Volume 259, number 2, 324-326 FEB 07986 January 1990

Electrogenesis associated with proton transfer in the reaction center protein of the purple bacterium *Rhodobacter sphaeroides*

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Received 19 October 1989

Electrogenic events in the photosynthetic reaction center complex (RC), accompanying single- and two-electron reduction of the secondar quinone acceptor Q_B, were investigated. In the presence of inhibitors of electron transfer via the bc_1-complex, the kinetics of formation of the transmembrane electric potential difference induced by two successive light flashes exhibit a few phases. Besides the fast phase A which is due to the charge separation between the bacteriochlorophyll dimer P and primary quinone acceptor Q_A, two slower atrazine-sensitive phases, BI and BII, were observed. Phase BI is suggested to be due to proton transfer between the amino acid residues of the reaction center protein, and phase BII due to proton uptake during the second flash-induced formation of ubiquinol. A possible model of electrogenesis in the acceptor moiety of the RC is discussed.

Reaction center; Quinone acceptor; Electrogenesis; (*Rhodobacter sphaeroides*)

1. INTRODUCTION

The acceptor moiety of the photosynthetic reaction center of non-sulphur purple bacteria contains two ubiquinone molecules, a one-electron primary acceptor (Q_A) and a two-electron secondary acceptor (Q_B) [1]. Following a flash excitation of the photochemically active pigment, a long-lived species Q_B^- is produced. After the second flash, it traps two H^+ ions and converts to a fully reduced ubiquinol molecule, Q_BH_2 [2-5]. We have earlier observed that at neutral pH values the protonation of the secondary quinone, following the second flash, is accompanied by the appearance of a new phase in the kinetics of generation of the transmembrane electric potential difference ($\Delta\psi$) [3,6]. In a study using isolated reaction center preparations it has been revealed that at acidic and neutral pH values H^+ binding occurs in responce to even-numbered flashes and at alkaline pH values proton uptake takes place in response to every flash [3-5,7]. Because the secondary quinone is in anion-radical form after odd-numbered flashes it has been suggested that the uptake of protons may take place as a consequence of protonation of a protein H^+ acceptor group [4,5,7,8] associated with the transfer of a negative charge to Q_B.

In the present work we investigated the phases of $\Delta\psi$ generation in chromatophores, a reflection of electron transfer and proton uptake by the RC in response to the first and second laser flashes at different pH values.

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2. MATERIALS AND METHODS

Cells of *Rb. sphaeroides* (wild-type, strain R1) were grown and chromatophores containing no cytochrome c_2 were isolated from ultrasonically ruptured cells as described elsewhere [9]. Chromatophores were associated with a flat phospholipid-impregnated collodion film and transmembrane potential was measured electrometrically as in [6,10]. The kinetic curves were analyzed using a modification of the DISCRETE program [11].

All the experiments were conducted in the presence of the electron transfer inhibitors, antimycin A (5 μM) and myxothiazol (3 μM). The incubation medium contained 20 mM of one of the following pH buffers: MES, MOPS, tricine, *bis-tris*-propane and CAPS. Each buffer was used at a pH value to its pK.

3. RESULTS

Presented in fig.1 are photoelectric responses of chromatophores at pH 7.5 (A) and pH 9.0 (B) induced by the first (curve 1) and second (curve 2) flashes in the presence of myxothiazol and antimycin A, which completely inhibit electron transfer within the bc_1-complex.

The fast phase (hereafter referred to as phase A), whose rise time is shorter than the resolution time of the equipment ($\tau < 0.1$ μs), was observed both after the first and second flash. The phase originates from charge separation between the bacteriochlorophyll dimer P and Q_A [6].

A slower phase (phase BI) was also observed after the first flash. Its amplitude is much larger at pH 9.0 (fig.1B) than at pH 7.5 (fig.1A). Since chromatophores obtained by ultrasonic treatment are devoid of cytochrome c_2, it is most probable that phase BI arises from the electrogenic reactions in the complex of quinone acceptors.

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00145793/90/$3.50

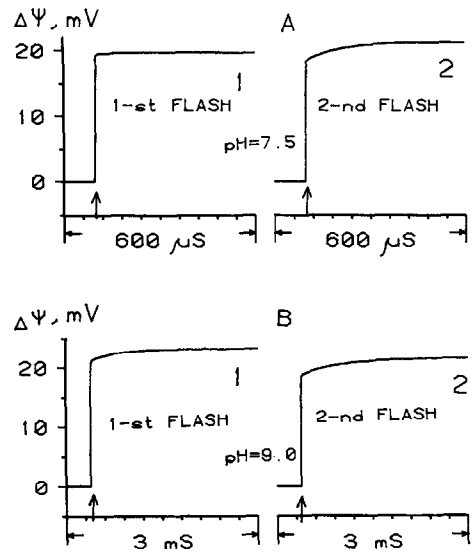

Fig.1. Generation of electric potential by *Rhodobacter sphaeroides* chromatophores on the first (1) and second (2) laser flashes at pH 7.5 (A) and pH 9.0 (B). The incubation medium contained 1 mM ferrocyanide, 50 μM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, 5 μM antimycin A, 3 μM myxothiazol and 20 mM buffer (HEPES at pH 7.5 and *bis-tris*-propane at pH 9.0). The arrows indicate laser flashes. The second flash was given 2 s after the first.

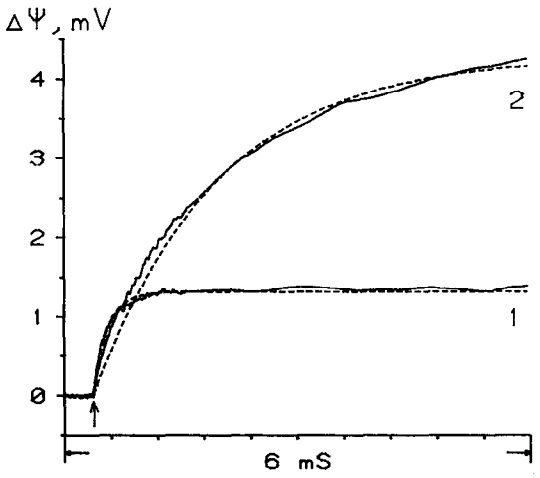

Fig.2. Kinetic curves obtained by subtracting the photoelectric response induced by the first flash in the presence of atrazine from the response induced by the first flash (1) or by the second flash (2) in the absence of the inhibitor. Conditions as in fig.1, pH 9.5. The dashed lines represent mono-exponential curves with a rise time of 0.18 ms (1) and 1.6 ms (2).

Another relatively slow phase was observed after the second flash (referred to as BII). Its amplitude at pH 7.5 was 15–20% of the amplitude of phase A. As has been demonstrated in [6], responsible for phase BII is electrogenic protonation of doubly reduced secondary quinone in response to the second flash.

In the presence of atrazine which inhibits the Q_A to Q_B electron transfer, only phase A is generated on the first flash. As shown in [4,5] the flash-induced proton uptake could be observed to some extent in the Q_B-depleted chromatophores. But it is noteworthy, that we failed to detect any flash-induced electrogenic reactions (besides phase A) in the Q_B-depleted chromatophores.

The differences in the photoelectric responses on the first and second flash in the absence of atrazine and the responses to the first flash in its presence enable the separation of the phases BI and BII. These differences are given in fig.2. It is seen that phase BII (curve 2) has a higher relative amplitude that the faster phase BI (curve 1). The kinetics of each of the two phases are well described by the mono-exponential curves (fig.2, dashed lines).

Fig.3 shows the pH dependencies of the amplitude and rise time for the phases BI and BII. The amplitude of phase BI (fig.3A, triangles) has a fairly small and constant value (2–3% of the phase A amplitude) at acidic and neutral pH values and reaches its maximal value (about 7% of the phase A amplitude) at pH 9.5. With further alkalinization, its amplitude drops again. The pH dependence of the first flash-induced proton uptake amplitude by the isolated RCs [8] is presented in fig.3A (open circles: the data were normalized to the BI phase amplitude at pH 9.5). The amplitude of phase BII (fig.3A, rectangles) constitutes 16–18% of that of phase A over the pH region 5.5–9.0. With further increasing pH, its amplitude decreases sharply.

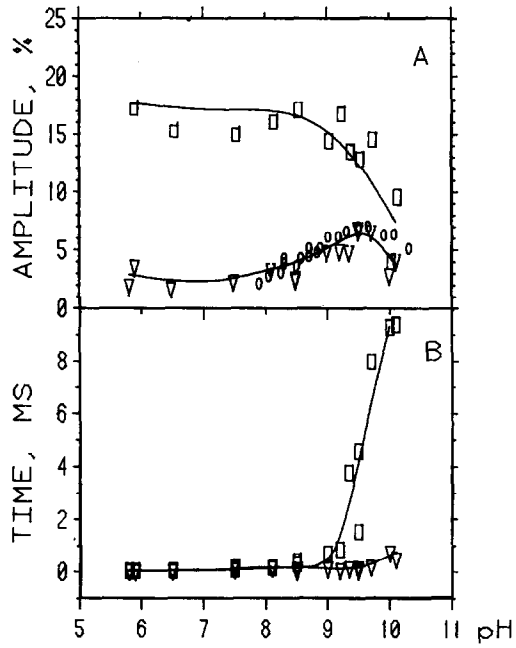

Fig.3. pH dependencies of the amplitudes (A) and rise times (τ) (B) of the electrogenic 'quinone' phases BI and BII induced by the first (triangles) and second (rectangles) flashes, respectively. The pH dependence of the first flash-induced proton uptake amplitude by the isolated RCs [13] is shown as open circles and normalized to the BI phase amplitude at pH = 9.5. The amplitudes of BI and BII phases are given in percents of phase A amplitude. Rise times were determined by decomposing the experimental curves with the DISCRETE routine [11]. Conditions as in fig.1, except for the buffers (see section 2).

The pH dependence of the rise time of the BI and BII phases are shown in fig.3B. At acidic and neutral pH values the rise times of the two phases are very similar in pattern (phase BI, triangles; phase BII, rectangles). In the alkaline pH region, phase BII becomes much slower than phase BI.

4. DISCUSSION

It follows from the X-ray crystallography of protein crystals of *Rb. sphaeroides* reaction centers [12,13] that the quinone acceptors Q_A and Q_B are located at approximately equal distances from the membrane surface. Hence, the transfer of electrons from Q_A to Q_B occurs along the membrane and may, therefore, only slightly contribute to the $\Delta\psi$ formation. It is possible that the small phase BI (about 2% of the phase amplitude, see fig.3A), which is observed at acidic and neutral pH values, is a reflection of the electrogenesis of the electron transfer from Q_A to Q_B. The projection of the vector of the P to Q_A charge transfer onto the normal of the membrane is 27 nm long. Consequently, the possible magnitude of the normal projection of the $Q_A \rightarrow Q_B$ vector (2% of 2.7 nm) is ~0.05 nm. The increase of the amplitude of phase BI under alkaline conditions (fig.3A) and the fact that the rate of its rise becomes pH-dependent suggest that under these conditions phase BI, at least partly, is associated with transmembrane proton transfer. The additional confirmation of the nature of the phase BI is the good correspondence between the pH-dependencies of the amplitudes of the first flash-induced proton uptake (fig.3A, circles) and of the phase BI (fig.3A, triangles).

The scheme in fig.4 explains the obtained results. X and Y are the amino acid residues of the RC along which the photo-induced proton transfer occurs. It is assumed that amino acid residue X is located on the membrane surface and that its pK is ca. 9.5 (see fig.3). Group Y is localized closer to Q_B and its pK depends on the charged state of Q_B (see fig.3A, triangles). When Q_B is oxidized (non-charged), the pK value (pK^0, fig.4) of amino acid residue Y is ~9.0. With Q_B being in the semiquinone form (charged negatively), the pK value (pK^1, fig.4) of Y is ~10. At pH <9, residue Y is in the protonated state and, hence, cannot trap a proton during electron transfer toward Q_B following the first flash. However, when the pH value of the incubation medium is higher than 9 but lower than 10, the arrival of an electron on Q_B causes proton transfer on group Y from group X, group X further accepting a proton from the environmental water phase. The transfer of a proton from X to Y has a component across the membrane dielectric and causes the appearance of the electrogenic phase. As residue X is proposed to be located on the membrane surface, its protonation does not cause the appearance of the electrogenic phase. Thus, the amplitude of phase BI is essentially determined by the

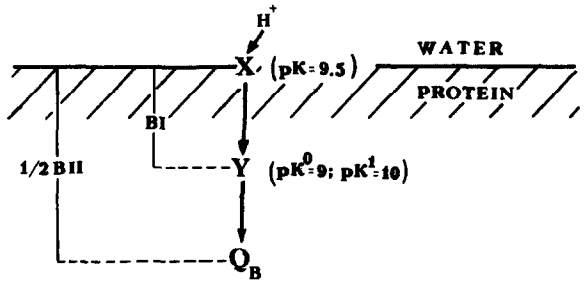

Fig.4. A schematic presentation of the electrogenesis associated with the secondary quinone acceptor functioning.

proton transfer between groups X and Y. Its rate does not depend on pH until group X is protonated under equilibrium conditions in the dark (it follows from fig.3B (triangles) that the pK value of group X is ~9.5). At pH values higher than the pK value, the rate of rise of phase BI will be determined by the rate of proton arrival from the surrounding medium to residue X and, hence, by the pH of the incubation medium. This is just what has been observed at pH >9.5. The mechanism of proton binding on the second flash, which causes the appearance of the electrogenic phase BII is not completely clear. Possible proton-transfer routes may be through the amino acid residues X and Y. A second proton arrives at Q_B via this same route. However, it is probable that there is an individual proton-transfer pathway toward each ubiquinone oxygen atom. Among the amino acid residues surrounding Q_B [13], arginine and tyrosine residues seem the most probable candidates for the role of the unidentified groups X and Y, bearing in mind that their pK values are in the alkaline region.

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