Biochimica et Biophysica Acta, 591 (1980) 298—311

BBA 47860

THE PHOTOSYNTHETIC ELECTRON TRANSFER CHAIN OF *CHROMATIUM VINOSUM* CHROMATOPHORES

FLASH-INDUCED CYTOCHROME *b* REDUCTION

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(Received September 19th, 1979)
(Revised manuscript received January 28th, 1980)

Key words: Bacterial photosynthesis; Cytochrome b reduction; Electron transfer; (Chromatium vinosum)

Summary

Reduction of a cytochrome *b* following excitation by a single, short, near-saturating light flash has been demonstrated in *Chromatium vinosum* chromatophores. The extent of reduction is increased by addition of antimycin. The cytochrome has an α-band maximum at 562 nm in the presence of antimycin.

The cytochrome *b* reduction is most readily observed in the presence of antimycin at high redox potential when cytochrome *c*-555 is oxidised before excitation. Under these conditions the half-time for reduction is about 20 ms, and the extent is about 0.5 mol of cytochrome *b* reduced per mol of reaction center oxidised. This extent of reduction is observed on the first flash-excitation from the dark-adapted state, and there was no indication that the reaction center quinone acceptor complex acted as a two-electron accumulating system. With cytochrome *c*-555 reduced before excitation, the extent of cytochrome *b* reduction is approximately halved. The factors which result in substoichiometric cytochrome *b* reduction are not yet understood.

Agents which appear to inhibit primary acceptor oxidation by the secondary acceptor (UHDBT, PHDBT, DDAQQ, HOQNO, *o*-phenanthroline), inhibit

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Abbreviations: DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl benzoquinone; DDAQQ, 6-*n*-dodecylamino-5,8-quinoline quinone; HOQNO, 2-*n*-heptyl-4-hydroxy-quinoline *N*-oxide; Mops, morpholinopropane sulfonic acid; PHDBT, 5-*n*-pentadecyl-6-hydroxy-4,7-dioxobenzothiazole; *P*-870, the primary electron donor of the photochemical reaction center; TTFA, 4,4,4-trifluoro-1-(2-thienoyl)-1,3-butanedione (thenoyltrifluoroacetone); UHDBT, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole.

reduction of the cytochrome *b*. DBMIB inhibits cytochrome *b* reduction but does not appear to inhibit primary acceptor oxidation.

These observations confirm that a cytochrome *b* receives electrons delivered from the primary acceptor complex, and indicate that the photoreduced cytochrome *b* is reoxidised via an antimycin-sensitive pathway.

Introduction

A great deal has been learnt about the immediate (microsecond) donors to the photo-oxidised reaction center in cells and chromatophores of the purple sulfur bacterium *Chromatium vinosum*. Two types of membrane-bound *c* type cytochrome are present, the low potential cytochrome *c*-552 ($E_{m(7.0)}$ 10 mV) and the high potential *c*-555 ($E_{m(7.0)}$ 350 mV) [1,2]. Both can donate electrons to the same oxidised reaction center, and both occur in a stoichiometry of at least two haems per reaction center [3—6]. At redox potentials when *c*-555 is reduced and *c*-552 is oxidised, *c*-555 is oxidised by P^+-870 in a monophasic reaction with a half-time at room temperature of 2—3 μs [7,8]. When *c*-552 is also reduced, it acts as the electron donor to P^+-870 rather than *c*-555, in a monophasic reaction with a half-time of 1 μs [3—5]. Both *c*-555 and *c*-552 are tightly bound to the reaction center in vivo and in isolated reaction centers [9,10].

Much less is known about the pathway of electron transport outside the cytochrome-reaction center complex. The reduction of c^+-552 after a flash is slow [1,2], and it appears that this cytochrome hardly contributes at all to cyclic photosynthetic electron transport at 'normal' light intensities. Van Grondelle et al. [11] have characterised a soluble *c*-type cytochrome (*c*-551) which appeared to act as the principal electron donor to c^+-555 in intact *C. vinosum* cells. It reduced c^+-555 with a half-time of 300 μs and was apparently present at a stoichiometry of 0.6—0.7 mol/mol reaction center. Their preliminary characterization of *c*-551 in chromatophores indicated a midpoint potential at pH 7.0 of 260 ± 10 mV. The c^+-551 was re-reduced partly by electrons delivered from the reaction center acceptor complex in an *o*-phenanthroline-sensitive cyclic pathway (k 10—15 s^{-1}) and partly by a donor pool, e.g. thiosulfate, in a slower reaction. Re-reduction of c^+-551 was inhibited strongly by HOQNO, but only slightly by antimycin. However, van Grondelle et al. [11] were unable to observe any involvement of a *b*-type cytochrome in cells. This is in contrast to the findings of Knaff and Buchanan [12], who demonstrated the presence of cytochrome *b* in *Chromatium* chromatophores by electrochemical redox titration, by protoheme formation, and by its reducibility in the presence of antimycin under continuous illumination. Its α band maximum was at 560 nm, and had a midpoint potential at pH 8.0 of —5 mV. Knaff et al. [13] have also recently isolated a complex containing cyt *c*-552 and cyt *b* from deoxycholate-treated *Chromatium* chromatophores.

Another candidate for a role in cyclic electron transport is the Rieske type $g_y = 1.90$ iron-sulfur center [14,15]. Some indication that the Rieske-type iron-sulfur center may be on the pathway of photosynthetic electron transport in *Chromatium* is suggested by the work of Evans et al. [14] who found that

it was oxidised after 3 min continuous illumination at room temperature in the absence of redox mediators. The high potential iron protein (HiPIP) is also oxidised under continuous illumination in *Chromatium* [16].

In *Rhodopseudomonas sphaeroides* Ga chromatophores we have recently demonstrated that the Rieske iron-sulfur center appears to act as a direct electron donor to photo-oxidised cytochrome c_2, and that the electron transfer is blocked by the quinone derivative, 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT), or its pentadecyl analogue (PHDBT) (Ref. 17, and Bowyer, J.R., Dutton, P.L., Prince, R.C. and Crofts, A.R., manuscript in preparation). In contrast, Halsey and Parson [18], and Parson (Parson, W.W., personal communication) found that in *C. vinosum* chromatophores, PHDBT had no effect on cyt *c*-555 oxidation or reduction on the first flash but dramatically decreased the extent of oxidation on a second flash delivered 1 ms later. Halsey and Parson [18] also found that another quinone derivative, DDAQQ, had a similar effect to PHDBT in *C. vinosum* chromatophores. They attributed the effect to an inhibition of electron transport from the reaction center primary acceptor quinone, designated Q_I, to the secondary acceptor quinone, designated Q_{II}. UHDBT inhibited cyt *b* reduction in *Rps. sphaeroides* Ga chromatophores but did not appear to inhibit electron transport from the primary to secondary acceptors (Bowyer, J.R. and Crofts, A.R., manuscript in preparation, and see also Ref. 19). It is generally considered that cyt *b* is reduced by electrons delivered from the secondary acceptor, with few if any electron carriers in between [20,21].

In this paper we report on cyt *b* reduction induced by flash excitation, both at high redox potential when cyt *c*-555 is oxidised, and at a lower redox potential. This cyt *b* reduction is enhanced by the addition of antimycin and is inhibited by a number of agents (e.g. *o*-phenanthroline, UHDBT) which block electron transfer from the primary quinone to the secondary acceptor.

We also confirm the observations of Halsey and Parson [18] on the effect of UHDBT (or PHDBT) on cyt *c*-555 oxidation and have extended their observations on inhibition of electron transfer from the primary quinone acceptor (Q_I).

Materials and Methods

Chromatium vinosum strain D (American Type Culture Collection Number 17899) was grown on a medium described by Hendley [22] in the light of air-cooled 150 W tungsten filament lamps. The culture used was kindly provided by Dr. Don DeVault. It had been subcultured about 200 times from the original ATC culture. Microscopic examination indicated that it was pure, but some experiments were repeated with a culture obtained from a single clone. Cells were cloned by serial dilution of a cell suspension in warm molten agar diluted with Hendley's medium. Cells were harvested after about 40 h growth. At this stage the appearance of the cells indicated that they were still utilising sulfur accumulated in the initial growth phase. Chromatophores were prepared as described in Ref. 21. The chromatophores were additionally washed once with 50 mM Mops, 100 mM KCl (pH 7.0). All the experiments

were performed at room temperature (23—25°C). The UHDBT and DDAQQ were gifts from Professor K. Folkers and the DBMIB a gift from Dr. P. Loach. Redox potentiometry was carried out as described in Ref. 23.

The kinetics of absorption changes induced by flash excitation were measured using a computer-linked single beam spectrophotometer as previously described [21]. Excitation was provided by a xenon flash lamp of pulse width 24 μs at half maximum intensity. This led to significant reaction center double turnover at potentials when cyt *c*-555 was reduced, as indicated by the effect of *o*-phenanthroline [11]. Multipulse excitation at E_h 430 mV indicated that the flash was 90% saturating.

Kinetic traces for the time-resolved spectra were measured automatically using a PDP-11 assembly language program to control the computer-linked flash-kinetic spectrophotometer. A file of kinetic traces, one recorded at each wavelength, was stored on a magnetic diskette for processing into time-resolved spectra by a Fortran program. At intervals during the experiment, a measurement was made at an appropriate reference wavelength to check for decay of the response. The spectra were plotted on a Tektronix 4662 Interactive Digital Plotter (Tektronix, Inc., P.O. Box 500, Beaverton, OR 97077).

Results

Flash-excitation induced cyt b reduction at high redox potential

At sufficiently high redox potential, cyt *c*-555, the immediate electron donor to *P*-870, is oxidised at equilibrium, so that, following a flash, absorption changes due to its oxidation and the concomitant carotenoid response [24] are not seen. The major absorption changes which occur are due to photo-oxidation of the primary electron donor (*P*-870) and the considerably slower redox changes of components involved in the cyclic system returning the electron to P^+-870.

Figs. 1A and B show the flash-induced changes recorded at 605 nm, and at 562—572 nm. The latter show an absorption increase which is enhanced by addition of antimycin. Antimycin blocks re-oxidation of cyt *b* in mitochondria and in chromatophores of *Rps. sphaeroides* and *Rhodopseudomonas capsulata* [25—27]. The closed circles in Fig. 2 show a spectrum of the flash-induced absorption change recorded in the presence of antimycin A, and the open triangles a spectrum recorded in the presence of DDAQQ. The band centered at 562 nm in the presence of antimycin A is clearly missing in the presence of DDAQQ. The open triangles in Fig. 3 show the difference between the two spectra after normalizing at 605 nm. It shows clearly that a cyt *b* was reduced. The half-time for the reduction of the cyt *b* in the presence of antimycin A was about 20 ms. Using an extinction coefficient for cyt *b* of 10 $mM^{-1} \cdot cm^{-1}$ at 562—572 nm (based on measurements on a cytochrome *b* complex isolated from *Rps. sphaeroides* Ga (Prince, R.C., personal communication) and 19.5 $mM^{-1} \cdot cm^{-1}$ for the reaction center at 605 nm (based on measurements made on *Rps. sphaeroides* Ga chromatophores [28]), gives a value for cyt *b* reduced/reaction center oxidised of about 0.5.

Fig. 4 shows the reaction center spectrum over a greater wavelength range to include the characteristic bleaching of the 600 nm band. The isosbestic

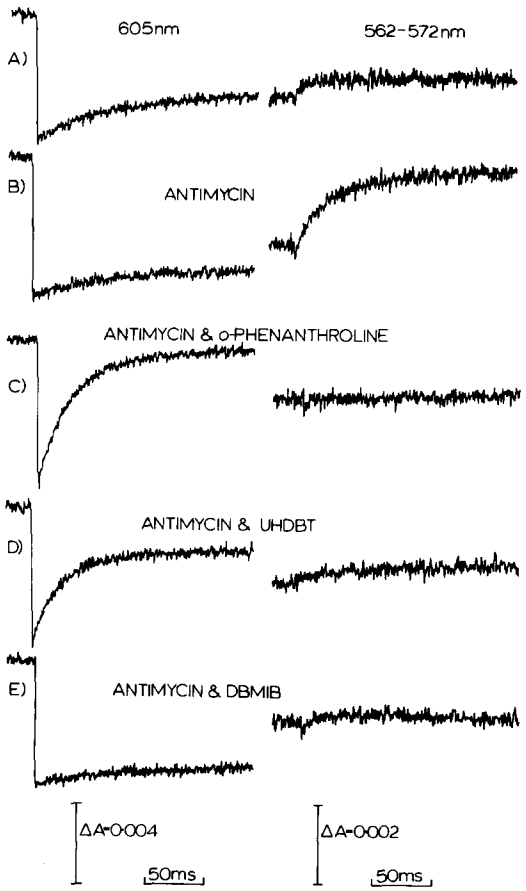

Fig. 1. Effect of various inhibitors on the re-reduction kinetics of P^+-870 and on cyt *b* reduction following a single flash excitation. Chromatophores were suspended to an absorbance at 880 nm of 1.36 in 50 mM Mops, 100 mM KCl (pH 7.0), containing 1 mM each of potassium ferricyanide and ferrocyanide, and 2 μM valinomycin in a stirred anaerobic redox cuvette. The E_h was 430 ± 3 mV. The traces are an average of 4, and the instrument response time was 200 μs. Inhibitors were added to the following concentrations: Antimycin, 20 μM; *o*-phenanthroline, 1 mM; DBMIB, 100 μM; UHDBT, 25 μM.

point at 586 nm and the minimum at 604 nm correspond almost exactly to those in the light-induced difference spectrum of a fairly pure preparation of reaction center particles from *C. vinosum* published by Romijn and Amesz [10]. The chromatophore reaction center difference spectrum is very similar to that obtained in *Rps. sphaeroides* and *Rps. capsulata* under the same conditions (Bowyer, J.R., unpublished observations and Ref. 28), although the broad peak centered at 545 nm is more pronounced in *Chromatium*.

Effects of various inhibitors on cyt b reduction

Figs. 1C—E show that DBMIB, *o*-phenanthroline, UHDBT (or PHDBT, not shown) inhibited cyt *b* reduction at $E_h \sim 430$ mV. HOQNO at 200 μM, and DDAQQ at 100 μM had a similar effect to UHDBT. When the electron is trapped on the primary acceptor it returns to P^+-870 by a back reaction at a

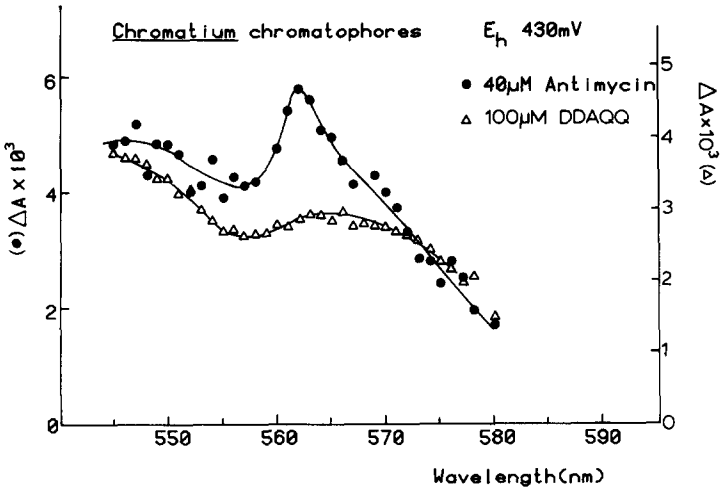

Fig. 2. Light-minus-dark difference spectra showing absorption changes due to reaction center and cytochrome *b* redox transitions. The spectra were obtained by recording the absorption change induced by flash excitation at a series of wavelengths. (•) Chromatophores were suspended to an absorbance at 880 nm of 1.36 in 50 mM Mops, 100 mM KCl (pH 7.0), containing 10 μM DAD, 40 μM antimycin A, 1 mM each of potassium ferricyanide and ferrocyanide, and 2 μM valinomycin in an unsealed unstirred cuvette. The E_h was 430 mV. Excitation was provided by 4 flashes spaced 40 ms apart. The instrument response time was 5 ms and traces were not averaged. The time between each measurement was 60 s. The points show the change recorded 40 ms after the last flash in the train at each wavelength. (△) Conditions as above except that the cuvette contained 100 μM DDAQQ and not antimycin A. The points show the change recorded 10 ms after a single flash. Traces used for the spectrum were an average of 4, with 30 s between each measurement. The instrument response time was 1 ms.

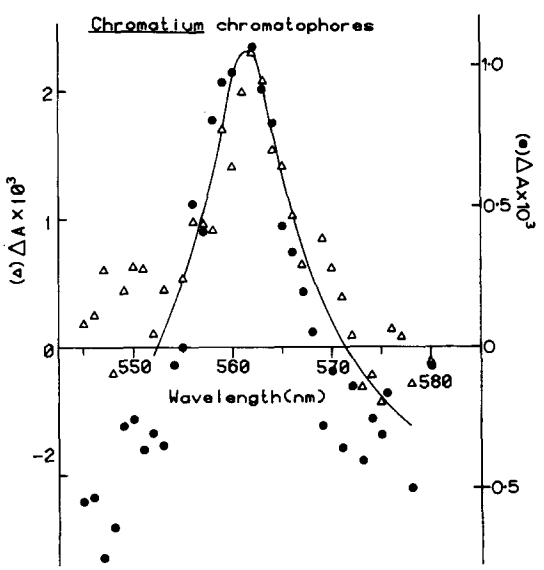

Fig. 3. Resolved light-minus-dark difference spectra attributed to reduction of cytochrome *b*. (△) Data obtained from Fig. 2. The points obtained in the presence of DDAQQ (open triangles of Fig. 2) were first normalised to the same extent of absorbance change at 605 nm as that in the spectrum obtained with antimycin present (closed circles of Fig. 2). The normalised DDAQQ values were then subtracted from the appropriate antimycin values. (•) Data obtained from Fig. 7. The points obtained in the presence of UHDBT (crosses) were first normalised to the same extent of absorbance change at 555 nm as that in the spectrum obtained with antimycin alone present (open triangles). The normalised UHDBT values were then subtracted from the appropriate antimycin values.

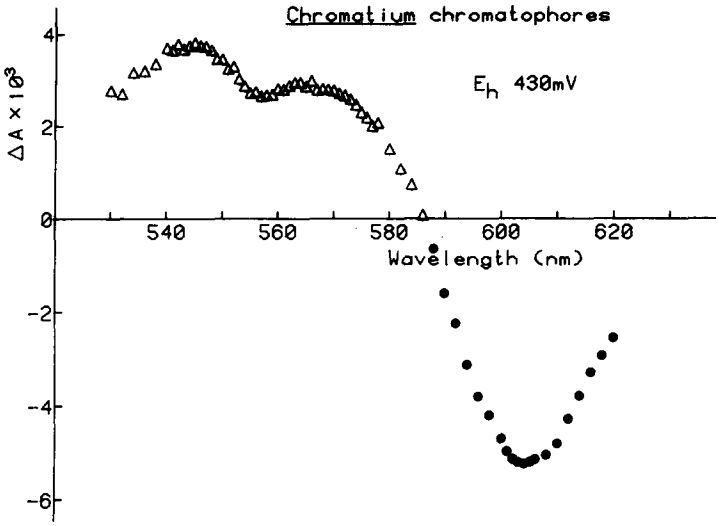

Fig. 4. Difference spectrum attributed to reaction center oxidation. (△) Conditions as for the spectrum obtained with DDAQQ present in Fig. 2. (●) Conditions as in Fig. 2 except that 1 mM *o*-phenanthroline was present instead of 100 μM DDAQQ.

rate considerably faster than the rate at which the electron traverses the cycle, as is interpreted from Figs. 1C and D. It is notable that whereas *o*-phenanthroline caused almost 100% rapid P^+-870 re-reduction, UHDBT, DDAQQ and HOQNO only caused rapid re-reduction of about 50% of the P^+-870. Higher concentrations tended to diminish the total extent of *P*-870 oxidation rather than change the pattern of re-reduction; this decrease was partly over-come by decreasing the exposure time to the measuring beam before excitation. 2-*n*-Heptyl-4-hydroxyquinoline *N*-oxide had a similar effect to UHDBT, whereas Halsey and Parson [18] found that 7-*n*-heptyl-8-hydroxyquinoline *N*-oxide had no effect. DBMIB inhibited cyt *b* reduction but did not accelerate the re-reduction of P^+-870. TTFA, which appears to inhibit reduction of ubisemiquinone to ubiquinol but not reduction of ubiquinone to ubisemiquinone in resolved mitochondrial succinate ubiquinone reductase complex [29], had

TABLE I

COMPARISON OF THE EFFECTS OF VARIOUS AGENTS ON ELECTRON TRANSFER FROM THE PRIMARY TO THE SECONDARY ACCEPTOR, IN *CHROMATIUM VINOSUM* AND *RHODOPSEUDOMONAS CAPSULATA* CHROMATOPHORES

The concentrations of inhibitors used are those listed in the legend to Fig. 1. + indicates inhibition, as demonstrated by accelerated P^+-870 re-reduction at E_h 430 mV.

Agent	*Chromatium*	*Rps. capsulata*
UHBDT	+	—
o-Phenanthroline	+	+
HOQNO	+	—
DDAQQ	+	—
DBMIB	—	—

TABLE II

COMPARISON OF THE EFFECTS OF VARIOUS AGENTS ON CYTOCHROME *b* REDUCTION IN CHROMATOPHORES OF *CHROMATIUM VINOSUM* AND *RHODOPSEUDOMONAS CAPSULATA*.

The cyt *b* reduction was induced by flash excitation in the presence of antimycin A at E_h 430 mV. The concentrations of inhibitors used are those listed in the legend to Fig. 1. + indicates inhibition.

Agent	*Chromatium*	*Rps. capsulata*
UHDBT	+	— *
o-Phenanthroline	+	+
HOQNO	+	—
DDAQQ	+	—
DBMIB	+	+

* In *Rps. capsulata*, UHDBT only slightly inhibits cyt *b* reduction at high redox potential, but becomes much more effective as the potential falls below about 400 mV.

no effect at 100 μM concentration on either reduction of cytochrome *b* or on oxidation of the primary acceptor.

Effects of electron transfer inhibitors with cyt c-555 reduced

When cyt *c*-555 is reduced before flash excitation, it rapidly re-reduces the photooxidised reaction center ($t_{1/2}$ 2—3 μs). The photo-reduced primary acceptor quinone (Q_I^-) is re-oxidised by the secondary electron acceptor(s) with a half-time of about 60 μs [30]. This means that some of the reaction centers would be capable of turning over again before the flash used in these experiments had diminished to a low intensity. A second turnover would then result in further cyt *c*-555 oxidation, since there are at least two *c*-555 haems per reaction center. Inhibition of primary acceptor oxidation should prevent this double turnover and hence reduce the extent of cyt *c*-555 oxidation (for example, see Ref. 11). Fig. 5C shows that, after collapse of the initial transient, which is due to the decay of the carotenoid response accelerated by valinomycin [3], UHDBT had this effect. HOQNO also had a similar effect, confirming the observations made at higher redox potential. Fig. 5B shows that antimycin appeared to slow the rate of cyt c^+-555 reduction.

The traces recorded at 563 nm (Fig. 6) show at least two overlapping absorption changes. Comparison of Fig. 6 with the time-resolved spectra of Ref. 31, Fig. 4 and Fig. 5, suggest that the initial rapid absorbance decrease was due to the carotenoid shift linked to cyt *c*-555 oxidation, and the subsequent slower absorbance increase represents relaxation of the carotenoid change revealing the residual cyt *c*-555 change, together with other changes shown in Fig. 7. The initial downward transient did not occur on the third and fourth flashes. However, the traces recorded at 605 nm indicate that after the third and fourth flashes, the photo-oxidised reaction center was only partially rapidly re-reduced. Fig. 4 shows that at 563 nm, this would lead to a net absorbance increase which probably negates the absorbance decrease due to the carotenoid response.

Because of the large absorbance changes due to cyt *c*-555 oxidation and the carotenoid response, it was difficult to resolve the cyt *b* changes in the α band region. The kinetic traces recorded at 563 nm (Fig. 6) show an antimycin-

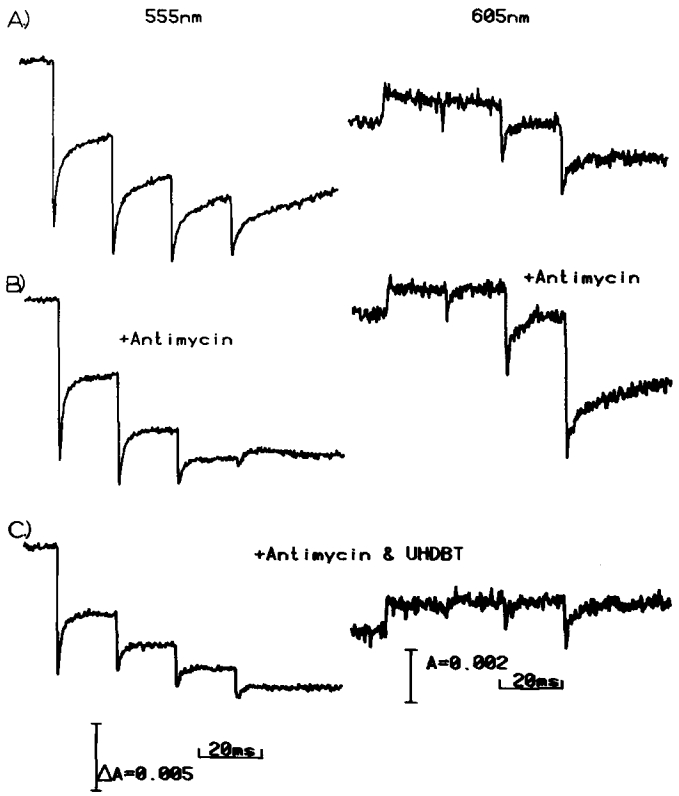

Fig. 5. Multipulse kinetics of *P*-870 and cyt *c*-555. Chromatophores were suspended to an absorbance at 880 nm of 1.36 in 50 mM Mops, 100 mM KCl (pH 7.0), containing 2 μM valinomycin, and 10 μM each of 1,4-naphthoquinone, 1,2-naphthoquinone, 2-hydroxy-1,4-naphthoquinone and DAD in an anaerobic stirred cuvette. The redox potential was 170 ± 10 mV. The traces recorded at 555 nm are an average of 2, those at 605 nm an average of 4. The instrument response time was 100 μs. Chromatophores were subjected to 4 flashes with 20 ms between flashes. (A) No additions. (B) Antimycin added to 20 μM. (C) Antimycin added to 20 μM and UHDBT added to 50 μM.

enhanced net absorbance increase which is inhibited by UHDBT, strongly suggestive of the involvement of cyt *b*. Fig. 7 shows two spectra of the flash-induced change recorded after the complete collapse of the carotenoid response, one in the presence of antimycin, and the other with UHDBT also present. The latter data were normalised at 555 nm. The closed circles in Fig. 3 show the difference between the two spectra. Clearly cyt *b* was reduced in the presence of antimycin and this reduction was again inhibited by UHDBT. The extent of cyt *b* reduction was only about half that recorded at 430 mV, even though the reaction center turnover was 25% greater.

The traces recorded at 605 nm (Figs. 5A—C) indicate an absorbance increase, probably associated with cyt *c*-555 oxidation, which was superimposed on the absorbance decrease due to *P*-870 oxidation. They also demonstrate the inhibitory effect of antimycin on cyclic electron transport, since in the presence of antimycin the donor pool to *P*-870 was more effectively emptied on multipulse excitation. The traces recorded in the presence of UHDBT

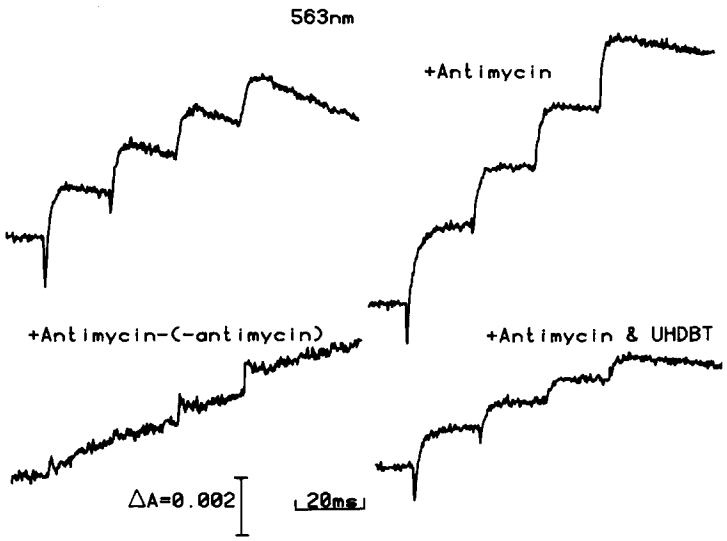

Fig. 6. Multipulse kinetics of the change at 563 nm. Conditions as in Fig. 5.

again indicate its inhibitory effect on turnover of the primary photochemical reactions.

Involvement of cyt c-551

Van Grondelle et al. [11] demonstrated that in whole cells of *C. vinosum*, ferricyt *c*-555 was re-reduced by a *c*-type cytochrome (*c*-551) which was

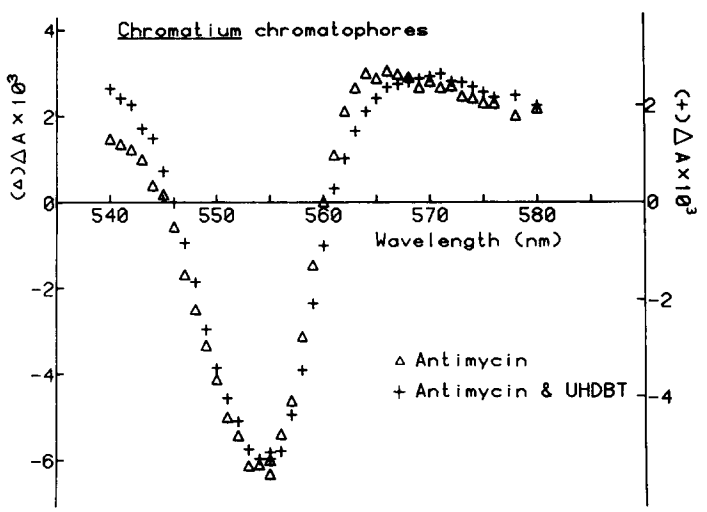

Fig. 7. Spectra of the flash-induced change with cyt *c*-555 reduced before flash excitation. Conditions were as in Fig. 5. At each wavelength of measurement, chromatophores were subjected to a single xenon flash-excitation. The time between measurements was 30 s. The points are from traces that were an average of 2. (Δ) 20 μM antimycin present. (+) 20 μM antimycin and 50 μM UHDBT present. Both spectra show the flash-induced change, recorded 15 ms after the flash.

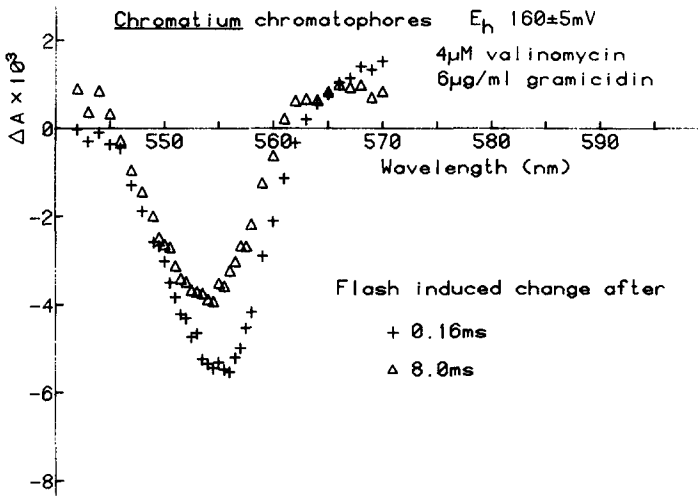

Fig. 8. Time-resolved spectra of cytochrome changes induced by a single flash excitation. Chromatophores were suspended to an absorbance at 880 nm of 1.36 in 50 mM Mops, 100 mM KCl (pH 7.0), containing 4 μM valinomycin, 6 μg/ml gramicidin, 1 μM nigericin, 20 μM DAD, and 10 μM *N*-methylphenazonium methosulfate in a stirred anaerobic cuvette. The redox potential was 160 ± 5 mV. The points are from traces that were an average of 8, with 30 s between measurements. The instrument response time was 20 μs. The spectra show the change 0.16 ms (+) and 8.0 ms (△) after the flash.

able to interact with more than one ferricytochrome *c*-555 molecule. This was shown in part by a detailed analysis of the α-band absorption changes. In their measurements on whole cells, the electrochromic carotenoid response appeared to be very small (Fig. 5, in Ref. 11).

The occurrence of the relatively large contribution of the carotenoid response to absorption changes in the cytochrome α-band region makes it difficult to study the involvement of cyt *c*-551 in *C. vinosum* chromatophores. Fig. 8 shows time-resolved difference spectra of the absorption changes following a single flash-excitation of chromatophores in the presence of sufficient valinomycin and gramicidin to reduce the carotenoid response to a very low level. It shows a slight shift of the α-peak towards lower wavelength with time, which may be attributable to cyt *c*-551 oxidation by cyt c^+-555. It is possible that since cyt *c*-551 is a soluble protein, a considerable amount is lost during chromatophore preparation.

Discussion

Our results reveal a number of interesting similarities and differences between *C. vinosum* and *Rps. capsulata* (and *Rps. sphaeroides*).

As in *Rps. capsulata*, it seems that the cyt *b* is reduced by electrons delivered from the primary acceptor complex. However, the pattern of cyt *b* reduction in *Chromatium* differs from that in *Rps. capsulata*, in chromatophores poised at high redox potential (300 mV $< E_{h(7.0)} <$ 400 mV), and dark-adapted for 5 min prior to excitation by consecutive flashes spaced at 10 s. In *Rps. capsulata* chromatophores, very little cyt *b* is reduced on the first flash in the

presence of antimycin under these conditions (0.2 mol cyt *b* per mol reaction center, using an $\epsilon^{red\text{-}ox}_{560\text{-}570\,nm}$ of 10 $mM^{-1} \cdot cm^{-1}$), but a considerable amount (0.8 mol per mol reaction center) is reduced on a second flash given 10 s later (after the slow re-reduction of P^+-870). This was interpreted as reflecting a charge accumulating two-electron gate operating at the level of the secondary acceptor Q_{II} [21,32,33]. In such a mechanism, the secondary acceptor quinone has to be reduced to the quinol form by two successive turnovers of the reaction center (requiring two flash-excitations) before further electron transfer (e.g. to cyt *b*) can occur. In *C. vinosum* chromatophores, the extent of cyt *b* reduction in the presence of antimycin appeared to be 0.5 mol per mol reaction center on the first and second flashes.

The detailed mechanism of cyt *b* reduction in *Rps. capsulata* chromatophores is not fully understood, and any explanation for the substoichiometric cyt *b* reduction in *C. vinosum* would be highly speculative. Possible causes include a limitation on the amount of available photoreducible cyt *b*, a hypothesis which could be tested by exciting with subsaturating flashes; competition for electrons by the pool of ubiquinone present [18]; or some other mechanistic limitation, as would be provided, for example, by a Q-cycle process [34,35]. The lack of variation in extent of cyt *b* reduction with flash number in *C. vinosum* chromatophores may indicate that the secondary acceptor quinone does not function as a two-electron gate, although Wraight [36] has reported semiquinone formation on odd-numbered flashes in *C. vinosum* subchromatophore particles, indicative of a charge-accumulating mechanism.

The reaction between the primary and secondary acceptor in *Chromatium* is more susceptible to quinone analogs than in *Rps. capsulata* (and *Rps. sphaeroides*) (Fig. 1 and Table I). At present, the molecular basis of the inhibitory effects is not understood. The primary acceptor quinone in *Chromatium* is menaquinone, probably menaquinone-7 [10,37] whereas in *Rps. sphaeroides* (and almost certainly in *Rps. capsulata*) it is ubiquinone-10 [38,39]. Apart from the different number of isoprenoid units in the side chain, the two molecules differ in that menaquinone lacks the methoxy groups at positions 4 and 7 on the benzenoid ring. The primary acceptors also have different thermodynamic properties, the $E_{m(7.0)}$ for the one-electron reduction of Q_I being 75 mV lower in *C. vinosum* in comparison to that in *Rps. sphaeroides* [40].

The secondary acceptor of *C. vinosum* appears to be different from that in *Rps. capsulata* only in that the ubiquinone homologue is ubiquinone-7 (7 isoprenoid units in the side chain) and not ubiquinone-10 [41—44]. The thermodynamic properties of the secondary acceptor in chromatophores of *C. vinosum* are not yet understood [45], but information is now available on *Rps. viridis* and *Rps. sphaeroides* (Refs. 46—48, and Rutherford, A.W. and Evans, M.C.W., manuscript in preparation).

The diminution in the extent of flash-induced *P*-870 oxidation at high concentrations of UHDBT, DDAQQ and HOQNO might be attributed to prior reduction of a fraction of the primary acceptor due to a low level of photochemistry elicited by the measuring beam, and the inhibition of primary acceptor reoxidation. Another possibility is that high concentrations of quinone analogue inhibit the reduction of the primary acceptor following flash excitation, possibly by a competitive displacement. A similar effect has

been observed by Bering and Loach [49] with high concentrations of DBMIB on chromatophores from several species.

Since DBMIB did not accelerate the return of the electron to P^+-870 in *C. vinosum* chromatophores, we suspect that it may inhibit cyt *b* reduction by acting as an alternative electron acceptor either from Q_I or Q_{II}. This is different from the mode of action proposed by Bering and Loach [49] who found that DBMIB at 100—200 μM reduced the efficiency of electron transfer to the primary acceptor, Q_I, in *Rhodospirillum rubrum*.

Our results indicate that the inhibition of cyt *c*-551 reduction by HOQNO observed by van Grondelle et al. [11] may be attributable at least in part to an inhibition of electron flow from the primary to the secondary acceptor. The lack of demonstration of the presence of photoreducible cyt *b* in whole cells by van Grondelle et al. [11] and the rather small inhibitory effects of antimycin may both be attributable to the poor ability of antimycin to penetrate the cell envelope as is observed in *Rps. capsulata* cells (our unpublished observations). Our results (Figs. 6 and 7) show that, even in chromatophores, the reduction of cyt *b* is not easily measured unless cyt *c*-555 is pre-oxidised.

With *Chromatium* chromatophores, none of the effects of UHDBT indicated an inhibition of electron transport at the Rieske iron-sulfur center, in contrast to the effects observed with chromatophores from *Rps. sphaeroides* and *Rps. capsulata* (Refs. 17,19 and Bowyer, J.R., Dutton, P.L., Prince, R.C. and Crofts, A.R., in preparation). It is possible that the iron-sulfur center reduces cyt c^+-551, which shuttles electrons to cyt c^+-555. Depletion of cyt *c*-551 in chromatophore preparations would tend to mask any inhibitory effects of UHDBT. After collapse of the carotenoid absorption change at 555 nm, the residual absorption change (due mainly to photo-oxidised cyt *c*-555) decayed on a seconds time scale, again reflecting the inefficiency of the cyclic system in these chromatophores.

Acknowledgements

This work was supported by United States Public Health Service Grant No. GM 26305-01. We would like to thank Dr. Don DeVault for advice on growing the *Chromatium*, and Mr. Ralph Tanner for advice and assistance on single cell cloning. We would also like to thank Ms. Beth Ransdell for general technical assistance, and Mr. G.V. Tierney for designing, constructing and maintaining peripheral devices associated with the computer-linked spectrophotometers.

References

1 Duysens, L.N.M. (1965) Arch. Biol. (Liege) 76, 251—275
2 Olson, J.M. and Chance, B. (1960) Arch. Biochem. Biophys. 88, 26—53
3 Parson, W.W. and Case, G.D. (1970) Biochim. Biophys. Acta 205, 232—245
4 Seibert, M. and DeVault, D. (1970) Biochim. Biophys. Acta 205, 220—221
5 Parson, W.W. (1968) Biochim. Biophys. Acta 189, 397—403
6 Tiede, D.M., Prince, R.C. and Dutton, P.L. (1976) Biochim. Biophys. Acta 449, 447—460
7 Parson, W.W. (1968) Biochim. Biophys. Acta 153, 248—259
8 DeVault, D. and Chance, B. (1966) Biophys. J. 6, 825—847

9 Case, G.D., Parson, W.W. and Thornber, J.P. (1970) Biochim. Biophys. Acta 223, 122—128
10 Romijn, J.C. and Amesz, J. (1977) Biochim. Biophys. Acta 461, 327—338
11 Van Grondelle, R., Duysens, L.N.M., van der Wel, J.A. and van der Wal, H.N. (1977) Biochim. Biophys. Acta 461, 188—201
12 Knaff, D.B. and Buchanan, B.B. (1975) Biochim. Biophys. Acta 376, 549—560
13 Knaff, D.B., Worthington, T.M., White, C.C. and Malkin, R. (1979) Arch. Biochem. Biophys. 192, 158—163
14 Evans, M.C.W., Lord, A.V. and Reeves, S.G. (1974) Biochem. J. 138, 177—183
15 Rieske, J.S., MacLennan, D.H. and Coleman, R. (1964) Biochem. Biophys. Res. Commun. 15, 338—344
16 Dutton, P.L. and Leigh, J.S. (1973) Biochim. Biophys. Acta 314, 178—190
17 Bowyer, J.R., Dutton, P.L., Prince, R.C., Porter, T.H., Folkers, K. and Crofts, A.R. (1979) in Abstracts of the International Workshop on Membrane Bioenergetics (I.U.B. Symposium No. 93) (Lee, C.P., org.), p. 11, Detroit, MI
18 Halsey, Y.D. and Parson, W.W. (1974) Biochim. Biophys. Acta 347, 404—416
19 Bowyer, J.R. and Crofts, A.R. (1978) in Frontiers of Biological Energetics, Vol. 1 (Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), pp. 326—333, Academic Press, New York
20 Petty, K.M. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 346—353
21 Bowyer, J.R., Tierney, G.V. and Crofts, A.R. (1979) FEBS Lett. 101, 201—206
22 Hendley, D.D. (1955) J. Bacteriol. 70, 625—634
23 Prince, R.C. and Dutton, P.L. (1977) Biochim. Biophys. Acta 462, 731—747
24 Case, G.D. and Parson, W.W. (1973) Biochim. Biophys. Acta 325, 441—453
25 Slater, E.C. (1973) Biochim. Biophys. Acta 301, 129—154
26 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
27 Dutton, P.L. and Jackson, J.B. (1972) Eur. J. Biochem. 30, 495—510
28 Dutton, P.L., Petty, K.M., Bonner, H.S. and Morse, S.D. (1975) Biochim. Biophys. Acta 387, 536—556
29 Trumpower, B.L. (1978) in Frontiers of Biological Energetics, Vol. 2 (Dutton, P.L., Leigh, J.S. and Scarpa, A., eds.), pp. 965—973, Academic Press, New York
30 Parson, W.W. (1969) Biochim. Biophys. Acta 189, 384—396
31 Bowyer, J.R. and Crofts, A.R. (1979) Arch. Biochem. Biophys., in the press
32 Vermeglio, A. (1977) Biochim. Biophys. Acta 459, 516—524
33 Wraight, C.A. (1977) Biochim. Biophys. Acta 459, 525—531
34 Mitchell, P. (1976) J. Theor. Biol. 62, 327—367
35 Trumpower, B.L. (1976) Biochem. Biophys. Res. Commun. 70, 73—80
36 Wraight, C.A. (1979) Photochem. Photobiol. 30, 767—776
37 Okamura, M.Y. (1976) Biophys. J. 16, p. 223A
38 Okamura, M.Y., Isaacson, R.A. and Feher, G. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 3491—3495
39 Prince, R.C. and Crofts, A.R. (1973) FEBS Lett. 35, 213—216
40 Prince, R.C. and Dutton, P.L. (1976) Arch. Biochem. Biophys. 172, 329—334
41 Lester, R.L. and Crane, F.L. (1959) J. Biol. Chem. 234, 2169—2175
42 Fuller, R.C., Smillie, R.M., Rigopoulos, N. and Yount, V. (1961) Arch. Biochem. Biophys. 95, 197—202
43 Carr, N.G. and Exell, A. (1965) Biochem. J. 96, 688—692
44 Peters, G.A. and Cellarius, R.A. (1972) Biochim. Biophys. Acta 256, 544—547
45 Case, G.D. and Parson, W.W. (1971) Biochim. Biophys. Acta 253, 187—202
46 Rutherford, A.W. and Evans, M.C.W. (1979) FEBS Lett. 100, 305—308
47 Rutherford, A.W. and Evans, M.C.W. (1979) FEBS Lett. 104, 227—230
48 Rutherford, A.W., Heathcote, P., and Evans, M.C.W. (1979) Biochem. J. 182, 515—523
49 Bering, C.L. and Loach, P.A. (1977) Photochem. Photobiol. 26, 607—615