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The pathway of cyclic electron transport in chromatophores of *Chromatium vinosum*. Evidence for a Q-cycle mechanism

J.M.C.C. Coremans [a], H.N. van der Wal [a], R. van Grondelle [b], J. Amesz [a] and D.B. Knaff [a,*]

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

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Chromatophores of the purple sulfur bacterium *Chromatium vinosum* were shown to contain a cytochrome similar to cytochrome c_1 and two *b* cytochromes. Cytochrome *b* can be accumulated in the reduced form upon illumination at an ambient redox potential of +415 mV in the presence of the electron transport inhibitors antimycin A or HOQNO. The reductions of cytochrome *b*, of the high-potential cytochrome c_{555} and of the primary electron donor P-870 are all inhibited by myxothiazol. Dark-adapted *C. vinosum* chromatophores show little cytochrome *b* reduction on the first flash. Considerable cytochrome *b* reduction (1 cytochrome *b*: 8 P-870 present) is observed on the second flash. This observation and the 1:1 stoichiometry observed between cytochrome *b* reduction and P-870$^+$ reduction after the second flash support a Q-cycle model for cyclic electron flow in *C. vinosum*.

Introduction

At one time, the cyclic electron transport chain of photosynthetic purple sulfur bacteria such as *Chromatium vinosum* was thought to differ significantly from the cyclic pathways of photosynthetic purple non-sulfur bacteria like *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum*. More recently, *C. vinosum* (like these purple non-sulfur bacteria) was shown to have a membrane-bound, photoreducible *b*-type cytochrome [1–3], a membrane-bound cytochrome similar to mitochondrial cytochrome c_1 [4] and a soluble cytochrome related to mitochondrial cytochrome *c* [5–8]. Additional similarities between *C. vinosum* and purple non-sulfur bacteria are found in the presence of a membrane-bound Rieske iron-sulfur protein [9–11] and in the effects of the electron transport inhibitor antimycin A [1–3, 12]. The stoichiometry of the electron transport components was reported to be highly variable in different species of purple bacteria. *Rps. sphaeroides* seems to contain about 0.7 cytochrome $b \cdot c_1$ complexes per reaction center [13]. In *R. rubrum* the amount of cytochrome $b \cdot c_1$ complexes per reaction center may even be as low as 0.1–0.2 [14], whereas Bowyer and Crofts reported data consistent with 0.5 cytochrome $b \cdot c_1$ complex per reaction center for *C. vinosum* [3]. In the light of the similarities, it seemed of interest to explore the possibility that cyclic electron flow in *C. vinosum* can be explained in terms of a Q-cycle

* To whom reprint requests should be addressed at (permanent address): Department of Chemistry, Texas Tech. University, Lubbock, TX 79409, U.S.A.

Abbreviations: BChl, bacteriochlorophyll; HOQNO, 2-n-heptyl-4-hydroxyquinoline-*N*-oxide; juglone, 5-hydroxy-1,4-naphthoquinone; PES, phenazine ethosulfate, PMS, phenazine methosulfate; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Mops, 3-(*N*-morpholino)propanesulfonic acid; Q_A, Q_B, primary and secondary acceptor quinones.

[15] model, as has recently been successfully done for *Rps. sphaeroides* [16,17] and *R. rubrum* [14] and to reinvestigate the cytochrome $b \cdot c_1$ complex: reaction center stoichiometry in *C. vinosum*.

Materials and Methods

C. vinosum cells were grown as described previously [5]. 'Green' *C. vinosum* cells with greatly reduced carotenoid content were grown in red light in the same medium containing 70 μM diphenylamine to inhibit carotenoid biosynthesis [18]. Cells were washed and suspended in 50 mM Tris-HCl buffer (pH 8.0) and chromatophores were isolated by differential centrifugation after cell breakage in a French Press cell at a pressure of $4.4 \cdot 10^8$ Pa ($N \cdot m^{-2}$). After addition of 50% glycerol, chromatophores were stored at a bacteriochlorophyll (BChl) concentration of 2 mM at -20°C until use. All experiments were conducted in the presence of 2 μM valinomycin and potassium chloride. Kinetic measurements after xenon flash ($t_{1/2} = 10$ μs) excitation and the spectra of Fig. 1B and Fig. 5 were obtained with a computer-interfaced, single-beam spectrophotometer as described previously [5,14]. The flash passed through Schott RG715 (5 mm) and RG780 (3 mm) filters. Flashes were saturating. The potentiostat cuvette had a volume of 25 ml, and the optical pathlength was 1 cm. The difference spectrum of Fig. 1A was measured at a spectral resolution of 0.25 nm using a Cary model 219 spectrophotometer interfaced with a HP 85 computer. The BChl concentration in a chromatophore suspension was estimated from the absorbance at 850 nm using an extinction coefficient of 100 $mM^{-1} \cdot cm^{-1}$. This extinction coefficient was determined by chlorophyll extraction. Oxidation-reduction potentials were determined as described in Ref. 14.

Valinomycin, HOQNO, PES, PMS, duroquinone, sodium ascorbate and 5-hydroxy-1,4-naphthoquinone (juglone) were obtained from Sigma Chemical Co, antimycin A from Nutritional Biochemicals and pyocyanine from Mann Research Laboratories. Anthraquinone-2,6-disulfonic acid was obtained from J.T. Baker. Myxothiazol was a generous gift from Dr. W. Trowitzsch. All inhibitors were prepared as concentrated stock solutions in ethanol. The final concentration of ethanol never exceeded 3.5% (v/v). At this concentration ethanol itself had no effect on electron transport.

Results

The only report of the presence of a cytochrome similar to cytochrome c_1 in *C. vinosum* chromatophores, relied largely on spectral measurements with a detergent-solubilized fraction from the chromatophore membranes [4]. Because it was possible that the spectral characteristics of this cytochrome could be altered by detergent, it seemed important to determine whether the cytochrome could be detected in untreated chromatophore membranes. Fig. 1A shows a chemically induced difference spectrum (hydroquinone minus no addition) of *C. vinosum* chromatophores in the cytochrome α-band region. Hydroquinone ($E_m = +230$ mV at pH 8.0) is capable of reducing the high potential *C. vinosum* cytochromes such as cytochrome *c*-555, which has $E_m = +340$ mV [19,20], and cytochrome c_1, estimated $E_m = +245$ mV [4]. The difference spectrum of Fig. 1A is characterized by a peak at 555 nm, that can be attributed to cytochrome *c*-555 [1–3,5,19,20], and a shoulder at 552 nm. The shoulder at 552 nm cannot be due to the low-potential ($E_m = +10$ mV) membrane-bound cytochrome *c*-552, which is not reduced by hydroquinone [4,9,19,20]. In addition we cannot attribute it to soluble cytochrome *c*-550 ($E_m = +240$ mV, Refs. 7, 8) trapped inside the chromatophore membranes during cell breakage, since we have shown (see below) that chromatophores used in these studies contain no detectable cytochrome *c*-550. Thus we conclude that the shoulder in Fig. 1A can be assigned to cytochrome c_1 and that *C. vinosum* chromatophore membranes do indeed contain two high-potential (i.e., hydroquinone-reducible) *c*-type cytochromes: Cytochrome *c*-555 and a cytochrome with an α-band maximum similar to those of cytochrome c_1 of mitochondria and purple non-sulfur bacteria.

Electron transport involving *b*-type cytochromes exhibits fundamental similarities in mitochondria, higher plant chloroplasts, cyanobacteria and purple non-sulfur bacteria [21,22]. From each of these systems [22], a multiprotein complex containing cytochrome c_1, the Rieske iron-sulfur protein and cytochrome *b* can be iso-

lated. In all these systems the cytochrome $b : c_1$ stoichiometry is 2 : 1 and the two *b*-type cytochromes differ in E_m and α-band spectra [22]. To date, only a single *b* cytochrome ($E_m = +30$ mV at pH 7.0, α-band maximum at 560 nm) has been detected in *C. vinosum* [1–3,12]. However, in the light of the many other similarities, it seemed reasonable to expect that *C. vinosum* might also contain two different *b* cytochromes in this region of its electron transfer pathway. To explore this question, chemically induced difference spectra of the *C. vinosum* membrane-bound low-potential cytochromes were measured. Essentially identical spectra were obtained using dithionite-reduced minus ascorbate/PMS-reduced difference spectra or by subtracting spectra obtained at defined E_h values during the course of oxidation-reduction titrations. Difference spectra obtained with 'red' chromatophores prepared from normally grown *C. vinosum* cells were qualitatively similar to those obtained with 'green' chromatophores prepared from *C. vinosum* cells grown in the presence of diphenylamine. However, the spectra obtained with 'green' chromatophores showed less noise because of their lower carotenoid-cytochrome ratio. Fig. 1B shows a typical example of a low-potential difference spectrum obtained with 'green' *C. vinosum* chromatophores in the cytochrome α-band region. The main peak is due to cytochrome *c*-552 ($E_m = +10$ mV, Refs. 9, 19, 20) and additional shoulders at 560 and 566 nm suggest the presence of either two *b* cytochromes or a single *b*-type cytochrome with a split α-band. For comparison, a reduced minus oxidized difference spectrum of cytochrome *c*-552 alone, obtained from photo-oxidation experiments with anaerobic *C. vinosum* cells is included. Although a cytochrome *b* in *C. vinosum* with an α-band maximum of 560 nm has been reported [1–3,12], no component with an absorbance maximum at 566 nm has previously been observed. An oxidation-reduction titration of the absorbances at 560 and 566 nm suggested that, if there are two distinct *b* cytochromes in *C. vinosum* chromatophores, they both have an E_m between 0 and +50 mV at pH 7.0.

We next turned to an investigation of the effects of inhibitors on the electron transfer reactions after a flash. Fig. 2 shows the effects of antimycin A and myxothiazol on the reduction of

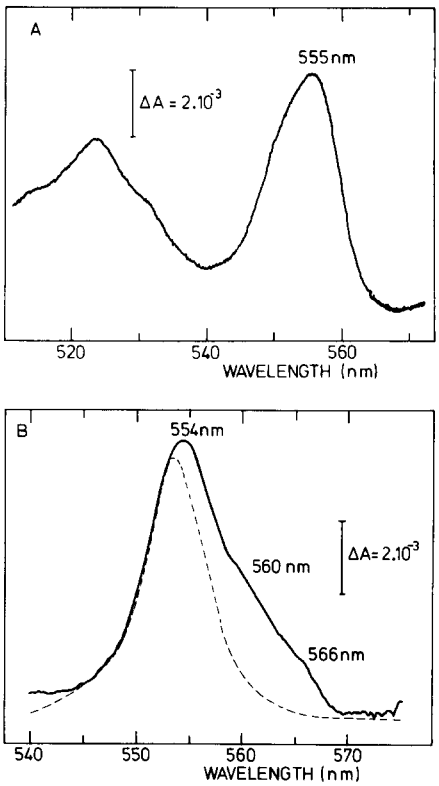

Fig. 1. Spectra of membrane-bound cytochromes of *C vinosum*. (A) Hydroquinone (400 μM) minus no addition difference spectrum. The spectrum represents an average of 16 spectra with the original baseline subtracted. Chromatophores prepared from cells grown in the presence of diphenylamine were suspended in 50 mM Tris-HCl buffer (pH 8.0) to 70 μM BChl in the presence of 2 μM valinomycin (B) $E_h = +139$ mV minus $E_h = -16$ mV difference spectrum (solid line). Chromatophores prepared from cells grown in the presence of diphenylamine were suspended in 20 mM Mes buffer (pH 5 5) containing 50 mM KCl to 100 μM BChl in the presence of 2 μM valinomycin. The reaction mixture also contained the redox mediators 5 μM PMS, 25 μM duroquinone and 25 μM anthraquinone-2,6-disulfonate The potential was adjusted by adding small volumes of concentrated dithionite solution The broken line shows the reduced minus oxidized spectrum of cyt *c*-552 measured by photooxidation of the cytochrome in an anaerobic suspension of 'green' *C vinosum* cells grown in the presence of diphenylamine. The cells were placed in a closed cuvette in medium without thiosulfate and, 30 min before the start of the measurement, sodium thiosulfate was added to a final concentration of 2 mM. Time between flashes was 180 s. The spectrum represents the average of four spectra The spectrum was normalized at 552 nm

cytochrome(s) *b* (measured at 430 nm, Ref. 2) and P-870 (measured at 604 nm, Ref. 3). The experiments were performed at $E_h = +415$ mV where

cytochrome *c*-555 is more than 90% oxidized and P-870 more than 90% reduced prior to the flash. At this redox potential, in the absence of antimycin A, no cytochrome *b* reduction was observed after a flash. Addition of antimycin A resulted in a detectable cytochrome *b* reduction after a flash with $t_{1/2} = 75$ ms. Cytochrome *b* reduction in the presence but not in the absence of antimycin A could also be observed in the α-band region, as has previously been reported [2,3]. Maximal cytochrome *b* reduction was observed at 4 μM antimycin A, with half-maximal reduction obtained at 0.6 μM antimycin A. A Scatchard plot [23] of the flash-induced cytochrome *b* reduction as a function of the antimycin A concentration (assuming one binding site) showed a $K_d = (6 \pm 1) \cdot 10^{-7}$ M, which is similar to a K_d of $(3.5\text{–}7.3) \times 10^{-7}$ M obtained by Takamiya [12]. Antimycin A addition resulted in some inhibition of $P\text{-}870^+$ rereduction after the flash, but the effect was less than that reported by Bowyer and Crofts [3]. HOQNO, another inhibitor of cyclic electron flow in *C. vinosum* [1,5,24] behaved in a manner similar to antimycin A at a concentration of 5 μM (data not shown). At higher concentrations we observed that HOQNO also inhibits between the primary and secondary acceptor quinones Q_A and Q_B [3,21], as indicated by the appearance of a relatively rapid ($t_{1/2} = 30$ ms) reduction of $P\text{-}870^+$ due to the back reaction between $P\text{-}870^+$ and Q_A^- [3]. Neither antimycin A nor myxothiazol showed any sign of inhibition of Q_A^- to Q_B electron transfer by this criterion.

Of particular interest was the effect of myxothiazol, a newly characterized inhibitor of electron transport in mitochondria [25,26] and

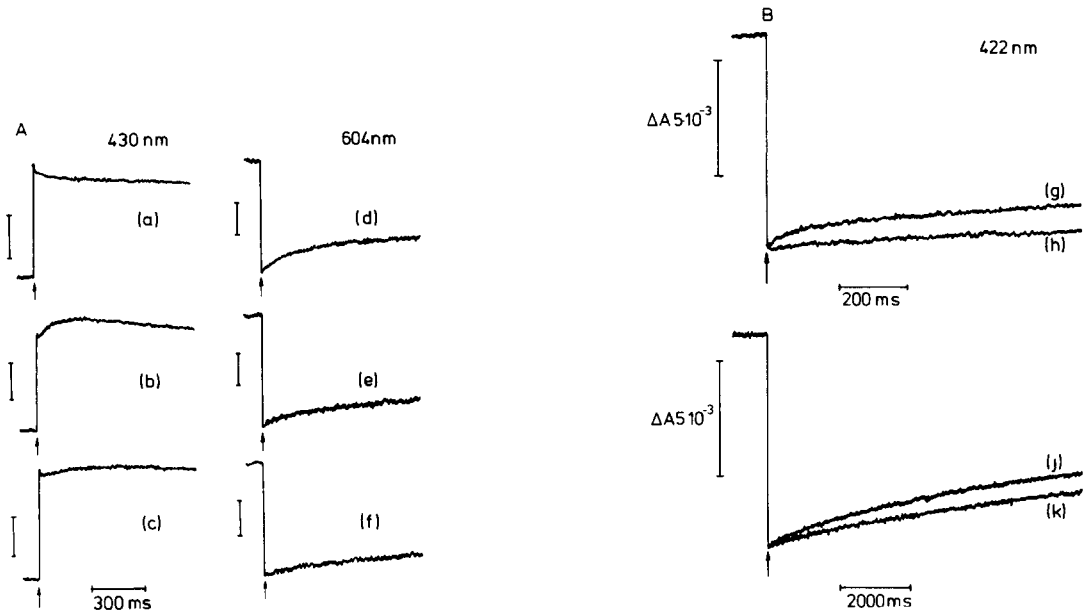

Fig 2. The effect of electron-transport inhibitors on the absorption changes of cytochrome *b* (measured at 430 nm) and P-870 (measured at 604 nm) at $E_h = +411$ mV and on cytochrome *c*-555 (measured at 422 nm) at $E_h = +200$ mV. (A) Chromatophores from *C vinosum* were suspended to 14 μM BChl in 50 mM Mops buffer (pH 7.0)/100 mM KCl. The reaction mixture also contained 40 μM potassium ferricyanide, 40 μM potassium ferrocyanide and 2 μM valinomycin. Traces at 430 nm are an average of 30 measurements. Traces at 604 nm are an average of 60 measurements. Time between two flashes, 30 s. The bars at 430 nm correspond to a ΔA of $5 \cdot 10^{-4}$ and the bars at 604 nm correspond to a ΔA of $2 \cdot 10^{-4}$. (a, d) In the absence of inhibitors, (b, e) in the presence of 5 μM antimycin A and (c, f) after 4 μM myxothiazol was added to the sample used for traces (b, e). (B) Chromatophores from *C vinosum* were suspended to 14 μM BChl in the same buffer used in (A) in the presence of 10 μM juglone, 1 μM PES, 1 μM pyocyanine, 0.1 μM PMS and 2 μM valinomycin. Traces are an average of two measurements. The time between two flashes was 180 s. The bars at 422 nm correspond to a ΔA of $5 \cdot 10^{-3}$. (g) In the absence of inhibitors, (h, j) in the presence of 5 μM antimycin A and (k) with 4 μM myxothiazol added to the sample (j)

photosynthetic purple non-sulfur bacteria [16,17, 27,28], since a myxothiazol-binding protein has recently been isolated from *C. vinosum* [29]. As can be seen in Fig. 2A, myxothiazol inhibits the reduction of *C. vinosum* cytochrome *b* observed in the presence of antimycin A. More than 90% inhibition of cytochrome *b* reduction occurred at myxothiazol concentrations of 6 μM or more, with 50% inhibition occurring at 1.25 μM. Myxothiazol, in this concentration range, also inhibited P-870$^+$ reduction. Similar effects of myxothiazol on cytochrome *b* photoreduction had previously been obtained in chromatophores from purple non-sulfur bacteria [15–17,27].

Fig. 2B shows the effects of antimycin A and myxothiazol on the reactions of cytochrome *c*-555, monitored at 422 nm. This series of experiments was conducted at $E_h = +200$ mV, a potential where the cytochrome is reduced prior to illumination. Van Grondelle et al. [5] have reported a reduction of photooxidized cytochrome *c*-555 with $t_{1/2} \approx 300$ μs in intact cells of *C. vinosum*, with a soluble *c*-type cytochrome serving as the reductant for the membrane-bound cytochrome *c*-555. Experiments similar to those of Fig. 2B, but on a faster time scale, showed no 300 μs reduction of cytochrome *c*-555, suggesting that *C. vinosum* chromatophores prepared as described in Materials and Methods had lost more than 80% of the soluble cytochrome *c*-550. The slower cytochrome *c*-555 reduction, that occurs on the millisecond time-scale, is inhibited by antimycin A, as was previously reported by Bowyer and Crofts [3], and is also inhibited by myxothiazol with or without antimycin A present. Similar results were obtained when cytochrome *c*-555 reactions were monitored at 555 nm. Unfortunately it was not possible to measure the effects of antimycin A and myxothiazol on cytochrome *b* reduction at $E_h = +70$ to $+200$ mV, because little photoreduction of the cytochrome was observed in this potential range. As will be discussed below, the low amount of cytochrome *b* reduction at $E_h = +70$ to $+200$ mV may possibly be related to the absence of the soluble cytochrome *c*-550 in the *C. vinosum* chromatophores.

As mentioned in the Introduction, modifications of the Q-cycle model originally proposed by Mitchell [15] for the mitochondrial cytochrome $b \cdot c_1$ complex have been successfully applied to electron transport in purple non-sulfur bacteria [14,16,17]. Fig. 3 shows a possible Q-cycle model for the cyclic electron transport chain of *C. vinosum*. The scheme of Fig. 3 allows one to make certain predictions about the behavior of cytochrome *b* reduction at high ambient oxidation-reduction potentials (where all components of the chain except for P-870 are oxidized prior to illumination) that can be readily tested. For example, the scheme of Fig. 3 predicts that because fully reduced quinol is the reductant for cytochrome *b* reduction, no cytochrome *b* reduction should be observed after one single-turnover flash, in rigorously dark-adapted chromatophores. Only after a second flash delivers a second electron to the acceptor pool, forming QH_2, should cytochrome *b* reduction be observed. Such 'periodicity of two' has been observed in chromatophores of the purple non-sulfur bacterium *Rhodopseudomonas capsulata* [30] but not with *C. vinosum* chromatophores [3]. Our own initial measurements (Fig. 2) showed considerable cytochrome *b* reduction on the first flash. However, these flashes were given with relatively short intervals of 20–30 s, because of the signal-averaging procedures used, and a relatively intense continuous measuring beam was used that might produce a considerable amount of Q_B^- prior to the flash. When precau-

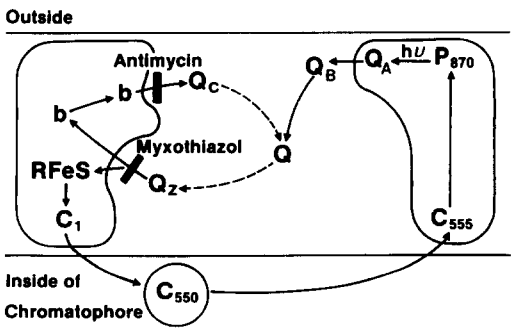

Fig 3 Q-Cycle scheme for cyclic electron flow in *C vinosum*. *c*-550, c_1 and *c*-555 refer to the three *c*-type cytochromes described in the text, RFeS to the Rieske iron-sulfur protein, *b* to *b*-type cytochrome(s), Q_A and Q_B to, respectively, the primary and secondary acceptor quinones and Q to the quinone pool Q_z and Q_c represent sites on the cytochrome $b \cdot c_1$ complex where quinol is oxidized and quinone is reduced, respectively HOQNO is thought to act at the same site as antimycin A.

tions were taken to dark-adapt the chromatophores between measurements, the pattern shown in Fig. 4A was observed. Little cytochrome *b* reduction was now observed on the first flash, while substantial reduction occurred on the second flash. Spectra of the flash-induced absorbance changes are shown in Fig. 5. Maxima were observed at 563 nm in the α-band region and at 431 nm in the Soret band region. A similar spectrum of the Soret band region, though smaller in amplitude, was obtained from the absorbance changes after the first flash. A spectrum of cytochrome *b* reduction in the α-band after a second flash showed the same features and was of the same size as the spectrum of Fig. 5 where flashes were given with relatively short intervals. Using extinction coefficients of 100 $mM^{-1} \cdot cm^{-1}$ at 430 nm and 20 $mM^{-1} \cdot cm^{-1}$ at 562 nm for cytochrome(s) *b* and 20 $mM^{-1} \cdot cm^{-1}$ at 604 nm for P-870, values of 1 cytochrome *b* per 6 to 12 P-870 present were calculated from the flash-induced cytochrome *b* reduction in the α-band and Soret band (Figs. 4, 5). Unfortunately, the spectral resolution obtainable in the measurements of Fig. 5 does not allow any firm conclusion to be drawn as to whether a single species of cytochrome *b* accumulates in the

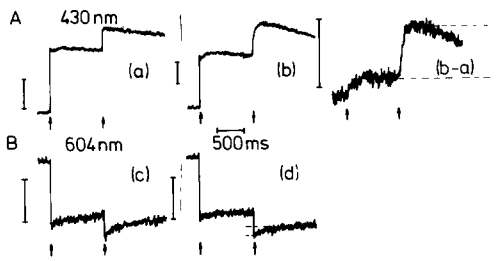

Fig. 4. Kinetics of the absorbance changes in chromatophores of *C. vinosum* after two consecutive flashes. The chromatophores were exposed to the measuring beam 10 s prior to the flash, with pairs of flashes, spaced 1 s apart, given at 120 s intervals The bars correspond to a ΔA of $3 \cdot 10^{-4}$. Chromatophores (14 μM BChl) were suspended in 50 mM Mops buffer (pH 7.0)/100 mM KCl in the presence of 2 μM valinomycin, 40 μM potassium ferrocyanide and 40 μM potassium ferricyanide ($E_h = +415$ mV). (A) Absorbance changes at 430 nm. Trace (b−a) shows the reduction of cytochrome *b* corrected for contributions by the reaction center to the absorbance change. (B) Absorbance changes at 604 nm. Traces (a, c) in the absence of inhibitors, (b, d) in the presence of 5 μM antimycin A Traces are an average of 20–30 measurements.

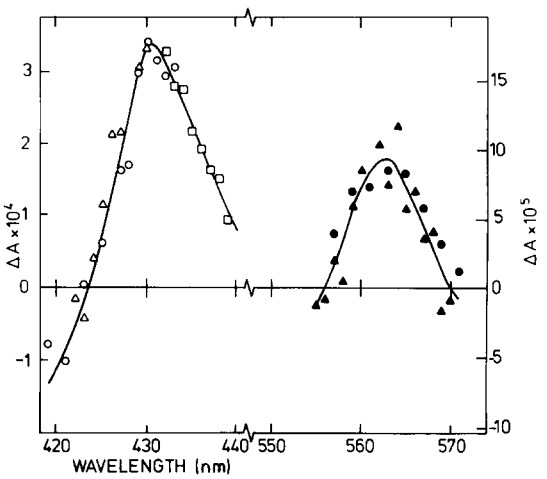

Fig 5. Spectrum of the reduction of cytochrome *b* in chromatophores of *C. vinosum*. Conditions as in Fig. 4. The concentration of antimycin A, if present, was 5 μM. The spectral resolution of the apparatus was 1.6 nm. Different symbols refer to different samples. Left-hand side: Soret band spectrum; measured 350 ms after the second of three consecutive flashes, spaced 1 s apart The time interval between two flash-trains was 120 s. Right-hand side· α-Band spectrum; measured 200 ms after a single flash. The absorbance change at each wavelength represents the average of 50–60 measurements. Time between two flashes was 25 s. Cytochrome *b* reduction in sample ▲ was measured at a BChl concentration of 8 μM. In the spectrum the absorbance changes are normalized to a BChl concentration of 14 μM. Both spectra were obtained by subtracting the absorbance changes with and without antimycin A

reduced form after a flash in the presence of antimycin A or whether the single electron delivered to cytochrome(s) *b* equilibrates between two approximately isopotential forms of cytochrome *b* with α-band maxima at 560 and 566 nm. Moreover, comparison with the chemically induced difference spectrum of Fig. 1B is complicated by the red shift(s) of the α-band(s) of the *b*-cytochrome(s) that may be expected in the presence of antimycin A [12].

A second major prediction of the Q-cycle model is that for each electron going to cytochrome *b* from quinol, the other electron from quinol must go to the high-potential pool of acceptors. This prediction has recently been tested with chromatophores of *R. rubrum* [14]. Comparison of the kinetics of $P\text{-}870^+$ reduction (Fig. 4B) with those of cytochrome *b* reduction shows that the same is true for *C. vinosum*. In the presence of antimycin

A the amount of P-870$^+$ rereduced in the first 300 ms after a flash was nearly the same as that of cytochrome *b* reduced. The ratio of cytochrome *b* reduced. The ratio of cytochrome *b* reduced: P-870$^+$ reduced is 1.06, or 1.0 within the experimental uncertainties. As predicted by the scheme of Fig. 3, cytochrome *b* reduction and P-870$^+$ reduction after the second flash were both inhibited by myxothiazol.

Discussion

We have confirmed the presence of cytochrome c_1 in *C. vinosum* chromatophore membranes and obtained evidence that the membranes may contain two spectrally distinct forms of cytochrome *b* rather than the single *b* cytochrome formerly thought to be present [1–3]. Thus the cytochrome $b \cdot c_1$ region of the *C. vinosum* electron transport chain now seems more similar to those of purple non-sulfur bacteria, mitochondria, chloroplasts and cyanobacteria than was thought earlier [21,22]. If, in fact, *C. vinosum* contains two *b* cytochromes, one significant difference between the *C. vinosum* *b* cytochromes and those in the cytochrome $b \cdot c_1$ complexes of mitochondria and purple non-sulfur bacteria is that the *C. vinosum* cytochromes appear to be approximately isopotential. In contrast, the two different *b* cytochromes of mitochondria and purple non-sulfur bacteria differ in E_m by between 60 and 140 mV [22].

It should be noted that as a consequence of the number of reaction centers per cytochrome *b* (in the experiment of Fig. 5), a saturating flash yielded approx. 8 electrons per cytochrome *b* and therefore all *b*-type cytochromes involved in cyclic electron transport will have been reduced. This means that the spectra may well be composed of the reduced minus oxidized spectra of two *b*-type cytochromes.

Additional similarities between the cytochrome *b*-containing region of the *C. vinosum* electron transport chain and that of purple non-sulfur bacteria and mitochondria extend to the site of action of three specific inhibitors of the cytochrome $b \cdot c_1$ complex. Antimycin A appears to inhibit electron transport by blocking the oxidation of reduced cytochrome(s) *b*. Apparently the rate of cytochrome *b* oxidation in uninhibited chromatophores is sufficiently rapid (compared to the rate of cytochrome *b* reduction) that little or no reduced cytochrome accumulates in the absence of antimycin A. HOQNO, at low concentrations, appears to act in a very similar manner to antimycin A. According to the Q-cycle scheme of Fig. 3, antimycin A would not be expected to inhibit P-870$^+$ or ferricytochrome *c*-555 reduction on the first turnover. We attribute the slight inhibition observed (see Fig. 2) to that small portion of electron flow that involves multiple turnovers of the cytochrome $b \cdot c_1$ complex.

Myxothiazol inhibits the reduction of both cytochromes *b* and *c*. As indicated in Fig. 3, a likely site for myxothiazol inhibition could be the oxidation of quinol by the Rieske iron-sulfur protein. Inhibition at this point would prevent electrons from being delivered to the *b* cytochromes and (via cytochrome *c*-550) to ferricytochrome *c*-555 and P-870$^+$.

The much larger yield of cytochrome *b* reduction on the second flash compared to the first flash (see Fig. 4A) indicates that a two-electron reductant is required for the reduction of cytochrome *b* in *C. vinosum*. These results and the observation (see Fig. 4) that a 1 : 1 stoichiometry exists at $E_h = +415$ mV between electrons delivered to cytochrome *b* and P-870$^+$ are consistent with a Q-cycle mechanism for cytochrome *b* reduction at high ambient potentials. At lower ambient potentials ($E_h \approx +70$ mV) where the quinone pool would be expected to be substantially reduced prior to the flash, the role of the reaction center in reducing cytochrome *b* would be to oxidize the Rieske iron-sulfur protein, thus initiating the two-electron reduction of this iron-sulfur center plus ferricytochrome *b* by quinol. In *C. vinosum*, oxidizing equivalents from P-870$^+$ can only reach the Rieske iron-sulfur protein via cytochrome *c*-555 and the soluble cytochrome *c*-550. Our failure to observe significant cytochrome *b* reduction at low potential may be due to the loss of most of the soluble cytochrome *c*-550 during chromatophore preparation. Thus, under these conditions, the remaining cytochrome *c*-550 may be unable to compete effectively with other electron donors for P-870$^+$. Reports from other laboratories [2,3] of cytochrome *b* reduction at ambient potentials near 100 mV may reflect the higher cytochrome *c*-550

content of the chromatophores used in these laboratories [3].

An interesting point is the stoichiometry. In Table I we list our data together with data calculated from the literature to compare the amounts of BChl per reaction center (photosynthetic unit size) and cytochrome *b* per reaction center. It appears that a significant variability exists between the unit sizes, presumably as a result of different light intensities during growth, as was observed by Aagaard and Sistrom for *Rps. sphaeroides* [31]. During the growth of the cells used in this work the light regime was 1 mW/cm^2. A batch grown in stronger light (approx. 20 mW/cm^2) had an antenna of 240 BChl per reaction center.

Connected to the unit size is a variation of the cytochrome *b* : reaction center ratio which seems to be smaller in cultures with a large photosynthetic unit. This phenomenon is also found in green plants [32,33].

Table I also shows that the total amount of cytochrome *b* estimated from chemical determinations (column 'dark') is generally higher by a factor of 2–4 than the amount of cytochrome *b* estimated from light-induced redox changes. It appears that a large part of the available cytochrome *b* does not participate in photosynthetic electron transfer which is analogous to the situation observed in *R. rubrum* (Van der Wal, H.N., unpublished data) and *Rps. sphaeroides* [34]. Absorption difference spectra of cytochrome *c*-555 that are of sufficient precision to obtain an accurate determination of the amount of cytochrome c_1 from Fig. 1A are, unfortunately, not available in the literature.

Much still remains unknown about the details of electron transport through the *C. vinosum* cyclic

TABLE I

OBSERVED REACTION CENTER/CYTOCHROME *b* AND REACTION CENTER/BChl RATIOS AND PHOTOSYNTHETIC UNIT SIZE IN CHROMATOPHORES OF *C VINOSUM*

Column 'light': determined from light-induced redox changes; 'dark': from chemical determinations. The extinction coefficients we used to calculate the amounts of the reduced and oxidized components are as follows. for BChl, $\epsilon_{850} = 100$ mM$^{-1}\cdot$cm^{-1}; for P-870, $\epsilon_{604}^{red\text{-}ox} = 20$ mM$^{-1}\cdot$cm^{-1} and $\epsilon_{430}^{red\text{-}ox} = 26$ mM$^{-1}\cdot$cm^{-1}; for cytochrome *b*, $\epsilon_{430}^{red\text{-}ox} = 100$ mM$^{-1}\cdot$cm^{-1} and $\epsilon_{560}^{red\text{-}ox} = \epsilon_{562}^{red\text{-}ox} = 20$ mM$^{-1}\cdot$cm^{-1}. RC, reaction center; DPA, diphenylamine.

Source		[BChl] (μM)	[RC] (nM)	[cyt *b*] (nM)	[BChl]/[RC]	[cyt *b*]/[RC]	
						light	dark
Ref. 1,	Fig. 4	30	140 [a]	50	214	0.36	
	Fig. 1			250			1.8
							1 3 [b]
Ref. 3,	Fig. 1	20 [c]	300	115	67	0.38	
Ref. 2,	Fig. 1.	12	~ 70 [d]	10 [e]	~ 180	~ 0.14	
	Fig. 1			20 [e]		~ 0.28	
	Fig. 4	65		225	~ 180		0.62
Ref. 12,	Fig. 2	44		33	~ 180	0.13	
This work	Fig. 5	14	40	3.3 [e]	350	0.08	
	Fig. 5			4.2 [e]		0.10	
	DPA [f]	20	35	3.6	570	0.10	
				5.0		0.14	
	Fig. 1B	100		122	~ 570		0.70

[a] Assuming 2 molecules of cytochrome *c*-555 oxidized per reaction center and an $\epsilon_{555}^{red\text{-}ox}$ cytochrome *c*-555 = 20 mM$^{-1}\cdot$cm^{-1}.

[b] Cytochrome *b* determined by protoheme analysis; based on a unit size of 214.

[c] Calculated assuming an $A_{850}/A_{880} = 1.5$.

[d] Calculated from the rapid phase of the absorbance change at 430 nm.

[e] Calculated from the absorbance changes at 430 nm and 560 nm, respectively.

[f] From an experiment similar to Fig. 4B performed on 'green' chromatophores. Cytochrome *b* measured at 430 nm and at 560 nm, respectively

electron transport chain. However, it is becoming increasingly clear that this pathway is fundamentally similar to that of purple non-sulfur bacteria and, like the latter, shares many similarities with mitochondrial electron transport in the cytochrome $b \cdot c_1$ region. Although additional data at lower redox potentials are desirable, the data obtained for *C. vinosum* at +415 mV strongly support a Q-cycle mechanism for cyclic electron flow in this purple sulfur bacterium.

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