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Flash-induced electrogenic events in the photosynthetic reaction center and bc_1 complexes of *Rhodobacter sphaeroides* chromatophores

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Electrogenic events in *Rb. sphaeroides* chromatophores have been studied (i) electrometrically in the chromatophore/phospholipid-impregnated collodion film system and (ii) spectrophotometrically by measuring the electrochromic spectral shift of carotenoids. Under the conditions when the bc_1 complex and ubiquinone pool were oxidized at pH 7.5, the second flash was shown to give rise to at least two additional electrogenic phases of τ values approx. 0.2 and approx. 20 ms, which were not induced by the first flash. The fast phase was resistant to the inhibitors of the bc_1 complex, antimycin A and myxothiazol. It seems to be due to the protonation of reduced Q_B in the RC complex. The slow phase was partly inhibited by antimycin A and completely by subsequent addition of myxothiazol. The antimycin-sensitive constituent of the slow phase was $\tau \approx 40$ ms and its rise was non-exponential. The antimycin-insensitive, myxothiazol-sensitive constituent was $\tau \approx 7$ ms. A comparison of (i) the kinetics of cytochrome b_h redox conversion induced by the first and second flashes and (ii) the electrogenic reactions sensitive to the Q-cycle inhibitors suggests that the myxothiazol-sensitive electrogenic phase is associated with the reduction of cytochrome b_h (b-561). The antimycin-sensitive electrogenic phase apparently results from the protonation of reduced Q in the quinone-reducing center of the bc_1 complex. Reduction of ubiquinone to ubisemiquinone by b_h seems to be electrically silent, since there is no electrogenic phase to follow the kinetics of this process. Myxothiazol added in the absence of antimycin A induced a negative electrogenic phase with an opposite polarity ($\tau \approx 2.5$ ms) after the second flash. This phase, completely abolished by the addition of antimycin A, is assumed to be due to the electrogenic deprotonation of the RC-reduced QH_2 which combines with center C in the bc_1 complex. The data obtained by the electrometric and spectrophotometric methods appear to be very similar, though the electrometric method is more sensitive because of the much higher signal-to-noise ratio.

Introduction

The cytochrome bc_1 complex is a functionally active entity in the coupling membranes of most biological systems. In photosynthetic bacteria, it is involved in the cyclic electron flow and serves to oxidize the reaction center ubiquinol and to reduce cytochrome c_2, an electron donor for the RC [1–7].

Although the electron-transfer sequence in the bc_1 complex has been clarified somewhat of late, the mechanism whereby electron transport gives rise to a transmembrane proton electrochemical potential ($\Delta\mu_{H^+}$) is not yet clear [1–7].

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 [8–13] which accompany the generation of a transmembrane electric potential ($\Delta\psi$). Using this method, it was shown that electrogenic reactions in the bc_1 complex are partly suppressed by antimycin A and completely by myxothiazol, indicating

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Abbreviations: E_h, redox potential of the medium; $\Delta\tilde{\mu}_{H^+}$, transmembrane proton electrochemical potential difference; $\Delta\psi$, transmembrane electric potential; bc_1 complex, QH_2:ferricytochrome c_2 oxidoreductase; b-561 (b_h) and b-566 (b_l), high- and low-potential hemes of cytochrome b, respectively; RC, photosynthetic reaction center complex; P870, the primary electron donor in RC; Q_A, Q_B, primary and secondary quinone acceptors in the RC; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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that there are two electrogenic stages in the bc_1 complex [12]. However, this method has low sensitivity (signal averaging is necessary), so that quantitative characteristics of these stages cannot be revealed. Moreover, it cannot be applied to carotenoidless species. We developed an alternative method to study electrogenesis in the bc_1 complex, i.e., direct (voltmeter) measurement of $\Delta\psi$ generation in the chromatophore adsorbed onto the surface of a phospholipid-impregnated collodion film [14–16]. Offering a large signal-noise ratio, the method enables the measurement of photoelectric responses without averaging. With this method, the electrogenic activity of electron transfer from P870 to the primary quinone acceptor [15], and from cytochrome c_2 to P870 [17] has been measured. Moreover, an additional electrogenic stage was detected associated with secondary quinone-acceptor protonation [18]. In the presence of TMPD and sodium ascorbate, the photoelectric response of *Rhodobacter* (previously *Rhodopseudomonas) sphaeroides* chromatophores was found to be sensitive to antimycin A [19].

The previous investigations into the electrogenic reaction of the bc_1 complex were carried out usually at a rather low redox potential of the medium when most quinone molecules were reduced. Under such conditions, the QH_2 efflux from the RC may be ignored [6,10,12,13], with the electron-transfer reactions in the bc_1 complex being initiated by cytochrome c_2 oxidation. The electrogenic events taking place in the bc_1 complex may thus be analyzed only with the aid of inhibitors.

Ubiquinol formation is another way of initiating electron transfer in the bc_1 complex [20]. At high redox potentials, all the ubiquinone molecules in the membrane are oxidized. Consequently, the bc_1 complex remains inoperative after the photoinduced oxidation of cytochrome c_2. Semiquinone Q_B^- produced by the first flash is not capable of serving as an electron donor for the cyclic electron flow [1,21,22,23]. It is only after the second flash, when ubiquinol is formed, that the bc_1 complex can be reduced [20,23]. Thus, under such conditions, it appears possible to study the electrogenic reactions of the bc_1 complex by comparing the photoelectric responses produced by the first and second flashes.

The binary $\Delta\psi$ oscillations in chromatophores of purple bacteria were observed in earlier investigations by monitoring the electrochromic absorption changes of carotenoids [23] and by using lipophilic ions [24,25], as well as their sensitivity to antimycin A [23,25] and myxothiazol [25]. However, no detailed kinetic analysis of the observed oscillations was made.

In the present work we have investigated the electrogenic reactions of the *Rb. sphaeroides* bc_1 complex under RC binary operation and oxidizing conditions.

Materials and Methods

Cells of *Rb. sphaeroides* (wild type, strain RI) were grown and chromatophores were isolated by French-press treatment (700 kg/cm^2) as described elsewhere [22,26].

Photoelectric responses of chromatophores were measured electrometrically with the use of a phospholipid-impregnated collodion film as described in Refs. 14–16. Made of 1% nitrocellulose solution in amylacetate on water surface, the film was dried for at least 1 h, impregnated with a decane solution of asolectin (100 mg/ml) and ubiquinone-10 (20 mg/ml), which was added to prevent secondary quinone extraction during the association of chromatophores with the artificial membrane (see Ref. 18). The film separated the two electrolyte-containing compartments of a dismountable Teflon chamber.

A chromatophore suspension was added into one of the compartments (the final concentration of bacteriochlorophyll in the cuvette was approx. 10 μM). To induce adsorption of chromatophores on the film surface 20 mM $CaCl_2$ was added. After 3–4 h an excess of chromatophores not associated with the membrane was removed. To this end, the incubation medium was replaced, with the help of a peristaltic pump, by another one that contained neither chromatophores nor Ca^{2+}.

Ag|AgCl electrodes were used to measure the electric potential across the chromatophore membrane. The voltage output was coupled via an operational amplifier (Burr Brown 3554BM, U.S.A.) to a DL-1080 transient recorder (Data Lab, U.K.) and then to a Nova-3D minicomputer (Data General, U.S.A.).

Light absorption measurements were made on a single-beam differential spectrophotometer with a resolution time of 1 μs. A KGM-9-75 lamp was employed as a source of measuring beam. Saturating flashes ($\lambda = 530$ nm; 15 ns, 20 mJ) were given from a Quantel Nd laser. A shutter was used to prevent the dark-adapted samples (dark-adaptation time 5 min) from the effect of measuring light. Measurement was taken immediately after removing the shutter and stabilization of the direct current through the photomultiplier. The kinetic curves were analyzed on a Nova-3D computer using a modification of the DISCRETE program [27] suggested by Dr. A.L. Drachev. In Fig. 3a–d, and during the subtraction of kinetical curves, the data were normalized according to the amplitude of the fast ($\tau < 100$ ns) electrogenic phase.

The bacteriochlorophyll concentration was determined from absorption at 375 nm using an absorption coefficient of 100 mM$^{-1} \cdot$ cm^{-1} [28]. The substances used in the experiment were Hepes, antimycin A (Serva) asolectin, ubiquinone-10, TMPD and sodium ascorbate (Sigma). Myxothiazol was kindly provided by Prof. W. Trowitzsch.

Results

As demonstrated in Refs. 4, 22, 23, 29, 30 and 33, distinct binary oscillations of the Q_BH_2 formation in chromatophores can be observed under certain conditions, namely in the presence of an efficient electron donor for P870 and an electron acceptor capable of oxidizing Q_B^- in between the series of flashes. These two functions can be performed by reduced and oxidized forms of the redox mediator TMPD [4,22,29].

Fig. 1 shows binary flash-induced 452 nm absorption changes in the *Rb. sphaeroides* chromatophore suspension which indicate the binary oscillations of the semiquinone [23,31–34]. The first flash produces a semiquinone species Q_B^- (absorption maximum at 450 nm). After the second flash, an electron is donated to Q_B^- so that ubiquinol, a species which hardly absorbs at 450 nm, is formed. The third flash produces the semiquinone anion Q_B^- again, and so on.

The Q_B^- produced after odd-numbered flashes remains bound to RC [1,22], whereas Q_BH_2 produced by even-numbered flashes is exchangeable with the membrane pool of ubiquinol [1,35]. The released ubiquinol can be oxidized by the bc_1 complex [20,30].

Presented in Fig. 2 are flash-induced redox changes of cytochrome *b*-561 (b_h) as recorded by absorption changes at 561 nm relative to 569 nm [13,20,30]. It can be seen that the amount of cytochrome b_h reduced after the first flash (Fig. 2a) is much smaller than it is after the second flash (Fig. 2b). Generation of ubiquinol by the first flash in some centers is attributed to measuring-beam light, as will be discussed below. Such a difference seems to increase in the presence of antimycin A, the inhibitor of cytochrome *b*-561 oxidation [7] (Fig. 2c,d). Hence, under conditions used, electrons are delivered mainly to the bc_1 complex after every even-numbered flash when ubiquinol is formed. In agreement with the data of Meinhardt and Crofts [36], 3 μM myxothiazol was shown to completely inhibit the cytochrome b_h reduction both in the presence and in the absence of antimycin A (data not presented).

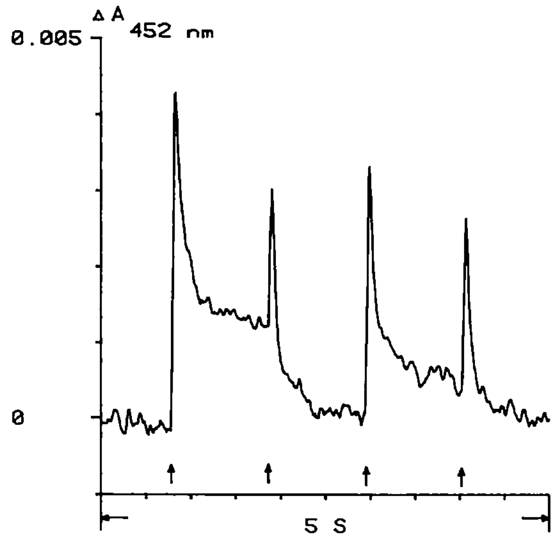

Fig. 1. The 452 nm absorption changes induced by a series of flashes in *Rb. sphaeroides* chromatophores, reflecting binary oscillations in the appearance and disappearance of the semiquinone. Incubation medium: 30 mM Hepes (pH 7.5), 50 μM TMPD, 2 mM potassium ferrocyanide, E_h, 300 mV. Bacteriochlorophyll concentration, 25 μM. Arrows represent laser flashes.

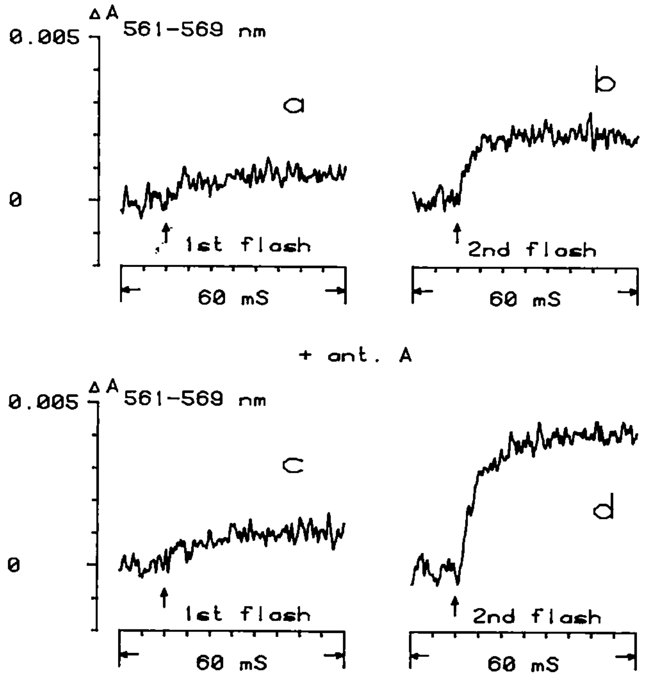

Fig. 2. Redox responses of cytochrome b_h in *Rb. sphaeroides* chromatophores to the first (a, c) and second (b, d) flashes in the absence (a, b) or in the presence (c, d) of 5 μM antimycin A. Incubation medium: 30 mM Hepes (pH 7.5), 50 μM TMPD, 1 mM potassium ferrocyanide, E_h, 320 mV. Bacteriochlorophyll concentration, 50 μM.

Fig. 3a shows the photoelectric responses of *Rb. sphaeroides* chromatophores absorbed on the surface of the phospholipid-impregnated collodion film. Oxidizing conditions were used ($E_h = 300$ mV). After the first flash, the electric potential increases rapidly ($\tau < 100$ ns) as a result of primary charge separation in RC. The relatively slow rise seems to be due to the ($\tau \approx 500$ μs) electrogenic reduction of $P870^+$ by cytochrome c_2 [17] (this phase will be considered in detail in a separate communication). The subsequent slow decay ($\tau \approx 500$ ms) represents the passive discharge of the membrane [15].

Following the second flash (Fig. 3a), two additional electrogenic phases appeared. To study the nature of these two phases, we used the differences in photopotentials induced by the second and first flashes (Fig. 3e). In the absence of inhibitors (Fig. 3e, curve 1), the

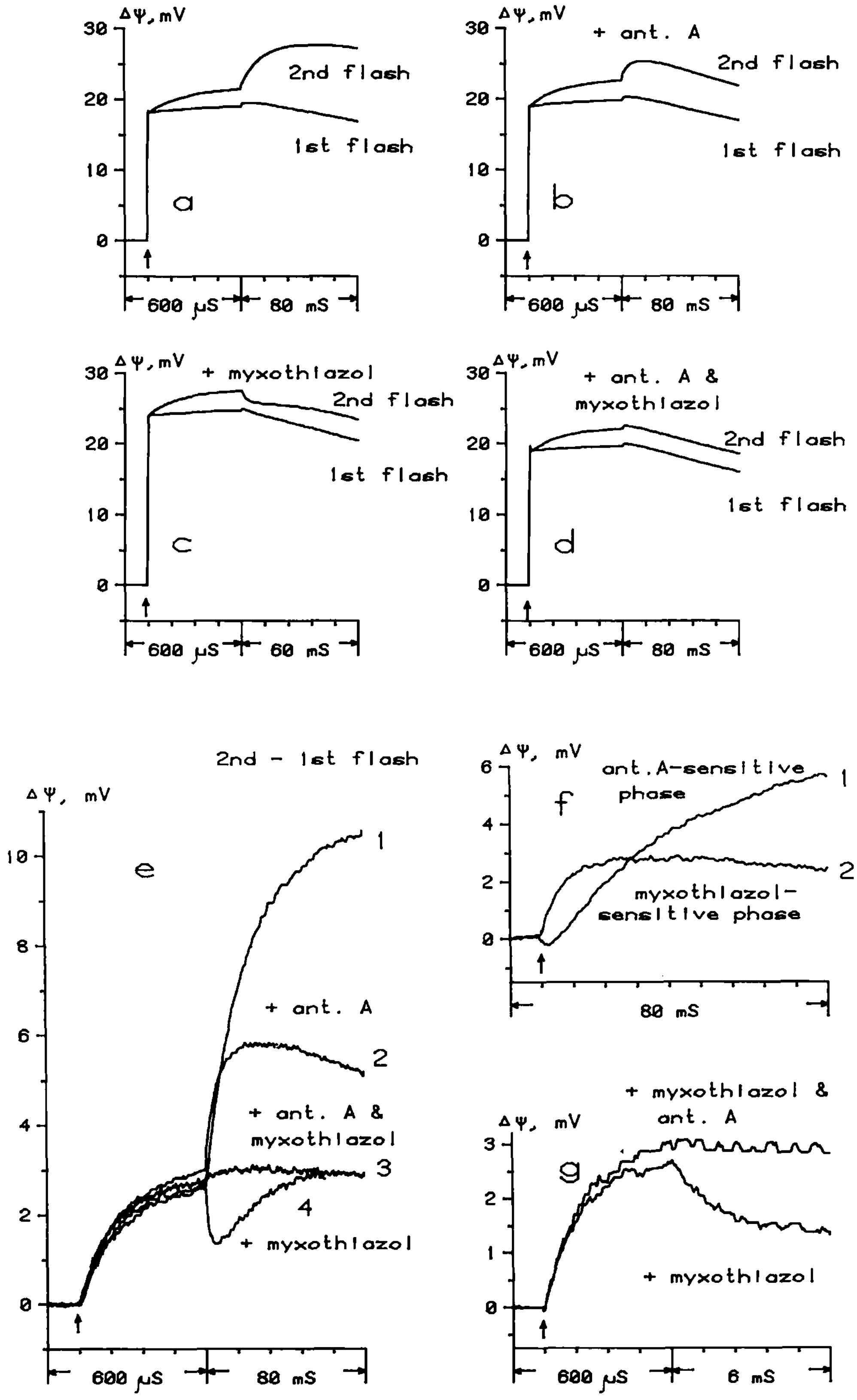

Fig. 3. Photoelectric responses of *Rb. sphaeroides* chromatophores, adsorbed onto the phospholipid-impregnated collodion film, to the first and second flashes. a, with no inhibitors; b, in the presence of 4 μM antimycin A; c, in the presence of 5 μM myxothiazol; d, in the presence of 4 μM antimycin A and 5 μM myxothiazol; e, 2nd minus 1st flash differences calculated from Fig. 3a–d; f, the kinetics of the antimycin-sensitive (curve 1) and antimycin-insensitive, myxothiazol-suppressed (curve 2) phases of $\Delta\psi$ generation, derived from data of Fig. 3e, as described in the text; g, 2nd minus 1st flash difference in the presence of 4 μM myxothiazol and after addition of 4 μM of antimycin A. Incubation medium as in Fig. 1.

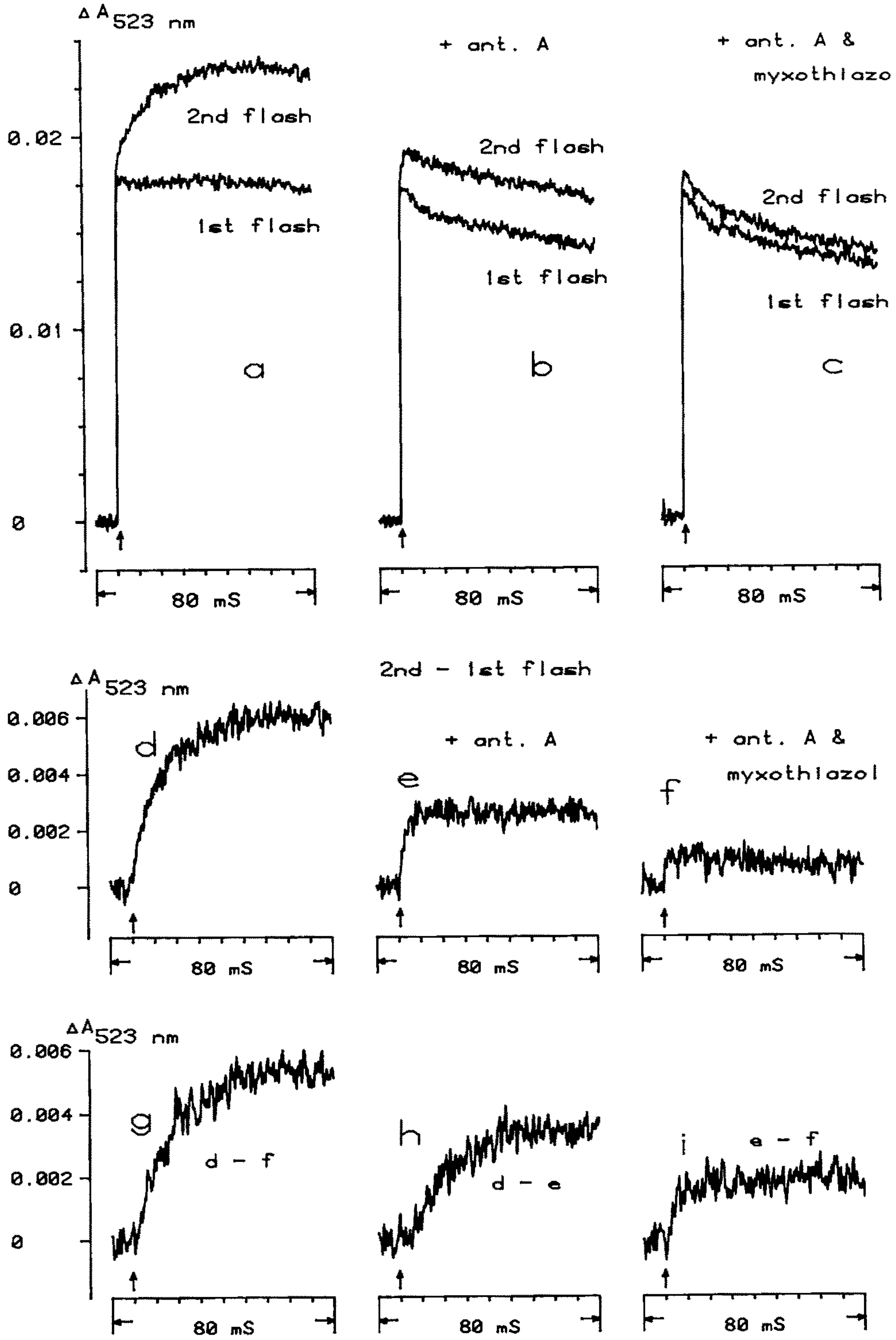

Fig. 4. First and second flash-induced absorption changes at 523 nm in *Rb. sphaeroides* chromatophores, measured with the electrochromic carotenoid bandshift. a, no inhibitors; b, in the presence of 5 μM antimycin A; c, in the presence of 5 μM antimycin A and 3 μM myxothiazol. d–f, 2nd minus 1st flash difference: d, in the absence of inhibitors; e, in the presence of 5 μM antimycin A; f, in the presence of 5 μM antimycin A and 3 μM myxothiazol. The kinetics of the 'slow' (g), antimycin-sensitive (h) and antimycin-insensitive, myxothiazol-suppressed (i) phases. The curves (g–i) are derived by subtracting curves d–f, as is indicated. Incubation medium, as in Fig. 2 (pH 7.8). Bacteriochlorophyll concentration, 25 μM.

fast phase can be approximated by a single exponential curve with a risetime ≈ 150 μs, and the slow phase by an exponential curve with $\tau \approx 20$ ms.

As shown in Figs. 3b and 3e (curve 2), antimycin A has hardly any effect on the second flash-induced fast phase but causes a decrease of the slow-phase amplitude and of its risetime to approx. 7 ms. Subtraction of the slow phase of the photoresponse in the presence of antimycin A (Fig. 3e, curve 2) from the slow phase in the absence of inhibitors (Fig. 3e, curve 1) yields an electrogenic phase sensitive to antimycin A (Fig. 3f, curve 1). The characteristic time of this phase is approx. 40 ms. The addition of myxothiazol (the inhibitor of the ubiquinol-oxidizing center of the bc_1 complex) in the presence of antimycin A causes a further decrease of the second flash-induced photoresponse (Fig. 3d) and disappearance of the slow phase (Fig. 3e, curve 3). By subtracting curve 3 from curve 2 in Fig. 3e, we obtain an electrogenic phase insensitive to antimycin A but inhibited by myxothiazol ($\tau \approx 7$ ms) (Fig. 3f, curve 2).

Thus, the second flash-induced slow electrogenic phase, which is sensitive to both antimycin A and myxothiazol, seems to result from the functioning of the bc_1 complex.

Fig. 3c shows the photoresponses in the presence of myxothiazol without antimycin A. It is evident that, after the second flash, myxothiazol induces an additional transient electrogenic phase which has opposite polarity to all the phases discussed above. The corresponding difference between the second and first flash-induced $\Delta\psi$ change is shown in Fig. 3e (curve 4) and in the more rapid time-scale in Fig. 3g. The addition of antimycin A completely inhibits this negative phase (Fig. 3g). The duration characteristic of this phase is about 2.5 ms at pH 7.5.

The fast phase, insensitive to the inhibitors used, is connected with the events in RC, namely, with reduction of Q_B^- by Q_A^- followed by protonation of the formed Q_B^{2-} (for discussion, see Ref. 37). The risetime of the fast phase (150 μs at pH 7.5) coincides with that of the second flash-induced electron transfer from the primary to secondary quinone [38,39], and also with the time of proton uptake by an isolated RC after even-numbered flashes [34,40].

The data obtained by direct electrometry agree reasonably well with those of carotenoid absorption band-shift monitoring.

Fig. 4a shows the electrochromic absorption changes induced at 523 nm by the first and second flashes. After the first flash (Fig. 4a), the electrochromic shift proceeds very fast. Its subsequent recovery is slow and takes hundreds of milliseconds. Following the second flash, a pronounced additional slow phase appears (Fig. 4a).

The addition of antimycin A causes the slow phase to decrease in amplitude (Fig. 4b).

With the addition of antimycin A and myxothiazol, the slow-phase amplitude decreases much more (Fig. 4c). We found that, in the presence of antimycin and myxothiazol, the absorbance change after the second flash is somewhat larger than after the first flash. Most probably, this is due to the fast electrogenic phase associated with the protonation of Q_B^{2-}.

The obvious influence of the inhibitors of the bc_1 complex on 523 nm absorbance after the first flash (Figs. 4a–4c) suggests that some bc_1 complexes are activated even after the first flash, despite the large dark-adaptation period. The probable cause of this may be the effect of the monitoring light. In the 2–3 s time interval between the opening of the shutter and the stabilization of direct current through the multiplier, the secondary acceptors in some of the RCs may be converted to the semiquinones before the first flash. In the case of electrometry where no measuring beam is used, the Q_B molecules are fully oxidized before the first flash, as shown by the absence of the fast electrogenic phase in the first-flash response. It is not surprising, therefore that in this case, the inhibitors of the bc_1 complex exert no effect on the photoelectric responses induced by the first flash (Figs. 3a–3d).

The 2nd minus 1st flash absorption changes at 523 nm are shown in Fig. 4d–f. The lower line of Fig. 4 represents the kinetic curves derived from those in the upper line. The electrogenic phase shown in Fig. 4g corresponds to the slow electrogenic phase in Fig. 3e, curve 1. Also, one can see the kinetics of the two phases of the electrochromic carotenoid shift – one sensitive to antimycin A (Fig. 4h) and the other insensitive to antimycin A, but sensitive to myxothiazol (Fig. 4i). Notably, there is some lag in the rise of the phase suppressed by antimycin A (Fig. 4h).

The results thus obtained clearly indicate that, under our conditions, the generation of the electric potential in the bc_1 complex takes place only after every second flash of two successive flashes whenever ubiquinol is formed in an RC.

The data obtained by the two methods are qualitatively identical, but the electrometric method is more suitable because of the very much larger signal-to-noise ratio.

Discussion

Within the widely accepted conceptual framework, the photosynthetic electron transport in *Rb. sphaeroides* chromatophores at high E_h values can be described by a diagram presented in Fig. 5.

According to the Q-cycle concept, the oxidation of the ubiquinol molecule formed in RC after two flashes (states a–c) and transferred to the ubiquinol-oxidizing center Z of the bc_1 complex (state d) is organized in such a way that one electron returns via the Rieske iron-sulfur protein (FeS) and cytochromes c_1 and c_2 to P870, and the other is passed via the two-heme cytochrome *b* to the ubiquinone-reducing center C to reduce a ubiquinone molecule which originates from the ubiquinone pool. It has been suggested that the semiquinone formed remains bound to center C [13] (state e).

As RCs in chromatophores are in two-fold excess over bc_1 complexes and because of the need to fill in all the electron vacancies on the reducing side of the RC,

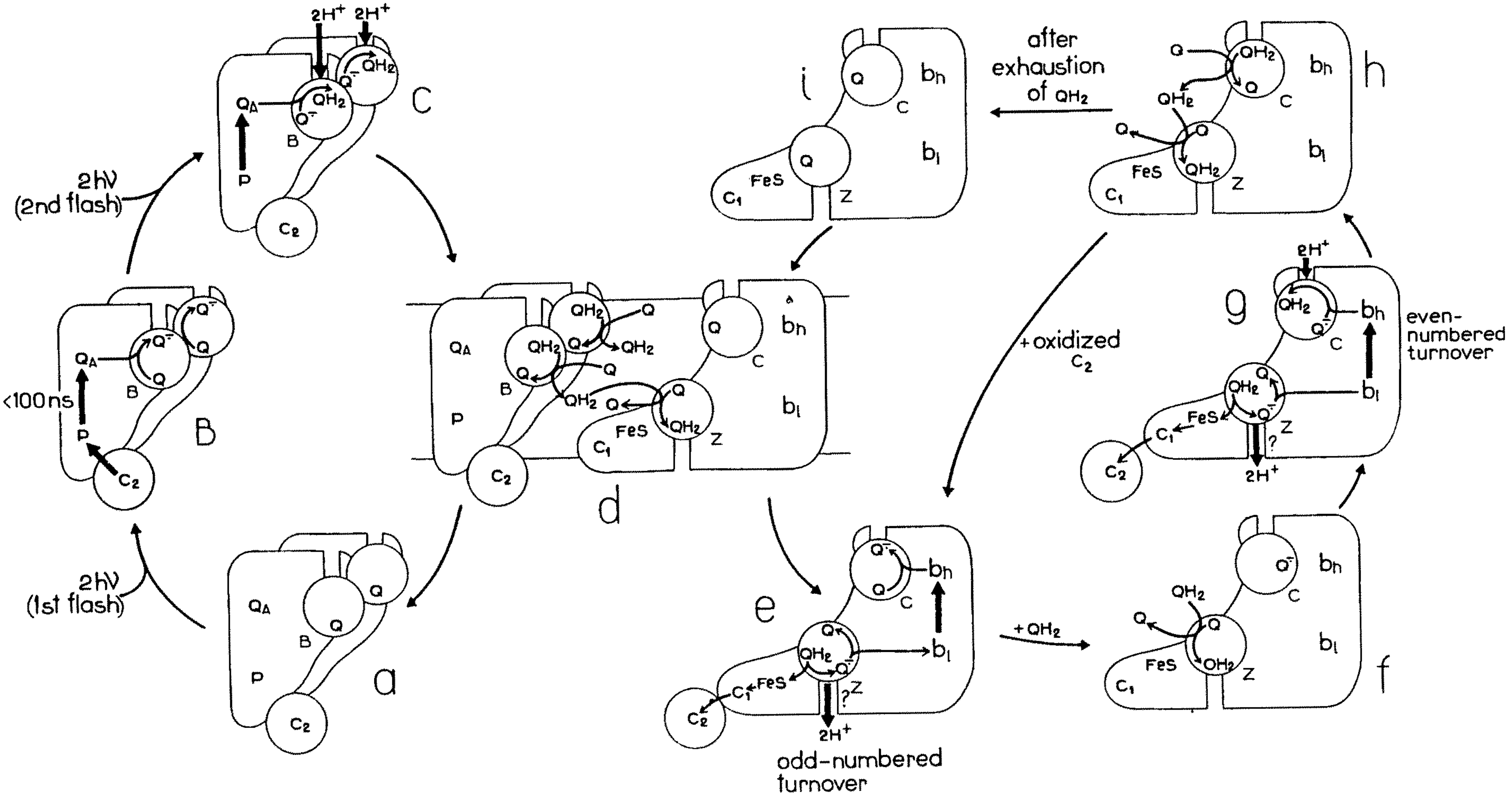

Fig. 5. A schematic presentation of electron transport and formation of a transmembrane proton concentration gradient in *Rb. sphaeroides* chromatophores. Bold arrows represent electrogenic stages. For explanation, see the text.

which are available after the first saturating flash, the bc_1 complex, in the next turnover, oxidizes the rest of the ubiquinol population (states f–g). During this turnover, the center C semiquinone is reduced to QH_2. QH_2 is mobile and can be released from the center C (state h). At high E_h, the cytochrome c_1 and the Rieske center are in the oxidized state. Hence, the ubiquinol molecules formed in center C have to undergo a fairly fast oxidation in the next turnovers of the bc_1 complex (states e–h). After the exhaustion of the ubiquinol, the bc_1 complex returns to the initial state i.

The following mechanisms may be responsible for electrogenic events in the bc_1 complex.

(1) Electron transfer between the hemes of b-566 (b_l) and b-561 (b_h);

(2) electron transfer between the heme b_h and a ubiquinone molecule in center C;

(3) proton uptake coupled to the redox reactions of center C quinone;

(4) a proton release upon oxidation of ubiquinol in center Z.

The myxothiazol-sensitive and antimycin-insensitive electrogenic phase seems to result from mechanism (1) [12], since its τ value and that of cytochrome b_h reduction are identical [12,13] (compare Fig. 2d with Fig. 3f, curve 2 and Fig. 4i). The antimycin-sensitive electrogenic phase is assumed to be coupled to cytochrome b_h oxidation, i.e., to mechanism (2) [9,12,13]. To verify the validity of this view, we compared the kinetics of the electrogenic phases of the bc_1 complex (Figs. 3 and 4) and those of cytochrome b redox reactions (Fig. 2). In the absence of antimycin A, only a portion of the cytochrome b_h population is reduced after the second flash. Its subsequent recovery is slow and takes several hundred milliseconds. This portion of cytochrome is presumably associated with bc_1 complexes, the last turnover of which was odd-numbered because of collisional interaction with ubiquinol molecules. Owing to this, a slowly relaxing quasi-equilibrium is established between cytochrome b_h and Q_C^-, whose midpoint potentials at neutral pH are nearly the same [44]. From a comparison of the data in Fig. 2b and 2d, it follows that in the absence of antimycin A, redox changes of more than half of the cytochrome b_h population are not observable: these are cytochromes b associated with bc_1 complexes, the last turnover of which was even-numbered and led to ubiquinol formation in center C. Under these conditions, most cytochromes b are apparently oxidized faster than they are reduced, and so their redox responses escape observation.

As for the antimycin-sensitive phase, had it been due to the electrogenic oxidation of cytochrome b_h (mechanism 2), its risetime would have been similar to the rate of cytochrome b_h oxidation. Hence, the kinetics of the antimycin-sensitive electrogenic phase may be expected to be similar to the antimycin-insensitive, myxothiazol-suppressed electrogenic phase. However, the fact that the former is much slower than the latter, (Fig. 3f and Fig. 4h,i) suggests that the electron transfer between b_h and center C ubiquinone is non-electrogenic, and implies that some subsequent reaction is electrogenic. This means that the electron transfer between the b_h heme and center C ubiquinone proceeds parallel to the membrane plane. The antimycin-sensitive electrogenic phase is most probably due to the protonation of Q_C that accompanies the reduction of ubiquinone (mechanism (3)), and also due to the electrogenic reactions that occur during repetitive turnovers of the bc_1 complex.

The topography of the membrane bc_1 complex suggests [6] that the release of proton(s) as a result of ubiquinol oxidation in center Z is also electrogenic (mechanism 4). Under the conditions investigated, it is most likely to be coupled to cytochrome b_h reduction and thus the corresponding electrogenic stage is part of the antimycin-insensitive, myxothiazol-suppressed electrogenic phase. We cannot exclude the possibility of a slow proton release from center Z as a consequence of some kinetical constraints or coupling between the pK of the protolytic group in center Z that accepts a proton(s) during ubiquinol oxidation [45] and surplus negative electric charge(s) that arrives at cytochrome b hemes and Q_C, as has been suggested earlier [46,47]. In the latter case, the kinetic coupling between proton release in center Z and antimycin-sensitive phase may exist.

The negative electrogenic phase induced by myxothiazol and suppressed by antimycin A reflects, most probably, electrogenic events in center C. Since, at neutral pH values, cytochrome b reduction via center C does not take place [48], this phase can be explained by the binding of ubiquinol formed after the second flash in center C and its further deprotonation ($QH_2 \rightleftarrows QH^- + H^+$).

The proton release can take place directly or via a special proton-accepting group similar to the one believed to be present in the ubiquinol-oxidizing center Z [45]. The data presented suggest also that proton exchange in the center C is electrogenic.

It is worth mentioning that there is a small negative spike in the kinetics of the antimycin-sensitive phase (curve 1 in Fig. 3f). Thus we can suggest that, even in the absence of any inhibitors, a part of the ubiquinol formed in RC binds in center C with subsequent proton release. Antimycin A suppresses this reaction, and thus the antimycin-sensitive phase possibly represents electrogenic events in center C caused both by the protonation of ubiquinol molecules formed in this center and by the much faster, and possibly reversible, deprotonation of some ubiquinol molecules formed in RC.

The characteristics of this negative electrogenic phase observed at neutral pH values and its relation to the electrogenic cytochrome b_h reduction via center C at alkaline pH values [49] will be discussed in a separate publication.

So far, direct electrometry of flash-induced electrogenic reactions has been restricted to RC. As for the bc_1-complex-linked $\Delta\psi$ generation, it was measured by means of carotenoid shift only. The very fact that the times of $\Delta\psi$ generation in the bc_1 complex obtained by the direct electrometry and carotenoid method proved to be similar shows that electrometry may be used for studying fast electrogenic reactions induced by $\Delta\tilde{\mu}_{H^+}$ generators other than the photosynthetic reaction centers.

The high sensitivity of direct electrometry allows us to observe minor electrogenic events (e.g., the negative electrogenic phase at neutral pH values) which cannot be studied by the carotenoid method or any other technique of $\Delta\psi$ measurement.

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