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ON THE MECHANISM OF PHOTOSYNTHETIC ELECTRON TRANSFER IN *RHODOPSEUDOMONAS CAPSULATA* AND *RHODOPSEUDOMONAS SPHAEROIDES*

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(1) Current models for the mechanism of cyclic electron transport in *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* have been investigated by observing the kinetics of electron transport in the presence of inhibitors, or in photosynthetically incompetent mutant strains. (2) In addition to its well-characterized effect on the Rieske-type iron sulfur center, 5-(*n*-undecyl)-6-hydroxy-4,7-dioxobenzothiazole (UHDBT) inhibits both cytochrome b_{50} and cytochrome b_{-90} reduction induced by flash excitation in *Rps. sphaeroides* and *Rps. capsulata*. The concentration dependency of the inhibition in the presence of antimycin (approx. 2.7 mol UHDBT/mol reaction center for 50% inhibition of extent) is very similar to that of its inhibition of the antimycin-insensitive phase of ferricytochrome *c* re-reduction. UHDBT did not inhibit electron transfer between the reduced primary acceptor ubiquinone (Q_I^-) and the secondary acceptor ubiquinone (Q_{II}) of the reaction center acceptor complex. A mutant of *Rps. capsulata*, strain R126, lacked both the UHDBT and antimycin-sensitive phases of cytochrome *c* re-reduction, and ferricytochrome b_{50} reduction on flash excitation. (3) In the presence of antimycin, the initial rate of cytochrome b_{50} reduction increased about 10-fold as the $E_{h(7.0)}$ was lowered below 180 mV. A plot of the rate at the fastest point in each trace against redox potential resembles the Nernst plot for a two-electron carrier with $E_{m(7.0)} \approx 125 \pm 15$ mV. Following flash excitation there was a lag of 100–500 μs before cytochrome b_{50} reduction began. However, there was a considerably longer lag before significant reduction of cytochrome *c* by the antimycin-sensitive pathway occurred. (4) The herbicide ametryne inhibited electron transfer between Q_I^- and Q_{II}. It was an effective inhibitor of cytochrome b_{50} photoreduction at $E_{h(7.0)}$ 390 mV, but not at $E_{h(7.0)}$ 100 mV. At the latter E_h, low concentrations of ametryne inhibited turnover after one flash in only half of the photochemical reaction centers. By analogy with the response to *o*-phenanthroline, it is suggested that ametryne is ineffective at inhibiting electron transfer from Q_I^- to the secondary acceptor ubiquinone when the latter is reduced to the semiquinone form before excitation. (5) At $E_{h(7.0)} > 200$ mV, anti-

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Abbreviations: Mops, 4-morpholinepropanesulfonic acid; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; PMS, *N*-methylphenazonium methosulfate; PES, *N*-ethylphenazonium ethosulfate; PYO, 1-hydroxy-*N*-methylphenazonium methosulfate; UHDBT, 5-(*n*-undecyl)-6-hydroxy-4,7-dioxobenzothiazole; $(BChl)_2$, reaction center bacteriochlorophyll dimer; $(BChl)_2^+$, oxidized dimer; Q_I, primary acceptor ubiquinone; Q_{II}, secondary acceptor ubiquinone; Q_Z, ubiquinone molecule involved in electron transfer through the cytochrome b-c_2 oxido-reductase; $Q^-(H^+)$, semiquinone form in which protonation state is uncertain; Rieske Fe-S center, Rieske type iron sulfur center; $E_{h(x)}$, the oxidation-reduction potential referred to the standard hydrogen electrode at pH*x*; $E_{m(x)}$, the E_h at which a redox couple is half reduced at pH*x*; *n* value, number of electrons transferred per molecule reduced.

mycin had a marked effect on the cytochrome b_{50} reduction-oxidation kinetics but not on the cytochrome *c* and reaction center changes or the slow phase III of the electrochromic carotenoid change on a 10-ms time scale. This observation appears to rule out a mechanism in which cytochrome b_{50} oxidation is obligatorily and kinetically linked to the antimycin-sensitive phase of cytochrome *c* reduction in a reaction involving transmembrane charge transfer at high E_h values. However, at lower redox potentials, cytochrome b_{50} oxidation is more rapid, and may be linked to the antimycin-sensitive reduction of cytochrome *c*. (6) It is concluded that neither a simple linear scheme nor a simple Q-cycle model can account adequately for all the observations. Future models will have to take account of a possible heterogeneity of redox chains resulting from the two-electron gate at the level of the secondary quinone, and of the involvement of cytochrome b_{-90} in the rapid reactions of the cyclic electron transfer chain.

Introduction

In the purple non-sulfur photosynthetic bacteria *Rhodopseudomonas sphaeroides* and the closely related *Rhodopseudomonas capsulata*, the light-activated reaction center drives an electron from a high potential donor ($(BChl)_2$) to a low potential acceptor (Q_I/Q_I^-) (see Ref. 1 for a recent review). Normally, the electron on Q_I^- is delivered to a secondary acceptor ubiquinone (Q_{II}) in the reaction center complex with a halftime in the range 30–60 μs [2,3]. Recently, Rutherford and Evans [4] have shown in *Rps. sphaeroides* that Q_{II} forms a stable semiquinone on equilibrium redox titration, the $E_{m(8.0)}$ being 40 mV for the quinone/semiquinone couple, and −40 mV for the semiquinone/quinol couple. In isolated reaction centers, and in chromatophores under certain conditions, when Q_{II} is oxidized before excitation, metastable Q_{II}^- is generated following flash-excitation (halftime for decay approx. 1 min). It is further reduced to the quinol form by a second turnover of the reaction center and is subsequently re-oxidized, probably to the quinone form. In this way, Q_{II} acts as a two-electron gate [3,5–8].

The photo-oxidized reaction center, $(BChl)_2^+$, is re-reduced by a *c*-type cytochrome in a reaction which is normally complete within about 1 ms. Cytochrome c_2 ($E_{m(7.0)}$ 360 ± 15 mV in both *Rps. sphaeroides* and *Rps. capsulata* [9], λ_{max} of the α band in the reduced minus oxidized difference spectrum at 550.5 ± 0.5 nm [10]) appears to be the direct electron donor to $(BChl)_2^+$ [11–14]. Another species of cytochrome *c*, designated c_b (for bound *c*) ($E_{m(7.0)}$ 290 mV, λ_{max} of the α band in the reduced minus oxidized difference spectrum at 552.0 ± 0.5 nm [15]) is only photo-oxidizable in the presence of cytochrome c_2 [11,12,14,15] and probably represents the 295 mV component in earlier titrations of photo-oxidizable cytochrome *c* [16,17]. It seems likely that cytochrome c_2 (analogous to mitochondrial cytochrome *c*) acts to carry electrons between cytochrome c_b (analogous to mitochondrial cytochrome c_1; see Ref. 18) and the reaction center. We will refer to the pool of cytochrome c_b and c_2 as cytochrome *c*.

The electron donor to ferricytochrome *c* appears to be the Rieske Fe-S center [19–21]. Cytochrome *c* re-reduction by the Rieske Fe-S center occurs with a halftime of less than 1 ms, but is incomplete due to the redox equilibrium and apparent stoichiometry. The electron transfer is inhibited by 5-(*n*-undecyl)-6-hydroxy-4,7-dioxobenzothiazole (UHDBT), but is insensitive to antimycin [19,22]. Complete cytochrome *c* re-reduction is achieved in a slower reaction ($t_{1/2}$ 1–2 ms under optimal conditions) which is both antimycin- and UHDBT-sensitive [19,23,24]. This slower reaction is attributed to a bound ubiquinone molecule, Q_z ($E_{m(7.0)} \approx 155$ mV for the couple Q_z/Q_zH_2 in *Rps. sphaeroides* and very similar in *Rps. capsulata* [25–27]). It is currently considered that Q_zH_2 reduces cytochrome *c* via the Rieske center [19], transiently generating an unstable semiquinone ($Q_zH^\cdot$ of Q_z^-) in a reaction which is inhibited, probably indirectly, by antimycin [28].

Another component known to be involved in the electron transfer system is cytochrome b_{50} ($E_{m(7.0)}$ 50 mV [29], λ_{max} for reduced-oxidized difference spectrum, 560.5 nm [14]). Following flash excitation in the presence of antimycin, cytochrome b_{50} is reduced at a rate and to an extent dependent on redox potential [29,30]. In the absence of antimycin, the apparent extent of flash-induced cytochrome b_{50} reduction is greatly diminished, and at lower redox

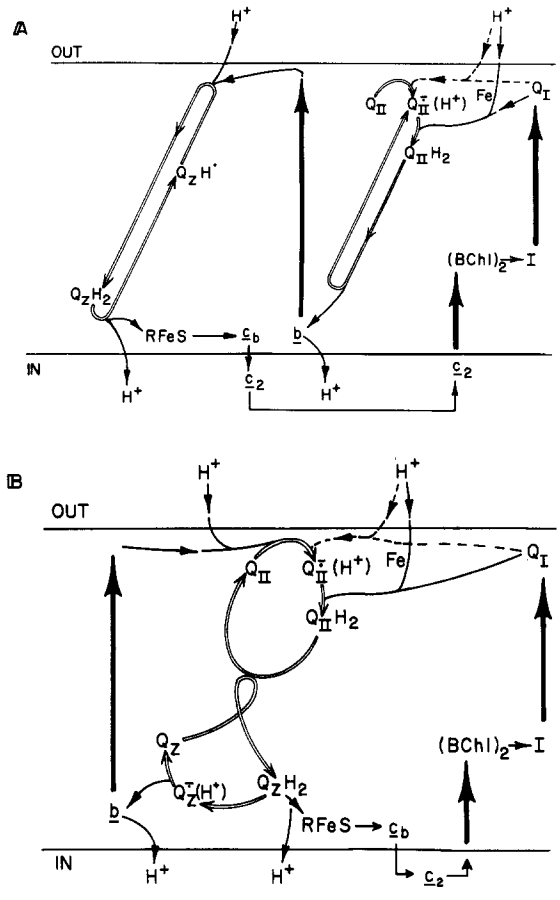

Fig. 1. Schemes for the cyclic electron transfer mechanism in *Rps. capsulata* and *Rps. sphaeroides*. OUT and IN refer to the aqueous phases on the outside and inside of the chromatophore membrane, respectively. The thick arrows represent transmembrane electron transfer reactions. The double-line arrows represent ubiquinone redox reactions outside the reaction center complex. Single arrows represent other electron, hydrogen, or proton transfer processes. The proton release steps are those thought to occur at pH 7.0. $Q_I^{\cdot-}$ is able to reduce both Q_{II} (- - - →) and $Q_{II}^{\cdot-}$ (H^+) (——→), depending on the redox state of Q_{II} before flash excitation. (A) Linear model; (B) Q-cycle model.

potentials, the cytochrome is rapidly and completely re-oxidized [23,31]. The mechanism of cytochrome b_{50} reduction and oxidation is not yet understood. Crofts and co-workers have favored a linear scheme (eg., see Ref. 23) (Fig. 1A) in which cytochrome b_{50} is reduced by electrons delivered from the reaction center, with no coupling to redox events at the oxidizing end of the chain. In this model, the ubisemiquinone $Q_zH^{\cdot}$ generated during oxidation of Q_zH_2 acts as an oxidant for ferrocytochrome b_{50} in an antimycin-sensitive reaction involving transmembrane charge transfer. Other workers have favored a Q-cycle scheme (Fig. 1B) in which cytochrome b_{50} reduction is obligatorily linked to re-reduction of cytochrome *c* [28,32,33]. It is proposed that ubisemiquinone $Q_z^{\cdot-}$ (H^+) generated during oxidation of Q_zH_2 reduces ferricytochrome b_{50} in an antimycin-insensitive process.

We present here studies using inhibitors of cytochrome *c* re-reduction and $Q_I^{\cdot-}$ re-oxidation which reveal effects not previously considered, and suggest that neither scheme accounts adequately for the observations.

Materials and Methods

Rps. sphaeroides Ga and *Rps. capsulata* strains N22 and Ala pho$^+$ were grown and chromatophores were prepared as described previously [3]. *Rps. capsulata* R126, a strain unable to grow photosynthetically but which possesses active reaction centers, and *Rps. capsulata* MR126, a photosynthetically competent strain derived from strain R126 by treatment with gene transfer agents, were grown in 1% oxygen [34]. Cells grown in this way were stored in 20 mM Mops/100 mM KCl/10% glycerol, pH 7.0 at −70°C, shipped in dry ice, and chromatophores were prepared by the usual procedure. *Rps. capsulata* R126 and MR126 were provided by Dr. B. Marrs et al. [34].

Detergent-solubilized reaction centers from *Rps. sphaeroides* R26 were provided by Dr. R.E. Overfield. UHDBT and 6-(*n*-dodecyl)amino-5,8-quinoline quinone were gifts from Prof. Karl Folkers, piericidin A was a gift from Dr. C.J. Coles, and ametryne was from CIBA-GEIGY and was kindly made available to us by Dr. C.J. Arntzen. All inhibitors were added as solutions in ethanol.

Redox poising of samples in a stirred anaerobic redox cuvette for kinetic measurements was carried out as described previously [3]. Valinomycin was routinely added to prevent the build up of a membrane potential during flash excitation, and to diminish electrochromic absorption changes. The computer-linked single beam kinetic spectrophotometer equip-

ped with a shutter over the measuring beam is described elsewhere [14,35]. All measurements were performed at room temperature. Flash-excitation was provided by a Phase-R DL-1200 dye laser, by a General Radio Stroboslave xenon flash lamp, or by a home-made xenon flash lamp using a flash tube made to design by T.W. Wingent Ltd., Cambridge, U.K., with respective durations at half-maximal intensity of 300 ns, approx. 5 μs and 24 μs. Chromatophore concentration was adjusted to give a flash saturation of approx. 90% in each case.

Extinction coefficients used to estimate cytochrome *c* and $(BChl)_2^+$ were 19.0 $mM^{-1} \cdot cm^{-1}$ at 550–540 nm and 10.3 $mM^{-1} \cdot cm^{-1}$ at 540 nm, respectively [16] after normalisation to appropriate values for the wavelengths used, 551–542 nm for cytochrome *c* and 542 nm for $(BChl)_2^+$. No reliable extinction coefficient for cytochrome *b* is available; a value of 13.2 $mM^{-1} \cdot cm^{-1}$ at 560–570 nm was used [3].

Results and Discussion

Effect of UHDBT on light-induced cytochrome b reduction. Fig. 2 shows spectra of the absorption changes in *Rps. sphaeroides* Ga induced by two excitations, spaced at 20 ms, in the presence of antimycin, before and after addition of UHDBT. In the absence of UHDBT, the spectra, after correction for the reaction center change, indicate contributions from cytochrome *c* and cytochrome *b* (Fig. 2A). After further correction for the cytochrome *c* change (not shown), the residual spectrum after the first flash resembles that normally attributed to cytochrome b_{50}, although small contributions from other components are apparent as a broadening of the spectrum and a shoulder at 566 nm [14]. The spectrum of the change induced by the second flash resembles that of cytochrome b_{-90} ($E_{m(7.0)} \approx -90$ mV; split α band with λ_{max} at 558.5 and 566 nm in the reduced-oxidized difference spectrum [14]), providing evidence that the low potential cytochrome b_{-90} is available to accept electrons from the photosynthetic system on a millisecond time scale. By appropriate choice of measuring wavelength, and by subtraction of absorbance changes due to known components, kinetic changes attributable to cytochrome b_{-90} can be resolved on both the first and second flashes. These show a half-time for reduction in the presence of antimycin at

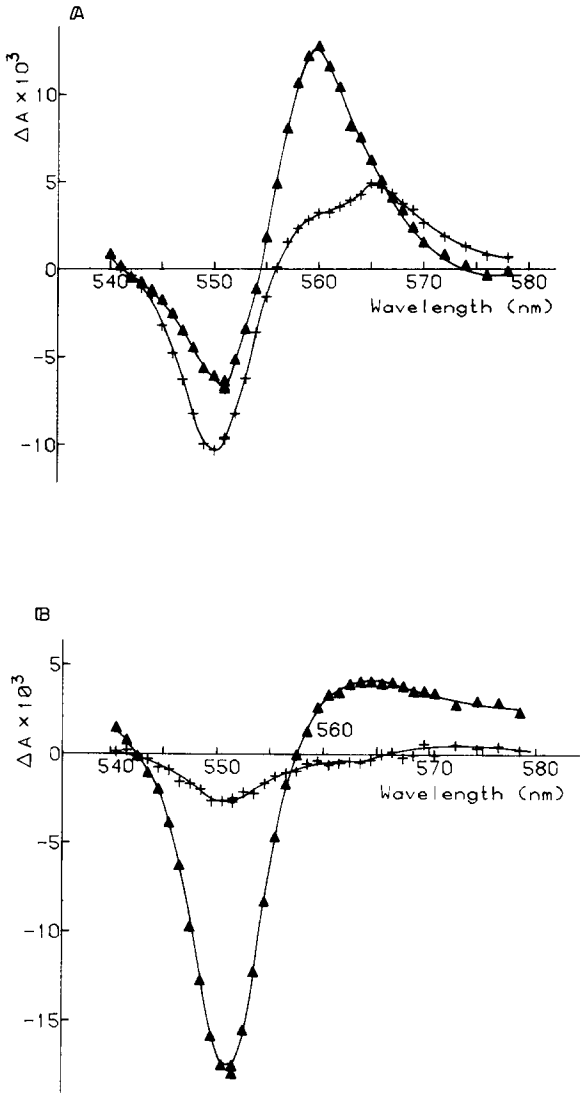

Fig. 2. Light-minus-dark differences spectra of cytochrome changes in *Rps. sphaeroides* Ga chromatophores in the presence and absence of UHDBT. (A) Chromatophores were suspended to 1.2 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing 10 μM each of DAD, 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone; 1 μM valinomycin and 4 μM antimycin at E_h 190 ± 5 mV. At each wavelenth, chromatophores were subjected to two 24-μs xenon flashes with 20 ms between each flash. Signals were not averaged. 33 s elapsed between each measurement. (▲) The change 13 ms after the first flash; (+) The change 5 ms after the second flash, using the point 17.5 ms after the first flash as the baseline. The contribution of the reaction center change was removed by subtraction of the appropriately normalised change recorded at 603 nm. (B) As in A but with 25 μM UHDBT added.

$E_{h(7.0)} \approx 50$ mV of ≈400 μs [36]. Similar changes have been measured in chromatophores from *Rps. capsulata*.

The spectra recorded in the presence of UHDBT show the inhibition of cytochrome *c* re-reduction following flash one (as indicated by an increased level of oxidation) and the consequent diminution of cytochrome *c* oxidation on the second flash (see also Fig. 11C, G). They also show that cytochrome *b* reduction is fully inhibited on both flashes (see also Fig. 11D, H). Equilibrium redox titrations indicated that UHDBT does not have a marked effect on the E_m values of the cytochrome *b* complement (Meinhardt, S.W. and Crofts, A.R., unpublished results). UHDBT also inhibited cytochrome b_{50} reduction under similar conditions in *Rps. capsulata*.

The concentration dependencies of the inhibition by UHDBT of the reduction of cytochromes *c* and *b* following a single flash in the presence of antimycin are very similar (Fig. 3). About 2.7 mol UHDBT/mol reaction center were required to inhibit the extents of both reactions by 50%, and about 20 mol/mol reaction center were required to completely inhibit both processes. UHDBT also inhibited cytochrome *b* reduction in the absence of antimycin [37]. UHDBT became less effective at inhibiting cytochrome b_{50} reduction in *Rps. sphaeroides* at $E_{h(7.0)}$ greater than approx. 380 mV (i.e., at the potential range over which the Rieske Fe-S center becomes oxidized in the presence of UHDBT [11,19]).

Effect of UHDBT on electron transfer from the reaction center. A possible way in which UDHBT could inhibit cytochrome *b* reduction might be by blocking electron transfer between the reaction center primary and secondary acceptor quinones, as is the case in *Chromatium vinosum* [38,39]. Inhibition of electron transfer from Q_I^- to Q_{II} can be demonstrated in two ways: (a) if the primary acceptor remains reduced following a flash excitation, but the reaction center is rapidly re-reduced by cytochrome *c*, turnover on a second flash is prevented; (b) if cytochrome *c* is oxidized before flash-excitation (high redox potential), the photo-oxidized reaction center is re-reduced slowly (halftime of several seconds). If electron transfer from Q_I^- to Q_{II} is inhibited, $(BChl)_2^+$ is re-reduced with a halftime of approx. 60 ms by a back reaction from Q_I^- [40]. UHDBT elicited neither of these effects (results not shown).

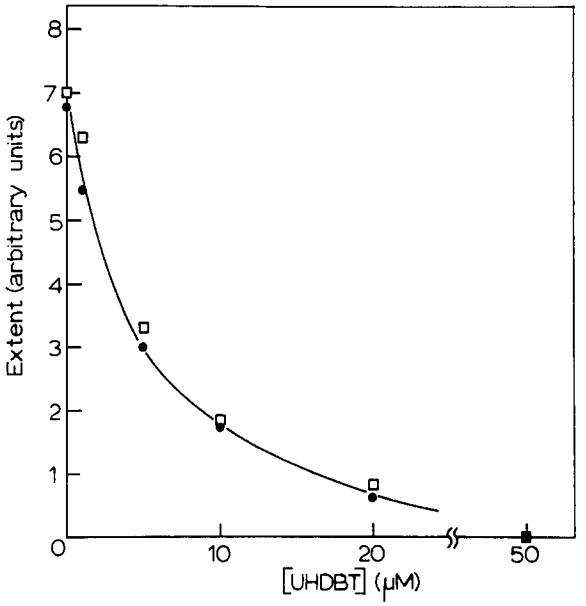

Fig. 3. Effects of UHDBT on cyclic electron transport. (•) Antimycin-insensitve cytochrome *c* re-reduction. Chromatophores of *Rps. capsulata* Ala pho$^+$ (1.5 μM reaction center) were suspended in 50 mM Mops/100 mM KCl, pH 7.0 in the presence of 2 μM valinomycin and 5 μM antimycin in a stirred anaerobic cuvette. No redox mediators were present, but the rate of cytochrome *c* re-reduction before addition of antimycin indicated an E_h of about 100 mV. UHDBT was added to increasing concentration. Measurements were made at least 5 min after each addition of UHDBT to allow time for removal of any oxygen dissolved in the ethanolic UHDBT solution. Chromatophores were subjected to single 24-μs xenon flash excitations. The trace of the excitation-induced change recorded at 551–543 nm at each UHDBT concentration was subtracted from the trace obtained at 551–543 nm with 50 μM UHDBT present. No further increases in the extent of cytochrome *c* oxidation occurred at UHDBT concentrations in excess of 50 μM. The points show the extent of the UHDBT-sensitive change 18 ms after the excitation. The traces were an average of four, and the instrument response time was 20 μs. (□) Cytochrome b_{50} reduction in the presence of antimycin. The conditions were as in (•). The points show the extent of the single flash-excitation-induced change at 560–570 nm recorded 18 ms after the flash, at each UHDBT concentration.

In experiments with detergent-solubilized reaction centers partially depleted of secondary acceptor quinone Q_{II}, addition of excess ubiquinone-10 slows the rate of $(BChl)_2^+$ re-reduction by acting as a sink for electrons which would otherwise back-react from Q_I^- to $(BChl)_2^+$. UHDBT could not replace ubiquinone

in this reaction, neither did it prevent ubiquinone from slowing the back reaction. In addition, UHDBT had no effect on the binary oscillation in semiquinone anion formation in chromatophores [3,7,8], attributed to the two-electron gate function of Q_{II} (results not shown). It therefore seems clear that UHDBT does not inhibit cytochrome *b* reduction by an inhibition of electron transfer from Q_I^-, but in some way disrupts electron transfer, direct or otherwise, between Q_{II} and cytochrome *b*.

The quinone analogue piericidin A also inhibited cytochrome *c* reduction and cytochrome *b* reduction in the presence of antimycin, but much higher concentrations (approx. 400 μM) were required for full inhibition. 6-(*n*-Dodecyl)amino-5,8-quinoline quinone, a potent inhibitor of Q_I^- to Q_{II} electron transfer in *C. vinosum* [38,39], had no effect on cytochrome *c* re-reduction, cytochrome *b* reduction, or Q_I^- oxidation by Q_{II} in *Rps. sphaeroides* and *Rps. capsulata*. A similar derivative, 6-(*n*-pentadecyl)amino-5,8-quinoline quinone was previously shown to be a poor inhibitor of electron transfer in the cytochrome *b*-c_1 segment of yeast mitochondria, whereas UHDBT was very effective [41].

Effect of UHDBT on photoredox reactions in Rps. capsulata R126. Rps. capsulata R126 shows no light-induced cytochrome *b* reduction and lacks the antimycin-sensitive slow phase of cytochrome *c* reduction (Ref. 34, and Prince, R.C., Dutton, P.L., Zannoni, D. and Marrs, B., unpublished observations). The electron transfer system in this mutant was also totally insensitive to UHDBT, and comparison of the extents of ferrocytochrome *c* oxidation and $(BChl)_2^+$ reduction indicated that the phase of cytochrome *c* re-reduction attributed to Rieske Fe-S center oxidation is missing in the mutant. The Rieske Fe-S center is, however, present in its membranes (Dutton, P.L., Prince, R.C. and Marrs, B., personal communication).

Rps. capsulata R126 showed the UHDBT-insensitive oscillation in semiquinone anion formation with flash number (result not shown) and its cytochrome *b* complement appears to be similar to that of a photosynthetically competent strain (*Rps. capsulata* MR126), derived by treatment of R126 with gene transfer agents, and grown under similar conditions (Meinhardt, S.W., Zannoni, D., Prince, R.C., Marrs, B. and Dutton, P.L., unpublished observations). The behavior of the R126 mutant therefore appeared to be very similar to that of UHDBT-treated wild-type chromatophores.

Interpretation of the effect of UHDBT. The inhibition of both cytochrome *c* and cytochrome *b* reduction by UHDBT, with a similar titration for inhibition of the two processes, suggests that they may be mechanistically linked as in the Q-cycle mechanism. However, a large molar excess of UHDBT is required and even if inhibition of both processes required addition of only one UHDBT molecule per electron transfer chain, it may have diverse effects on independent reactions arising from conformational changes

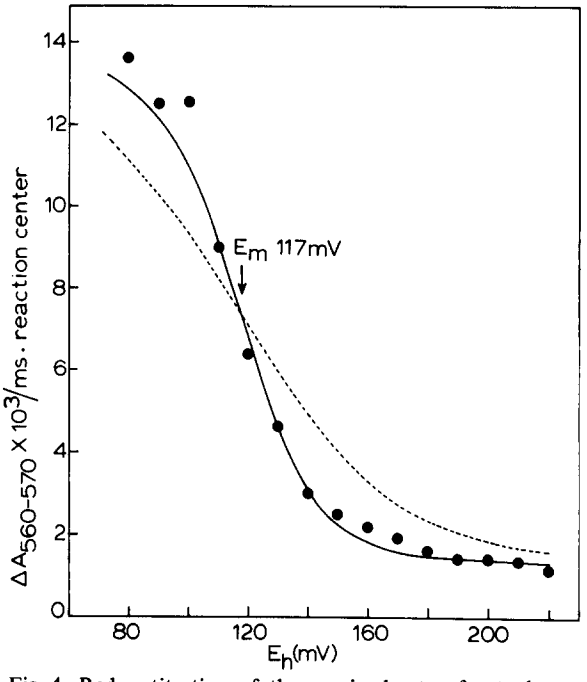

Fig. 4. Redox titration of the maximal rate of cytochrome b_{50} reduction in the presence of antimycin. *Rps. sphaeroides* chromatophores were suspended to 0.43 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing 3 μM PMS and PES, 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone and DAD, 1 μM PYO, 2 μM TMPD, 2 μM antimycin, and 2 μM valinomycin. Chromatophores were subjected to a single 5-μs xenon flash. The points show the fastest rate of cytochrome b_{50} reduction measured at 560–570 nm. Traces were an average of four. The maximal rate for E_h values below 130 mV was normalised to account for the decreased extent of cytochrome b_{50} reduction. Superimposed are theoretical Nernst curves for two (———) and one (- - - - -) electron carriers, with an E_m value of 117 mV.

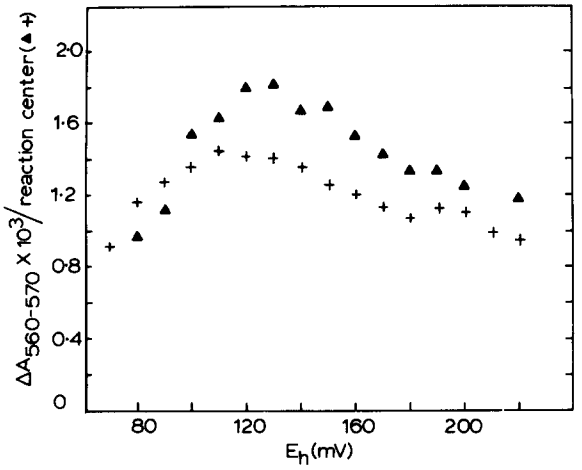

Fig. 5. Redox titrations of the maximal extents of cytochrome b_{50} reduction in the presence of antimycin. (▲) Data for *Rps. sphaeroides* Ga from the experiment of Fig. 4; (+) Data for *Rps. capsulata* N22. *Rps. capsulata* N22 chromatophores were suspended to 0.4 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing 3 μM PMS and PES; 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinine, 2-hydroxy-1,4-naphthoquinone and DAD; 2 μM antimycin, 4 μM valinomycin and 80 μg/ml gramicidin. Chromatophores were subjected to a single laser flash. Traces for the titration were an average of two or four and the instrument response time was 5–50 μs depending on the sweep time used.

induced in a multicomponent complex, as appears to be the case for antimycin. Similarly, the behavior of the photosynthetic mutant *Rps. capsulata* R126, in which both cytochrome *c* and cytochrome *b* reduction are blocked, cannot be taken as definitive evidence for an obligatory linkage, since it is not yet known what component or components are affected. On the other hand, the dual effect of UHDBT cannot be easily explained by a linear scheme for electron transfer unless two distinct sites of action are proposed. Such an explanation does not readily account for the pattern of electron transfer in strain R126.

Kinetics of cytochrome b_{50} reduction in the presence of antimycin. It has previously been reported that in *Rps. capsulata* chromatophores, the rate of cytochrome b_{50} reduction following laser excitation in the presence of antimycin accelerates as the $E_{h(7.0)}$ is lowered from 130 to 80 mV, and that the extent of cytochrome b_{50} reduction doubles between $E_{h(7.0)}$ 220 and 130 mV [30].

Fig. 4 shows a redox titration of the maximal rate of cytochrome b_{50} reduction following flash excitation in the presence of antimycin in *Rps. sphaeroides* Ga chromatophores. The data fit better to a two-electron Nernst curve than to a one-electron curve, with a midpoint potential for the acceleration of 125 ± 15 mV at pH 7.0. Almost identical results were obtained with *Rps. capsulata* N22.

The extent of cytochrome b_{50} reduction increased by 35% as the $E_{h(7.0)}$ was lowered from 240 to 120 mV, and then subsequently decreased, over a slightly lower potential range in *Rps. capsulata* than in *Rps. sphaeroides* (Fig. 5). The maximal extent of cytochrome b_{50} reduction per reaction center was 20% greater in *Rps. sphaeroides* Ga (about 0.7 mol/mol reaction center). The decrease in extent of cytochrome b_{50} reduction at lower E_h is presumed to reflect equilibrium reduction before excitation. On the assumption that the flash-excitation-induced reduction of cytochrome b_{50} involves a collisional reaction (discussed by Van den Berg et al. [28]), the maximal reduction rates at E_h values lower than that at which the maximal extent was observed were normalised to this maximal extent. The rates at higher E_h values were not normalised, on the assumption that the

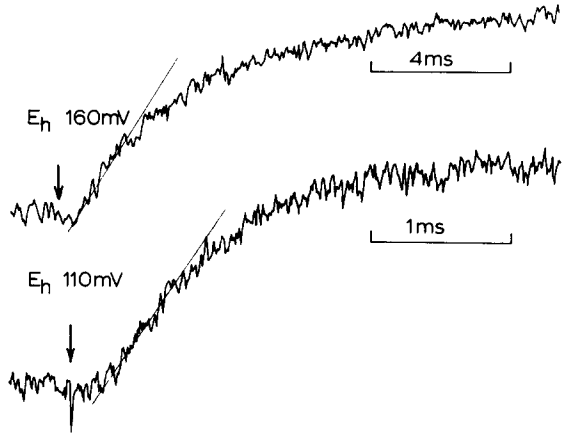

Fig. 6. Reduction kinetics of cytochrome b_{50} following a single flash excitation in the presence of antimycin in *Rps. sphaeroides* Ga chromatophores. The traces are from the experiment in Fig. 4. The trace recorded at E_h 160 mV is an average of four, instrument response time 50 μs, that at E_h 110 mV is an average of four, instrument response time 5 μs. The vertical arrows indicate when the flash occurred. The diagonal lines indicate how the rate of reduction was measured.

decreased extent reflected a characteristic of the reduction mechanism, rather than the availability of ferricytochrome b_{50}.

Fig. 6 shows sample kinetics of the cytochrome b_{50} reduction in the presence of antimycin at $E_{h(7.0)}$ 110 and 160 mV in *Rps. sphaeroides* Ga. Similar results were obtained with *Rps. capsulata* N22. There was a time lag before reduction began; 100–300 μs at $E_{h(7.0)}$ 110 mV and 500–600 μs at $E_{h(7.0)}$ 160 mV. These lag times are much longer than the time for Q_I^- to Q_{II}^- (H^+) transfer in *Rps. capsulata* ($t_{1/2} \approx$ 30 μs at $E_{h(7.0)}$ 390 mV [3]), but are similar to the rate of the UHDBT-sensitive electron transfer from the Rieske Fe-S center to cytochrome *c* [19], and the faster rates of binding of H_{II}^+ (the antimycin-sensitive H^+ binding) [42]. In chromatophores from both species, the maximal rate before normalisation to extent increased about 10-fold as the potential was lowered. Adjustment of the rates at $E_{h(7.0)} < 100$ mV to compensate for the decreased extent leads to an even greater rate increase. After normalisation to the same reaction center concentration, the rate was faster in *Rps. sphaeroides* Ga than in *Rps. capsulata* N22 over the whole potential range.

Semilogarithmic plots of the reduction kinetics indicate that the reaction is first-order after the lag. It is possible that the reaction is rate-limited by a first-order process following the 'collision' between cytochrome b_{50} and its reductant. Halftimes determined from semilogarithmic plots do not include the lag; for example, in *Rps. sphaeroides*, at $E_{h(7.0)}$ 200 mV, the $t_{1/2}$ was 5.2 ms, following a lag of 0.5 ms; at $E_{h(7.0)}$ 100 mV, the $t_{1/2}$ was 0.38 ms, following a lag of approx. 0.24 ms.

At pH 6.0, the acceleration in initial rate of cytochrome b_{50} reduction was less marked (in *Rps. sphaeroides* Ga, the initial rate was $1.31 \cdot 10^{-3}$ $\Delta A_{560-570nm}$/ms. reaction center at $E_{h(6.0)}$ 280 mV, and $7.0 \cdot 10^{-3}$ $\Delta A_{560-570nm}$/ms reaction center (after normalisation) at $E_{h(6.0)}$ 110 mV) and had a midpoint potential in the titration at 180 mV, i.e., 60 mV higher than that at pH 7.0. This suggests that the component involved in the acceleration requires the same number of protons bound as electrons for its equilibrium reduction between pH 6.0 and 7.0.

Redox centers which may affect the rate of cytochrome b_{50} reduction. The apparent *n* value of the titration of the maximal rate of cytochrome b_{50} reduction is the same as that of the Q_zH_2/Q_z component involved in the 1–2 ms phase of cytochrome *c* reduction [24] and that of the ubiquinone pool (19 molecules per reaction center with $E_{m(7.0)}$ 90 mV in *Rps. sphaeroides* Ga [43]). A Q-cycle mechanism, in which Q_z^- (H^+) was the reductant for cytochrome b_{50} (Fig. 1B), would provide a simple explanation for the acceleration. With Q_z reduced to Q_zH_2 before excitation, the rate of generation of Q_z^- (H^+) (by re-reduction of the photo-oxidized Rieske center) would be increased. With Q_z oxidized before excitation, the formation of Q_z^- (H^+) would presumably be limited by the rate at which electrons from the reaction center could reduce Q_z to Q_zH_2, with the redox equilibrium between Q_z and its reductant limiting the extent of cytochrome b_{50} reduction. However, as pointed out by Crofts et al. [23], and more recently by Van den Berg et al. [28], the kinetics and extent of cytochrome b_{50} reduction and cytochrome *c* re-reduction in the presence of antimycin are not compatible with a Q-cycle mechanism if antimycin simply acts to prevent cytochrome b_{50} re-oxidation. The Q-cycle mechanism of Fig. 1B indicates that the kinetics of reduction of cytochrome b_{50} in the presence of antimycin should reflect the kinetics of oxidation of Q_zH_2. The kinetics of oxidation of Q_zH_2 have been measured as the slower phase of cytochrome *c* reduction. In the absence of antimycin, this rate (minimum $t_{1/2}$ 1–2 ms) is considerably slower than the fastest rate of cytochrome b_{50} reduction ($t_{1/2} < 0.5$ ms) due mainly to the different lag times involved. In the presence of antimycin, the rate is of course much slower (>200 ms). The fastest rate of cytochrome b_{50} reduction in the presence of antimycin is similar to the apparent rate of the antimycin-insensitive rate of cytochrome *c* reduction. However, this phase is attributed to electron donation from the Rieske iron sulfur center, and there is as yet no convincing evidence that Q_zH_2 is involved, at least when all the reaction centers are activated by the flash. In addition, the apparent midpoint potential for the acceleration (approx. 125 ± 15 mV at pH 7.0) is somewhat lower than that of Q_z/Q_zH_2 ($E_{m(7.0)}$ 155 mV).

It seems likely that the reduction of cytochrome b_{50} in the presence of antimycin is mediated by electrons delivered directly from the reaction center quinone acceptor complex (probably from $Q_{II}H_2$ or by way of a component of equivalent stoichimetry) and

that any dependence on the redox state of Q_z is indirect. Removal of the ubiquinone pool and Q_z (as indicated by the loss of the 1–2 ms antimycin-sensitive phase of cytochrome *c* reduction) does not prevent flash-excitation induced cytochrome b_{50} reduction [27,44], but the detailed redox potential dependency of the rate and extent of cytochrome b_{50} reduction in these depleted chromatophores is not yet known. It is possible that the ubiquinone pool (or a fraction of the pool close to the reaction center quinone) may compete with cytochrome b_{50} as an electron acceptor from $Q_{II}H_2$, and would be unavailable as it became reduced before excitation. This would require that the pool molecules could interact with $Q_{II}H_2$ on a millisecond time scale, and it would become necessary to explain how any cytochrome b_{50} reduction is observed, in view of the lower midpoint potential of this component.

Another redox component which may be involved in the increased rate and extent of cytochrome b_{50} reduction is the Q_{II}/Q_{II}^{-} (H^+) couple. The data of Rutherford and Evans [4] suggest that $E_{m(7.0)}$ for Q_{II}/Q_{II}^{-} (H^+) is 100 mV, and that of Q_{II}^{-} $(H^+)/Q_{II}$ H_2 is 20 mV. Reduction of Q_{II} to Q_{II}^{-} (H^+) before excitation would mean that Q_{II} H_2 rather than Q_{II}^{-} (H^+) is generated following the flash and this would be a stronger reductant. However, computer simulation and other data presented later indicate that the redox state of Q_{II} under the conditions of these experiments is not a reflection of the equilibrium midpoint potentials of the Q_{II}/Q_{II}^{-} (H^+) and Q_{II}^{-} $(H^+)/Q_{II}$ H_2 couples. In addition, if Q_{II}/Q_{II}^{-} (H^+) could act as a reductant for cytochrome b_{50}, it would be re-oxidized at $E_{h(7.0)} > 200$ mV as cytochrome b_{50} became re-oxidized, and would then not act as a metastable intermediate in a two-electron gate mechanism, as is observed at $E_{h(7.0)} > 300$ mV. Furthermore, a dependency on the redox state of the Q_{II}/Q_{II}^{-} (H^+) couple before flash excitation would lead to an $n = 1$ redox potential dependency rather than $n = 2$. A resolution of this problem will require knowledge of the redox state of Q_{II} before excitation in these experiments, and of the reaction mechanism leading to cytochrome b_{50} reduction. The possible involvement of cytochrome b_{-90} in the reactions of the secondary acceptor pools will be discussed extensively elsewhere [36].

Inhibition of electron transfer from Q_I^{-} using ametryne. Ametryne (2-methylymercapto-4-ethylamino-6-isopropylamino-1,3,5-triazine) is a herbicide which appears to inhibit electron transfer from the reduced primary acceptor quinone to the oxidized secondary acceptor of the Photosystem II reaction center in chloroplasts [45,46] and in detergent-solubilized reaction centers from *Rps. sphaeroides* R26 (Stein, R.R. and Wraight, C.A., personal communication). The upper set of traces in Fig. 7 shows that ametryne had this effect in *Rps. capsulata* N22 chromatophores, as indicated by the acceleration in the re-reduction of $(BChl)_2^+$ when cytochrome *c* was largely oxidized before flash excitation. At this high redox potential ($E_{h(7.0)}$ 390 mV), cytochrome b_{50} reduction was markedly inhibited by ametryne. The lower set of traces in Fig. 7 shows the cytochrome b_{50} change in the presence of antimycin, recorded following a flash excitation given 8 s after the first flash from the dark-adapted state (sufficient time for complete $(BChl)_2^+$ re-reduction). In the absence of ametryne, the extent of cytochrome *b* reduction on the second flash is normally greater than that on the first, probably owing to the operation of the gating mechanism in the reaction center quinone acceptor

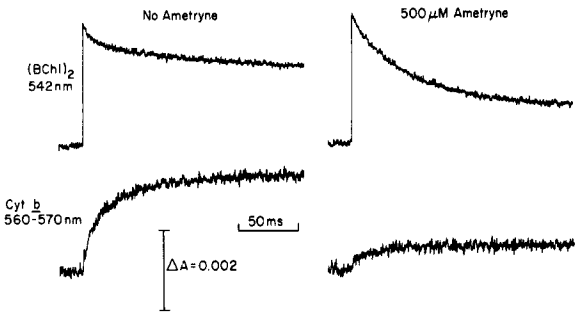

Fig. 7. Effects of ametryne on flash-induced cytochrome b_{50} reduction and on $(BChl)_2^+$ re-reduction at high E_h. *Rps capsulata* N22 chromatophores were suspended to 0.71 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing a total of 1.5 mM potassium ferrocyanide/ferricyanide, 10 μM DAD, 2 μM antimycin, 2 μM valinomycin and 1 μM nigericin, at E_h 390 mV. Chromatophores were subjected to two 24-μs xenon flashes with 8 s between flashes (long enough for $(BChl)_2^+$ re-reduction and cytochrome b_{50} re-oxidation) following at least 3 min dark-adaptation. The top traces show the effect of 500 μM ametryne on $(BChl)_2^+$ re-reduction following the first flash. The lower two traces show the effect of ametryne on cytochrome b_{50} reduction on the second flash. The traces were not averaged, and the instrument response was 100 μs.

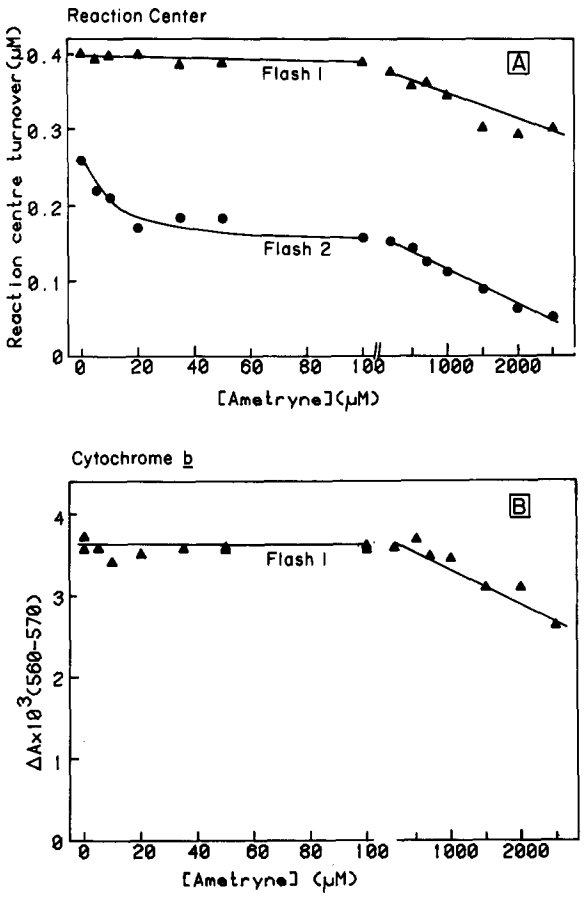

Fig. 8. Concentration dependencies of the effects of ametryne (A) on reaction center turnover on two closely spaced flash excitations, and (B) on cytochrome b_{50} reduction following a single flash. (A) *Rps. capsulata* N22 chromatophores were suspended to 0.4 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing 3 μM each of PMS and PES; 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, and DAD; 2 μM antimycin, 25 μM UHDBT and 1 μM valinomycin at E_h 105 mV. Chromatophores were subjected to a laser flash (flash 1) and a 24-μs xenon flash (flash 2) 5.3 ms later. The points show the maximal extent of the reaction center turnover on flash 1 (▲) and on flash 2 (●). Reaction center turnover was obtained by summing, after appropriate normalisation, the extent of stable reaction center oxidation (measured at 542 nm) and cytochrome *c* oxidation (measured at 551–542 nm) (in the presence of UHDBT to block rapid re-reduction) 0.8 ms after each of the two flashes. (B) *Rps. capsulata* N22 chromatophores were suspended as in (A), except that UHDBT was not added. Chromatophores were subjected to a single 24-μs xenon flash. The points show the maximal extent of cytochrome b_{50} reduction in the presence of antimycin, monitored at 560–570 nm.

complex [3]. In the presence of ametryne, a major fraction of the electrons on Q_I^- after the first flash returned to $(BChl)_2^+$ by back reaction (see above), so that in most centers Q_{II} remained oxidized after the first flash.

At $E_{h(7.0)}$ 100 mV, when the rate of cytochrome b_{50} reduction is close to its maximum, a different pattern is observed. Fig. 8A shows the effect of increasing ametryne concentration on the extent of reaction center turnover on each of two excitations spaced 5.3 ms apart. Reaction center turnover was estimated by adding the extent of cytochrome *c* oxidized to the amount of reaction center remaining stably oxidized, in the presence of UHDBT. Fig. 8A shows that in 35–40% of the reaction centers, Q_I^- re-oxidation was inhibited by relatively low concentrations (<100 μM) of ametryne, as indicated by the diminution in turnover on the second flash. At higher concentrations, the extent of the remaining reaction center turnover on the second flash diminished with a dependency very similar to that on the first. The reaction center turnover on the second flash in the absence of ametryne was lower than that on the first due to incomplete $(BChl)_2^+$ re-reduction following the first flash. Experiments performed in the absence of UHDBT but in the presence of antimycin, using the fast phase of the carotenoid spectral response to monitor reaction center turnover, gave essentially the same result.

Fig. 8B shows that the maximal extent of cytochrome b_{50} reduction following a single 24 μs xenon flash, recorded in the presence of antimycin, showed no decrease at the low concentrations of ametryne which partially inhibited Q_I^- re-oxidation. These low concentrations (<100 μM) of ametryne had no significant effect on the kinetics of cytochrome b_{50} reduction. Higher concentrations of ametryne diminished the extent of cytochrome *b* reduction roughly in proportion to the inhibition of reaction center turnover on the first flash seen in Fig. 8A. Control experiments performed at a few selected ametryne concentrations indicated that the use of different flash durations in these experiments was not a significant variable.

Interpretation of the effects of ametryne. The results suggest that at $E_{h(7.0)}$ 100 mV, the reaction center acceptor complexes are heterogeneous in the sensitivity to ametryne of primary to secondary qui-

non electron transfer, and furthermore, that cytochrome b_{50} reduction is considerably more sensitive to ametryne at $E_{h(7.0)}$ 390 mV than at 100 mV. A similar diminution in the inhibition of cytochrome b_{50} reduction with decreased redox potential is seen with *o*-phenanthroline (not shown). Evidence is now accumulating that *o*-phenanthroline inhibits electron transfer from Q_I^- to Q_{II}, but not from Q_I^- to Q_{II}^-, both in reaction centers and in situ [8,47]. Preliminary experiments (Stein, R.R. and Wraight, C.A., personal communication) indicate that in reaction centers, ametryne has a similar effect. The equilibrium redox titration data of Rutherford and Evans [4] suggest that in the dark state, Q_{II} would be fully oxidized at $E_{h(7.0)}$ 390 mV, and 50% reduced to the Q_{II}^- (H^+) state at $E_{h(7.0)}$ 100 mV. At $E_{h(7.0)}$ 390 mV, it appears that cytochrome b_{50} is reduced by $Q_{II}H_2$ which is generated following two excitations [3]. On the first excitation in the presence of ametryne at $E_{h(7.0)}$ 390 mV, Q_I^- would be formed, but back-reacts with $(BChl)_2^+$, and this process would be repeated on the second excitation, so that no cytochrome b_{50} reduction occurs. At $E_{h(7.0)}$ 100 mV, about 50% of the centers would be in the Q_{II}^- (H^+) state before excitation, and would thus be converted to $Q_{II}H_2$ following the flash, and reduce cytochrome b_{50}. If ametryne behaved in chromatophores in the same way as *o*-phenanthroline, this process would be unaffected by ametryne. Centers in the Q_{II} state (fully oxidized) before excitation would not contribute to b_{50} reduction on the first flash, but electron transfer from Q_I^- to Q_{II} would be inhibited by ametryne and prevent turnover in these centers on the second flash.

The situation is more complex, however. At the concentrations of redox mediators used in millisecond kinetic experiments, Q_{II} appears to become at least partially reduced to the semiquinone form at considerably higher potentials than would be predicted from equilibrium potentiometric data. Thus, the binary oscillation in cytochrome b_{50} reduction and semiquinone anion formation disappears as the $E_{h(7.0)}$ is lowered below 300 mV in *Rps. capsulata* Ala pho$^+$, and at $E_{h(7.0)}$ 210 mV cytochrome b_{50} reduction was considerably less sensitive to ametryne than at E_h 390 mV. Both observations may be attributed to the presence of a significant amount of Q_{II}^- (H^+) before excitation. The semiquinone oscillation has been reported at considerably lower potentials in the presence of concentrations of redox-mediating dyes (50 μM TMPD, approx. 500 μM DAD) [7,8] much greater than those normally used in kinetic studies (Rutherford, A.W., personal communication), and it was shown that the oscillation in the extent of cytochrome b_{50} reduction may be observed at considerably lower potentials ($E_{h(6.0)}$ 250 mV) than previously using 50 μM TMPD as redox mediator. This suggests that Q_{II}/Q_{II}^- (H^+) does not readily come to equilibrium with the levels (<10 μM) of redox-mediating dyes normally used in kinetic experiments.

An alternative explanation based on a Q-cycle mechanism may also be suggested for the decreased efficiency of ametryne in inhibiting cytochrome b_{50} reduction at $E_{h(7.0)}$ 100 mV. At this potential, Q_z would be reduced before excitation and would be expected to reduce cytochrome b_{50} in a reaction linked to cytochrome *c* re-reduction. This reaction would not be affected by an inhibition by ametryne of Q_I^- oxidation. At $E_{h(7.0)}$ 390 mV, reduction of Q_z to Q_zH_2 would depend on electron delivery from Q_I^- to both Q_{II} and Q_{II}^-, so that cytochrome b_{50} reduction would be fully inhibited. At $E_{h(7.0)}$ 210 mV, Q_zH_2 would only be generated in those centers containing Q_{II}^- before excitation, and these would be uninhibited by ametryne. However, such an explanation suffers from the kinetic difficulties mentioned earlier.

Relationship between cytochrome b_{50} oxidation and reduction and cytochrome c re-reduction

(a) Q_z reduced before excitation.

When Q_z is reduced before flash excitation, the kinetics of the resolved 1–2 ms phase of cytochrome *c* re-reduction, phase III of the carotenoid response to membrane potential formation, and cytochrome b_{50} oxidation, are very similar and are all inhibited by antimycin. They all accelerate as Q_zH_2 titrates [23, 48]. On the basis of these observations, Crofts et al. [23] proposed a linear mechanism (Fig. 1A) in which the unstable oxidant for ferricytochrome b_{50} ($Q_zH^\cdot$) was a product of the one-electron transfer reaction between Q_zH_2 and ferricytochrome *c*. The model predicts that the re-reduction of $Q_zH^\cdot$ by ferrocytochrome b_{50} must occur in order to achieve net cytochrome *c* reduction, owing to the high midpoint potential of $Q_zH^\cdot/Q_zH_2$. Inhibition of either reaction by antimycin would then inhibit both.

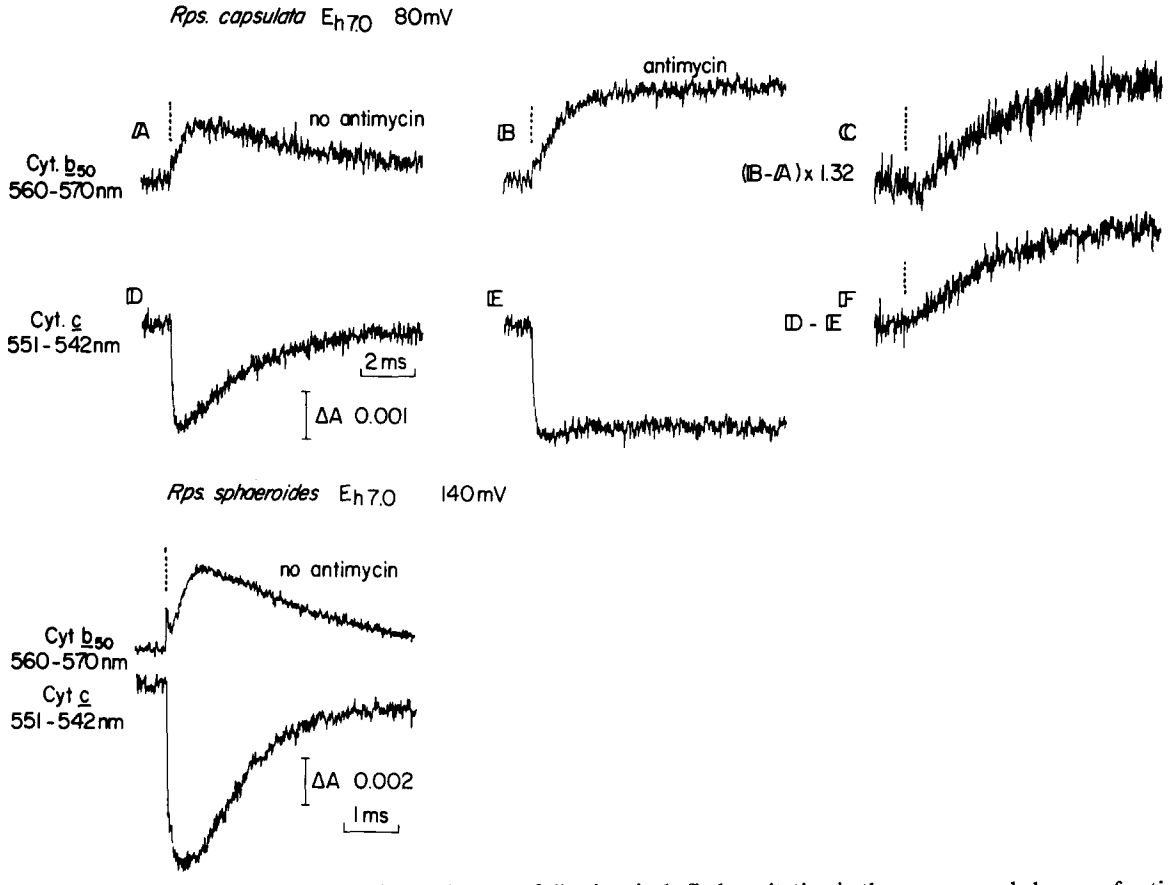

Fig. 9. Kinetics of cytochrome b_{50} and cytochrome c following single flash excitation in the presence and absence of antimycin. *Rps. capsulata* N22. (Traces A–F). Chromatophores were suspended to 0.41 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing 3 μM PMS and PES, 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone and DAD, 100 μM potassium ferrocyanide, 2 μM valinomycin, 1 μM nigericin and 4 μg/ml gramicidin, at E_h 80 mV. Chromatophores were subjected to a 24-μs xenon flash. The traces are an average of 16, and the instrument response time was 10 μs. Antimycin was added to 2 μM (traces B and E). The two traces at the right (C and F) show the antimycin-sensitive phases of the cytochrome c and b_{50} kinetics. They have been normalised to approximately the same extent. The vertical dashed lines indicate when the flash occurred. The initial rapid rise in the changes monitored at 560–570 nm is not attributable to cytochrome *b*. *Rps. sphaeroides* Ga. Chromatophores were suspended to 1.2 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0 containing 2 μM PMS, 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone; 10 μM carbonylcyanide-*p*-trifluoromethoxylphenylhydrazone, 4 μM valinomycin and 1 mM $MgCl_2$ at E_h 140 mV. They were subjected to a single 24-μs xenon flash. Measurements at 560 and 570 nm were an average of 64, those at 551 and 542 nm an average of 16. The instrument response time was 5 μs.

Crofts et al. [23] noted that the transient reduction of cytochrome b_{50} following excitation appeared to be a much faster process than the reoxidation reaction or the re-reduction of cytochrome c. This is seen clearly in the kinetic traces of Fig. 9, and the time-resolved spectra of the light-induced change (Fig. 10). The latter figure shows that the overall redox state of cytochrome c did not change significantly between 0.24 and 0.79 ms after the flash, whereas that of cytochrome b reduced during this time. Correction for the cytochrome c change indicates that the main cytochrome b species becoming reduced ($\lambda_{max} \approx 560$ nm) was cytochrome b_{50}.

The kinetic matching between ferrocytochrome b_{50} oxidation and ferricytochrome c re-reduction was achieved by subtracting the kinetics of the change

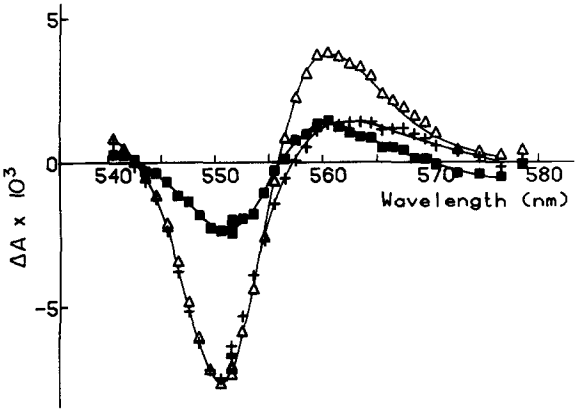

Fig. 10. Time-resolved spectra of single-turnover cytochrome *b* and cytochrome *c* changes in *Rps. sphaeroides* Ga in the absence of antimycin. Chromatophores were suspended to 1.2 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0, containing 2 μM PMS, 10 μM each of 1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone; 10 μM carbonylcyanide-*p*-trifluoromethoxylphenylhydrazone, 4 μM valinomycin and 1 mM $MgCl_2$ at E_h 140 mV. At each wavelength, chromatophores were subjected to a single 24-μs xenon flash-excitation. Kinetic traces were an average of eight, with 4 s between each measurement. The instrument response time was 100 μs. The reaction center contribution was subtracted out using the change at 603 nm as the reaction center reference trace. The spectra show the corrected absorption change induced by the flash after 0.24 ms (+), 0.79 ms (△) and 2.44 ms (■).

with and without antimycin (Refs. 23, 31, and Fig. 9). In *Rps. capsulata* chromatophores, such a kinetic matching occurs over the E_h range that Q_z titrates, and below [49]. In *Rps. sphaeroides* Ga chromatophores, the transient change measured at 560–570 nm in the absence of antimycin appears to have a smaller maximum amplitude than is observed at similar potentials in *Rps. capsulata* (compare Fig. 11B and 9), and net cytochrome *b* photo-oxidation is sometimes observed at potentials greater than would be expected if cytochrome b_{50} was the electron source. The apparent kinetics of the antimycin-sensitive oxidation of cytochrome b_{50} (Fig. 11F) can then be considerably faster than those of the antimycin-sensitive phase of cytochrome *c* reduction (Fig. 11E). Traces A, C, and E in Fig. 11 show that there is a lag of 0.6 ms in the antimycin-sensitive cytochrome *c* re-reduction kinetics, with a $t_{1/2}$ for the subsequent reduction of 0.84 ms leading to an overall $t_{1/2}$ of 1.24 ms. In terms of the linear scheme, the lag would reflect the availability of ferrocytochrome b_{50} as the electron donor to Q_zH^-. In contrast, the apparent halftime for cytochrome *b* oxidation is 0.56 ms following a lag of less than 200 μs, giving an overall halftime of 0.76 ms (Fig. 11F). However, the apparent kinetics of cytochrome *b* oxidation are fairly similar to the overall kinetics of electron flow to cytochrome *c* and reaction center, measured by summing (after normalisation) the difference kinetics for the two components with and without UHDBT (Fig. 11I).

(b) Q_z oxidized before excitation.

The traces in Fig. 12 show that on a 10-ms time scale at $E_{h(7.0)}$ 210 mV, when Q_z is oxidized before excitation, antimycin had a negligible effect on the cytochrome *c* and carotenoid shift phase III changes (and the $(BChl)_2^+$ change, not shown), but had a marked effect on the cytochrome *b* redox change. At $E_{h(7.0)}$ 380 mV, when cytochrome *c* is largely oxidized, antimycin had a negligible effect on $(BChl)_2^+$ re-reduction on a 10-ms time scale on either the first or second flashes following dark-adaptation, but again had a marked effect on the cytochrome b_{50} redox changes (not shown). At both potentials, antimycin appeared to increase the maximal steady extent of cytochrome b_{50} reduction following flash-excitation.

Mechanism of cyclic electron transfer. Although the antimycin-sensitive phases of cytochrome *c* reduction and cytochrome b_{50} oxidation may match kinetically, as predicted by the linear model of Fig. 1A, there is no mechanistic requirement that they should match in terms of the number of electrons involved in the two phases. This is because about 50% of the electrons delivered from Q_zH_2 are used to re-reduce the Rieske Fe-S center oxidized in the unresolved phase of electron transfer to cytochrome *c*, and a few percent are delivered through to $(BChl)_2^+$.

As shown in Fig. 11E and F, a kinetic matching between the apparent antimycin-sensitive phases of cytochrome *b* oxidation and cytochrome *c* reduction is not always observed when Q_z is reduced before excitation in *Rps. sphaeroides* Ga chromatophores. When net photo-oxidation of cytochrome *b* occurs, the 'net' ferricytochrome *b* is re-reduced on a seconds time scale, and on this basis and on the redox potential dependency, Dutton and Jackson [50], and Dutton and Prince [33] suggested that the component

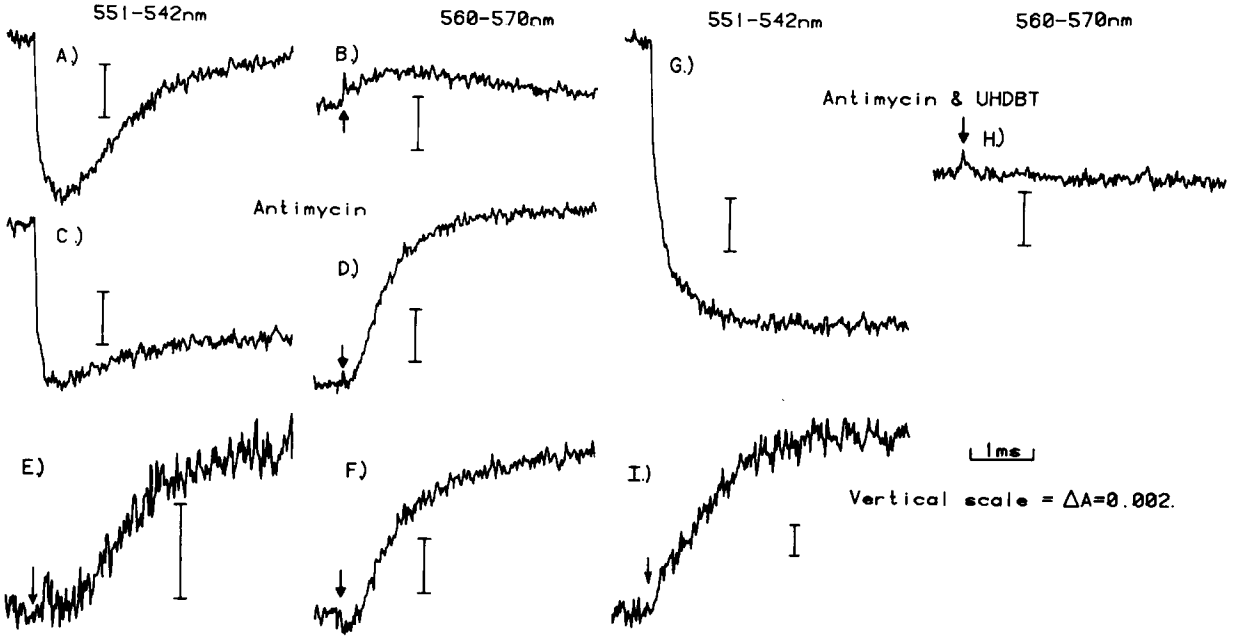

Fig. 11. Fast kinetics of cytochrome *c* (traces A, C, E, and G in columns 1 and 3) measured at 551–542 nm and cytochrome b_{50} (traces B, D, F, and H in columns 2 and 4) measured at 560–570 nm following a single 24-μs xenon flash excitation in *Rps. sphaeroides* Ga chromatophores. Chromatophores were suspended to 0.7 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0, containing 5 μM each of PMS and PES; 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone and 2-hydroxy-1,4-naphthoquinone; 2 μM valinomycin and 1 μM nigericin. Antimycin was added where indicated to 2 μM (C, D, G, H) and UHDBT to 25 μM (G, H). The $E_{h(7.0)}$ was 100 mV. The traces at 551–542 nm are an average of four, those at 560–570 nm an average of eight, and the instrument response time was 10 μs. The time between each measurement was 45 s. The vertical arrows indicate the time at which the xenon flash-excitation occurred. Traces E and F show the difference between the change recorded in the presence and absence of antimycin, normalised to the same extent, and in the same direction, for easier comparison. Trace I was obtained as follows:

(Change at 551–542 nm – Change at 551–542 nm with antimycin and UHDBT)

$$+\left[(\text{Change at 542 nm} - \text{Change at 542 nm with antimycin and UHDBT}) \times \frac{18.1}{10.8}\right]$$

The second term gives the kinetics and extent of the UHDBT (and antimycin)-sensitive phase of $(BChl)_2^+$ re-reduction. The subtraction is reversed to account for the opposite direction of the absorption change on re-reduction of $(BChl)_2^+$ in comparison to cytochrome *c* at the measuring wavelength used. Normalisation by 18.1/10.8 approximately accounts for the difference in extinction coefficients for cytochrome *c* at 551–542 nm and $(BChl)_2^+$ at 542 nm.

involved, proposed to be cytochrome b_{155} ($E_{m(7.0)}$ 155 mV [50]), could be oxidized in an antimycin-sensitive reaction by ferricytochrome *c*, but could not accept electrons rapidly from the reaction center quinone complex. This reaction would diminish the observable extent of the transient cytochrome b_{50} reduction, and would be consistent with the much smaller effect and considerably lower level of cytochrome b_{155} in *Rps. capsulata* chromatophores than in *Rps. sphaeroides* Ga. However, such an explanation could not explain the kinetic discrepancy unless the electrons delivered from cytochrome b_{155} to cytochrome *c* bypassed the rate-limiting reaction between Q_zH_2 and Rieske center, and re-reduced the Rieske center (or cytochrome *c*) at a rate similar to the rate of electron transfer from the Rieske center to cytochrome *c*. Time-resolved spectra of the net cytochrome *b* oxidation in *Rps. sphaeroides* Ga show a greater bandwidth than that of cytochrome b_{50} reduced in the presence of antimycin, and a contribution from

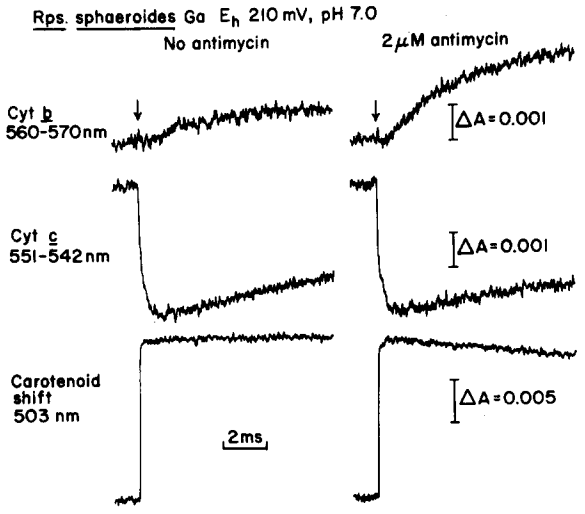

Fig. 12. Effect of antimycin on the kinetics of cyclic electron transfer with Q_z oxidized before flash excitation. *Rps. sphaeroides* Ga chromatophores were suspended to 0.43 μM reaction center in 50 mM Mops/100 mM KCl, pH 7.0, containing 5 μM PMS and PES; 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone and DAD, 2 μM TMPD and 2 μm valinomycin at E_h 210 mV. Antimycin was added where indicated to 2 μM. Chromatophores were subjected to a single 5-μs xenon flash excitation. The traces are an average of four, except those at 503 nm, which were not averaged. The instrument response time was 10 μs. The vertical arrows indicate when the flash occurred.

cytochrome b_{155} could not be ruled out [14]. Clearly, more experiments are required to determine the involvement of cytochrome b_{155} as an electron donor to the Rieske center or cytochrome *c*.

The kinetics of cytochrome b_{50} and cytochrome *c* when Q_z is oxidized before excitation are not readily explained by schemes in which cytochrome b_{50} is reoxidized at all potentials via transmembrane electron transfer leading to carotenoid shift phase III, concomitant with the antimycin-sensitive phase of cytochrome *c* reduction. A possible interpretation of the effect is that antimycin, as well as blocking rapid cytochrome b_{50} re-oxidation associated with transmembrane charge transfer when Q_z is reduced before excitation, also shifts the redox equilibrium between cytochrome b_{50} and its reductant in favor of cytochrome b_{50} reduction. Thus, when the rate of cytochrome b_{50} re-oxidation is intrinsically very slow (Q_z oxidized before excitation), a simple enhancement in the level of cytochrome b_{50} reduction is observed. This may be achieved either by raising the E_m of cytochrome *b*, or by lowering that of its reductant. Current evidence suggests that antimycin does not raise the E_m value of any of the cytochrome *b* species in *Rps. sphaeroides* or *Rps. capsulata* (Ref. 28, and Meinhardt, S.W. and Crofts, A.R., unpublished results) and no firm information is available on the effect of antimycin on ubiquinone redox properties in chromatophores. Ohnishi and Trumpower [51] have, however, observed that antimycin destabilizes a ubisemiquinone species in isolated mitochondrial succinate-cytochrome *c* reductase, and this could arise from a lowering of the E_m value for the $QH^{\cdot}/Q$ couple relative to that of $QH_2/QH^{\cdot}$. Alternatively, when Q_z is oxidized before excitation, it is possible that cytochrome b_{50} is in equilibrium with an optically invisible component, such as the ubiquinone pool, in a reaction which is sensitive to antimycin but which does not involve transmembrane charge transfer. At lower redox potentials, this process would be unable to compete effectively with the considerably faster antimycin-sensitive re-oxidation by $Q_zH^{\cdot}$ associated with transmembrane charge transfer. This would be consistent with the observation that depletion of the Q-pool, but apparently not Q_z, had a marked effect on cytochrome b_{50} oxidation but not on cytochrome *c* re-reduction, under conditions in which the cytochrome *c* re-reduction was slow [44].

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References

1 Dutton, P.L., Prince, R.C. and Tiede, D.M. (1978) Photochem. Photobiol. 28, 939–949

2 Crofts, A.R., Cogdell, R.J. and Jackson, J.B. (1971) in Energy Transduction in Respiration and Photosynthesis (Quagliariello, E., Papa, S. and Rossi, C.S., eds.), 883–901, Adriatica Editrice, Bari, Italy

3 Bowyer, J.R., Tierney, G.V. and Crofts, A.R. (1979) FEBS Lett. 101, 201–206

4 Rutherford, A.W. and Evans, M.C.W. (1980) FEBS Lett. 110, 257–261

5 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525–531

6 Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516–524

7 DeGrooth, B.G., van Grondelle, R., Romijn, J.C. and Pulles, M.P.J. (1978) Biochim. Biophys. Acta 503, 480–490

8 Vermeglio, A., Martinet, T. and Clayton, R.K. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1809–1813

9 Pettigrew, G.W., Meyer, T.E., Bartsch, R.G. and Kamen, M.D. (1976) Biochim. Biophys. Acta 430, 197–208

10 Bartsch, R.G. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 475–495, Antioch Press, Yellow Springs

11 Bowyer, J.R. (1980) Ph.D. thesis, University of Bristol

12 Wood, P.M. (1980) Biochem. J. 192, 761–764

13 Prince, R.C., Baccarini-Melandri, A., Hauska, G.A., Melandri, B.A. and Crofts, A.R. (1975) Biochim. Biophys. Acta 387, 212–217

14 Bowyer, J.R., Meinhardt, S.W., Tierney, G.V. and Crofts, A.R. (1981) Biochim. Biophys. Acta 635, 167–186

15 Wood, P.M. (1980) Biochem. J. 189, 385–391

16 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536–556

17 Prince, R.C. and Dutton, P.L. (1977) Biochim. Biophys. Acta 459, 573–577

18 Trumpower, B.L. and Katki, A.G. (1979) in Membrane Proteins in Energy Transduction (Capaldi, R.A., ed.), pp. 89–200, M. Dekker, New York

19 Bowyer, J.R., Dutton, P.L., Prince, R.C. and Crofts, A.R. (1980) Biochim. Biophys. Acta 592, 445–460

20 Prince, R.C. and Dutton, P.L. (1976) FEBS Lett. 65, 117–119

21 Prince, R.C., Leigh, J.S. and Dutton, P.L. (1974) Biochem. Soc. Trans. 2, 950–953

22 Bowyer, J.R., Tierney, G.V. and Crofts, A.R. (1979) FEBS Lett. 101, 207–212

23 Crofts, A.R., Crowther, D. and Tierney, G.V. (1975) in Electron Transfer Chains and Oxidative Phosphorylation (Quagliariello, E., Papa, S., Palmieri, E., Slater, E.C. and Siliprandi, N., eds.), pp. 233–241, North Holland, Amsterdam

24 Prince, R.C. and Dutton, P.L. (1977) Biochim. Biophys. Acta 462, 731–747

25 Crofts, A.R., Crowther, D., Bowyer, J.R. and Tierney, G.V. (1977) in Structure and Function of Energy-Transducing Membranes (van Dam, K. and van Gelder, B.F., eds.), pp. 139–155, Elsevier/North Holland, Amsterdam

26 Prince, R.C., Bashford, C.L., Takamiya, K-I, van den Berg, W.H. and Dutton, P.L. (1978) J. Biol. Chem. 253, 4137–4142

27 Takamiya, K.I., Prince, R.C. and Dutton, P.L. (1979) J. Biol. Chem. 254, 11 307–11 311

28 Van den Berg, W.H., Prince, R.C., Bashford, C.L., Takamiya, K.I., Bonner, W.D. and Dutton, P.L. (1979) J. Biol. Chem. 254, 8594–8604

29 Petty, K.M. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 346–353

30 Evans, E.H. and Crofts, A.R. (1974) Biochim. Biophys. Acta 357, 89–102

31 Prince, R.C. and Dutton, P.L. (1975) Biochim. Biophys. Acta 387, 609–613

32 Dutton, P.L. and Prince, R.C. (1978) FEBS Lett. 91, 15–20

33 Dutton, P.L. and Prince, R.C. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 525–570, Plenum Press, New York

34 Marrs, B. (1978) Curr. Top. Bioenerg. 8, 261–297

35 Jackson, J.B. and Crofts, A.R. (1971) Eur. J. Biochem. 18, 120–130

36 Crofts, A.R., Meinhardt, S.W. and Bowyer, J.R. (1981) in Function of Quinones in Energy Conserving Systems (Trumpower, B.L., ed.), Academic Press, New York, in the press

37 Bowyer, J.R. and Crofts, A.R. (1978) in Frontiers of Biological Energetics (Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), Vol. I, pp. 326–333, Academic Press, New York

38 Halsey, Y.D. and Parson, W.W. (1974) Biochim. Biophys. Acta 347, 404–416

39 Bowyer, J.R. and Crofts, A.R. (1980) Biochim. Biophys. Acta 591, 298–311

40 Blankenship, R.E. and Parson, W.W. (1979) Biochim. Biophys. Acta 545, 429–444

41 Roberts, H., Choo, W.M., Smith, S.C., Marzuki, S., Linnane, A.W., Porter, T.H. and Folkers, K. (1978) Arch. Biochem. Biophys. 191, 306–315

42 Petty, K.M., Jackson, J.B. and Dutton, P.L. (1977) FEBS Lett. 84, 299–303

43 Takamiya, K.-I. and Dutton, P.L. (1979) Biochim. Biophys. Acta 546, 1–16

44 Bowyer, J.R., Baccarini-Melandri, A., Melandri, B.A. and Crofts, A.R. (1978) Z. Naturforsch. 33c, 704–711

45 Audus, L.J. (1976) in Herbicides, Vol. 1, p. 608, Academic Press, New York

46 Pfister, K. and Arntzen, C.J. (1979) Z. Naturforsch. 34c, 996–1009

47 Wraight, C.A. and Stein, R.R. (1980) FEBS Lett. 113, 73–77

48 Bashford, C.L., Prince, R.C., Takamiya, K.-I. and Dutton, P.L. (1979) Biochim. Biophys. Acta 545, 223–235

49 Crowther, D. (1977) Ph.D. thesis, University of Bristol

50 Dutton, P.L. and Jackson, J.B. (1972) Eur. J. Biochem. 30, 495–510

51 Ohnishi, T. and Trumpower, B.L. (1980) J. Biol. Chem. 255, 3278–3284