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TWO REGIMENS OF ELECTROGENIC CYCLIC REDOX CHAIN OPERATION IN CHROMATOPHORES OF NON-SULFUR PURPLE BACTERIA

A STUDY USING ANTIMYCIN A

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Summary

Antimycin A causes a biphasic suppression of the light-induced membrane potential generation in *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides* chromatophores incubated anaerobically. The first phase is observed at low antibiotic concentrations and is apparently due to its action as a cyclic electron transfer inhibitor. The second phase is manifested at concentrations which are greater than 1—2 μM and is due to uncoupling that may be connected with an antibiotic-induced dissipation of the electrochemical H^+ gradient across the chromatophore membrane. The inhibitory effect of antimycin added at low concentrations under aerobic conditions is removed by succinate to a large extent. It is expected that the electrogenic cyclic redox chain in the bacterial chromatophores incubated under conditions of continuous illumination may function at two regimes: (1) as a complete chain involving all the redox components, and (2) as a shortened chain involving only the *P*-870 photoreaction center, ubiquinone and cytochrome c_2.

Introduction

Membrane potential generation in *Rhodospirillum rubrum* chromatophores has been shown [1,2] to be caused by electron transfer through two sites of the cyclic redox chain, one site being associated with the bacteriochlorophyll photoreaction center and the other with the dark, antimycin A-sensitive electron transfer between cytochromes *b* and *c* (for example, upon the oxidation

of succinate by ferricyanide). Data on the two electrogenic stages of the cyclic electron transfer were then also obtained with the chromatophores of other non-sulfur purple bacteria in the experiments, including studies of flash-induced redox changes, pH changes and electrochromic carotenoid absorption band shifts [3—7].

Under conditions of flash illumination, the H^+/e^- ratio is equal to 2 in the native *Rhodopseudomonas sphaeroides* chromatophores and becomes equal to 1 upon addition of antimycin A [8]. However, antimycin-insensitive H^+ binding is observed only in response to the first 3 single turnover flashes: photooxidized cytochrome c_2 is reduced slowly in the presence of antimycin, and this leads to the accumulation of cytochrome c_2 and bacteriochlorophyll dimer as the primary electron donor in the oxidized state and stops further light-induced electron transfer [8].

These data may be interpreted to indicate the impossibility of an operation of the system 'cytochrome c_2-photoreaction center-ubiquinone' as an autonomous electrical generator (independent of *b* type cytochromes) in the native chromatophores. However, such a system of the shortened cyclic transfer of the reducing equivalents has a functional activity in reconstituted membranes.

Proteoliposomes containing purified bacteriochlorophyll reaction center complexes were shown to generate the transmembrane electrical potential difference upon illumination [9,10]. Photoelectrical responses in this system that does not contain *b* type cytochromes are dependent upon ubiquinone (or 1,4-naphthoquinone) and cytochrome *c* (or *N,N,N',N'*-tetramethyl-*p*-phenylenediamine).

Based on the findings of this report we assume that the similar system of the shortened redox chain generating the membrane potential operates in the native chromatophores incubated without artificial dyes which appear to bypass the cytochrome *b* site (see Ref. 11). This idea is in agreement with data [12] on a biphasic inhibition of photophosphorylation by antimycin A in *R. rubrum* chromatophores, as the antibiotic concentration increases. We also report that antimycin may act depending on a concentration used as an electron transfer inhibitor and an uncoupler of photophosphorylation in the chromatophores.

Methods

Cells of *Rhodospirillum rubrum* (wild type strain No. 1 MGU) and *Rhodopseudomonas sphaeroides* (wild type strain No. 1 MGU) were grown in the light under anaerobic conditions in the medium of Bose et al. [13] in the presence of malate and yeast extract. The chromatophores were isolated by ultrasonication and by consequtive centrifugation at 40 000 × *g* for 15 min and at 165 000 × *g* for 60 min as described previously [1]. The bacteriochlorophyll content of chromatophores was estimated using a molar extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$ at 772 nm in vitro [14].

The uptake of penetrating tetraphenylborate anions by chromatophores was monitored using a phospholipid (azolectin) membrane [1] separating two compartments of a Teflon cuvette with the solution. Incubation mixture contained 250 mM sucrose, 50 mM Tris-HCl buffer (pH 7.6), 10^{-6} M sodium tetraphenyl-

borate and chromatophores with bacteriochlorophyll content 14 μM. Some experiments were carried out in anaerobic cuvettes: 0.17 mg/ml glucose oxidase (EC 1.1.3.4) and 0.17 mg/ml of catalase (EC 1.11.1.6) were added to both compartments with the solution supplemented with 30 mM glucose (the concentration of sucrose declined to 220 mM in these experiments). Paraffin oil (6—8 mm in thickness) was layered on top of the aqueous phase. The solution in the experimental cell was mixed by means of a magnetic stirrer. Actinic light of saturating intensity ($\lambda > 660$ nm) was used for the illumination of the chromatophore suspensions.

Results

As shown in Fig. 1A, illumination causes an uptake of penetrating tetraphenylborate anions by *R. rubrum* chromatophores against the concentration gradient. This indicates the generation of electrical potential difference across the chromatophore membrane with a positive charge inside the vesicles [1,2]. A level of the tetraphenylborate uptake by the chromatophores incubated aerobically in the light reaches a maximum and decreases then slowly. It was previously shown [15] that this spontaneous decline is due to photooxidase

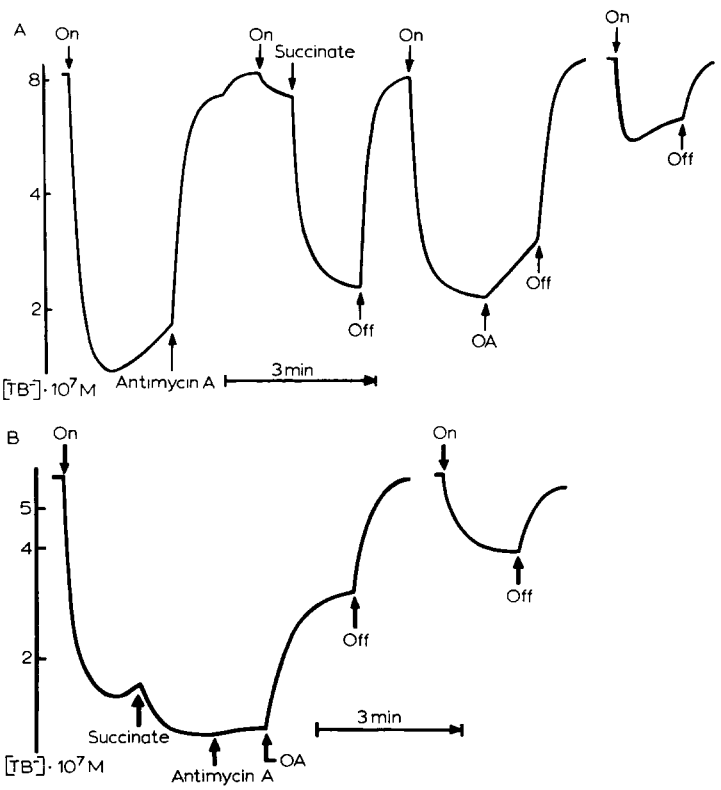

Fig. 1. Effect of antimycin A, succinate and oxaloacetate on the light-induced uptake of tetraphenylborate (TB^-) anions by *R. rubrum* chromatophores incubated aerobically. Additions: $2 \cdot 10^{-6}$ M in experiment A and $1 \cdot 10^{-6}$ M antimycin A in experiment B, 5 mM Tris-succinate and 1 mM Tris-oxaloacetate. On and Off, switching on and off of the light, respectively.

activity that leads to the oxidation of cyclic redox chain components. Antimycin A causes a rapid inhibition of the light-induced tetraphenylborate uptake by the chromatophores. The inhibitory effect of antimycin is removed by succinate to a great extent. A subsequent addition of oxaloacetate that is a competitive inhibitor of succinate dehydrogenase leads to the return of the inhibition of the tetraphenylborate response. Oxaloacetate does not influence the tetraphenylborate uptake in the absence of succinate and antimycin (data not shown).

Fig. 1B illustrates that succinate prevents the spontaneous decline and raises the light-induced tetraphenylborate response. The inhibitory effect of antimycin added against the background of succinate is observed only in the presence of oxaloacetate.

As the antimycin concentration is increased, a biphasic inhibition of the tetraphenylborate uptake is found in the chromatophores incubated anaerobically in the light (Fig. 2). The tetraphenylborate responses are maintained at a relatively constant level in the range of the concentrations from 0.2 to 2 μM and inhibited upon a further increase in the concentration of antibiotic. A plateau level varies between 80 and 40% for different chromatophore preparations. Similar data on the action of antimycin A were previously obtained by

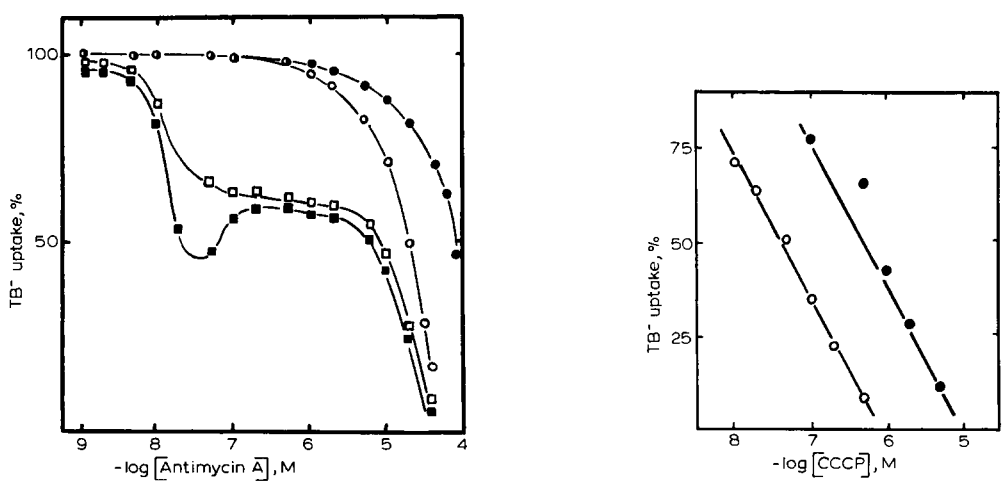

Fig. 2. Effect of antimycin A on the uptake of tetraphenylborate anions (TB^-) by energized *R. rubrum* chromatophores under various conditions. Conditions: ■———■, without additions, anaerobiosis, in the light, 100% of TB^- uptake level corresponds to electrical potential difference across the measuring phospholipid membrane of 62 mV; □———□, 10 mM Tris-succinate, anaerobiosis, in the light, 100% of TB^- uptake level corresponds to 65 mV; ●———●, 5 mM Tris-ascorbate, 0.1 mM diaminodurene, 2 mM methyl viologen, aerobiosis, in the light, 100% of TB^- uptake level corresponds 120 mV; ○———○, 3 mM $MgCl_2$, the successive additions of $5 \cdot 10^{-5}$ M inorganic sodium pyrophosphate, aerobiosis, in the dark, 100% of TB^- uptake level corresponds to 70 mV.

Fig. 3. Effect of *m*-chlorocarbonylcyanide phenylhydrazone (CCCP) on the uptake of tetraphenylborate anions (TB^-) by aerobic *R. rubrum* chromatophores energized by the light or inorganic pyrophosphate in the dark. Conditions: ●———●, 5 mM Tris-ascorbate, 0.1 mM diaminodurene, 2 mM methyl viologen, in the light, 100% of TB^- uptake level corresponds to electrical potential difference across the measuring phospholipid membrane of 114 mV; ○———○, 3 mM $MgCl_2$, the successive additions of $5 \cdot 10^{-5}$ M inorganic sodium pyrophosphate, 100% of TB^- uptake level corresponds to 59 mV.

Jones and Vernon [12] in studies of photophosphorylation in *R. rubrum* chromatophores. In the absence of succinate, an establishment of the plateau is preceded by a pit corresponding to the antimycin concentration about of $5 \cdot 10^{-8}$ M.

A monophasic inhibition appearing at an antimycin concentrations above 2 μM is found at the tetraphenylborate responses induced by inorganic pyrophosphate in the dark as well as in the light-induced tetraphenylborate responses in chromatophores incubated aerobically with ascorbate, diaminodurene (2,3,5,6-tetramethyl-*p*-phenylenediamine) and methyl viologen (Fig. 2). The non-cyclic electron transfer from diaminodurene to methylviologen and O_2 was proposed to take place without a cytochrome participation [15].

Based on the data of Fig. 2 it is believed that the tetraphenylborate response inhibition observed at high concentrations of antimycin is due to an uncoupling action of antibiotic. Antimycin at high concentrations was shown to be an uncoupler of photophosphorylation in chloroplasts [16].

The differences of the antibiotic concentrations at which the uncoupling takes place in the cyclic and non-cyclic electron transfer systems as well as in the inorganic pyrophosphate hydrolysis system (Fig. 2) may be due to the differences of the power of these systems. As seen from Fig. 3, the concentrations of *m*-chlorocarbonylcyanide phenylhydrazone (a typical protonophorous uncoupler) required to inhibit the tetraphenylborate responses upon non-cyclic electron transfer from diaminodurene to methylviologen and O_2 is 10-fold higher than those upon inorganic pyrophosphate hydrolysis in the dark.

Results similar to those for *R. rubrum* were obtained also with *Rps. sphaeroides* chromatophores. Fig. 4 shows that a level of the light-induced tetraphenylborate uptake by the aerobic chromatophores of *Rps. sphaeroides* is declined under the action of antimycin upon the cyclic electron transfer and restored upon the subsequent addition of succinate. An increase in the antibiotic concentration leads to the biphasic inhibition of the tetraphenylborate responses (Fig. 5). The inhibitory effect of antimycin increases when the intensity of the actinic light is declined. A plateau in Fig. 5 for *Rps. sphaeroides* is manifested to a lesser degree than in Fig. 2 for *R. rubrum*.

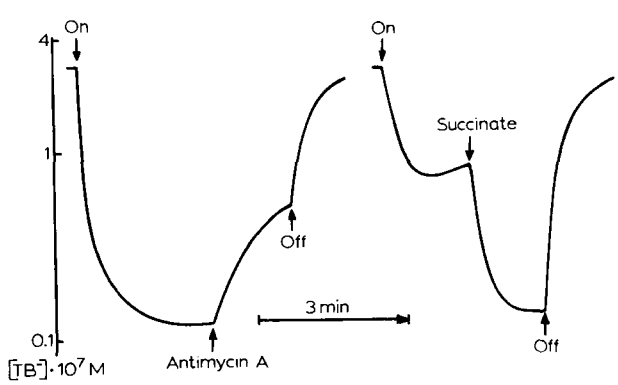

Fig. 4. Effect of antimycin A and succinate on the light-induced uptake of tetraphenylborate anions (TB^-) by *Rps. sphaeroides* chromatophores incubated aerobically. Additions: $1 \cdot 10^{-6}$ M antimycin A and 5 mM Tris-succinate.

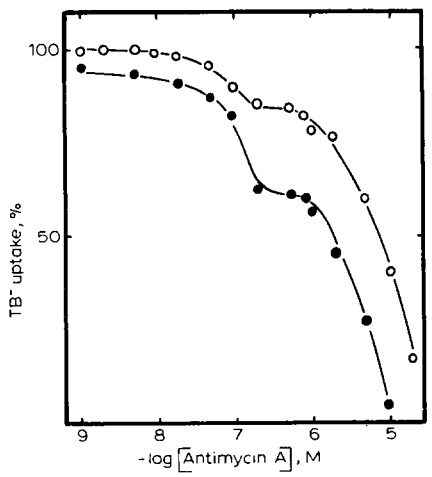

Fig. 5. Effect of antimycin A on the light-induced uptake of tetraphenylborate anions (TB^-) by *Rps. sphaeroides* chromatophores incubated anaerobically. Conditions: o———o, in the light of saturating intensity, 100% of TB^- uptake level corresponds to electrical potential difference across the measuring phospholipid membrane of 110 mV; •———•, in the light of half-saturating intensity, 100% of TB^- uptake level corresponds to 56 mV.

Discussion

Results obtained show that antimycin A causes the biphasic inhibition of the light-induced membrane potential generation in bacterial chromatophores incubated anaerobically. A similar effect of antimycin on photophosphorylation in *R. rubrum* chromatophores was described previously [12]. The first phase of the inhibition is observed at antibiotic concentrations below 0.1 μM, the second phase at concentrations above of 1—2 μM (Figs. 2 and 5).

Most apparently, the second phase of the inhibition is due to an uncoupling action of antimycin since the antibiotic at high concentrations inhibits also: (1) the membrane potential generation induced by inorganic pyrophosphate hydrolysis in the dark, and (2) the membrane potential generation upon the light-induced non-cyclic electron transfer that appears to take place without a participation of cytochromes (see Ref. 15). This means that antimycin in high concentrations may act as a protonophorous uncoupler of the photophosphorylation.

As for the first phase of the inhibition by antimycin, it is proposed to be connected with a change-over of the electrogenic cyclic electron transfer from one regime to the other so that the H^+/e^- ratio equal to 2 in the native chromatophores becomes equal to 1 upon the addition of antibiotic. Such a change-over can arise from a change in the mechanism of ubiquinone oxidation-reduction as shown in Fig. 6.

At $H^+/e^- = 2$, ubiquinone is subjected to two successive one-electron reductions by a component b_2 and by the primary electron acceptor accordings to Mitchell's protonmotive ubiquinone cycle [17] (see also Ref. 8) on the external side of the chromatophore membrane. Its protonation and transmembrane movement (an alternative way may be presented as the relay transfer of

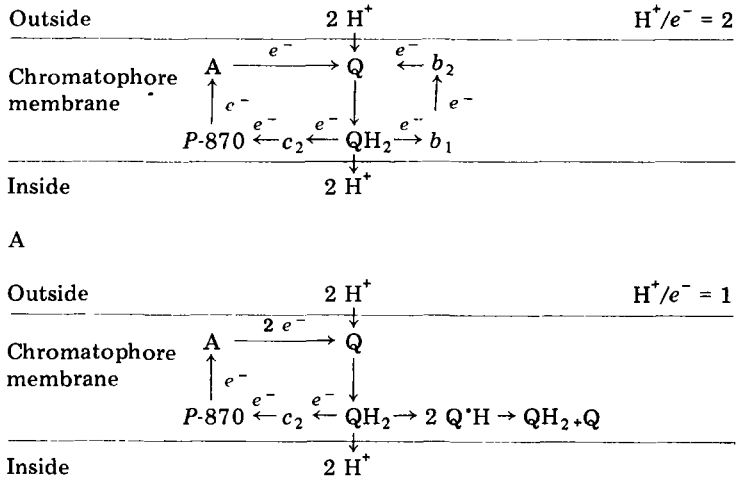

Fig. 6. Two regimes of electrogenic cyclic redox chain operation in the bacterial chromatophores. (A) Mitchell's protonmotive ubiquinone cycle. (B) Shortened reducing equivalent transfer system without participation of *b* type cytochromes. *P*-870, bacteriochlorophyll reaction center; A, electron acceptor (ferroquinone or primary, tightly-bound ubiquinone); Q, secondary, loosely bound ubiquinone; b_1 and b_2, components corresponding to mitochondrial cytochromes b_T and b_K; c_2, cytochrome c_2.

H-atoms around a chain of ubiquinone molecules), its consequent oxidation by cytochrome c_2 and another component b_1 on the internal side of the membrane lead to the transmembrane transfer of two H^+ on per quantum of light exciting the *P*-870 reaction center basis.

At $H^+/e^- = 1$, ubiquinone gains a pair of electrons from *P*-870 (see Refs. 18, 19) and a pair of H^+ on the external side of the chromatophore membrane. Hydroubiquinone QH_2 is oxidized by cytochrome c_2 on the internal side of the membrane giving ubiquinone semiquinone Q·H and one free H^+. Antimycin appears to prevent the oxidation of Q·H by *b* type cytochrome and cause a local accumulation of semiquinone under conditions of continuous illumination. The accumulation of Q·H is apparently suppressed when the actinic light intensity is declined (Fig. 5) and may be lacking under conditions of the flash illumination. A dismutation reaction and the oxidation of QH_2 (a product of this reaction) by cytochrome c_2 apparently cause the release of the second H^+. This pathway of the reducing equivalent transfer around a shortened variant of the cyclic chain is independent of *b* type cytochromes and may function, bypassing the antimycin-sensitive site located at the cytochrome *b* level in the chromatophore redox chain.

Electrogenic activity of such a system was demonstrated previously in 'the isolated form' by experiments with reconstituted membranes (proteoliposomes) containing purified bacteriochlorophyll reaction center complexes from *R. rubrum* [9].

It is not improbable that this shortened system of the membrane potential generation functions also in the bacterial cells. This means that the isolated chromatophores are likely to be represent a mixture of two types of membrane vesicles of which one part contains and another part does not contain *b*

type cytochromes or is deficient in these components. The manifestation of the first phase of the light-induced electrogenic activity inhibition at antimycin concentrations that are below or comparable to chromatophore redox chain concentrations may be explained in the light of this suggestion. The bacteriochlorophyll concentration in experimental samples was $1.4 \cdot 10^{-5}$ M. Inasmuch as a molar ratio between the *P*-870 reaction centers (or redox chains) and the bulk of lightharvesting bacteriochlorophyll in *R. rubrum* and *Rps. sphaeroides* chromatophores is approximately equal to 1 : 50, the antimycin concentration should be no less than $3 \cdot 10^{-7}$ M even under conditions of complete and specific antibiotic binding. However, the first phase of the inhibition in *R. rubrum* chromatophores (Fig. 2) reaches a maximum already at the antimycin concentration equal to 10^{-7} M and in *Rps. sphaeroides* chromatophores (Fig. 5) at $2 \cdot 10^{-7}$ M.

Variations in the plateau level (the first phase of the inhibition by antimycin) observed in different membrane preparations can be also explained in the light of the suggestion of the chromatophore heterogeneity.

Antimycin as an inhibitor of the electron transfer in the chromatophores incubated aerobically is more effective than in the anaerobic chromatophores. This appears to be connected with photooxidase activity that causes the oxidation of components of the cyclic redox chain and the inhibition of its electrogenic function. Antimycin [20] as well as 2-nonyl and 2-heptyl 4-hydroxyquinoline-*N*-oxide [21,22] has been reported to stimulate photooxidase activity that is competitive with the cyclic electron transfer system. An addition of succinate leads to the reduction of redox chain components and to the appearance of electrogenic activity that may be caused by the operation of the shortened electron transfer system without the *b* type cytochrome participation.

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