Biochimica et Biophysica Acta, 725 (1983) 94–103
Elsevier

BBA 41375

FLASH-INDUCED ELECTRON TRANSPORT IN *b*- AND *c*-TYPE CYTOCHROMES IN *RHODOSPIRILLUM RUBRUM*

EVIDENCE FOR A Q-CYCLE

H.N. VAN DER WAL [a] and R. VAN GRONDELLE [a,b]

[a] *Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden and* [b] *Department of Biophysics, Physics Laboratory of the Free University, De Boelelaan 1105, 1081 HV Amsterdam (The Netherlands)*

(Received March 22nd, 1983)

Key words: Cytochrome b; Electron transport; Q-cycle; Bacterial photosynthesis; Cytochrome c; (R. rubrum)

Flash-induced electron flow in the cyclic chain in chromatophores of the purple photosynthetic bacterium *Rhodospirillum rubrum* was studied in order to obtain information about the reactions of various redox components and to test the validity of the Q-cycle model for electron transport. Redox reactions of the *b*-type cytochromes *b*-433 and *b*-428, the *c*-type cytochrome *c*-420 and the reaction center bacteriochlorophyll P-870 were observed at redox potentials between −50 and +350 mV in the presence and absence of the electron-transport inhibitors antimycin and *n*-heptadecylmercapto-6-hydroxy-5,8-quinolinequinone (HMHQQ). At all redox potentials, the reduction of the *b*-type cytochromes in the presence of antimycin was linked to the reduction of an equal amount of oxidized cytochrome *c*-420 or P-870$^+$. These results are interpreted as evidence for the functioning of a Q-cycle in the cyclic electron transport. The Q-cycle is inhibited by the quinone analogue HMHQQ. At potentials around 20 mV, in the absence of antimycin, cytochrome *b*-433 was reduced in a flash and subsequently oxidized with a simultaneous net oxidation of the dark-reduced cytochrome *b*-428 and reduction of the flash-oxidized cytochrome *c*-420. We conclude therefore that cytochrome *b*-433 has a lower midpoint potential than cytochrome *b*-428. Presumably, the midpoint potential of cytochrome *b*-428 is around 50 mV and the midpoint potential of cytochrome *b*-433 is below 0 mV. The concentration of the *b*-type cytochromes involved in light reactions was low. There seemed to be approximately one of each per six or eight reaction centers.

Introduction

In purple bacteria two protein complexes are involved in cyclic electron flow during photosynthesis. The first one is the photosynthetic reaction center where on illumination the primary charge separation takes place (for a recent review, see Ref. 1). The second complex involved is the Qbc complex which was only recently isolated [2,3] and which can be compared to the mitochondrial ubiquinol:cytochrome *c* oxidoreductase ('Complex III'). In photosynthetic bacteria this complex has been extensively studied in situ, especially in the species *Rhodopseudomonas sphaeroides*, *Rps. capsulata* and *Rhodospirillum rubrum*. The isolated complex contains one or two bound quinones, two *b*-type cytochromes, a Rieske iron-sulfur and a

---

Abbreviations: HMHQQ, *n*-heptadecylmercapto-6-hydroxy-5,8-quinolinequinone; BHQ, benzohydroquinone; P-870, the primary electron donor of the reaction center; UHDBT, 5-(*n*-undecyl)-6-hydroxy-4,7-dioxobenzathiole; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; DQ, 2,3,5,6-tetramethyl-2,5-cyclohexadiene-1,4-dione; BChl, bacteriochlorophyll; Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Qbc, ubiquinol:ferricytochrome *c* oxidoreductase.

c-type cytochrome [2,3]. Dark titrations of chromatophores of *Rps. sphaeroides* showed the presence of three *b*-type cytochromes with midpoint potentials near −90, +50 and +150 mV at pH 7.0 which were called cytochrome b_{-90}, b_{50} and b_{150}, respectively [4]. The bound cytochrome *c* was found to have an $E_{m,7} \simeq 290$ mV, the Rieske iron-sulfur $E_{m,7} \simeq 285$ mV [5].

Most of the known facts about the electron flow in the Qbc complex in purple bacteria have been obtained by flash spectroscopy. The oxidation and reduction kinetics of cytochrome *b* were studied extensively in *Rps. sphaeroides* and *Rps. capsulata*. The following properties relevant to the present work were established:

(i) The antimycin-sensitive oxidation kinetics of the high-potential cytochrome *b* (b_{50}) are identical to the antimycin-sensitive reduction kinetics of cytochrome *c* [6].

(ii) In the presence of antimycin, the rate of reduction of cytochrome b_{50} depends on the ambient redox potential [7]. The half-maximal rate is at $E_{m,7} = 117$ mV in *Rps. capsulata* and *Rps. sphaeroides* [8]. This nearly coincides with the midpoint potential for the reduction rate of cytochrome *c* in the absence of antimycin ($E_{m,7} \simeq 125$ mV) [8].

(iii) UHDBT inhibits the reduction of both *b*- and *c*-type cytochromes in the presence of antimycin [8,9].

(iv) The reduction of cytochrome *b* depends on the flash number at high potentials. A period of two is observed.

(v) Recently, the flash-induced reduction of the low-potential cytochrome b_{-90} in the presence of antimycin was observed by Bowyer and Crofts [8].

It has been suggested that the reduction of cytochrome b_{50} is connected to the oxidation of the secondary acceptor Q_B of the reaction center [10] or alternatively to the reduction of the Rieske iron-sulfur protein [1] but in fact no clear stoichiometric or kinetic link to support this view has been found.

The mechanism of the electron transport through the Qbc complex which should follow from the effects mentioned above is far from established, but several models have been suggested. Dutton and Prince [11] suggested that cytochrome b_{50} is reduced by the ubiquinone called Q_z, when the quinone is in the state Q_zH_2 or $Q_z^{\cdot}H$. The latter state would be generated from the state Q_zH_2 after oxidation by oxidized cytochrome *c*. This mechanism would involve a Q-cycle in which a quinone (or quinones) transports electrons and protons from the acceptor side of the reaction center located on the outside of the chromatophore membrane to the *b*-cytochromes and to the components at the donor side at the inside of the chromatophore membrane. Two Q-cycle models depending on whether or not the semiquinone of Q is a stable entity are described in Ref. 12. According to Dutton and Prince the oxidation of cytochrome b_{50} also involves $Q_z^{\cdot}H$ which then would act as a one-electron carrier transporting electrons from reduced cytochrome *b* to oxidized cytochrome *c*. Recently, Crofts et al. [13,14] suggested that a Q-cycle mechanism for the reduction of the *b*-type cytochromes could explain all the pertinent experimental data.

The stoichiometry of the electron-transport components varies in different species of purple bacteria. *Rps. sphaeroides* seems to contain about 0.7 Qbc complex per reaction center [10,15]. In *R. rubrum* the amount of Qbc complex may even be as low as 0.1–0.2 per reaction center (this work). In connection with these values it seems unlikely that a tight association between the Qbc complex and the reaction center is needed in order to perform efficient electron transport. To transport electrons between the two complexes the diffusable cytochrome *c* at the inside of the chromatophores and the quinones inside the membrane could be of primary importance, but the exact arrangement of the various components and the role of the Q-pool remain to be solved.

The present paper reports the results of a study of the redox properties and light-induced reactions of the *b*- and *c*-type cytochromes and the stoichiometries of these components in *R. rubrum*. By measuring the electron flow to the *b*-type cytochromes and to the reaction center we were able to conclude that in chromatophores of this species a Q-cycle model may account for nearly all of the data obtained in the presence and absence of inhibitors.

Materials and Methods

Cells of *R. rubrum* strain S1 were cultured in light in a medium described by Slooten [16]. The cells were harvested in the exponential phase, and were disrupted using a French press at $3.4 \cdot 10^8$ N/m^2 [17]. The broken cells were separated from the unbroken ones by centrifugation at $26000 \times g$ for 5 min. Large cell fragments were removed by centrifugation of the supernatant at $30000 \times g$ for 20 min. Chromatophores were then separated from the supernatant by centrifugation at $200000 \times g$ for 90 min. The chromatophores were suspended in a buffer containing 50 mM Tes at pH 7.8. Chromatophores were stored at −20°C after addition of 50% glycerol until use.

Electron transport was activated by a flash of light from a xenon lamp. The duration of the flash was 13 μs and it passed through Schott RG 780 (3 mm) and RG 715 (4 mm) filters. The flash saturated the charge separations to only 30% to limit the number of turnovers of the Qbc complex, and to reduce artefacts. The potentiostat cuvette had a volume of 25 ml, and the optical path length was 1 cm. The sample contained 50 mM Tes buffer at 7.8, and chromatophores to a concentration of 7 μM BChl, estimated using an extinction coefficient of 140 mM$^{-1} \cdot$ cm^{-1} at 880 nm [18]. Gramicidin was added to 1 μM to avoid the formation of membrane potentials in all experiments. The extinction coefficients used to calculate the amounts of the reduced and oxidized components are as follows: for P-870$^+$, $\epsilon_{red-ox} = 20$ mM$^{-1} \cdot$ cm^{-1} at 604 nm, −20 mM$^{-1} \cdot$ cm^{-1} at 421 nm, −24 mM$^{-1} \cdot$ cm^{-1} at 428 nm and −26 mM$^{-1} \cdot$ cm^{-1} at 433 nm [19–21]; for cytochrome *b*-428, $\epsilon_{red-ox} = 100$ mM$^{-1} \cdot$ cm^{-1} at 428 nm and approx. 0 at 421 nm [20,22]; for cytochrome *b*-433: $\epsilon_{red-ox} = 100$ mM$^{-1} \cdot$ cm^{-1} at 433 nm, −20 mM$^{-1} \cdot$ cm^{-1} at 421 nm [21]; and for cytochrome *c*-420, $\epsilon_{red-ox} = 60$ mM$^{-1} \cdot$ cm^{-1} at 421 nm, 25 mM$^{-1} \cdot$ cm^{-1} at 428 nm and approx. 0 at 433 nm [20,21,23]. In some cases total cytochrome *b* reduction was measured at 431 nm where we assumed an average extinction coefficient for cytochromes *b*-428 and *b*-433 of 80 mM$^{-1} \cdot$ cm^{-1}.

HMHQQ was a gift from J.A. Berden; UHDBT was a gift from B.L. Trumpower.

Results

Cytochrome c-420

The amount of cytochrome *c*-420 in whole cells depends on the culturing conditions. In chromatophores it depends on the method of preparation [17]. In the chromatophores used here, the ratio of cytochrome *c*-420 to reaction center was 1 : 5 and, since the reduced cytochrome *c*-420 is mobile [20], a large fraction of it was oxidized by even a weak flash.

The redox titration of the extent of flash-induced cytochrome *c*-420 oxidation is shown in Fig. 1. The oxidation of cytochrome *c*-420 is at half maximum at 250 ± 10 mV, and the shape of the titration curve is markedly different from that expected from an $n = 1$ Nernst curve (see Fig. 1). In a dark titration (not shown) of cytochrome *c*-420 oxidation at pH 7.8 a midpoint potential of about 275 ± 10 mV was found, the titration curve for this case being very close to an $n = 1$ Nernst curve (Van der Wal, H.N., Van Grondelle, R. and Gorter, P.Y., unpublished results). The latter result is very close to the value given for a dark titration of cytochrome *c*-420 oxidation in *R. rubrum* at pH

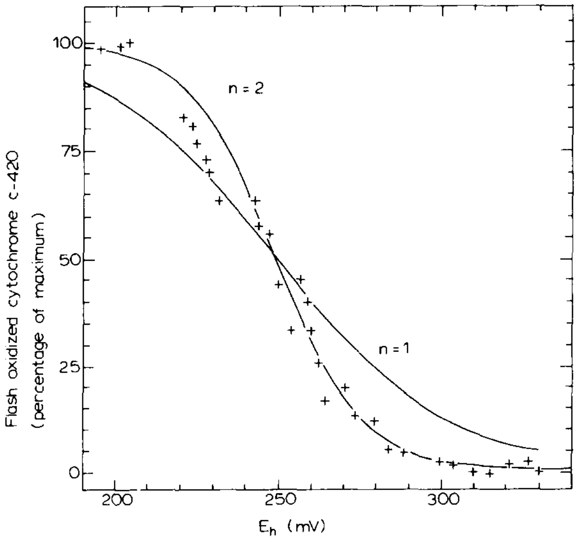

Fig. 1. The amount of flash-oxidized cytochrome *c*-420 in chromatophores of *R. rubrum* measured as the absorbance change at 420 nm at different redox potentials. The drawn lines show Nernst plots for one- and two-electron donors with midpoint potential $E_m = 255$ mV. 10 μM DAD and 10 μM BHQ were present as redox mediators.

7.2 by Dutton and Jackson [4], who obtained $E_{m,7.2} = 293$ mV ($n = 1$). The small difference may be due to some experimental error or the weak pH effect on the midpoint potential for cytochrome *c*-420 oxidation [24]. The lower midpoint for cytochrome *c*-420 oxidation in chromatophores of *R. rubrum* compared to the in vitro value ($E_{m,7} \simeq 330$ mV [24], and Van der Wal, unpublished observations) has been ascribed to preferential binding of the oxidized species [12]. The midpoint for the flash-induced cytochrome *c*-420 oxidation is very close to that observed for the analogous mobile *c*-type cytochrome in *Chromatium vinosum* [25]. The remarkable flash-induced titration curve for cytochrome *c*-420 cannot easily be explained and deviates strongly from that observed for other purple bacteria [12,19] (see Discussion).

The oxidation of cytochrome *c*-420 after a flash proceeded in a single phase with a half-time of about 300 μs. At redox potentials above 150 mV this phase corresponded to the rereduction of a stoichiometric amount of photooxidized P-870. The addition of UHDBT or HMHQQ [26] did not significantly increase the amount of cytochrome *c*-420 oxidized by a flash as is observed in *Rps. sphaeroides* [8].

The rate of reduction of oxidized cytochrome *c*-420 depended on the ambient redox potential, E_h (Fig. 2, curve A). Below 120 mV the reduction rate was independent of E_h. A half-time of reduction of 5 ms was found which is somewhat faster than the half-time of 10 ms observed in whole cells [27]. The titration curve A of Fig. 2 can be explained by assuming a two-electron carrier as the reductant of cytochrome *c*-420, analogous to the quinone Q_zH_2 identified in *Rps. sphaeroides* and *Rps. capsulata* [11–13].

We have tried to find evidence for a second *c*-type cytochrome involved in the electron transport as was found in *Rps. sphaeroides* [28]. The only indication we found was a shift in the negative peak in the α-region of the difference absorbance spectrum of cytochrome *c*. When saturating flashes were used, the peak was at 551 nm, whereas in weak flashes it shifted to 552 nm, indicating that the cytochrome *c* may not be homogeneous. No kinetic evidence for the presence of two kinds of *c*-type cytochrome was obtained.

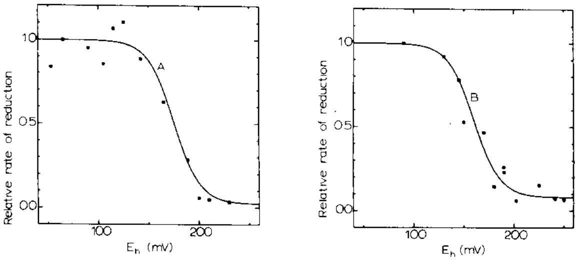

Fig. 2. The relative rates of reduction in chromatophores of *R. rubrum* after a flash, of cytochrome *c*-420 (A) in the absence of antimycin and the *b*-cytochromes (*b*-433 + *b*-428) (B) in the presence of 1 μM antimycin. Curves A and B are are Nernst curves ($n = 2$) with $E_m = 180$ and 165 mV. The rates of reduction were normalized to the same value at 80 mV. The maximum rate or reduction of cytochrome *c*420 was 0.7 μM/s per mg BChl; that of cytochrome *b* 0.8 μM/s per mg BChl.

The b-type cytochromes and cytochrome c-420 in the presence of antimycin

Fig. 3 shows the kinetics of the flash-induced redox reactions of P-870, cytochrome *c*-420 and cytochrome *b* at two potentials. At 310 mV no change of cytochrome *c*-420 is observed. The reduction of cytochrome *b* proceeds with a half-time of about 40 ms and the same phase can be perceived in the reduction of P-870$^+$. From these kinetics one can calculate the amounts of cytochrome *b* and P-870$^+$ reduced between 5 and 150 ms as 1.7 and 1.4 nM/mg BChl (see Table I, first row). At lower potentials the rate of reduction of cytochrome *b* increased and the amount which was reduced became slightly larger. At potentials around and below 290 mV there appeared a net oxidation of cytochrome *c*-420 by P-870$^+$. The

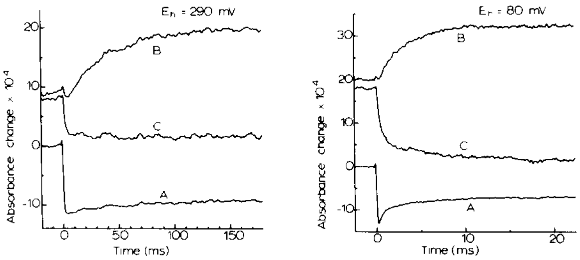

Fig. 3. Kinetics of absorbance changes induced by a flash of light measured at different redox potentials in the presence of 1 μM antimycin. Curves A show the kinetics of P-870$^+$ measured at 604 nm. Curves B show the reduction of the cytochromes *b* at 431 nm. Curves C show cytochrome *c*-420 measured at 421 nm.

kinetics of cytochrome *c*-420 and P-870 can now be explained as the combined effects of a shift of electron from cytochrome *c*-420 to P-870$^+$ and the net reduction of the donor side of the reaction center (i.e., P-870 + cytochrome *c*-420). Table I gives the calculated quantity of reduced compounds at different potentials from which can be seen that (in the presence of antimycin) the reduction of cytochrome *b* is kinetically and stoichiometrically linked to electron transfer to P-870$^+$ and oxidized cytochrome *c*-420.

The flash-induced absorbance change of cytochrome *b* at $E_h = 310$ mV has a spectrum (Fig. 4) of which the widths of the peaks suggest two components. Analysis of the flash-induced kinetics at 428 and 433 nm (Fig. 5) shows an equal size at $E_h = 295$ mV. At $E_h = 20$ mV the change at 428 nm is less by 40%, indicating the disappearance of the component with a maximum absorbance change around 428 nm (cytochrome *b*-428). At high potentials two cytochromes *b* are present in the spectrum, conveniently called cytochromes *b*-433 and *b*-428. Cytochrome *b*-433 has been described before in chromatophores of *R. rubrum* [21]; cytochrome *b*-428 has been described in whole cells [27]. The amount of cytochrome *b* reduction observed at 80 mV (Table I, third row) corresponded to one cytochrome *b* for three or four flash-oxidized reaction centers which means one cytochrome *b*-428 and one cytochrome *b*-433 for six or eight flash-oxidized reaction centers. This is in agreement with observations in whole cells of *R. rubrum* where the abundance of cytochrome *b*-428, named *c*-560 in Ref. 27, was estimated as one in six reaction centers. The maximum amount of cytochrome *b*-433 observed by us (at low flash intensities and low E_h) was one per four photo-oxidized reaction centers.

TABLE I

The amount of electrons in chromatophores of *R. rubrum* transported to the donor side and to the *b*-type cytochromes at different redox potentials measured in the time intervals after a flash indicated in the second column. The electron flow was calculated from the kinetics of the three components using the extinction coefficients mentioned in Materials and Methods.

E_h (mV)	Time interval (ms)	Reduced equivalents (nM/mg BChl)		
		c-420	P-870	*b*-428 + *b*-433
310	0 -150	0.0	1.4	1.7
180	0 - 2	−3.1	3.6	0.4
	2 -150	−0.3	2.0	2.0
60	0 - 0.6	−2.4	2.6	0.6
	0.6- 12.5	−0.9	2.6	1.7

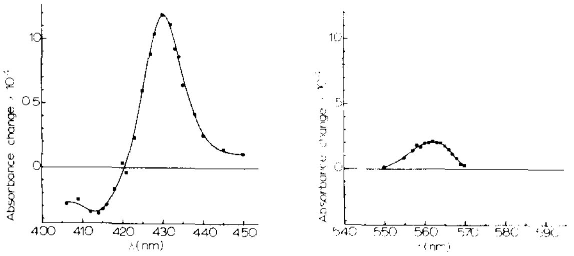

Fig. 4. Spectrum of the reduction of cytochrome *b* measured between 5 and 150 ms after a flash at $E_h = 300$ mV in the presence of 1 μM antimycin. Redox mediators were 10 μM BHQ and 10 μM ferricyanide. The spectrum was corrected for the absorbance changes caused by redox changes of P-870$^+$.

A titration of the rate of reduction of cytochrome *b* after a flash is shown in Fig. 2, curve B, indicating a two-electron donor with $E_{m,7.8} = 160$ mV, only slightly lower than that found for the reductant of cytochrome *c*-420 in the absence of antimycin (curve A). The difference may be an indication that the rate-limiting step in the electron flow through the Qbc complex is not the oxidation of Q_zH_2 but the reduction of Q_z to Q_zH_2 or the reoxidation of the *b*-type cytochro-

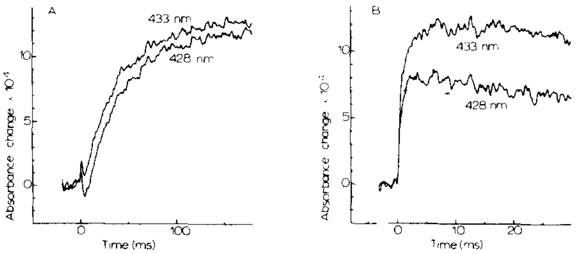

Fig. 5. Kinetics at two different wavelengths of flash-induced cytochrome *b* reduction in chromatophores of *R. rubrum*. (A) $E_h = 295$ mV, (B) $E_h = 20$ mV, both in the presence of 1 μM antimycin. Redox mediators in A: 10 μM DAD, 10 μM BHQ. Redox mediators in B: 1 μM *N*-ethylphenazonium ethosulfate, 1 μM *N*-methylphenazonium methosulfate, 1μM pyocyanine and 10 μM DQ.

mes. At this point it should be realized that in order to reduce fully the flash-oxidized donor side of the reaction center, the Qbc complex has to complete several cycles of reduction and oxidation due to the fact that there is only one Qbc complex per 6–8 reaction centers.

Cytochrome reactions in the presence of antimycin and HMHQQ

The quinone analogue HMHQQ has effects like those of UHDBT, used by Bowyer and Crofts [8] in *Rps. sphaeroides*. Since it inhibits the antimycin-insensitive reduction of cytochrome *b*, its site of action involves either Q_zH_2 or the Rieske iron-sulfur center [26,29]. In the presence of 1 μM antimycin and 40 μM HMHQQ no light-induced reduction of cytochrome *b* was observed, and in the kinetics of cytochrome *c*-420 and P-870$^+$ only the shift of electrons from the first to the second was observed but no net reduction of the donor side took place on a time scale of tens of milliseconds. This supports the view expressed in the preceding paragraph that the reduction of the *b*-type cytochromes and P-870$^+$ are coupled phenomena, the reductant being the two-electron carrier Q_zH_2. The absorption difference spectrum in the presence of both HMHQQ and antimycin showed no reduction of the *b*-type cytochromes (Fig. 6). The amount of cytochrome *c*-420 oxidized was not greatly enhanced (less than 10%) by the addition of HMHQQ and corresponded to the amount of P-870$^+$ reduced in the same time interval.

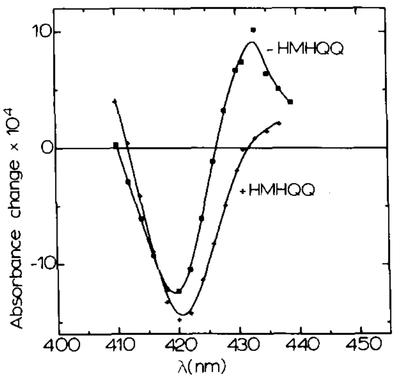

Fig. 6. Difference spectrum measured in chromatophores of *R. rubrum* 75 ms after a flash at E_h = 200 mV. Curve A in the presence of 1 μM antimycin and B in the presence of 20 μM HMHQQ. Note that HMHQQ inhibits the reduction of *b*-type cytochromes, as indicated by the absence of a positive band at 431 nm.

Cytochrome reactions in the absence of inhibitors

In contrast to the results discussed so far, in the absence of antimycin the reoxidation of the *b*-type cytochromes occurs at a rate comparable to their reduction. Therefore, the net reduction of the *b*-type cytochromes is only visible at low potentials where the reduction is faster than the reoxidation. The latter appeared to proceed at the same rate at different potentials. In the potential range 50–100 mV the transient reduction of cytochrome *b*-433 is apparent. In that range, the half-time of the reduction was about 1 ms, and that of the reoxidation 5 ms.

At potentials below 50 mV the net oxidation of cytochrome *b*-428 is observed (Figs. 7 and 8). The half-time for the oxidation was about 5 ms, which is somewhat faster than that observed in whole cells. The amount of cytochrome *b*-428 oxidized by a flash was about one per ten reaction centers. The rereduction was slow (as in whole cells) and depended on the redox potential ($t_{1/2} \simeq 30$ ms at $E_h = -50$ mV to $t_{1/2} \simeq 1$ s at $E_h = +50$ mV).

Fig. 7 shows the kinetics at 428, 421 and 604

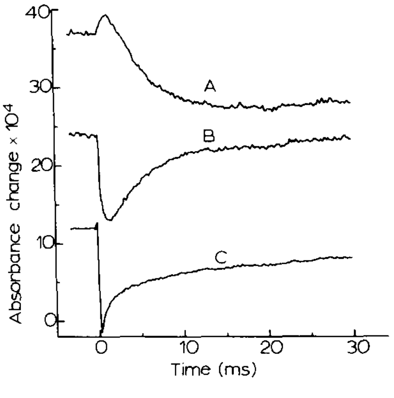

Fig. 7. Kinetics of the absorbance changes in chromatophores of *R. rubrum* after a flash at E_h = −40 mV in the absence of electron-transport inhibitors. Trace A, measured at 428 nm, shows the net oxidation of cytochrome *b*-428. Trace B (421 nm) shows the oxidation and reduction of cytochrome *c*-420. Both traces were corrected for the contributions of P-870$^+$ to the absorbance change, as indicated in the text. Trace C (604 nm) shows the oxidation and reduction of the reaction center bacteriochlorophyll.

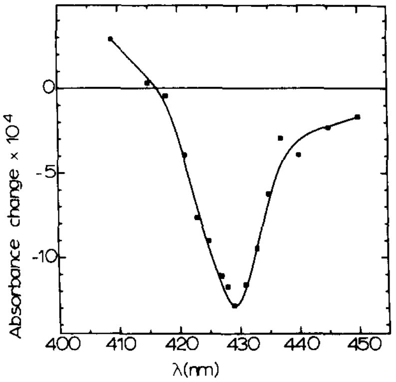

Fig. 8. Spectrum of the absorbance changes in chromatophores of *R. rubrum* 15 ms after a flash showing the net oxidation of cytochrome *b*-428. Conditions as in Fig. 7.

nm (at $E_h = -40$ mV). The kinetics at 421 and 428 nm were corrected for the contribution of P-870, those at 428 nm for the contribution of cytochrome *c*-420 (see Materials and Methods). The initial absorbance increase at 428 nm is due to the reduction of cytochrome *b*-433. The kinetics of cytochrome *b*-433 reduction are clearly separated from cytochrome *c*-420 oxidation and P-870$^+$ reduction ($t_{1/2} \approx 0.3$ ms) [20,21]. Following the initial reduction of cytochrome *b*-433 , the oxidation of both dark-reduced cytochrome *b*-428 and flash-reduced cytochrome *b*-433 takes place (Fig. 7, trace A). The kinetics at 433 nm (not shown) display a larger initial reduction compared to those at 428 nm (see, e.g., Fig. 5), a smaller net oxidation and about the same half-times. Surprisingly the oxidation kinetics of both *b*-type cytochromes are identical within experimental error, indicating that their oxidation is a coupled process. Moreover, the halftime for the reduction of flash-oxidized cytochrome *c*-420 is also equal to that observed for the oxidation of the *b*-type cytochromes. The kinetics at 604 nm show that a fraction (approx. 20%) of P-870$^+$ decays with the 5 ms phase and that a large part (30–40%) remains oxidized long after the flash, indicative of chromatophores totally devoid of mobile cytochrome *c*-420.

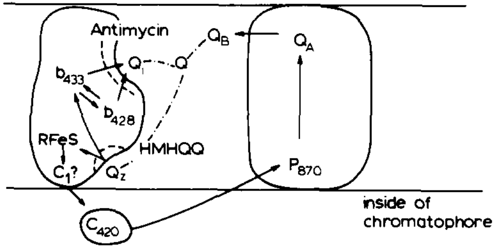

Fig. 9. Reaction scheme showing the Q-cycle in chromatophores of *R. rubrum* and the supposed site of inhibition of the inhibitors antimycin and HMHQQ. The solid arrow represents electron-transfer reactions, the broken arrow between Q_B and Q_zH_2 represents the diffusion of the quinones. RFeS, Rieske iron-sulfur center.

These observations suggest that cytochrome *b*-428 can be identified with the high-potential cytochrome b_{50} ($E_{m,7} = 50$ mV) which was observed by Niederman et al. [30] in dark titrations. Apparently, cytochrome *b*-433 has a much lower midpoint potential and may therefore be equivalent to Niederman's low-potential cytochrome b_{-90} [30].

Discussion

The Q-cycle scheme of Fig. 9 summarizes the main conclusions of this paper. It shows that the electron flow to the donor side of the reaction center is coupled to the electron flow to the cytochromes *b*. No stable ubisemiquinone $Q_z^{\cdot}H$ is admitted which would destroy the kinetic and stoichiometric link between the two electron flows as observed in this work (see Table I). Also, no direct reduction of the *b*-type cytochromes by the acceptor side of the reaction center seems to occur.

Earlier work indicated a very low stoichiometry of Qbc complexes to reaction center in *R. rubrum* [21,27]. Here we confirm these unequal abundances of both complexes and this seems to exclude a special association of Qbc complexes and reaction centers, in contrast to suggestions in this direction for *Rps. sphaeroides* (see, e.g. Ref. 1, p. 238). In our opinion, such an association is unnecessary in view of the high mobility of the electron carriers between the complexes, the quinones in the hydrophobic space inside the membrane and the mobile cytochromes in the periplasmic space outside the membrane [13,27]. A special association of the electron-transfer complexes would be hard to conform to our observation that even using strong flashes, a major fraction of P-870$^+$ and oxidized cytochrome *c*-420 is reduced via the HMHQQ-sensitive pathway.

Cytochrome c-420

The experiments described in this work confirm the properties of cytochrome *c*-420 established in earlier work, i.e.:

(i) Cytochrome *c*-420 is a mobile electron carrier which reduces P-870$^+$ [20].

(ii) A large fraction of cytochrome *c*-420 is lost upon preparation of the chromatophores.

(iii) At all potentials the oxidation of cytochrome *c*-420 is slow (300 μs) compared with species like *Rps. sphaeroides* (2 μs) [12].

The evidence for flash-induced redox reactions of a bound *c*-type cytochrome in *R. rubrum* is not convincing, apart from the small shift in the cytochrome *c*-420 oxidation difference spectrum observed with saturating flashes as described in Results. The findings that the $E_{m,7.8}$ of flash-oxidized cytochrome *c*-420 (255 mV) is significantly lower than that obtained from a dark titration ($E_{m,7.8} \simeq$ 275 mV) and that the shape of the titration curve deviates strongly from an $n = 1$ Nernst curve are difficult to explain. A possibility is that the reduced form of mobile cytochrome *c*-420 binds strongly to the Qbc complex with the bound cytochrome *c* oxidized, assuming the latter to have a lower E_m than the mobile cytochrome *c*-420 [13]. This hypothesis may be supported by the observation that in a mixed system of isolated reaction centers and mitochrondrial Qbc complexes, tight binding of one molecule of reduced cytochrome *c* per Qbc complex was observed [29]. However, at present most explanations on this point are rather speculative and we will discuss these effects extensively elsewhere (Van der Wal, H.N., Van Grondelle, R. and Gorter, P.Y., unpublished observations).

The Rieske iron-sulfur center

Several authors have provided strong evidence for the involvement of the Rieske iron-sulfur center in the electron-transport chain of photosynthetic bacteria [9]. One of the arguments is the increase in the amount of oxidized cytochrome *c* after a flash on the addition of UHDBT in *Rps. sphaeroides* [8]. It was suggested that a part of the positive charge on the electron donor side of the reaction center resides on the Rieske iron-sulfur center. The addition of UHDBT would inhibit the oxidation of the Rieske iron-sulfur center and the positive charge would then be located on the cytochrome *c*. Our work with HMHQQ and UHDBT does not confirm this role for the Rieske iron-sulfur center in *R. rubrum*. Neither inhibitor gives a strong stimulation of the amount of cytochrome *c*-420 oxidized after a flash (see Fig. 6). The phase in the reduction of P-870$^+$ attributed to the oxidation of Q_zH_2 is abolished by HMHQQ and at the same time the reduction of the two *b*-type cytochromes is inhibited. We can accurately account for the numbers of electrons delivered to P-870$^+$ by the sum of cytochrome *c*-420 oxidation and cytochrome *b* reduction which suggests that at any moment following a flash no large net reduction or oxidation of the Rieske iron-sulfur center takes place. In fact, results obtained by Bowyer and Crofts showed that in *Rps. sphaeroides* the increase in the oxidation of cytochrome *c* induced by UHDBT was for a large part at the expense of the reduction of cytochrome *b* (see Fig. 2 of Ref. 8), although this point was not explicitly mentioned in that paper. This would argue for only a small fraction of the positive charges stored on the Rieske iron-sulfur center under these conditions.

Q_z

We have given evidence that in *R. rubrum* a two-electron carrier exists with properties analogous to those of Q_z in *Rps. sphaeroides* and *Rps. capsulata*. The observed kinetics of cytochrome *b* reduction and reduction of the donor side of the reaction center showed that a Q-cycle model can describe the kinetic behavior of Q_z. Assuming that both the reduction rate of the cytochrome *b* in the presence of antimycin and the reduction rate of cytochrome *c*-420 reflect the redox state of Q_z, we have two independent indications for the midpoint potential of Q_z. The two values thus obtained are different by 15 mV (Fig. 2A and B). A similar difference is observed in *Rps. sphaeroides*, and could be explained by the notion that in the absence of antimycin the rate-limiting step for reduction of cytochrome *c* is not the oxidation rate of Q_zH_2 as it is in the presence of antimycin. It should be noted that the midpoint potential for Q_z found in *R. rubrum* ($E_{m,7.8} \simeq$ 170 mV) is significantly higher than that found in *Rps. sphaeroides* ($E_{m,7.8} \simeq$ 110 mV [8]). This difference can be explained by taking the quinone pool into consideration [13]. If one supposes (1) that the midpoint potential obtained from a titration of the reduction rate of cytochrome *b* (Fig. 2B) is the potential at which there is approximately one reduced quinone in the Q-pool for every Qbc complex, and (2) that the size of the Q-pool is about the same in *Rps. sphaeroides* and *R. rubrum* [31], then it follows that due to the higher concentration of Qbc complexes the midpoint of Q_z would be lower in *Rps. sphaeroides.*

The b-type cytochromes

The reduction at high potentials and the oxidation at $E_h < 50$ mV of cytochrome *b*-428 are shown in this work. The kinetic and spectral properties of cytochrome *b*-428 agree with those found in whole cells [27] where under anaerobic conditions cytochrome *b*-428 is oxidized after a flash ($t_{1/2} = 6$ ms).

The dependence of the kinetics of cytochrome *b*-428 on the redox potential suggests that cytochrome *b*-428 corresponds to cytochrome b_{50} with an $E_{m,7} = 50$ mV identified by Niederman et al. [30]. The maximum amount of cytochrome *b*-428 estimated in chromatophores (this work) and whole cells [27] is one per six to eight reaction centers. The oxidation after a flash of cytochromes *b*-428 and *b*-433 occurs more or less simultaneously. No clear distinction between the oxidation kinetics of the two *b*-type cytochromes can be observed (Fig. 6), in contrast to observations by Meinhardt and Crofts in *Rps. sphaeroides* (Fig. 6 of Ref. 14), who found different rates of oxidation for the two *b*-type cytochromes. Given the large difference in midpoint potentials one would expect a vast difference in initial oxidation rates if the two *b*-type cytochromes were in rapid equilibrium. It seems therefore that, at least in *R. rubrum*, no such rapid equilibrium exists. The oxidation of both cytochromes is inhibited by antimycin, suggesting that the Q_i site is involved in the oxidation of both *b*-type cytochromes [13].

There is no straightforward explanation for the large net oxidation of cytochrome *b*-428 at low potentials. With regard to the mechanism of oxidation of the *b*-type cytochromes several possibilities have been discussed in recent papers (see, e.g., Ref. 1). One possibility is that cytochrome *b*-428 is the reductant of $Q_z^{\cdot}H$ formed after transfer of one electron from Q_zH_2 to the oxidized donor side, as was suggested by Dutton and Prince [11]. This would explain the identical oxidation kinetics of cytochrome *b*-428 and reduction kinetics of cytochrome *b*-420. However, since several turnovers of the Qbc complex are required to reduce the oxidized donor side completely, a second turnover will again reduce cytochrome *b*-428 via the oxidation of Q_zH_2 not resulting in net cytochrome *b*-428 oxidation. Alternatively, it may be speculated that the *b*-type cytochromes are in rapid equilibrium with the Q-pool via the Q_i site [13]. A net oxidation of the pool after a flash results in the oxidation of both *b*-type cytochromes, cytochrome *b*-433 to its original dark level, cytochrome *b*-428 below the dark level. To explain the observations no rapid equilibrium between the reduced acceptor side of the reaction center and the *b*-type cytochromes should occur.

We remark that both the reduction of the *b*-type cytochromes in the presence of antimycin and the reduction of flash-oxidized cytochrome *c*-420 in the absence of antimycin are about 4-times slower in *R. rubrum* than in *Rps. sphaeroides*. This might be interpreted as a rate limitation of the cytochrome reactions by the concentration of Qbc complexes.

Acknowledgements

We should like to thank J. Amesz and L.N.M. Duysens for critical reading of the manuscript, N.G. Holmes for help with preliminary experiments and M. Kengen for writing the computer programs.

References

1 Prince, R.C., O'Keefe, D.P. and Dutton, P.L. (1982) in Electron Transport and Photophosphorylation (Barber, J., ed.), pp. 197–248, Elsevier, Amsterdam

2 Gabellini, N., Bowyer, J.R., Hurt, E., Melandri, B.A. and Hauska, G. (1982) Eur. J. Biochem. 126, 105–111

3 Takamiya, K.-I, Doi, M. and Okimatsu, H. (1982) Plant Cell Physiol. 23, 987–997

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

5 Prince, R.C., Lindsay, J.G. and Dutton, P.L. (1975) FEBS Lett. 51, 108–111

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

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

8 Bowyer, J.R. and Crofts, A.R. (1981) Biochim. Biophys. Acta 636, 218-233

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

10 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

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

12 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

13 Crofts, A.R., Meinhardt, S.W., Jones, K.R. and Snozzi, M. (1983) Biochim. Biophys. Acta 723, 202-218

14 Meinhardt, S.W. and Crofts, A.R. (1983) Biochim. Biophys. Acta 723, 219-230

15 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

16 Slooten, L. (1972) Biochim. Biophys. Acta 256, 452-466

17 Del Valle-Tascon, S., Van Grondelle, R. and Duysens, L.N.M. (1978) Biochim. Biophys. Acta 504, 26-39

18 Clayton, R.K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 495-500, Antioch Press, Yellow Springs

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

20 Van Grondelle, R. (1978) Thesis, University of Leiden

21 Van der Wal, H.N., Giménez-Gallego, G., Van Grondelle, R. and Duysens, L.N.M. (1981) Photobiochem. Photobiophys. 2, 149-158

22 Chance, B. (1952) Nature 169, 215

23 Horio, T. and Kamen, M.D. (1961) Biochim. Biophys. Acta 48, 266-286

24 Pettigrew, G.W. and Schejter, A. (1974) FEBS Lett. 43, 131-134

25 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

26 Zhu, Q.S., Berden, J.A., De Vries, S., Folkers, K., Porter, T. and Slater, E.C. (1982) Biochim. Biophys. Acta 682, 160-167

27 Van Grondelle, R., Duysens, L.N.M. and Van der Wal H.N. (1976) Biochim. Biophys. Acta 449, 169-187

28 Meinhardt, S.W. and Crofts, A.R. (1982) FEBS Lett. 149, 223-227

29 Zhu, Q.S., Van der Wal, H.N., Van Grondelle, R. and Berden, J.A. (1983) Biochim. Biophys. Acta 725, 121-130

30 Niederman, R.A., Hunter, C.N., Mallon, D.E. and Jones, O.T.G. (1980) Biochem. J. 186, 453-459

31 Takamiya, K.-I., Nishimura, M. and Takamiya, A. (1967) Plant Cell Physiol. 8, 79-86