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In vivo redox poising of the cyclic electron transport system of *Rhodobacter capsulatus* and the effects of the auxiliary oxidants, nitrate, nitrous oxide and trimethylamine *N*-oxide, as revealed by multiple short flash excitation

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Intact cells of *Rhodobacter capsulatus* in the presence of myxothiazol were exposed to trains of short flashes of saturating light and the pattern of the absorbance changes due to P870, cytochrome ($c_1 + c_2$) and the carotenoids that report on the membrane potential were monitored. Myxothiazol inhibits cyclic electron transport and therefore the extent to which electron donors and acceptors of the reaction centre are available for photochemistry is revealed. In darkened anaerobic suspensions of cells in the presence of myxothiazol, only the first two flashes in the train led to charge separation in the photosynthetic reaction centres. The results indicated that the quinone pool and quinone bound at the Q_B site in the reaction centre were extensively reduced and quinone bound at Q_A was partly reduced before initiation of flash excitation. Thus under these conditions, and in the absence of myxothiazol, cyclic electron transport would be restricted. In the presence of oxygen or the auxiliary oxidants trimethylamine *N*-oxide, NO_3^- or N_2O, the oxidation/reduction reactions and the electrochromic absorbance changes suggested that the pool and reaction centre quinones became more oxidised. Thus, the system was poised at a potential more conducive to optimal rates of photosynthetic electron transport. By reference to experiments on the growth of *Rb. capsulatus* (Richardson, D.J., King, G.F., Kelly, D.J., McEwan, A.G., Ferguson, S.J. and Jackson, J.B. (1988) Arch. Microbiol. 150, 131–137), it is argued that redox poising by the auxiliary oxidants is physiologically important, especially at low light intensities. Flash train experiments reveal that over-reduction of the quinones is more severe with succinate as a carbon source than with malate and this accounts for the observation that the rate of growth on succinate is decreased more strongly at low light intensities.

Introduction

The photosynthetic electron transport system of *Rhodobacter capsulatus* (formerly *Rhodopseudomonas capsulata*) is cyclic [1]. It comprises the photosynthetic reaction centre and cytochrome b/c_1 complexes, both of which are intrinsic membrane proteins, cytochrome c_2, which is located in the periplasm and a pool of ubiquinone, which is thought to diffuse freely in the hydrophobic domain of the cytoplasmic membrane. Light-driven oxidation of P870, a special pair of bacteriochlorophyll molecules in the reaction centre, leads to sequential reduction of bound ubiquinone at two distinct binding sites on the reaction centre (Q_A and Q_B) and subsequent oxidation of cytochrome c_2. This series of charge separation reactions spans the cytoplasmic membrane. Ubiquinol, released from the Q_B site of the reaction centre, and oxidised cytochrome c_2 serve as electron donor and electron acceptor, respectively, for the cytochrome b/c_1 complex. Discussions of the structure and operation of the cyclic electron transport chain of *Rhodobacter* sp. can be found in Refs. 2–4. At extremes of redox potential when P870 is held oxidised, or when bound, non-exchangeable quinone (Q_A) is reduced, cyclic electron transport is blocked [5]. At intermediate values of redox potential the net rate of cyclic electron transport is controlled by the redox state

Abbreviations: P870, bacteriochlorophyll special pair; Q_A, primary acceptor quinone; Q_B, secondary acceptor quinone; TMAO, trimethylamine *N*-oxide; DMSO, dimethylsulphoxide; Q_z, quinol oxidase site of the b/c_1 complex; Q_c, quinone reductase site of the b/c_1 complex.

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of the quinone (Q) pool [2,6]. The importance of a properly poised redox potential to the physiology of *Rb. capsulatus* and related organisms has long been appreciated [7–11].

Rb. capsulatus catalyses respiratory electron flow to a variety of electron acceptors. There are two terminal oxidases, cytochrome c_2 oxidase (cytochrome *b*-410) and 'alternative' oxidase (cytochrome *b*-260) which is believed to branch from the main chain at the level of the Q pool [12]. The Q pool can be fed with reducing equivalents from, for example, NADH dehydrogenase, succinate dehydrogenase or hydrogenase. NO_3^-, N_2O and trimethylamine *N*-oxide (TMAO) or dimethylsulphoxide (DMSO) can all serve as anaerobic electron acceptors [13]. The terminal NO_3^- reductase and TMAO/DMSO reductase both accept electrons by way of specific c-type cytochromes from the Q-pool without involvement of the cytochrome b/c_1 complex (unpublished observations of the authors and Refs. 13 and 14). Membrane-bound *b*-type cytochromes that are distinct from those [13] associated with the cytochrome b/c_1 complex may serve as quinol oxidases on these pathways. N_2O reductase is now thought to accept electrons via cytochrome c_2 and hence, at least in part from the b/c_1 complex [15]. In addition, some electrons can proceed via cytochrome c_2 to the N_2O reductase independently of the cytochrome b/c_1 complex [15]. Although some of these oxidants (N_2O, TMAO and DMSO) can support limited growth of *Rb. capsulatus*, under anaerobic conditions in darkened cultures with a number of carbon sources [16–19], their main function may be in facilitating certain modes of photosynthetic growth: (a) during anaerobic phototrophic growth on highly reduced carbon substrates, such as butyrate or propionate, an 'auxiliary' oxidant such as N_2O, NO_3^-, TMAO or DMSO is required as an electron sink [20]; (b) with more oxidised substrates, such as succinate or malate, the rate of phototrophic growth under anaerobic conditions at low light intensities is enhanced in the presence of an auxiliary oxidant [20]. A related observation is that in anaerobic, washed cell suspensions of *Rb. capsulatus* the photosynthetic electron transport chain is inhibited after addition of very low concentrations of phenazine methosulphate, apparently as a result of over-reduction brought about by equilibration of components of the chain with low potential reductants in the cell cytoplasm [11].

Photosynthetic electron transport in strains with appropriate pathways can be subsequently restored by addition of an auxiliary oxidant [11]. In another phototrophic bacterium, *Erythrobacter* sp. OCh 114, the problem of maintaining a properly poised redox potential for photosynthetic electron transport is especially acute. This organism can grow photosynthetically under aerobic conditions but under anaerobic conditions it requires an auxiliary oxidant, such as TMAO, for photosynthetic growth even with relatively oxidised carbon sources, such as malate [21]. Q_A in the reaction centre of *Erythrobacter* sp. OCh 114 has an unusually high midpoint potential and this may be the basis for the inability of this organism to maintain an optimal redox poise under anaerobic conditions [22]. In this report we describe the results of experiments in which trains of short flashes were used to monitor the effect of auxiliary oxidants on the redox state of the photosynthetic electron transport system of anaerobic intact cells of *Rb. capsulatus*.

Methods

Rb. capsulatus strain N22 was grown phototrophically under anaerobic conditions with malate as a carbon source in RCV medium [23] as described [24]. *Rb. capsulatus* strain N22DNAR$^+$, which is capable of nitrate respiration [25] was grown under similar conditions or with NO_3^- and butyrate replacing the malate as a carbon source as described [20]. Cells were harvested by centrifugation, then washed and resuspended in 10 mM sodium phosphate buffer (pH 7.6). Unless otherwise stated, experiments were performed in the same buffer, pre-gassed in argon containing less than 3 ppm oxygen. The cell suspension was magnetically stirred under argon for 30 min before experiments began. Absorbance changes due to P870, cytochrome ($c_1 + c_2$) and electrochromically sensitive carotenoids were recorded on a single-beam spectrophotometer with flash excitation as described [26]. At the concentration of bacteriochlorophyll used in these experiments, each flash was approx. 95% saturating on the basis of measurements of the extent of the electrochromic absorbance change in anaerobic intact cell suspensions at varying flash intensities.

Results and Discussion

The kinetics of oxidation and re-reduction of both P870 and cytochrome ($c_1 + c_2$), together with the electrochromic absorbance change corresponding to the development of a cytoplasmic membrane potential ($\Delta\psi$) during a train of single-turnover light pulses under anaerobic control conditions are shown in Fig. 1. In the absence of an electron transfer inhibitor acting on the cytochrome b/c_1 complex, net oxidation of P870 after each flash was small (Fig. 1a) because re-reduction by cytochrome ($c_1 + c_2$) was fast. Similarly, the full extent of oxidation of cytochrome ($c_1 + c_2$) was not observed because of their continued re-reduction by the Rieske Fe-S centre and the quinol oxidase (Q_z) site in the cytochrome b/c_1 complex (Fig. 1b). However, the electrochromic absorbance change (Fig. 1c) accumulated after successive flashes until, in the quasi-steady-state, reached after approxim. eight or nine flashes, the extent

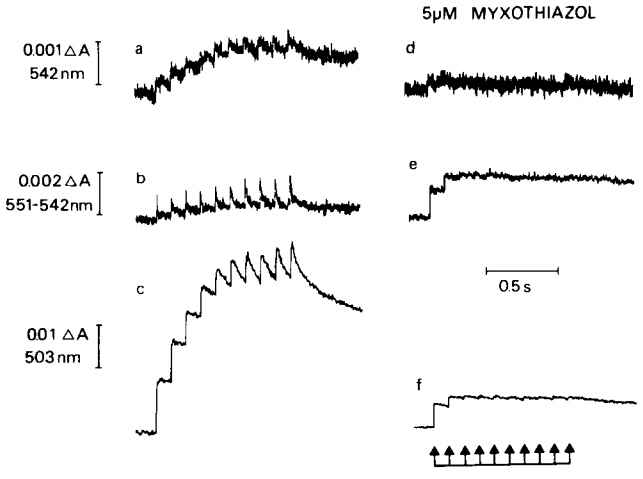

Fig. 1. Multiple flash excitation of anaerobic, intact cell suspensions of *Rb. capsulatus*. Experiments were performed as described in Methods at a concentration of bacteriochlorophyll of 20 μM. In all cases, the suspension was exposed to ten flashes fired at a frequency of 10 Hz. The absorbance change at 542 nm is attributed to P870, at 551–542 to cytochromes ($c_1 + c_2$), and at 503 nm to carotenoids sensitive to $\Delta\psi$. An upward deflection of a trace indicates net oxidation of the redox centre or an increase in the magnitude of $\Delta\psi$. Data recorded at 542 nm are the average of eight sweeps, at 503 nm the average of four sweeps and at 551–542 nm 2×8 sweeps, all at a sweep frequency of 0.0167 Hz. When present, myxothiazol (5 μM) was added at the start of the 30 min dark, anaerobic preincubation period.

of the change immediately after a flash was matched by the subsequent slower decay between the flashes, thus indicating continued turnover and electrogenic activity of the electron transport cycle.

In Fig. 1d–f the cells, again under strictly anaerobic conditions, were treated with a saturating concentration of myxothiazol to inhibit the Q_z site of the cytochrome b/c_1 complex [27,28]. Very little photooxidation of P870 was observed throughout the flash train (Fig. 1d) and oxidation of cytochrome ($c_1 + c_2$) was only prominent after the first two flashes. These data suggest that the components of the photosynthetic electron transport system on the acceptor side of the reaction centre were predominantly reduced: if the situation were otherwise we should have expected to observe progressive oxidation of P870 and cytochrome ($c_1 + c_2$) after the first few flashes in the train, since re-reduction of these components would be blocked by the inhibitor. The results can be explained if, before the first flash, Q_A was mostly oxidized, the second bound quinone, Q_B was mostly reduced and the Q-pool was almost completely reduced. Thus after the first flash, a single turnover of P870 to Q_A would have occurred, followed by electron transfer from cytochrome ($c_1 + c_2$) to $P870^+$ and, in those reaction centres with oxidised Q_B, electron transfer from Q_A to Q_B. Only in these latter reaction centres, where Q_A was re-oxidised, would there have been turnover following the second flash. After subsequent flashes the failure to clear reducing equivalents from the bound quinones Q_A and Q_B, because of the highly reduced Q pool, would have blocked photochemistry. This sequence of events in the presence of myxothiazol is also reflected by the electrochromic absorbance changes shown in Fig. 1f: there was extensive charge translocation through the reaction centre after the first flash in the train, some after the second flash, but very little after subsequent flashes.

Experiments with actimycin under conditions similar to those in Fig. 1d–f reinforced the conclusion that Q_B and the Q pool are largely reduced in dark anaerobic intact cell suspensions (data not shown). Differences in the kinetic data between experiments with myxothiazol and with antimycin could be readily explained by the fact that antimycin inhibits at the Q_c site of the cytochrome b/c_1 complex and that slow turnover of the Q_z site takes place even when Q_c is completely blocked [4]. Because myxothiazol inhibits at the Q_z site of the cytochrome b/c_1 complex, the effects of this inhibitor give a clearer indication than those of antimycin of the availability of oxidising equivalents in the acceptor pools. It is emphasised that the experiments in Fig. 1 were performed under strictly anaerobic conditions in the absence of any added electron acceptor. Thus, the addition of myxothiazol (or antimycin) would not have had any effect on the redox state of the components of the cyclic electron transport system in the cells in the dark before the flash commenced.

Since the overall rate of cyclic electron transport is critically dependent upon quinone and quinol diffusion between the reaction centre and the cytochrome b/c_1 complex [2,6], the conclusion that the Q pool and quinone at the Q_B site are extensively reduced in dark anaerobic suspensions of cells might have physiological implications. The organism will commonly be exposed to periods of quiescence under dark anaerobic conditions followed by periods of illumination. At the onset of illumination in these circumstances, electron transport through the reaction centre is expected to be limited by the rate at which quinone can be generated by oxidation of quinol at the Q_z at the site of the cytochrome b/c_1 complex and diffuse to the Q_B site of the reaction centre. Thus, in principle, the restriction in the rate of photosynthetic electron transport could limit the rate of growth. We have previously argued that the auxiliary oxidants, TMAO, DMSO, NO_3^- and N_2O may have a role in the maintenance of an optimal redox poise of the cyclic electron transport system [13]. Therefore we have tested the possibility that in intact cells of *Rb. capsulatus* in dark anaerobic conditions the presence of an auxiliary oxidant can lead to partial oxidation of the Q pool and bound quinones in the reaction centre and thus to a more rapid rate of photosynthetic electron transport. According to the arguments given above, the recordings of flash train oxidation of P870 and cytochrome ($c_1 + c_2$) and the related electrochro-

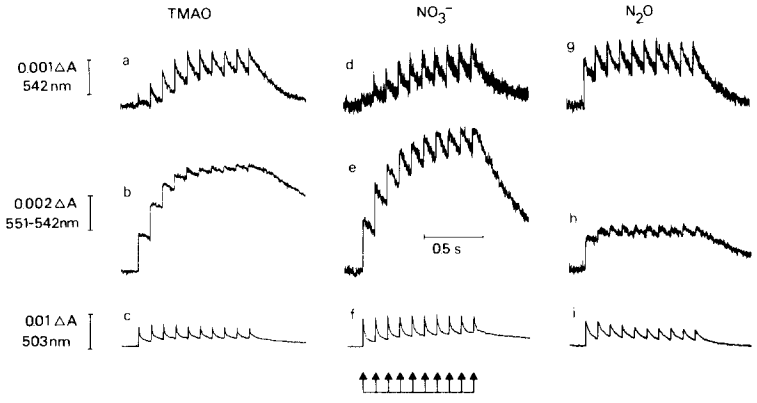

Fig. 2. The effect of TMAO, NO_3^- and N_2O on flash-induced absorbance changes. Experiments were performed as described in Methods and Fig. 1. Myxothiazol (5 μM) was present in all experiments. Where indicated, TMAO (4 mM), NO_3^- (0.4 mM) and N_2O (100 μl of an N_2O-saturated solution) were added at the end of the 30 min dark, anaerobic preincubation period. For all other details see the legend to Fig. 1. Note that controls for these experiments, in the absence of auxiliary oxidant but in the presence of myxothiazol, gave recordings that were practically indistinguishable from those in Figs. 1d–f. Note also that the addition of either TMAO, NO_3^- or N_2O led to a substantial increase in the membrane potential due to anaerobic respiration (see Ref. 11 but not shown in the figure) before flashing was commenced. Thus, after each flash in the train, the electrochromic absorbance change decayed with a considerably accelerated rate, relative to the control (Fig. 1f and see text).

mic absorbance changes in the presence of myxothiazol provide a simple qualitative indication of the redox state of Q_A, Q_B and the Q pool. For the experiments described below (Figs. 2, 3d–f and 4d–f) control experiments were routinely performed in the presence of myxothiazol, but in the absence of auxiliary oxidant. The results were not significantly different from those shown in Figs. 1d–f which can therefore be used for comparison.

When TMAO was added after the anaerobic preincubation period (in the presence of myxothiazol), but just before the flash excitation commenced, the results shown in Fig. 2a–c were obtained. Similar data were observed when the entire pre-incubation period was carried out in the presence of TMAO (not shown). These results can be explained on the assumption that TMAO increased the oxidation state of the Q pool and thus of the reaction centre quinones at sites Q_A and Q_B. Under these conditions the eventual restriction on turnover of the reaction centre arose, not from the limiting availability of oxidising equivalents in the acceptor pools, but from the availability of reducing equivalents in the donors to the reaction centre (cytochromes $(c_1 + c_2)$ and Rieske Fe-S). Thus the net amount of P870 remaining oxidised a few milliseconds after a flash increased with the flash number until the fifth or sixth flash in the train (Fig. 2a). When this quasi-steady-state was reached, the maximum amount of oxidised P870 was equivalent to about 8 nmol per μmol bacteriochlorophyll. Note that the apparent change in the extent of P870 oxidation was greater after the second, third and fourth flashes than after the first, but then subsequently declined until the quasi-steady-state was reached. This can be explained with reference to the data shown for cytochrome $(c_1 + c_2)$ oxidation under similar conditions (Fig. 2b). After flash 1 oxidation of cytochrome $(c_1 + c_2)$ was fast and extensive and, since myxothiazol was present to block the Q_z site, re-reduction of cytochrome $(c_1 + c_2)$ in the following dark period was inhibited (some re-reduction would have taken place, due to electron transfer from the Rieske Fe-S, but this was not detected because the reaction is fast [29]). Flash 2 and flash 3 also gave rise to fast cytochrome $(c_1 + c_2)$ oxidation. Progressively, however, the cytochrome $(c_1 + c_2)$ available for electron donation to the reaction centre was essentially exhausted and little further oxidation was evident after later flashes. Returning to the P870 trace in Fig. 2a, it is clear that after flash 1 very little net oxidation was observed because of fast, almost complete re-reduction by cytochrome $(c_1 + c_2)$. The amount of fast re-reduction of $P870^+$ by cytochrome $(c_1 + c_2)$ decreased after flashes 2 and 3 as the reduced cytochrome was depleted and the amount of $P870^+$ remaining a few milliseconds after the flash was therefore increased. The fact that P870 oxidation and slow re-reduction continued during the flash train beyond the time (about flash 4 or 5) after which reduced cytochrome $(c_1 + c_2)$ was apparently exhausted, is suggestive of a low-amplitude back reaction from Q_A to $P870^+$ ($t_{1/2} \approx 50$ ms) or from Q_B to $P870^+$ ($t_{1/2} \approx 1$ s) [1]. Thus, at this flash frequency, the combined rate at which QH_2 was generated by the reaction centre and by the respiratory dehydrogenases was greater than the rate at which the Q pool was oxidised by TMAO with the result that after several flashes in the presence of myxothiazol, reducing equivalents had accumulated in Q_A and Q_B. It is also possible that slow, myxothiazol-insensitive electron transport from the quinone pool to cytochrome c_2 [15] contributed to P870 re-reduction under these conditions.

The re-poising effect of TMAO (again in cells treated with myxothiazol) is shown clearly by the electrochromic absorbance changes (Fig. 2c). The extent of the absorbance change after a single flash (measured on a rapid timescale) was greater (routinely by 20–30%) in the presence of TMAO (compare Fig. 1f). This shows that the extent of charge separation through the reaction centre and across the cytoplasmic membrane after a single flash was increased by the auxiliary oxidant. More conspicuously, the extents of the electrochromic absorbance change after the second, third and subsequent flashes were enhanced in the presence of TMAO compared with the control. Again this signifies continued turnover of the reaction centre because reducing equivalents in Q_A and Q_B progress to the more oxidised Q pool. Parenthetically, the stimulated decay rate of the electrochromic absorbance change after each flash in the presence of TMAO (Fig. 2c compared with Fig. 1f) was a consequence of the increased absolute value of the membrane potential resulting from electrogenic electron transport to TMAO in the dark [30] before flashing commenced: the 'diodic' dependence of membrane conductance on $\Delta\psi$ leads to a significantly larger dissipative ionic current at high $\Delta\psi$ [31]. Similar enhanced decay rates of the electrochromic absorbance changes were also observed in the presence of other oxidants (see below, Figs. 2f, 2i and 3f) for the same reason. However, it is only the extents of the electrochromic absorbance changes immediately after the flashes (indicating electrogenic reactions) which are of significance in the present context.

Figs. 2d–f show that in anaerobic cells of *Rb. capsulatus* strain N22DNAR$^+$, which possesses a periplasmic, dissimilatory NO_3^- reductase [25], NO_3^- had a similar re-poising effect in the presence of myxothiazol to that of TMAO: the kinetics of P870 and cytochrome ($c_1 + c_2$) oxidation and reduction and the electrochromic absorbance change after multi-pulse excitation were very similar. The cells for this experiment were grown in the presence of butyrate and NO_3^- to induce high levels of NO_3^- reductase (Richardson, D.J., unpublished observations). The addition of NO_3^- to anaerobic cell suspensions of *Rb. capsulatus* strain N22, which lacks the dissimilatory NO_3^- reductase [25], had no repoising effect; the flash-induced absorbance changes (data not shown) were indistinguishable from those in the control experiments (Fig. 1d–f). Washed cells of *Rb. capsulatus* strain N22DNAR$^+$ grown in the presence of NO_3^- with malate as a carbon source, have a lower NO_3^- reductase activity than cells grown with butyrate. Experiments with such cells gave rise to results which were intermediate between those in Figs. 1d–f and those in Figs. 2d–f (not shown).

Rb. capsulatus strain N22 has a high level of N_2O reductase even in cells grown in the absence of N_2O [16]. N_2O respiration differs from nitrate and TMAO respiration because the cytochrome b/c_1 complex and cytochromes c_2 can act as components on the electron transport pathway [15]. Figs. 2g–i shows that the addition of N_2O to anaerobic cell suspensions treated with myxothiazol had a repoising effect (compare Fig. 1d and Fig. 1f). That re-poising with N_2O takes place in these conditions suggests that in the dark, even in the presence of myxothiazol, there is sufficient flux through the cytochrome b/c_1 complex and/or through the alternative pathway partially to oxidise the Q pool. In fact, the incomplete sensitivity of N_2O respiration to myxothiazol in several strains and the low rates of N_2O reduction in mutants of *Rb. capsulatus* deficient in the cytochrome b/c_1 complex was recently described and discussed [15]. The absorbance changes which occurred in response to the flash train with N_2O were different to those observed with NO_3^- or TMAO. The probable reason for this is that N_2O in the presence of myxothiazol causes substantial oxidation of cytochrome ($c_1 + c_2$) in anaerobic intact cells in the dark [15]. Thus cytochrome ($c_1 + c_2$) photo-oxidation during the flash train was less extensive in the presence of N_2O than in the presence of TMAO or NO_3^- (compare Fig. 2h with Fig. 2b and e). The corresponding pattern of P870 oxidation and reduction was also altered: in contrast to the observations of TMAO and NO_3^- (Fig. 2a and d) the re-reduction of P870$^+$ after the first flash was substantially incomplete, presumably because less reduced cytochrome ($c_1 + c_2$) was available as donor, and, for the same reason, the quasi-steady-state of P870 oxidation and reduction was reached after only three or four flashes in the presence of N_2O (Fig. 2g). Once again the extents of the electrochromic absorbance changes after the flashes (Fig. 2i) reflect the fact that addition of auxiliary oxidant (in this case N_2O) to the anaerobic suspension in the presence of myxothiazol (compare Fig. 1f) resulted in a larger capacity for electrogenic cyclic electron transport during multiple flash excitation.

Oxygen can also serve to re-oxidise the bacterial cyclic electron transport system after over-reduction under strictly anaerobic conditions. Data recorded in the presence of myxothiazol (for comparison with Fig. 1d–f) are shown in Fig. 3d–3f. Qualitatively, the results resemble those observed in the presence of N_2O (Figs. 2g–i), consistent with the evidence that O_2 and N_2O can oxidise both cytochrome ($c_1 + c_2$) and the ubiquinone pool. Compared with the results with N_2O, the photo-oxidation of cytochrome ($c_1 + c_2$) and the electrochromic absorbance changes during the flash train were slightly less extensive in the experiments performed after addition of O_2. This may be the result of a faster rate of oxidation of cytochrome ($c_1 + c_2$) by cytochrome *c* oxidase in these cells than by N_2O reductase. Also shown for comparison in Fig. 3a–c are the results of experiments performed in the presence of O_2 but in

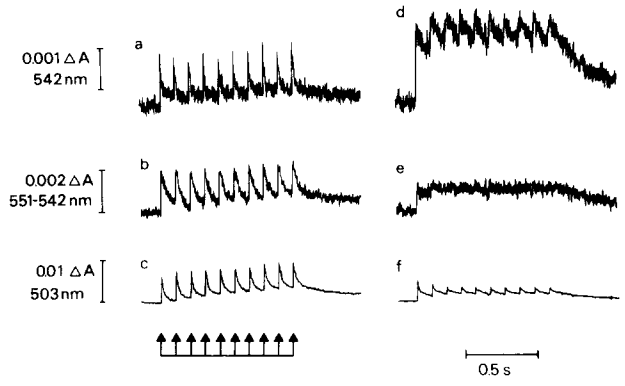

Fig. 3. The effect of O_2 on flash-induced absorbance changes. Experiments were performed as described in Methods in the absence (left) or presence (right) of (5 μM) myxothiazol (see also Fig. 1). At the end of the 30 min dark, anaerobic preincubation period samples were made aerobic by removing the stopper and gas trains and by bubbling water-saturated air through the sample via a hypodermic needle connected to a peristaltic pump. To ensure aerobic conditions were maintained throughout, air was bubbled through the sample during the 1 min dark period between successive flash trains.

the absence of myxothiazol. In these conditions P870 re-reduction and cytochrome ($c_1 + c_2$) re-reduction between the flashes were considerably faster than in the presence of myxothiazol and the presence of O_2 (Fig. 3d–f) but slower than in the absence of myxothiazol under anaerobic conditions (Fig. 1a–c). Analogous experiments performed under anaerobic conditions with auxiliary oxidant but in the absence of myxothiazol are not shown. The data closely resembled those shown in Fig. 3a–c.

Fig. 4 shows the results of an experiment designed to examine the effect of carbon sources, malate and succinate, on the poise of the photosynthetic electron transport components of anaerobic intact cells of *Rb. capsulatus*. Cells harvested from a culture grown in the presence of malate as a carbon source were resuspended under anaerobic conditions in growth medium lacking malate and exposed to flash excitation (Fig. 4a). Then either malate (Fig. 4b) or succinate (Fig. 4c) was added and the flash excitation was repeated. The train of flashes yielded the characteristic electrochromic response in Fig. 4a, $\Delta\psi$ reaching a quasi-steady-state level after the 7th or 8th flash. In the presence of malate (Fig. 4b), the quasi-steady-state was reached a little sooner and the maximum change in $\Delta\psi$ was slightly lower than in its absence (Fig. 4a). This can probably be attributed to improved poising of the photosynthetic electron transport chain and to a higher basal level of $\Delta\psi$ under dark anaerobic conditions before the flashing commenced. However, the most significant result was obtained in the presence of succinate (Fig. 4c). Here the absorbance changes after a flash were strongly attenuated and, in contrast to the results with malate, the electrochromic absorbance change had not reached a quasi-steady-state even after ten excitation flashes. A much decreased amplitude of the electrochromic absorbance changes in succinate media was also recorded in the presence of myxothiazol (Fig. 4d–f). The conclusion is that the redox state of the acceptor quinones bound to the reaction centre is even more unfavourable for rapid photosynthetic electron transport in succinate, than it is in the absence of added substrates (Figs. 1–3) or in the presence of malate. When the experiments shown in Fig. 4 were repeated, using cells grown on succinate, broadly similar results were obtained. The effect of adding succinate on the flash-induced electrochromic absorbance changes was only slightly less pronounced than that shown in Fig. 4 for the malate-grown cells (not shown). This suggests that even when the organism has the opportunity to adapt to growth on succinate its photosynthetic electron transport system, at least at the onset of illumination after a period in the dark, is limited by a degree of over-reduction. There are two complementary ways of looking at this phenomenon. First, it could be argued that the presence of succ-

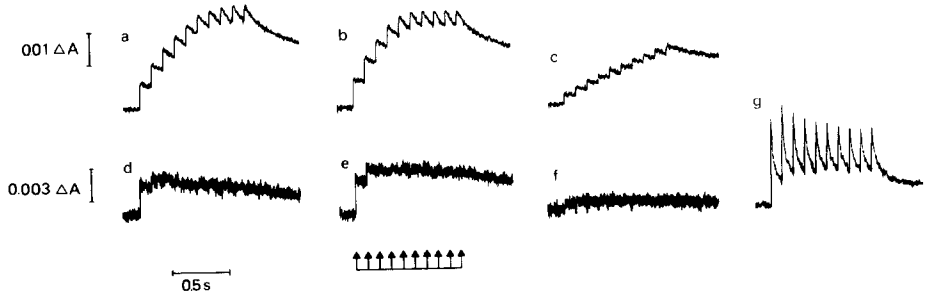

Fig. 4. The effect of carbon source on flash-induced electrochromic absorbance changes. Experiments were performed in fresh RCV (growth) medium [23] from which the carbon source had been omitted (see Methods and Fig. 1). In d, e, f and g 5 μM myxothiazol was added at the start of the 30 min preincubated period. Traces a and d were recorded at the end of the preincubation period. Then either 15 mM malate (in b and e) or 30 mM succinate (in c and f) was added and recordings were made after a further 15 min incubation. Trace g shows the effect of adding 4 mM TMAO to the sample used in the recording of trace f. All traces show the electrochromic absorbance change at 503 nm and are the average of eight sweeps at a frequency of 0.0167 Hz. Note the difference in the scale between the top and bottom rows.

cinate dehydrogenase, would increase the state of reduction of the Q pool and, hence, of Q_B and Q_A in the reaction centre. This effect would be particularly pronounced if the cytoplasmic fumarate level were low. Alternatively, it might be that, in cells grown on malate or incubated in the presence of malate, the generation of fumarate by fumarase would provide a convenient electron sink for removal, from the Q pool, of reducing equivalents introduced by the action of NADH dehydrogenase (even if the cytoplasmic pyridine nucleotide pool, $E_{m,7.0} \approx -0.3$ volt, were only partly reduced, the potential could be low enough, at equilibrium, to reduce extensively the reaction centre Q_A, $E_{m,7.0} \approx -0.02$ volt). However, after the addition of succinate to the incubation medium, the operating potential of the fumarate/succinate couple would be decreased to the point at which fumarate (derived from malate) would be unable to operate as an effective electron sink for the withdrawal of reducing equivalents from the Q pool and thus (Fig. 4) photosynthetic electron transport would be restricted.

Whether succinate exerts its effects in Fig. 4c and f by acting as a reductant of the Q pool or by acting to prevent fumarate from serving as an oxidant, it is evident from Fig. 4g that the effect can be overcome by the addition of the terminal oxidant TMAO. The addition of TMAO to the sample containing succinate in the presence of myxothiazol led to the generation of substantial electrochromic signals during the flash train. Consistent with the effects of auxiliary oxidants described above, it seems that TMAO even in the presence of succinate is able partially to oxidise the acceptor quinones and the Q pool to restore a redox poise which is suitable for rapid rates of photosynthetic electron transport. The addition of TMAO to cell suspensions containing malate and myxothiazol gave identical results to those in Fig. 4g for cells in the presence of succinate (data not shown).

General discussion

The results described above show that in washed intact cells of *Rb. capsulatus,* harvested from phototrophic cultures and resuspended in the dark in anaerobic buffer, the Q pool and reaction centre Q_B are extensively reduced and Q_A is slightly reduced. It is reasoned that under such conditions the rate of cyclic electron transport during a period of illumination would be restricted. The addition of auxiliary oxidant, such as TMAO, NO_3^- or N_2O, to organisms with an appropriate anaerobic respiratory chain led to partial oxidation of Q_B and the Q pool, circumstances, it was argued, which would lead to an acceleration of the photosynthetic electron transport rate. It was recently shown that auxiliary oxidants only stimulate the growth rate of *Rb. capsulatus* on carbon substrates such as malate and succinate when the incident light intensity is low; at high light intensities the auxiliary oxidants had no effect on growth with those substrates [20]. These observations can be reconciled if it is recognised that at high light intensities when the energy supply to the culture is more than sufficient, factors other than the rate of cyclic electron transfer limit the rate of growth. Thus, though the rate of cyclic electron transfer might increase in the presence of auxiliary oxidant, this would not have any significant effect on growth rate. However, at low light intensities the restriction in the rate of cyclic electron transfer becomes critical and growth becomes limited by the energy supply. Under these circumstances, an increase in the rate of cyclic electron transfer in the presence of auxiliary oxidant would lead to an increase in growth rate. Note that the experimental device of using flash trains to supply energy at a limited rate in some ways corresponds to the physiological condition of low incident light intensity. In anaerobic phototrophic cultures with malate or succinate as a carbon source, TMAO or NO_3^- are reduced at a greater rate towards the end of exponential growth when the effective light intensity decreases due to self-shading [20]. This is also consistent with the above conclusion. Thus the auxiliary oxidants are only consumed in the process of redox poising when the rate of electron transport becomes limiting for growth. Formerly [20], we provided a different explanation for the stimulatory effect of auxiliary oxidants on growth at low light intensities; it was suggested that at low light intensities it is energetically less expensive to reoxidise pyridine nucleotide generated by metabolism through anaerobic respiration than through CO_2 re-fixation in the Calvin cycle. However, at high light intensities the recovery of carbon, otherwise lost in decarboxylation reactions, is at a premium. These two explanations are not mutually exclusive and it might be supposed that their relative importance will depend precisely on the supply of light energy, reducing equivalents and carbon to the culture. The rate of energy supply to the cell will also alter the level of the protonmotive force across the cytoplasmic membrane of the cell and, by way of the electrogenic activity of the NADH dehydrogenase, this will also affect the distribution of reducing equivalents between the pyridine nucleotide and ubiquinone pools [11].

The finding (Fig. 4) that over-reduction of the reaction centre quinones is more severe in the presence of succinate than in the presence of malate, must be discussed in the context of the well-documented rapid growth of *Rb. capsulatus* on succinate as a carbon source. For example, at high-light intensities the growth rates on succinate and malate were similar [20]. Again it is argued that at high light intensities, factors other than the rate of photosynthetic electron transport are limiting for growth. At low light intensities the rate of growth on succinate is considerably less than that on

malate [20]. This is consistent with the general view developed above: under low light conditions, the operation of the photosynthetic electron transport chain becomes rate-limiting for growth and thus the restricted turnover of the cycle in the presence of succinate becomes more critical. The finding that addition of TMAO leads to similar patterns of electrochromic absorbance changes during a flash train, in the presence of either succinate or malate, parallels earlier data which show that at low light intensities growth rates of cultures in the presence of TMAO and using either succinate or malate as carbon sources are similar [20].

The maintenance of a suitable redox poise is an acute problem for phototrophic bacteria because the photosynthetic electron transfer chain operates as a cycle but, at the same time, shares components with respiratory electron donors and acceptors. It is likely that, in different species of bacteria, different mechanisms have evolved to control the redox poise of the electron transport components. These may include the use of metabolites such as fumarate, CO_2, etc., or the use of exogenous electron acceptors such as TMAO, NO_3^- and N_2O. Our experiments with *Rb. capsulatus* also show that O_2 can raise the redox state of the Q pool, in principle to permit more rapid electron transport. In this context, it is interesting that photosynthetic electron transport [22] and phototrophic growth [21] of *Erythrobacter* sp. OCh 114 are absolutely dependent on the presence of either O_2 or an auxiliary oxidant [32]. Though *Erythrobacter* OCh 114 and *Rb. capsulatus* are vulnerable to over-reduction of the cyclic electron transport system, in the latter organism some regulation of the redox poise can be achieved under anaerobic conditions, even in the absence of an auxiliary oxidant, provided that the carbon substrate is not highly reduced.

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