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Protonmotive activity of the cytochrome b/c_1 complex in chromatophores of *Rhodobacter capsulatus* in the presence of myxothiazol and antimycin A

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(1) The light-induced membrane potential measured by electrochromism in chromatophores and in intact cells of *Rhodobacter capsulatus* (formerly called *Rhodopseudomonas capsulata*) was reduced by about 70% with myxothiazol, but only by about 30% with antimycin. (2) The protonmotive current responsible for membrane potential formation was inhibited by more than 97% with myxothiazol and by approx. 89% with antimycin. It is suggested that the Cyt b/c_1 complex can catalyse a limited rate of electron transfer even when the quinol reductase site is completely blocked. (3) Titration with both inhibitors yielded a similar, diodic dependence of the dissipative membrane ionic current on the membrane potential. (4) The observations in (1) can be explained completely by (2) and (3). (5) When added alone, antimycin and low concentrations of uncoupling agent only slightly depressed the light-induced membrane potential in chromatophores, but together they had a marked effect. Synergistic depression of membrane potential was less evident with myxothiazol and uncoupling agent. These observations are explained by (a) the fact that the intrinsic membrane ionic conductance is membrane potential-dependent, but that due to uncoupling agent is membrane potential-independent; (b) photosynthetic (cf. respiratory) control is pronounced in the presence of partial titres of myxothiazol, but is absent in antimycin-treated chromatophores.

Introduction

The most widely accepted mechanisms of electron transport through the ubiquinol cytochrome *c* oxidoreductase (cytochrome b/c_1) complex of photosynthetic bacteria and mitochondria are variants of the protonmotive Q-cycle originally proposed by Mitchell [1–5]. Central to all models is the existence of separate ubiquinol oxidase [Q_z or Q_o] and ubiquinone reductase [Q_c or Q_i] sites within the oxidoreductase complex. Ubiquinol oxidation occurs by a concerted or consecutive [6] reaction in which one reducing equivalent passes to a high potential chain via the Rieske FeS centre and a second reducing equivalent proceeds to a low potential b-type cytochrome [6]. The modified Q-cycle proposed for *Rhodobacter sphaeroides* (formerly called *Rhodopseudomonas sphaeroides*) [7] accommodates a two electron gate as suggested by Garland [8] in which an electron waits in the *b*-cytochrome chain before a second transfer at Q_z occurs thus allowing quinone to be reduced to quinol at the Q_c site. Therefore, the Q_z site must turn over twice for each turnover of the Q_c site. In the light-driven cyclic electron-transfer chains of photosynthetic bacteria electrons on the high

Abbreviation: Cyt, cytochrome.

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potential side of the Q_z site return via the Rieske FeS centre and *c*-type cytochromes to the reaction centre complex.

During cyclic electron flow in photosynthetic bacteria two inwardly directed electron-transport events result in the generation of membrane potential $\Delta\psi$, [9]. These electrogenic steps give rise to the fast ($t \leqslant 100$ μs) and slow ($t \geqslant 2$ ms) phases of the electrochromic carotenoid absorbance change after single turnover light pulses. The fast electrogenic step is comprised of two partially transmembrane charge separations in the reaction centre, known as phase I and phase II associated with the oxidation of P-870 and the re-reduction of P-870 by cytochrome c_2, respectively [9]. These charge separations can also be identified with macroscopic electrodes and reconstituted proteins or membrane preparations [10,11]. The slow electrogenic phase III was long thought to be due to a single electrogenic event, the oxidation of cytochrome *b*-561 within the cytochrome b/c_1 complex [9]. Recent evidence suggests that phase III is comprised of two components with oxidation of cytochrome *b*-561 and cytochrome *b*-566 contributing approx. 50%–65% and 35–50% to the total [12].

Specific electron-transport inhibitors have been instrumental in establishing the sequence of electron flow through the cytochrome b/c_1 complex [13–15]. The distinct sites of action of the inhibitors antimycin and myxothiazol have been well documented, the antimycin-sensitive site being the ubiquinone reductase site, whilst myxothiazol is thought to bind at the ubiquinol oxidising site [13–15]. Remmenikov and Samuilov [16,17] found that non-uncoupling concentrations of antimycin only partly reduced light-induced uptake of tetraphenylboron by chromatophores and intact cells of *Rb. sphaeroides* and *Rhodospirillum rubrum* in steady state. Taking the uptake of tetraphenylboron as an indicator of membrane potential they concluded that the cyclic electron-transport chain of these organisms can operate in two electrogenic regimes: (1) as a complete chain involving all redox components; (2) as a shortened chain, insensitive to antimycin and involving only reaction centre, ubiquinone and cytochrome c_2. It was reasoned [16] that isolated chromatophores are a mixture of two types of membrane vesicle possessing either 'complete' or 'shortened' chains. The concept of vesicle heterogeneity was also extended to intact cells for which it was proposed that the intracytoplasmic membrane exists not as often supposed as a contiguous unit but as discrete internal vesicles [17]. It has been noted [18] that in general $\Delta\psi$ may be only slightly depressed even after pronounced inhibition of electron transport: because the ionic conductance of the chromatophore membrane is $\Delta\psi$-dependent a reduction in the rate at which $\Delta\psi$ is generated is partly compensated by a disproportionate decrease in the rate at which $\Delta\psi$ is dissipated. It is tempting to account for the incomplete sensitivity to antimycin of light-induced uptake of tetraphenylboron as described by Remmenikov and Samuilov [16,17] by this explanation and therefore avoid the assumption that the chromatophore population is heterogeneous. However, it can be concluded from the later experiments of Kotova et al. [19], who showed that myxothiazol is considerably more potent than antimycin in reducing the light-induced uptake of tetraphenylboron, that this explanation in itself is insufficient.

In this report, using electrochromic absorbance changes as an indicator of $\Delta\psi$ we confirm the observations of Samuilov and co-workers [16,17,19] that the magnitude of $\Delta\psi$ in steady-state light in both intact bacterial cells and chromatophores is more sensitive to myxothiazol than to antimycin. From measurements of the ionic flux across the membrane we conclude that there is indeed a component of electron transport which is insensitive to antimycin. Because of the dependence of membrane ionic conductance on $\Delta\psi$, the low rate of electron transport in the presence of antimycin is sufficient to generate a large $\Delta\psi$. To explain antimycin-insensitive electron flow we adapt an idea put forward by Rich [6] to account for a switch at the quinol oxidase site in the analogous Cyt b_6/f complex of chloroplasts. It is shown that in contrast to the complete pathway, antimycin-insensitive electron flow is not subject to feed-back control by $\Delta\psi$ and that this leads to a synergistic effect of antimycin and uncoupling agent on the magnitude of $\Delta\psi$.

Methods

The anaerobic, phototrophic growth of *Rb. capsulatus* strain N22 in RCV medium is described in Ref. 20. For experiments with intact cell suspensions, harvested bacteria were washed in a medium containing 10 mM Na_2HPO_4 (adjusted to pH 7.0 with H_3PO_4). The cells were stored on ice and used on the same day. Chromatophores were prepared as in Ref. 21, stored at 4°C and used within 5 days of preparation. In both cases bacteriochlorophyll was assayed in 7:2 acetone/methanol extracts [22].

Absorbance changes were measured in a laboratory constructed, cross-beam spectrophotometer as described earlier [23]. A microcomputer was used to control the opening and closing of an electronic shutter on the excitation beam. The 10×10 mm cuvette was completely filled with medium pregassed with argon. Bacterial cells or chromatophores were added (with or without inhibitor) to give a final bacteriochlorophyll concentration of 10 or 15 μM. The cuvette was stoppered and incubated in the dark for 30 min to ensure anaerobiosis. For experiments with intact cells fresh growth medium was used as described in Ref. 18. The medium used for chromatophore experiments was 10% sucrose/50 mM KCl/8 mM $MgCl_2$/50 mM tricine-KOH (pH 7.6)/0.5 mM NADH/0.5 mM K^+ succinate. In the conditions of the experiments $\Delta\psi$ was the sole contributer to the proton-motive force [18,23].

Membrane potentials were measured from electrochromic absorbance changes (see Refs. 18, 23 and 24) either at 503 nm or at 503–487 nm. All the measurement in this report relate to steady-state values ($\Delta\psi$ = constant) established within a few seconds (or less) of illumination: $\Delta\psi$ was taken as the difference between the darkened and illuminated absorbance values. Upon darkening, the initial rate of decay of the electrochromic absorbance change is a measure of the rate at which $\Delta\psi$ was being dissipated during illumination (J_{dis}). This can be calibrated by comparison with the electrochromic absorbance change generated by saturating single-turnover flashes in the presence of myxothiazol to disable completely the Cyt b/c_1 complex. Under these conditions a single charge is driven across the chromatophore membrane through each reaction centre: effectively, the membrane capacitance is calculated in the calibration procedure, so that the ionic current can be estimated from the rate of change of $\Delta\psi$ (see Ref. 18). In the steady state, $J_{dis} = J_E n$, where J_E is the electron transport rate and n is the H^+/e^- ratio [18].

Results

Myxothiazol depressed the steady-state level of $\Delta\psi$ as indicated by the electrochromic carotenoid absorbance change in illuminated chromatophores

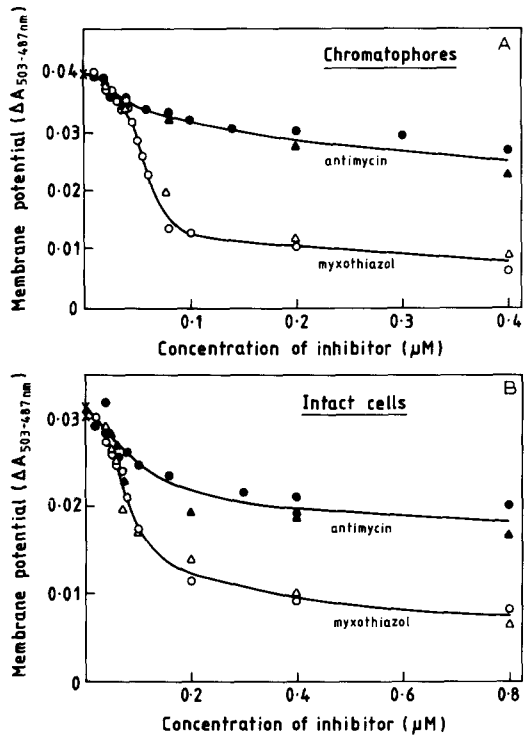

Fig. 1. The inhibition of light-induced $\Delta\psi$ by antimycin and myxothiazol. Chromatophores (A) were suspended in an argon-sparged medium. Whole cells (B) were suspended in argon-sparged RCV growth medium (pH 8.0). Bacteriochlorophyll concentration in all cases was 10 μM. All samples were pre-incubated in the dark with inhibitor for 30 min in stoppered cuvettes to ensure anaerobiosis. Membrane potential was measured at 503–487 nm in the illuminated steady state with respect to the dark level and is expressed in units of carotenoid absorption change. Closed symbols, antimycin; open symbols, myxothiazol. ×, uninhibited samples. Each symbol describes the result of a single experiment. The two symbol sets (○, ● and △, ▲) describe duplicate data from consecutive days's experiments.

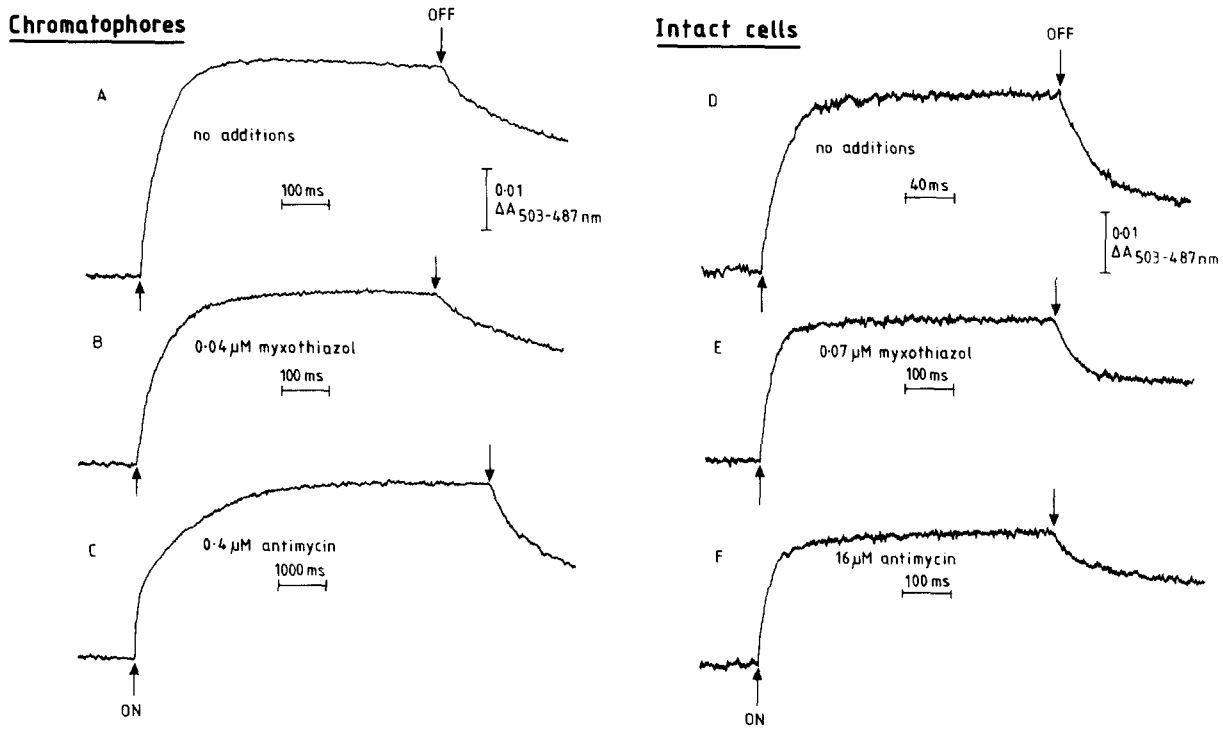

Fig. 2. The kinetics of membrane potential generation and dissipation. Conditions as in Fig. 1. Note the different time scales.

and cells considerably more than did antimycin (Fig. 1). This confirms the results of earlier experiments using uptake of tetraphenylboron as a measure of $\Delta\psi$ [16,17,19]. Inhibition was sigmoidal. In chromatophores, for both antimycin and myxothiazol, a component of inhibition was completed as the titre approached equivalence with the quantity of photosynthetic reaction centres (0.10 μM). In intact cells the titre was a little higher perhaps as a result of non-specific binding of the inhibitors to the bacterial cell envelope. Beyond saturation of the tight binding site for each inhibitor, $\Delta\psi$ declined only very slightly, possibly due to weak uncoupling activity of these reagents as suggested by Remmenikov and Samuilov [16]. On this basis, specific inhibition of the quinone reductase site by antimycin and of the quinol oxidase site by myxothiazol reduced the steady-state level of the light-induced $\Delta\psi$ by approx. 30% and 70%, respectively.

For intact cells and for chromatophores in the absence of inhibitors, the steady-state level of $\Delta\psi$ was reached within about 100 ms and 220 ms respectively of the start of illumination (Fig. 2D and A). After treatment of either intact cells or chromatophores with myxothiazol (Fig. 2E and B) the time taken to reach the maximum $\Delta\psi$ was slightly prolonged (e.g., a factor of approx. 2 for a 30% depression of $\Delta\psi$). Treatment with antimycin revealed a significant difference between intact cells and chromatophores (Fig. 2F and C): whereas in cells the time taken to reach the maximum $\Delta\psi$ was only slightly extended, in chromatophores the steady-state value of $\Delta\psi$ was not reached until several seconds of illumination had elapsed (e.g., approx. 5 s with 0.4 μM antimycin).

On earlier occasions were have described the dependence of the dissipative flux of ions across the bacterial or chromatophore membrane (J_{dis}) on the driving force, $\Delta\psi$ [23]. The steady-state of the electrochromic carotenoid absorption change during a brief period of illumination was used as a measure of $\Delta\psi$ and the initial rate of decay of the absorption change upon darkening as a measure of J_{dis}. In those experiments the value of $\Delta\psi$ was systematically varied by reducing the intensity of

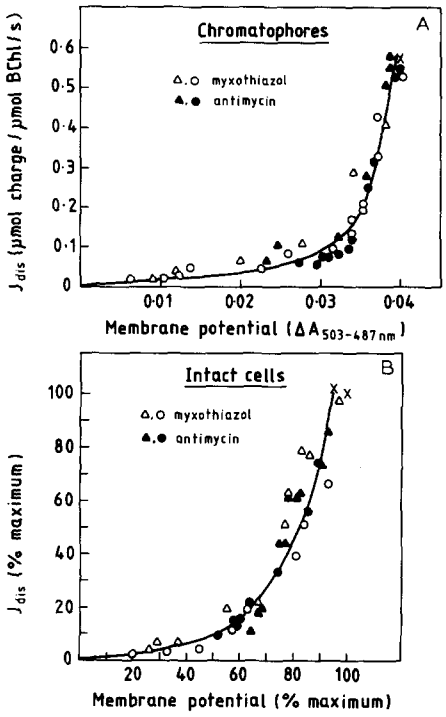

Fig. 3. $J_{dis}/\Delta\psi$ relationships revealed by titrations with antimycin and myxothiazol. Taken from the series of experiments described in Fig. 1 (A) chromatophores, (B) intact cells. J_{dis} was measured from the initial rate of decay of the carotenoid band shift upon extinguishing the actinic light after attaining the stady-state $\Delta\psi$. Closed symbols antimycin. Open symbols myxothiazol. ×, uninhibited samples. In chromatophores $\Delta\psi$ is expressed in units of carotenoid absorption change and J_{dis} is expressed in μmol charge per μmol BChl/s and was calculated as described in Ref. 18. In whole cells $\Delta\psi$ and J_{dis} are expressed as percentages of the control values in the absence of inhibitor.

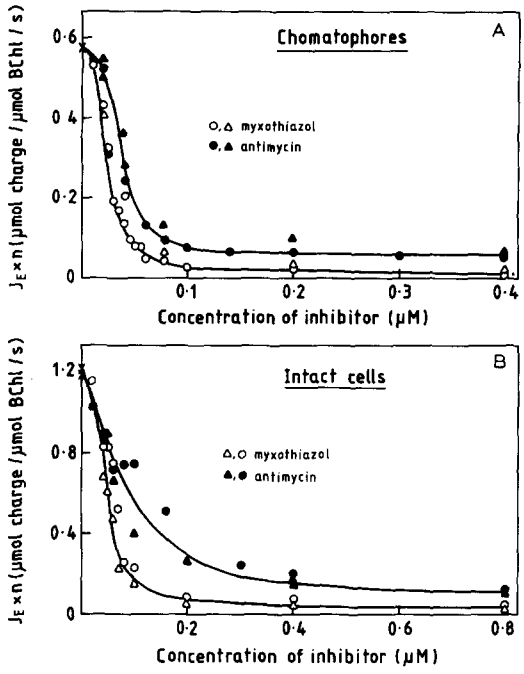

Fig. 4. Inhibition of the protonmotive current in steady-state light by antimycin and myxothiazol. Taken from the series of experiments described in Figs. 1 and 3. (A) Chromatophores; (B) whole cells. Closed symbols antimycin. Open symbols myxothiazol. ×, uninhibited samples. The protonmotive current ($J_E n$ – see text) is expressed in μmol chage per μmol BChl/s.

the actinic light. In Fig. 3 the dependence of J_{dis} on $\Delta\psi$ was followed in a series of experiments in which $\Delta\psi$ was decreased by treatment of intact cell and chromatophore suspensions with either antimycin or myxothiazol. J_{dis} was measured in the usual way upon promptly darkening the suspension. As shown in Fig. 1 antimycin was much less effective than myxothiazol in decreasing $\Delta\psi$. However, it is clear from Fig. 3 that the dependence of J_{dis} on $\Delta\psi$ was similar for both inhibitors. The relation reveals the diodic nature of the chromatophore membrane [18,23].

In steady-state the rate at which $\Delta\psi$ is generated (J_{gen}) is exactly balanced by the rate of $\Delta\psi$ dissipation ($J_{gen} = J_{dis}$). J_{gen} is the product of the photosynthetic electron-transport rate (J_E) and the stoichiometry (n) of protons translocated across the membrane per electron transferred. In Fig. 4 $J_E n$ is plotted as a function of the concentration of antimycin and myxothiazol. As with the effect on $\Delta\psi$ (Fig. 1) the inhibition of $J_E n$ by both antimycin and myxothiazol was sigmoidal. Maximal inhibition in chromatophores was achieved approximately at equivalence with the reaction centre content. In intact cells a 2–3-fold molar excess of inhibitor was required. The main point to emerge from Fig. 4 is that whereas inhibition of $J_E n$ by myxothiazol was virtually complete (more than 97%), a residual low rate, equivalent to a turnover of approx. 6 H^+ per reaction centre per s persisted in the presence of excess antimycin. This low residual rate and the diodic conductance properties of the membrane (Fig. 3) conspire to maintain a considerable membrane potential in the presence of a full titre of antimycin (Fig. 1).

It has been noted that a combination of an

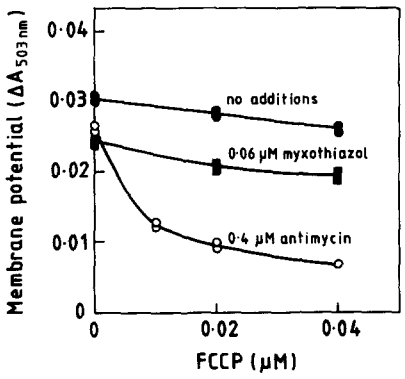

Fig. 5. The depression of $\Delta\psi$ in chromatophores by a combination of electron-transport inhibitor and uncoupling agent. Conditions as Fig. 1, but with a bacteriochlorophyll concentration of 15 μM. Membrane potential was measured at 503 nm in the illuminated steady-state and expressed in units of carotenoid absorption change. ●, No additions; ■, 0.06 μM myxothiazol; ○, 0.4 μM antimycin.

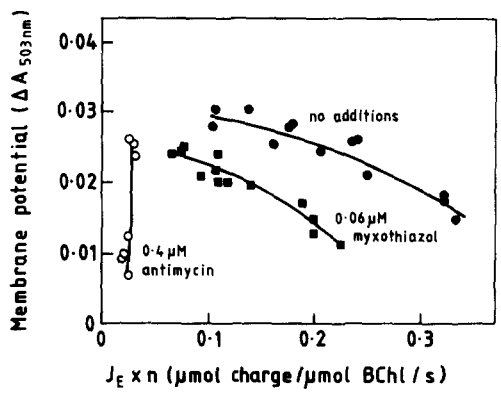

Fig. 6. Photosynthetic control in uninhibited chromatophores and in the presence of antimycin and myxothiazol. Conditions as in Fig. 5. The protonmotive current ($J_E n$) was measured as in Fig. 4. ●, No additions; ■, 0.06 μM myxothiazol; ○, 0.4 μM antimycin. Photosynthetic control was relieved by the addition of FCCP.

electron transport inhibitor and an uncoupling agent synergistically lowers the $\Delta\psi$ induced either by respiration or by light in intact cells of *Rb. capsulatus* [24]. In Fig. 5 an experiment with chromatophores shows that the synergistic effect was especially pronounced with antimycin as an inhibitor of photosynthetic electron transport. Alone, FCCP in the region of 0.02 μM, or a molar excess of antimycin over reaction centres, had only a small effect but in combination the two reagents almost completley abolished the light-induced $\Delta\psi$. The synergistic effect was less evident with myxothiazol; a concentration of myxothiazol was chosen to reduce $\Delta\psi$ to a value similar to that reached in the presence of antimycin and then a titration of $\Delta\psi$ with FCCP was carried out (Fig. 5). In contrast to the antimycin-inhibited chromatophores, the very low concentrations of FCCP applied to the myxothiazol-inhibited samples in Fig. 5 had only a slight effect on $\Delta\psi$.

Earlier it was argued that synergism between electron-transport inhibitor and uncoupler arises because the intrinsic conductance of the chromatophore membrane is diodic, whereas the conductance due to added FCCP is independent of $\Delta\psi$ [24]. In the Discussion of this article the same argument will be adopted; the difference in behaviour between antimycin and myxothiazol will be explained by the different degree of photosynthetic (cf. respiratory) control in the two situations described in Fig. 5. This is illustrated in Fig. 6. In this figure the value of $J_E n$ (measured as above) is plotted as a function of the steady-state $\Delta\psi$ during the course of a titration with FCCP. First, in the absence of inhibitor the value of $J_E n$ increased markedly when $\Delta\psi$ was decreased with uncoupling agent. At very low values of $\Delta\psi$, $J_E n$ appeared to approach a maximum although the rate measurement became increasingly difficult as the electrochromic absorption change decreased in extent. Taking n to be constant throughout this titration (see Discussion) the photosynthetic control ratio of uninhibited chromatophores was greater than 3.4. In inhibited chromatophores the response of $J_E n$ to the presence of increasing concentrations of uncoupler was dependent on

TABLE I

SYNERGISTIC DEPRESSION OF MEMBRANE POTENTIAL IN CHROMATOPHORES BY MYXOTHIAZOL AND FCCP

Conditions as Fig. 5 except that FCCP was present where shown at 0.16 μM and myxothiazol at 0.15 μM.

Additions	Steady-state membrane potential	%
None	0.037	100
FCCP	0.029	78
Myxothiazol	0.014	38
FCCP + myxothiazol	0.007	19

whether inhibition was achieved with antimycin or myxothiazol (Fig. 6). The residual electron transport rate in the presence of 0.4 μM antimycin was not stimulated by uncoupling agent. At the myxothiazol concentration (0.06 μM) which depressed $\Delta\psi$ by a similar amount to 0.4 μM antimycin, the photosynthetic electron transport rate was still heavily restricted by $\Delta\psi$: there was a substantial stimulation of the electron-transport rate when $\Delta\psi$ was dissipated with FCCP.

Synergistic lowering of $\Delta\psi$ by myxothiazol and uncoupling agent was evident but only at higher concentrations of inhibitor (Table I) and even then the effect was not as pronounced as with antimycin.

Discussion

Electron transport and proton translocation in the presence of antimycin

The finding that membrane potential in both intact cells and chromatophores of photosynthetic bacteria is more strongly depressed by myxothiazol than by antimycin [19] is confirmed using electrochromic measurements (Fig. 1). Other experiments show directly that an element of the protonmotive current across the bacterial membrane persists in the presence of sufficient antimycin to saturate completely its inhibitory site in the Cyt b/c_1 complex (Fig. 4). Instead of proposing that chromatophore vesicles and their counterparts in intact cells are heterogeneous, some with and some without operational *b*-type cytochromes [16,17], we suggest that chromatophores are homogeneous and that the Cyt b/c_1 complex can function in a limited capacity with the quinol reductase site completely inhibited by antimycin.

In a Q-cycle (e.g., Ref. 7) inhibition by antimycin at centre Q_c would result in a complete block in steady-state electron transport through the complex. In order to explain the lack of an electrogenic reaction in the cytochrome b_6/f complex operating between the two photosystems in chloroplasts from higher plants, Rich [6] suggested that under some conditions both electron equivalents from plastoquinol at centre Q_z can be directed to the Rieske FeS. Such a mechanism could account for the low rate of electron transport through the Cyt b/c_1 complex in the presence of antimycin in membranes from photosynthetic bacteria: normally a Q-cycle would prevail, but inhibition of the Q_c site and reduction of the *b*-type cytochromes would result in the diversion of both reducing equivalents from ubiquinol at Q_z to the Rieske FeS and hence to the reaction centre. Myxothiazol would inhibit both the normal and the re-directed operation of the Q_z site and therefore would completely block electron transport through the Cyt b/c_1 complex. Because electron exchange between reaction centres and Cyt b/c_1 complexes is mediated by diffusible pools of ubiquinone and Cyt c_2 [4,6,7] the steady-state rate of electron transport is not linearly related to the concentration of uninhibited components. The sigmoidal inhibition patterns displayed by antimycin and myxothiazol (Figs. 1 and 4) probably arise from the pool behaviour [25,26].

If the above reasoning is correct the H^+/e ratio (*n* in Fig. 4) of 2.0 for uninhibited electron transport would be reduced to 1.0 in the curtailed pathway in the presence of antimycin. On this basis we can calculate from Fig. 4 that the net rate of electron transport in saturating steady-state light in chromatophores is decreased from 0.29 μmol per μmol BCl/s in the absence to 0.06 μmol per μmol BChl/s in the presence of antimycin. Because it by-passes the electrogenic reactions of the Cyt b/c_1 complex and operates with a reduced H^+/e ratio the antimycin-insensitive pathway could be construed as a 'molecular slip' (see [27]) in the electron-transport-driven proton pump. This pathway could in principle serve to regulate the magnitude of $\Delta\psi$ in uninhibited, bacterial cells and chromatophores, although its contribution even at saturating light intensities would be rather less than 20% of the total turnover (calculation based on the assumption that flux through the pathway is maximal in the presence of antimycin). In earlier work with anaerobic chromatophore suspensions the H^+/e ratio in steady-state was found to be independent of the value of $\Delta\psi$ [18] which does indeed suggest that the contribution from slip pathways remains small. Interestingly, however, in a reconstituted system of photosynthetic reaction centres and Cyt b/c_1 complex from mitochondria there was evidence for a $\Delta\psi$-dependent decrease in the H^+/e ratio [28,29]. It would therefore be interesting to assess the relative con-

tributions of antimycin-sensitive and insensitive electron transport in the reconstituted membranes.

Whereas the steady-state data discussed above are similar in intact cells and chromatophores a clear difference was evident in the rate at which $\Delta\psi$ was generated in the approach to steady-state in the presence of antimycin (Fig. 2). In chromatophores treated with the inhibitor the time required for $\Delta\psi$ to reach steady-state after the sample was illuminated was considerably longer than in intact cells. This difference probably arises because significant quantities of Cyt c_2 are lost during chromatophore preparation. In order to divert both reducing quivalents from ubiquinol at Q_z into the Rieske FeS centre the latter must be maintained in a highly oxidised state [6]. Thus in our experiments with chromatophores with only limited quantities of Cyt c_2 present, it may take some time before Cyt c_1 and the FeS centre are sufficiently oxidised to catalyse the antimycin-insensitive reaction at a significant rate.

Ionic current / membrane potential relationships in inhibited intact cells and chromatophores

The dependence of the dissipative ionic current on its driving force, $\Delta\psi$, was not influenced by the manner in which $\Delta\psi$ was reduced: inhibition of electron transport by antimycin or by myxothiazol gave rise to an identical relationship (Fig. 3). Thus, in the conditions of these experiments there is no evidence for any direct interaction between the primary or secondary ion pumps or for any redox control over the dissipative ionic fluxes across the bacterial membrane. This applies to experiments with intact cells in which active ATP synthesis is taking place [30] and experiments with chromatophores in which a large contribution of the total ionic current also proceeds through F_0 [23]. As was found in experiments with reduced light intensity [23] the titrations with antimycin and with myxothiazol show a diodic dependence of J_{dis} on $\Delta\psi$. In a general way this relationship probably accounts in large measure for homeostatic control of the protonmotive force across the bacterial membrane [24] and in the present context it provides the explanation for why myxothiazol and antimycin have a smaller inhibitory effect on $\Delta\psi$ (Fig. 1) than on electron-transport rate (strictly $J_E n$ in Fig. 4). Thus, in chromatophores, although the residual value of $J_E n$ was only 20% of the maximum $\Delta\psi$ remained at 70% of the maximum in the presence of sufficient antimycin to inhibit completely the Q_c site. From Fig. 3 it is clear that this can be entirely explained by the non-ohmic properties of the chromatophore membrane: the pronounced inhibition of the rate at which $\Delta\psi$ is generated ($J_E n$) is largely compensated by the decrease in the rate at which $\Delta\psi$ is dissipated.

Photosynthetic control in the antimycin-sensitive and antimycin-insensitive electron-transport pathways of chromatophores: synergistic depression of $\Delta\psi$ by a combination of inhibitor and uncoupler

At maximum rates of electron transport (viz. at high light intensities in the absence of inhibitor) the build-up of $\Delta\psi$ is limited by high dissipative ionic currents due to the elevated membrane conductance. The extra dissipative ionic current resulting from the addition of low concentrations of uncoupler (in the region of 0.02 μM FCCP) is negligible in comparison with the intrinsic component and therefore lowers $\Delta\psi$ only but little (Fig. 5). As explained above the slightly lower $\Delta\psi$ in the presence of antimycin (in the absence of uncoupler) belies a large inhibitory effect on the net rate of electron transport and large decrease in J_{dis} due to the lowered membrane conductance. Under these conditions the extra dissipative proton current introduced by low concentrations of uncoupler is significant compared with the intrinsic J_{dis} and therefore leads to a marked drop in $\Delta\psi$ (Fig. 5), i.e., synergistic lowering of $\Delta\psi$.

The question therefore arises as to why when myxothiazol is added to give a similar $\Delta\psi$ to that achieved with antimycin, uncoupler fails to produce synergistic depression of $\Delta\psi$ (Fig. 5). Fig. 6 shows that during partial inhibition with myxothiazol, electron transport is still severely limited by $\Delta\psi$: the addition of low concentrations of uncoupler to dissipate $\Delta\psi$ leads to large increases in the rate of electron transport. Thus when low concentrations of FCCP are added to chromatophores partially inhibited by myxothiazol the tendency for $\Delta\psi$ to fall (as described above) is off-set by an accelerated rate electron-transport-driven proton translocation. The absence of 'photosynthetic' control in the

presence of antimycin (Fig. 6) means that the compensatory device fails and $\Delta\psi$ is depressed upon the addition of uncoupler.

The dependence of the uninhibited photosynthetic electron-transport rate ($J_E n$) on $\Delta\psi$, shown in Fig. 6, is broadly similar to the equivalent relation in mitochondrial respiratory electron transport [31]. In mitochondria there is a suggestion of a discontinuity between controlled and uncontrolled respiration at which kinetic factors in the respiratory chain become rate-limiting [32]. This is not evident in the experiments with chromatophores where control continues to be exerted by even quite low values of $\Delta\psi$. If respiratory and photosynthetic control are considered to be thermodynamic rather than kinetic, then their existence implies a measure of reversibility in an electrogenic reaction. In the absence of inhibitor or with low titres of myxothiazol this is probably the electrogenic reaction within the Cyt b/c_1 complex. In the presence of antimycin the only electrogenic reaction in the curtailed electron-transport pathway is that occurring across the photosynthetic reaction centre and therefore the lack of control by $\Delta\psi$ under these conditions suggests that this reaction operates irreversibly.

Acknowledgements

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