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FUNCTION AND PROPERTIES OF A SOLUBLE *c*-TYPE CYTOCHROME *c*-551 IN SECONDARY PHOTOSYNTHETIC ELECTRON TRANSPORT IN WHOLE CELLS OF *CHROMATIUM VINOSUM* AS STUDIED WITH FLASH SPECTROSCOPY

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SUMMARY

Changes in the absorption spectrum induced by 10-μs flashes and continuous light of various intensities were studied in whole cells of *Chromatium vinosum*.

This paper describes the role and function of a soluble *c*-type cytochrome, *c*-551, which was surprisingly found to act in many ways similar to the cytochrome *c*-420 in *Rhodospirillum rubrum*, described in a previous paper [1]

After the photooxidation of the membrane bound high potential cytochrome *c*-555 by a 10-μs flash, (the low potential cytochrome *c*-552 was kept permanently in the oxidized state) the oxidation of *c*-551 is observed ($t_{\frac{1}{2}} = 0.3$ ms). From a careful analysis of the absorbance difference spectrum and the kinetics it is concluded that there is approximately 0.6–0 7 *c*-551 per reaction center and that essentially all the c^+-555 is reduced via the cytochrome *c*-551. The oxidized–reduced difference spectrum of *c*-551 shows peaks at 551 and 421.5 nm. The reduction of c^+-551 following the flash-induced oxidation is strongly inhibited by HOQNO, but only slightly by antimycin A.

Cytochrome *c*-551 reduces only the oxidized high potential cytochrome *c*-555, which is probably located on the outside of the membrane, on the opposite side of the primary acceptor. The low potential cytochrome *c*-552 does not show any detectable interaction with cytochrome *c*-551. After the cells have been sonicated, no *c*-551 is photooxidized and at least part of the cytochrome occurs in the solution

Analysis of the reduction kinetics of c^+-551 in the absence and presence of external donors suggests that c^+-551 is partly reduced via a cyclic pathway, which is blocked by addition of *o*-phenanthroline, and partly via a non-cyclic pathway. The non-cyclic reduction rate of c^+-551 ($k = 6\ s^{-1}$) is increased approximately 5–10 times upon thiosulphate addition, suggesting a role for *c*-551 between the final donor pool and the oxidized membrane bound *c*-type cytochromes.

Abbreviations HOQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide, MOPS, morpholinopropane sulfonic acid

INTRODUCTION

In a previous paper [1] we have shown that in whole cells of *Rhodospirillum rubrum* a soluble *c*-type cytochrome, *c*-420, can reduce oxidized reaction center bacteriochlorophyll, P^+, ($t_{\frac{1}{2}} = 0.3$ ms). The P^+ reduction kinetics were explained by assuming that there is approximately 1 cytochrome *c*-420 per two reaction centers (1 *c*-420/2 reaction centers) and that one *c*-420 molecule is able to react with at least two P^+ molecules; a careful analysis of the kinetics indicated that *c*-420 was more or less freely diffusable in the cell, which is also in accordance with the easy removal of this cytochrome from the cell by sonic oscillation or French pressing [1, 2] The low potential cytochrome *c*-428 occurs in only a small fraction (about 5 %) of the reaction centers, which seem to be functionally different from those containing the high potential *c*-420.

In whole cells of *Rhodospirillum rubrum* the situation seems to be completely different from that encountered in *Chromatium vinosum* [3, 4] and *Rhodopseudomonas sphaeroides* [5]. In *C. vinosum* the oxidized reaction center bacteriochlorophyll P^+-870 is reduced also by a cytochrome, but with the following differences from *R. rubrum*:

1. Two types of membrane-bound *c*-type cytochromes are present, the low potential cytochrome *c*-552 and the high potential cytochrome *c*-555 [3, 6]. Both are able to donate electrons efficiently to the same oxidized reaction center bacteriochlorophyll and seem to occur in a stoichiometry of (at least) two per reaction center (refs. 7, 8, 9 and see also ref. 30).

2. When both *c*-552 and *c*-555 are in the reduced state before the flash, P^+-870 only oxidizes *c*-552. The reaction between P^+-870 and *c*-552 is monophasic and has a halftime of 1 μs at room temperature [7, 8, 9]; when *c*-552 is in the oxidized state before the flash, P^+-870 oxidizes *c*-555 The reaction is again monophasic, but now the halftime at room temperature is 2–3 μs [10, 11] Both *c*-552 and *c*-555 are tightly bound to the reaction center [12, 13], and even in highly purified reaction centers both cytochromes can still transfer electrons to P^+-870 at rates not much different from those observed in intact organisms.

The reduction of c^+-552 after a flash is slow [3, 6]. This means that the turnover number for *c*-552 is very low, and suggests that this cytochrome hardly contributes to electron transport at "normal" light intensities. *c*-552 is permanently in the oxidized state under donor-depleted conditions [14], or in the presence of oxygen [6].

In this paper we shall describe experiments which were done under conditions in which *c*-552 was oxidized so that no redox change of this cytochrome occurred. Looking for similarities between *R rubrum* and *C vinosum* we have found by a careful spectroscopic and kinetic analysis that in whole cells of *C. vinosum* a diffusable *c*-type cytochrome, *c*-551, is present which turns out to have properties analogous to *c*-420 in *R. rubrum*. We will describe the interaction between *c*-551 and c^+-555 in *C. vinosum* and show that it is similar to that between *c*-420 and P^+-870 in *R. rubrum*

Finally we have shown [1] that in *R. rubrum* part of the oxidized *c*-420 is reduced via a *b*-type cytochrome by some component on the acceptor side, the remainder is reduced via another pathway, possibly by a large donor pool A similar arrangement has been suggested for the reduction of c^+-555 in *C. vinosum* [15]. We have tried to obtain some insight into the identity and stoichiometry of the components involved in this reduction.

MATERIALS AND METHODS

Chromatium vinosum was cultured on a medium described by Hendley [16] in the light of fluorescent lamps (intensity approximately 200 lux) during 3–4 days, after which the suspension was more or less free of the molecular sulphur which was formed initially. Cells were harvested by centrifugation and put in a fresh medium without $Na_2S_2O_3$. In some experiments various concentrations of $Na_2S_2O_3$ were added about 10 min before the experiments were started. Chromatophores of *C. vinosum* were prepared using either sonic oscillation (2×30 s) or a French press. Cell debris was removed by centrifugation for 10 min at $20\,000 \times g$. The chromatophores were collected after further centrifugation for 90 min at $144\,000 \times g$. The chromatophores were suspended in a medium containing 50 mM MOPS and 50 mM potassium chloride, and continuously bubbled with pure nitrogen to obtain anaerobic conditions. Absorbance difference kinetics were measured with a split beam spectrophotometer described earlier [1], on-line connected to a PDP-9 computer, where data were stored and the necessary calculations could be done. Xenon flashes ($t_{\frac{1}{2}} = 10\ \mu s$) or shuttered continuous light were used as actinic illumination

In some experiments *o*-phenanthroline has been added to prevent double turnovers from one xenon flash. Cytochrome *c*-555 photooxidation was complete in 10 μs ($t_{\frac{1}{2}} = 3\ \mu s$) after a short laser flash, or after a xenon flash in the presence of *o*-phenanthroline. In the absence of *o*-phenanthroline approximately 30 % additional cytochrome was photooxidized, with a slower rate, probably due to a second turnover in the tail of the Xenon flash

RESULTS AND INTERPRETATION

Unless mentioned the experiments described in this section were performed after depletion of electron donors in the presence of molecular oxygen. Under these conditions *c*-552, the low potential cytochrome is permanently in the oxidized state and only the high potential cytochrome *c*-555 can be oxidized by P^+-870 after a flash Fig. 1 shows the rereduction of oxidized c^+-555 (measured at 422 nm) after a saturating xenon flash. The decay kinetics at this wavelength are clearly biphasic; the fast component has a halftime of approximately 0.3 ms (Fig. 1, insert). The slow component has a half time varying between 60–120 ms depending on the amount of molecular sulphur still present in the cells, the age of the cells and the oxygen tension.

Fig. 2 shows the spectral changes accompanying this 0.3 ms component. Fig. 2 left gives the results obtained in the Soret peak and Fig. 2 right for the α peak of the cytochrome spectrum. The open squares show the difference spectrum (light—dark) obtained 0.15 ms after a saturating flash and this clearly indicates the photooxidation of *c*-555. The α peak in the difference spectrum was found at 556 nm. This difference spectrum for *c*-555 photooxidation is essentially identical to the one obtained in *C. vinosum* chromatophores at a redox potential of approx 300 mV at which other cytochromes are presumably in the oxidized state so that only *c*-555 can reduce P^+-870. The closed circles give the difference spectrum (light—dark) 3 ms after the flash. In the soret region this spectrum is similar to the previous one (0.15 ms after the flash), although the isosbestic point shifts approximately 0.5 nm, and also the peak shifts to a slightly shorter wavelength. However in the α region the shape of the

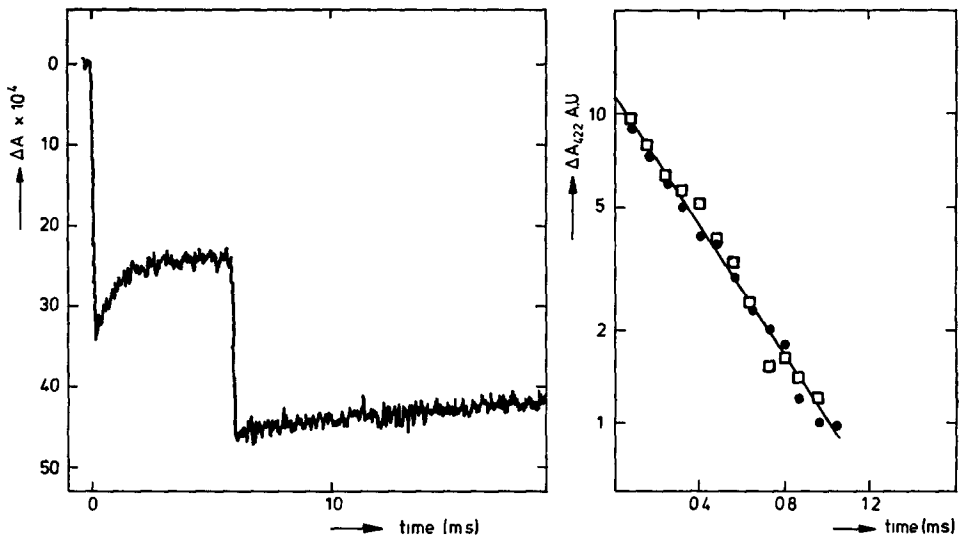

Fig. 1. Kinetics of absorbance changes at 422 nm in two consecutive saturating flashes given at a 6 ms time interval. The absorbance change reflects *c*-555 oxidation and reduction in whole cells of *C. vinosum*, under aerobic conditions without thiosulphate added. The absorbance of the sample is 0.25 at 880 nm, the optical pathlength was 5.0 mm, the flash frequency was 0.07 Hz Fig. 1, insert. Semilogarithmic plot of the fast ($t_{\frac{1}{2}} = 0$ 3 ms) phase of the absorbance increase at 422 nm after the first flash. □ and ● represent experiments with two different samples of one batch

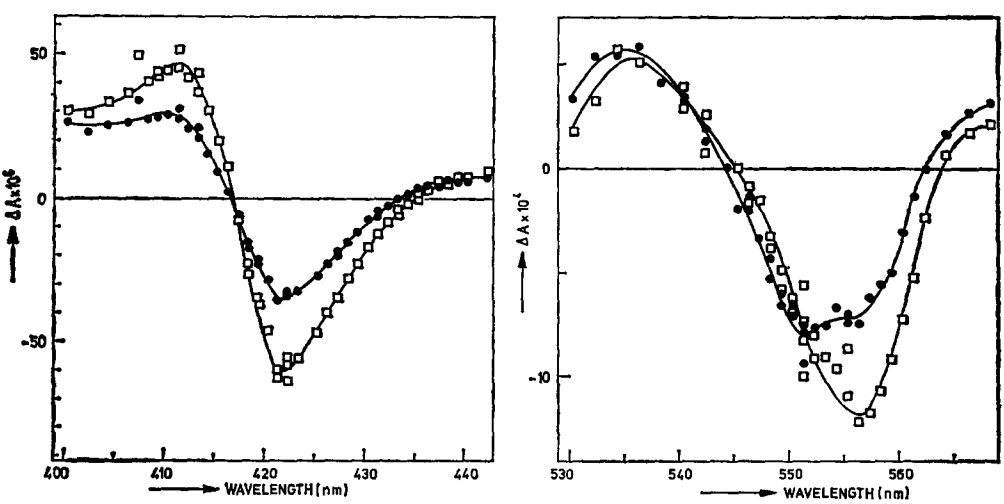

Fig. 2. Absorbance difference spectra in whole cells of *C. vinosum* induced by saturating xenon flashes in the regions 400–440 nm (left) and 530–570 nm (right) at 0.15 ms after the flash (□) and 3.0 ms after the flash (●). The absorbance of the sample was 0.5 at 880. The experiment was done in the presence of 1 mM *o*-phenanthroline (see Materials and Methods). Further conditions as in Fig. 1.

difference spectrum changes completely. Whereas the spectrum 0.15 ms after the flash (closed circles) indicates only *c*-555 oxidation, 3.0 ms after the flash, a new band has developed with the α peak around 551 nm while part of the c^+-555 seems to have been reduced within the same time. We shall call this new pigment showing an absorbance change at 551 nm "*c*-551" and we shall show in the following that *c*-551 is a *c*-type cytochrome with peaks in the oxidized—reduced difference spectrum at 421.5 nm and 551 nm, and that *c*-551 is oxidized by c^+-555.

A second flash given approximately 6 ms after the first again causes *c*-555 oxidation, however now the 0.3 ms component is absent, indicating that *c*-551 is still in the oxidized state and that its concentration is less than or equal to one per reaction center These results can be understood by assuming that *c*-551 can reduce part of c^+-555 with a halftime of approximately 0.3 ms; when a second flash causes again *c*-555 oxidation, no fast reduction occurs because all of the *c*-551 has been oxidized by the first flash.

If a scheme of electron transfer *c*-551 → *c*-555 → *P*-870 proposed in the preceding part is true, and if the redox potentials of the components in this chain increase in the direction of electron transport, one would expect that at low intensities of continuous background illumination, only *c*-551 will be in the oxidized state before the flash, giving rise to a decrease in the amplitude of the 0.3 ms component. At higher intensities *c*-555 also becomes oxidized, which would decrease the initial amplitude for *c*-555 oxidation in a flash. To check whether this is valid we have compared the total amplitude of the flash induced absorbance change at 422 nm with the amplitude of the 0.3 ms component for various intensities of the background illumination. The result is shown in Fig. 3: at low intensities of the background illumination (0.01–0.06 mW/cm^2, λ = 880 nm) the 0.3 ms reduction of c^+-555 by *c*-551 indeed disappears (Fig. 3,

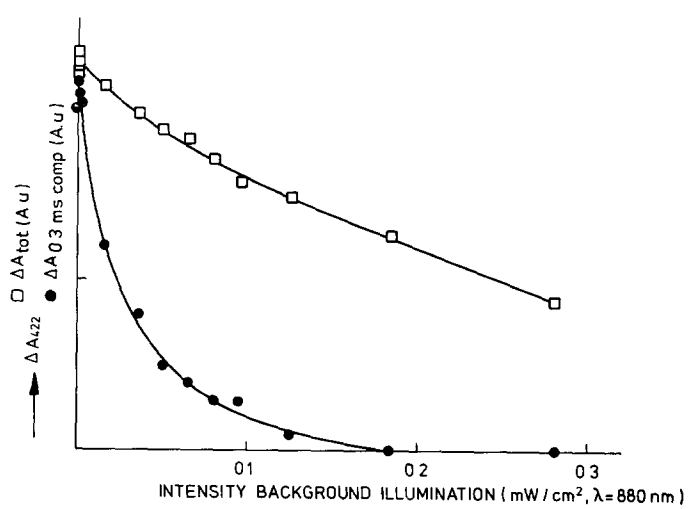

Fig 3 Amplitude of the total absorbance change 0 15 ms after a flash of saturating intensity (□, open squares) and the amplitude of the 0 3 ms component (●) measured at 422 nm as a function of the intensity of the background illumination in whole cells of *C vinosum* The background illumination was switched on 1 second before the flash was given. The absorbance of the sample was 0 25 at 880 nm, the wavelength of the background illumination is 880 nm. Further conditions as in Fig 1.

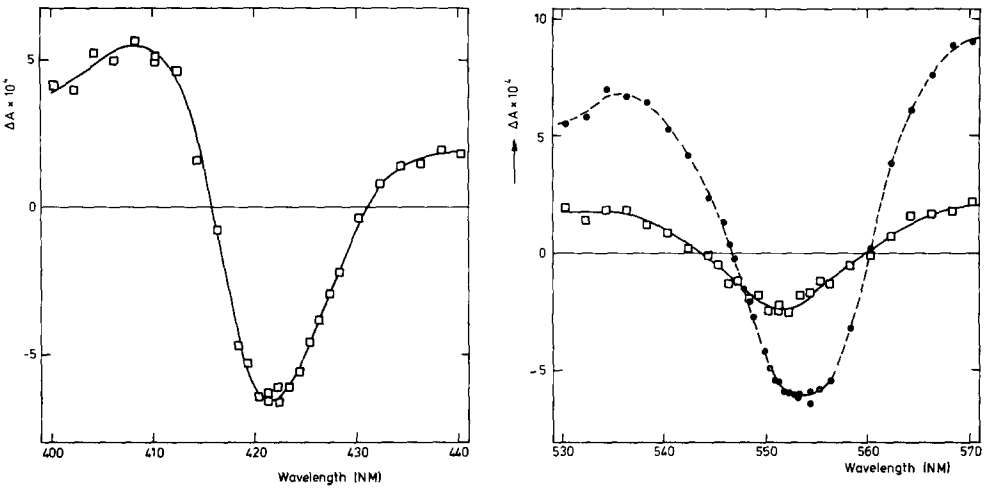

Fig. 4. Absorbance difference spectra (induced by 1 second of continuous illumination in whole cells of *C. vinosum* in the region 400–440 nm (left) and 530–570 nm (right). □, $I = 0\ 02$ mW/cm², $\lambda = 880$ nm, ●, $I = 0.2$ mW/cm², $\lambda = 880$ nm. The absorbance of the sample is 0 25 at 880 nm, further conditions as in Fig 1.

closed circles) while much higher intensities of background illumination are needed ($I > 0.1$ mW/cm², $\lambda = 880$ nm) to decrease the initial amplitude of *c*-555 oxidation (Fig. 3, open squares). From Fig. 3 it is clear that there is essentially no 0.3 ms c^+-555 reduction at a background illumination of 0 06 mW/cm² ($\lambda = 880$ nm), which means that this light intensity is sufficient to largely oxidize the component *c*-551, responsible for the rapid c^+-555 reduction. Fig. 4 shows the light–dark difference spectrum obtained after 1 s of continuous actinic illumination ($I = 0.02$ mW/cm², $\lambda = 880$ nm) and this clearly shows the photooxidation of a *c*-type cytochrome with peaks in the difference spectrum at 421.5 nm, the γ/α peak ratio is approximately 2.5. This γ/α peak ratio for the reduced minus oxidized difference spectrum is rather small but not much different from that obtained for the difference spectrum of *c*-420 in *R. rubrum* whole cells [1]. This result confirms our first tentative conclusion that the 0.3 ms reduction of c^+-555 is concomitant with the oxidation of a *c*-type cytochrome *c*-551. At increasing light intensities the α peak of the light-dark difference spectrum shifted to a longer wavelength; at high light intensities ($I = 0.2$ mW/cm², $\lambda = 880$ nm) the α peak was positioned at 554–555 nm (Fig. 4 right, black circles). This is also in accordance with previous measurements using continuous illumination under aerobic conditions in whole cells of *C. vinosum* [6].

We now felt able to analyze more accurately the difference spectrum shown in Fig. 2, assuming it to be composed of c^+-555, c^+-551 and some contribution from the carotenoid shift. This last change is probably small at these wavelengths, and we assume it to be constant. This is of course an approximation, but experiments in chromatophores of *C. vinosum* using valinomycin to increase the rate of decay of the transmembrane electric field [18], indicated no sharp bands in the difference spectrum in the region 540–560 nm which could be attributed to the carotenoid shift. From the difference spectrum registrated at different times after the flash, we then could calcu-

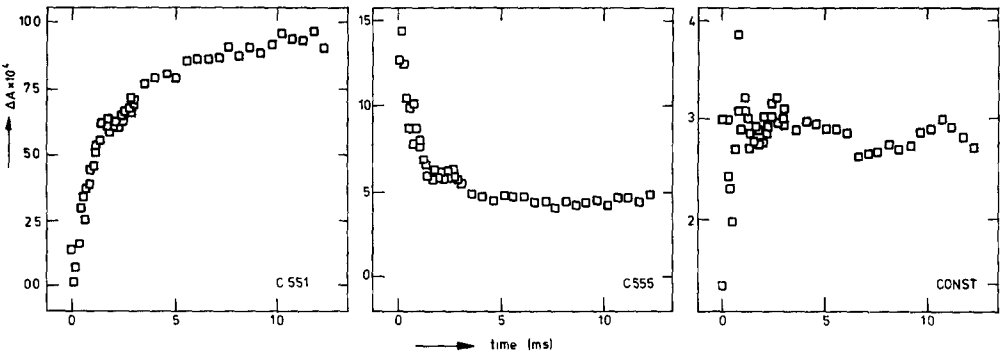

Fig. 5 Calculated kinetics of flash induced *c*-551 oxidation (left), c^+-555 reduction (middle) and the time course of a third component, which is approximately constant at various wavelengths (see text) as calculated from difference spectra shown in Fig 2, taken at several times after the flash. The difference spectra used to fit the experimental data were the difference spectrum of *c*-551 (light−dark) obtained after 1 s of weak (0.02 mW/cm², $\lambda = 880$) continuous illumination; the difference spectrum of *c*-555 (light−dark) was obtained in chromatophores of *C. vinosum* in the presence of 2 μM valinomycin and 10 mM KCl, 1 ms after a saturating flash at an ambient potential of 300 mV. We used the following method to calculate these results with the computer. The difference spectra for *c*-551, *c*-555 and the constant function of wavelength (all normalized to 1) were called $\vec{N}_1$, $\vec{N}_2$ and $\vec{N}_3$, respectively. The i'th element of each vector represents the absorbance change of each component of wavelength λ_i (i runs from 1 to the maximum number of wavelengths). The spectra measured were called $\vec{M}_t$, the i'th element of each spectrum represents the absorbance change measured at wavelength λ_i and at time t after the flash or after the continuous light is turned on Now at each time t the following relationship holds $\vec{M}_t = \vec{N}_1 \cdot x_t + \vec{N}_2 \; y_t + \vec{N}_3 \cdot z_t$, where x_t, y_t and z_t represent the amount of c^+-555, c^+-551 and constant function respectively at time t. These three numbers can be seen as the elements of a column vector $\vec{S}_t = \begin{bmatrix} x_t \\ y_t \\ z_t \end{bmatrix}$ and the three vectors $\vec{N}_i$ can be seen as the rows of the matrix $\vec{\vec{N}}$. The relationship can be written as $\vec{M}_t = \vec{N} \; S_t$ Because the number of equations (e g equal to the number of recordings, or the dimension of M_t) is in general much larger than the number of unknown variables (3) in most cases no exact solution of this problem exists. But an approached solution for $\vec{S}_t$ can be found in such a way that $\Sigma_i (\vec{M}_t - \vec{\vec{N}} \cdot \vec{S}_t)_i^2 =$ minimum using the method of calculation given in ref. 19.

late the kinetics of c^+-551 and c^+-555. Fig. 5 shows the result of this calculation; Fig. 5 left represents the concentration of c^+-555 as a function of time, the middle figure the concentration of c^+-551 and the right figure the carotenoid shift component. From these results it is obvious that the oxidation of *c*-551 is essentially concomitant with the partial rereduction of c^+-555, the third component shows no time dependence (on this time scale) which is confirmed by kinetic measurements at 490 nm. The calculated half time for c^+-555 reduction is approximately 0.4 ± 0.1 ms which is in accordance with the decay time at 422 nm (Fig. 1, insert). From the kinetics for *c*-551 oxidation and c^+-555 reduction we estimate that *c*-551 reduces 60–70 % of the c^+-555. Similar calculations as shown in Fig. 5 using difference spectra obtained after double flashes spaced 6 ms apart indicate that on the second flash again *c*-555 is oxidized, the amplitude is approximately 70 % of that on the first flash, but no *c*-551 oxidation is detectable. The result of the calculation is in good agreement with the experiment shown in Fig. 1. The kinetics displayed in Fig. 5 allow us to calculate the reduced minus oxidized

extinction coefficient of *c*-551: $\varepsilon(551)_{red\text{-}ox} = 13\ mM^{-1} \cdot cm^{-1}$ using an extinction coefficient $\varepsilon(556)_{red\text{-}ox} = 15\ mM^{-1} \cdot cm^{-1}$ for cytochrome *c*-555 [20].

The kinetics observed for the reaction between c^+-555 and *c*-551 are analogous to those obtained before [1] in *R. rubrum* whole cells for the reaction between P^+ and cytochrome *c*-420. There we found that the ratio *c*-420/P^+ was 0.5 and that each *c*-420 molecule was able to reduce at least two P^+ molecules (a detailed analysis of the kinetics suggested that the *c*-420 was more or less freely diffusable among 4 or more reaction centers). To check whether a similar model could account for the reaction between c^+-555 and *c*-551 we measured the difference spectrum with flashes of several light intensities. Fig. 6 shows the difference spectrum obtained after a weak flash, which oxidized approximately 25 % of the *c*-555 which could be oxidized after a saturating flash. This spectrum indicates that now all the c^+-555 is rereduced with a halftime of 0.3 ms by *c*-551; the difference spectrum taken 3 ms after the flash does not show an appreciable contribution of c^+-555, in contrast to the spectrum after a saturating (100 %) flash (Fig. 2, closed circles). Repeating this experiment using flashes of various intensities has enabled us to calculate the amount of c^+-555 which is still in the oxidized state 3 ms after the flash. Table I gives the results of these computations, which are analogous to the results obtained from a similar experiment we have performed with whole cells of *R. rubrum* [1], indicating again that in whole cells of *C. vinosum* one *c*-551 molecule can reduce at least two c^+-555 molecules located on different reaction centers If c^+-555 is reduced exclusively by *c*-551 the rate of the slow

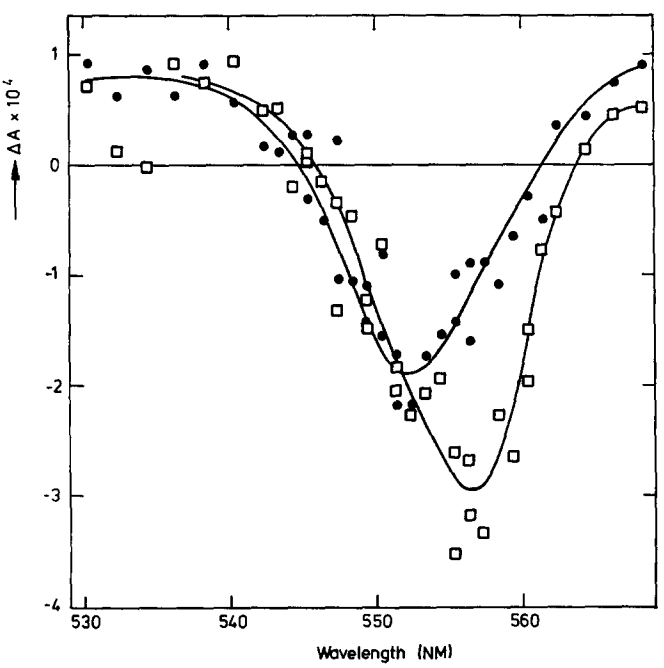

Fig. 6. Absorbance difference spectra induced by weak (2.6 %) xenon flashes in whole cells of *C. vinosum* in the region 530–570 nm, at 0 15 ms after the flash (□) and 3 0 ms after flash (●). The absorbance of the sample is 0.5 at 880 nm The experiments were done in the presence of 1 mM of *o*-phenanthroline. Further conditions as in Fig. 1.

TABLE I

c-555 and *c*-551 photooxidation in whole cells of *C vinosum* as a function of flash intensity The data have been calculated from the kinetics at a number of wavelengths in the region 530–570 nm after flashes of various intensities, analogous to the computations used for the results shown in Fig 5. The percentages are the number of oxidized cytochrome molecules per reaction center (in percent) First column flash intensity in percent of maximum; second column: amount of *c*-555 oxidized 0 15 ms after the flash, third column amount of *c*-555 still in the oxidized state 3.0 ms after the flash, fourth column· amount of *c*-551 oxidized 3 0 ms after the flash.

Flash intensity (%)	c^+-555 (t = 0.2 ms) (%)	c^+-555 (t = 3 ms) (%)	c^+-551 (t = 3 ms) (%)
100	100	37	63
8 4	72	20	52
5 1	51	12	39
2.6	24	1	23

phase in the reduction of c^+-555 after a strong flash will be limited by the rate of c^+-551 reduction and thus will be the same as the reduction rate of c^+-551 after a weak flash. This is indeed the case as Fig. 7 shows. The calculated c^+-555 reduction rate (Fig. 7, open squares) is identical to the rate of c^+-551 reduction after a weak flash (Fig. 7, closed circles).

Finally we have studied some of the kinetic properties of the *c*-551 cytochrome. The 0.3 ms reduction of c^+-555 by *c*-551 is not notably affected by *o*-phenanthroline, HOQNO and antimycin A The reduction of c^+-551 is strongly inhibited by HOQNO

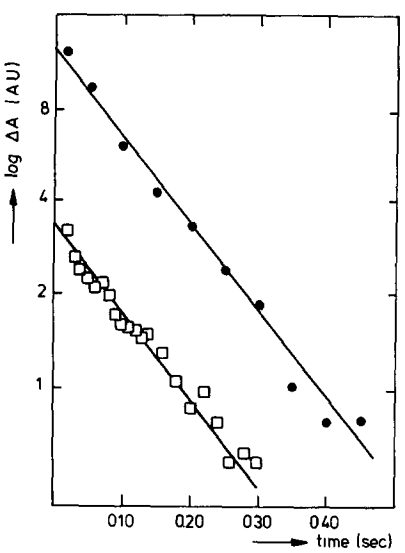

Fig 7. Semilogarithmic plot of the slow phase of the reduction of c^+-555 (□), calculated in a similar way as described in the legends of Fig. 5, and of the reduction of c^+-551 (●) after a weak (2.6 %) intensity flash, measured at 422 nm in whole cells of *C vinosum*. The absorbance of the sample was 0.5 at 880 nm. Experiments were done in the presence of 1 mM *o*-phenanthroline Further conditions as in Fig 1.

TABLE II

Initial rate of c^+-551 reduction as a function of concentration of HOQNO (A), antimycin (B) and $Na_2S_2O_3$ (C) calculated from the kinetics at 422 nm after weak (2 6 %) intensity flashes During the experiments reported under C, 1 mM *o*-phenanthroline was present in order to minimize cyclic reduction (via the primary electron acceptor) so it was possible to study exclusively the reaction of c^+-551 with the exogeneous donor (see text) If *o*-phenanthroline was left out, the initial rate of c^+-551 reduction in the absence of $Na_2S_2O_3$ was much higher (K_{in} = 10–20 s^{-1}), but the reaction was biphasic (slow phase k = 4–8 s^{-1}) Only the slow phase, which was still present after the addition of *o*-phenanthroline (see Fig. 8) was then stimulated by $Na_2S_2O_3$ addition

(A) HOQNO	$K_{in}(s^{-1})$	(B) antimycin	$K_{in}(s^{-1})$	(C) $Na_2S_2O_3$	$K_{in}(s^{-1})$
0	12 0	0	12 2	0	8 2
10^{-7}	12 8	10^{-7}	12 5	10^{-7}	8 3
$3 \cdot 10^{-7}$	13 0			$3 \cdot 10^{-7}$	8 3
10^{-6}	12 0	10^{-6}	11 8	10^{-6}	8 5
$3 \cdot 10^{-6}$	10.9			$3 \cdot 10^{-6}$	8.1
10^{-5}	8 1	10^{-5}	10 1	10^{-5}	8.7
$3 \cdot 10^{-5}$	5 2			$3\ 10^{-5}$	12 6
10^{-4}	1 9	10^{-4}	9 3	10^{-4}	20 8
$3\ 10^{-4}$	2 2			$3 \cdot 10^{-4}$	34.5
				10^{-3}	42 0
				$3\ 10^{-3}$	42.2

(Table II, column 1) but only very little by antimycin A (Table II, column 2). A possible reason for this may be that antimycin A is a specific inhibitor of cytochrome *b*-associated electron transport [21]. We have not been able to find a trace of cytochrome *b* oxidation/reduction under various conditions, within the limits of precision of our experiments (1 cytochrome *b* per 20 reaction centers might have been detected). HOQNO inhibits strongly the two reducing pathways of *c*-420 in *R. rubrum* [1] and appears to have a similar effect on the reduction of *c*-551 in *Chromatium vinosum*.

We have also investigated whether the *c*-555–*c*-551 chain is involved in the oxidation of a reduced sulphur compound like $Na_2S_2O_3$, via a non-cyclic pathway. Table II, column 3 shows that the initial rate of c^+-551 reduction increases with increasing concentrations of $Na_2S_2O_3$. The initial rate is not linear with $Na_2S_2O_3$ concentration which indicates that the c^+-551 does not react directly with the $Na_2S_2O_3$ but perhaps via an intermediate enzyme system. From our experiments it is not clear whether *c*-551 is also functioning in a cyclic pathway analogous to *c*-420 in *R. rubrum*. The only evidence for this so far comes from the reduction of c^+-551 in the absence (Fig. 8, trace A) and in the presence (Fig. 8, trace B) of *o*-phenanthroline. In the absence of *o*-phenanthroline and $Na_2S_2O_3$ we observe biphasic kinetics for c^+-551 reduction (fast phase, with k = 10–15 s^{-1}, slow phase with k = 4–8 s^{-1}), however in the presence of 10^{-3} M *o*-phenanthroline, which blocks electron transport from X^- towards a secondary acceptor [17, 22] only the slow (k = 4–8 s^{-1}) phase is present. Experiments with very low concentrations of $Na_2S_2O_3$ indeed indicated that it was this slow component which became faster after the addition of $Na_2S_2O_3$. These experiments indicate that *c*-551 is involved not only in non-cyclic, but also in cyclic electron transport via a secondary acceptor, probably ubiquinone [22]: At the moment we do not know whether there are other components involved in this cyclic pathway. Our experiments appear to exclude a cytochrome *b*, which operates in this cycle in

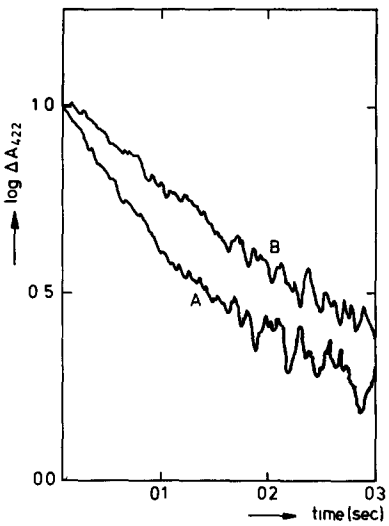

Fig. 8. Reduction of c^{+}-551 after a weak (2.6 %) intensity flash measured at 422 nm in the absence (trace A) and presence of 1 mM *o*-phenanthroline (trace B) in whole cells of *C vinosum*. The absorbance of the sample was 0 5 at 880 nm Further conditions as Fig. 1.

many other photosynthetic bacteria [1, 24, 25], and has also been suggested to function in *C. vinosum* chromatophores [26].

DISCUSSION

In Fig. 9 we have summarized the electron transport schemes for whole cells of *C. vinosum* (Fig. 9A) and for whole cells of *R. rubrum* (Fig. 9B). The scheme in Fig. 9A is based on results obtained by several authors. The conclusion that *c*-552 and *c*-555 were both directly oxidized by *P*-870 and that *c*-555 participated in non-cyclic electron transport was first drawn by Duysens [3] and confirmed by more direct kinetic evidence by Parson and coworkers [7, 9]. Other authors provided evidence for the functioning of the iron-quinone complex as the primary acceptor ([refs. 13, 27]; see for a review also [ref. 28]) and for the bacteriopheophytin as an intermediary acceptor [29, 30, 31]. Ubiquinone also functions as the secondary acceptor pool [23, 32]. The location of the electron carriers with respect to the membrane is as suggested by Dutton and Prince [33], who reinterpreted results obtained by Case and Parson [18] on carotenoid bandshifts. In this paper for the first time the role of a cytochrome *c*-551, with rather unusual properties, is decribed. Although it is present in a concentration of less than one per reaction center all electron transport via *c*-555 is mediated by this cytochrome, suggesting that *c*-551 is a mobile electron carrier. Cytochrome *c*-551 reacts both non-cyclic with $Na_2S_2O_3$ and cyclic. There were no indications for the participation of a b-type cytochrome in the photosynthesis of intact whole cells of *C. vinosum*. The location of *c*-551 with respect to the membrane is probably in the periplasmic space between the cell wall and the cell membrane as suggested by Prince et al. for *Rhodopseudomonas capsulata* [34] and similar to the position of *c*-420 in *R. rubrum*. The scheme in Fig. 9B is a copy of the scheme for whole cells of *R. rubrum*

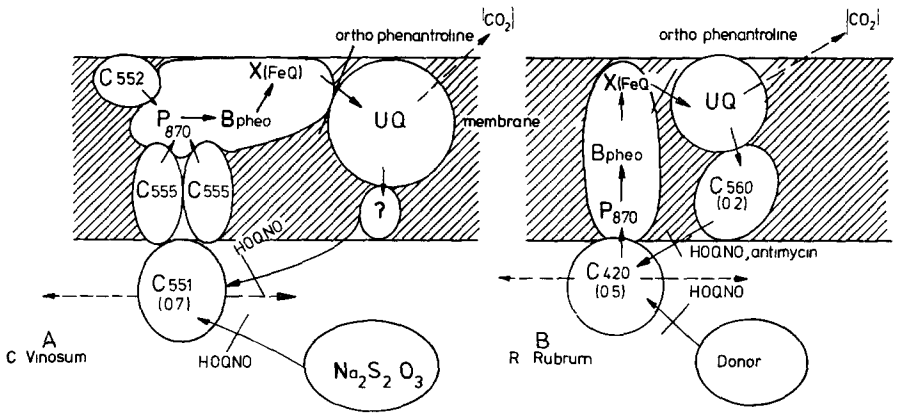

Fig 9 Comparison of the electron transport schemes for whole cells of *C vinosum* and *R rubrum* The construction of this figure is explained in the text. The second photosystem present in a small amount in *R rubrum* [1] has been omitted The function of *c*-552 is hitherto unknown It may function at very low light intensities as suggested for *c*-428 in the second photosystem of *R rubrum* The number written below *c*-551, *c*-560 and *c*-420 represents the number of cytochrome molecules present per reaction center

given in a previous paper [1] for the reaction center containing the high potential cytochrome *c*-420.

The electron transport schemes as shown in Fig. 9 for *R. rubrum* and *C. vinosum* appear in certain aspects rather similar. In both species there is a mobile cytochrome (*c*-551 and *c*-420) which is involved in both cyclic and non-cyclic electron transport. The turnover times for both cytochromes, which are determined by the rates of the cytochrome reducing reaction are, under optimal conditions (excess amount of donor present) similar and are approximately 20 ms at room temperature. Also the oxidation times are in the same time range: 0 2–0 3 ms A cytochrome having the same spectral properties as the *c*-551 functioning in intact cells can be isolated readily from *C. vinosum* [35]. When chromatophores are prepared by sonic oscillation, *c*-551 comes out, as does *c*-420 in *R. rubrum*.

However, there are also some differences between *c*-551 in *C. vinosum* and *c*-420 in *R. rubrum*. When the results in Table I are compared in detail with the analogous experiment for *R. rubrum*, one observes that in the experiments with *R. rubrum* the slow phase in the P^+-870 reduction disappears when approximately 35 % or less of the reaction centers are hit by the flash. This suggests that the reduced cytochrome *c*-420, which is present in a ratio of 0.5 *c*-420/reaction center can reach almost any reaction center rapidly (within 0.3 ms) In the extreme case where any cytochrome molecule has fast access to all reaction centers complete rapid P^+ reduction will occur if the ratio of the number of hits/reaction center is less than the number of *c*-420/RC. In that case for *R rubrum* the maximum flash intensity which would still give complete fast P^+ reduction, would photooxidize 50 % of the *P* present. If on the other hand each *c*-420 molecule has access to only a limited number of reaction centers it would be necessary to lower the flash intensity further to observe complete rapid reduction of the P^+ molecules, provided of course that each reaction center is accessible in principle to at least one *c*-420.

Thus we can see that the ratio $M_{c\text{-}420}$ of the maximum number of hits to give still a fast P^+ reduction and the number of reduced *c*-420 molecules before the flash (with $M \leqslant 1$) gives information about the mobility of the soluble cytochrome. For *R. rubrum* whole cells this comes out as $M_{c\text{-}420} = 0.35/0.5 = 0.7$. This value is close to that obtained from the freely diffusable cytochrome model. In the case of *C. vinosum* using the results given in Table I we can calculate for the reaction between *c*-551 and c^+-555 that the highest flash intensity at which almost complete fast c^+-555 reduction occurs, gives approximately 25 % hits. There was in the experiment of Table I 0.63 *c*-551 (in the reduced state)/reaction center so this gives $M_{c\text{-}551} = 0.25/0.63 = 0.4$. This is a much lower value than that obtained for *c*-420 in *R. rubrum*, which indicates that a smaller number of reaction centers are accessible to each *c*-551. This restricted accessibility may be caused by a stronger binding of *c*-551 to a limited part of the membrane. That *c*-551 is rather tightly bound to the membrane is also apparent when chromatophores are prepared using a French press; in *C. vinosum* approximately 80 % of the *c*-551 remains bound to the chromatophore membrane; in *R. rubrum* less than 20 % of *c*-420 remains bound.

The fact that *c*-551 can be retained in chromatophores of *C. vinosum* allows us to study the thermodynamic properties of this cytochrome as it presumably occurs in situ. We are currently investigating this and preliminary results indicate an $E_{m,7} = 260 \pm 10$ mV. This would enable the *c*-551 cytochrome to reduce the photooxidized c^+-555 ($E_{m,7} = 350$ mV [36]) by more than 90 %.

Because of the variability in concentration of an essential, mobile cytochrome like *c*-551 in *C. vinosum* and *c*-420 in *R. rubrum* in chromatophore preparations, with which many electron transport and phosphorylation experiments have been done, at least some of the earlier conclusions based on such preparations will have to be revised. Specifically most schemes based on such experiments (e.g. ref. 33 Fig. 6, or ref. 37 Fig. 49) lack the type of non-cyclic electron transport shown in Fig. 9. In the schemes for *C. vinosum* [33, 37] not only *c*-551 is absent which has a key role in the intact cells but in many cases a b-type cytochrome [26, 33] is placed in the cyclic chain for which we could get no evidence in the intact cells. More experiments have to be done to clarify the precise role of the soluble cytochromes in photophosphorylation.

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