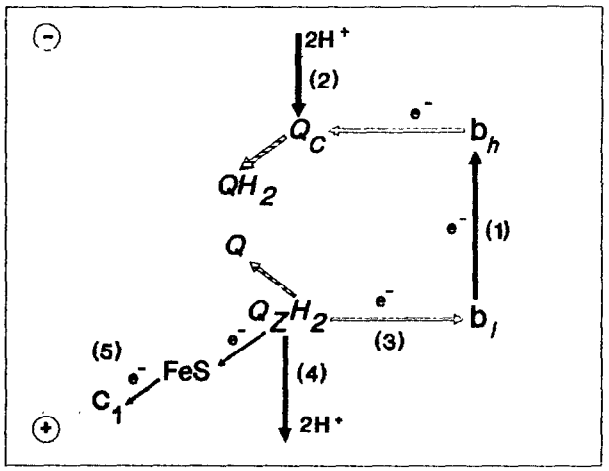

Fig. 1. A general scheme of charge transfer reactions and probable arrangement of redox centers in the chromatophore cytochrome bc_1 complex. The filled arrows indicate main electrogenic reactions. Figures in brackets refer to the numbered reactions (see the text). The pointed lines indicate the conditional 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^{2-} 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 qui-nol: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

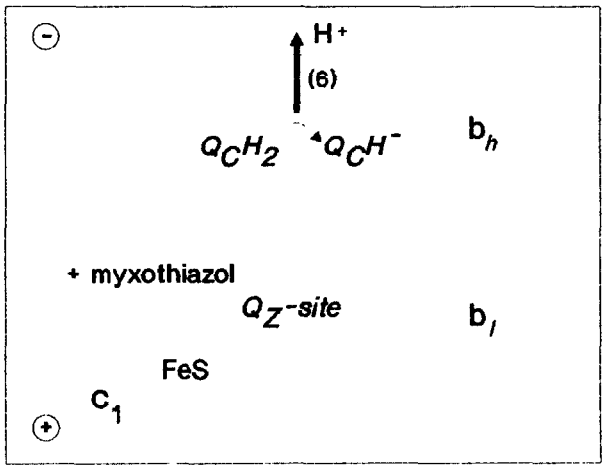

Fig. 2. The proposed scheme of the process leading to the negative electrogenic reaction, which is observed in the presence of myxothiazol.

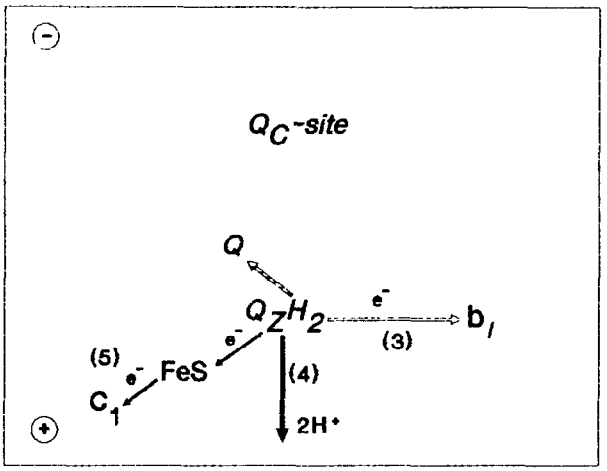

Fig. 3. The scheme of charge transfer reactions and location of redox centers in the cytochrome bc_1 complex of the H111N mutant chromatophores, lacking the high-potential *b* heme (see the text for details).

difference between the second and the first flash-induced $\Delta\psi$ kinetics reveals a fast phase (τ = 90 μs), reflecting protonation of the 'doubly reduced' Q_B^{2-} in the RC, and a slower phase (τ = 10 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 (τ = 10 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_CH_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 uniquinol 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 uniquinone at the Q_C site and with deprotonation of ubiquinol at the Q_Z site.

Acknowledgements: The author wishes to thank Prof. V.P. Skulachev and Dr. M.D. Mamedov for helpful discussions and critical reading of the manuscript.

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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	τ (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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