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THE CYTOCHROME SYSTEM OF HETEROTROPHICALLY-GROWN *RHODOPSEUDOMONAS SPHEROIDES*

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SUMMARY

Rhodopseudomonas spheroides was grown aerobically in the dark in continuous culture yielding cells with a low bacteriochlorophyll content. The supernatant obtained after cell breakage contained a mixture of cytochromes, apparently of the *c*-type; these were resolved by spectroscopy at 77°K into three components. The positions of the α, β and γ bands in reduced *minus* oxidised difference spectra at 77°K were 547, 520 and 417 nm; 549, 520 and 420 nm and 551, 521 and 421 nm, respectively. The 547-nm cytochrome was not readily reduced by ascorbate/tetramethyl-*p*-phenylene diamine (TMPD) and the 551-nm cytochrome was autoxidisable. Both the 547- and the 549-nm cytochrome were found in the particulate fraction and both were reduced by NADH or succinate in the aerobic steady state and became more oxidised in the presence of antimycin or 2-heptyl-4-hydroxyquinone-*N*-oxide (HQNO). CO difference spectra of the soluble fraction indicated that it also contained cytochromoid *c*; maxima were obtained at 569, 539 and 416 nm.

The particles were found to contain a mixture of cytochromes. Reduced *minus* oxidised spectra showed bands at 607 and 445 nm and there was a trough in CO difference spectra at 445 nm indicating the presence of an *a* type terminal oxidase. Components reacting more slowly with CO yielded maxima at 540 and 570 nm with a trough at 428 nm and sometimes a maximum at 416 nm. These may be due to a mixture of CO-binding pigments in the particles. The α-peak of the *b*-type cytochromes was asymmetrical in low-temperature difference spectra. Two components were separated spectroscopically; the main component was substrate-reducible and absorbed at 560 nm (557 nm at 77°K); a second component absorbed at 565 nm (562 nm at 77°K) and was found in dithionite-reduced *minus* substrate-reduced spectra.

The small amount of bacterial chlorophyll present in the particles was photosynthetically active. In the presence of antimycin, illumination caused *c*-oxidation and the reduction of both *b*-type components.

Abbreviations: TMPD, tetramethyl-*p*-phenylene diamine; DCIP, 2,6-dichlorophenolindophenol; HQNO, 2-heptyl-4-hydroxyquinone-*N*-oxide; TTFB, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole; DTFB, 4,5-dichloro-2-trifluoromethylbenzimidazole; MES, 2(*N*-morpholino)ethane sulphonic acid.

INTRODUCTION

The purple non-sulphur bacterium *R. spheroides* is a facultative photoheterotroph. When grown anaerobically in the light the cells contain bacteriochlorophyll and carotenoids associated with the numerous cytoplasmic vesicles (chromatophores) that are characteristic of the photosynthetic form; under aerobic conditions the number of pigment-containing vesicles declines and pigment synthesis is suppressed[1]. This organism is therefore useful in studying the adaptive formation of organelles concerned in photosynthesis. As a preliminary to such work we have examined the nature of the haem proteins present in *R. spheroides* grown aerobically in continuous culture.

Previous workers[2–4] have shown that the terminal oxidase of aerobically grown *R. spheroides* is of the *a*-type but it has recently been shown that under semi-aerobic conditions a CO-binding pigment of the *o*-type developed[5]. We have evidence for the presence of both these oxidases in addition to the well-characterised autoxidisable haem protein cytochromoid *c* and an autoxidisable cytochrome of the *c*-type that may be the same as that isolated by ORLANDO[6] and have also shown by spectroscopy at 77°K that the aerobically grown organism contains two other cytochromes of the *c*-type and several cytochromes of the *b*-type. The spectroscopic and electron transport properties of these pigments are described in this communication.

MATERIALS AND METHODS

Growth of organisms. R. spheroides, Strain 2.4.1, was grown in the dark, in continuous culture, in the medium of SISTROM[7]. The culture vessel was maintained at 30° and vigorously aerated and stirred. Under these conditions the cells still contained some bacteriochlorophyll, but less than 4 % of that present in illuminated anaerobically grown cultures and no vesicles were detected by electron microscopy. In order to prevent changes caused by anaerobic conditions in the collecting vessel this was maintained at −10°. Samples from the growth chamber were regularly checked for purity by aerobic growth on agar plates and by subsequent illuminated anaerobic growth.

Preparation of particulate and soluble fractions. Harvested cells were washed and resuspended in 100 mM KCl, 20 mM 2(*N*-morpholino)ethane sulphonic acid (MES), pH 6.8 and disrupted by two passages through a French pressure cell at 10 tons/inch^2. The extract was centrifuged at 20 000 × *g* for 10 min to remove unbroken cells and debris and the supernatant recentrifuged at 100 000 × *g* for 80 min. The clear supernatant was pipetted off and recentrifuged at 100 000 × *g* for 120 min to remove small contaminating particles; the main particulate fraction was washed once in 100 mM KCl, 20 mM MES (pH 6.8).

Bacteriochlorophyll was determined after extraction into acetone–methanol using the constants given by CLAYTON[8].

Protein was determined by the method of LOWRY *et al.*[9].

Enzyme assays. Oxidase activity was measured in 50 mM Tris–HCl (pH 8.0) using a Clark-type oxygen electrode in a 5-ml reaction vessel maintained at 22°. Cytochrome *c* reductase was measured at 22° as the intial rate of reduction of horse heart cytochrome *c*, in a dual-wavelength spectrophotometer at 551 *minus* 540 nm,

using a $\Delta\varepsilon_{mM}$ reduced *minus* oxidised of 19.1 (ref. 10). Reoxidation of reduced cytochrome *c* was prevented by adding 1.6 mM KCN to the 50 mM Tris–HCl (pH 8.0), used for the assay.

Absorption spectra. Difference spectra of pigments were obtained at room temperature and at 77°K using a split beam spectrophotometer constructed by Dr. P. B. Garland of this department. The monochromator had a reciprocal dispersion of 1.3 nm/mm slit width and the slits were normally kept at 1.0 mm. For spectroscopy at 77°K the light path was 2 mm, at room temperature it was 10 mm.

Dual-wavelength spectrophotometry. The apparatus was a modification of the basic design of CHANCE[11] to permit actinic illumination of the sample by far red light[12]. The full-scale response time of the Rikadenki potentiometric recorder used was 1 sec.

Chemicals. 4,5,6,7-Tetrachloro-2-trifluoromethylbenzimidazole (TTFB) and 4,5-dichloro-2-trifluoromethylbenzimidazole (DTFB) were gifts from Fisons Pest Control Ltd., piericidin A was a gift from Dr. K. Folkers.

RESULTS

Difference spectra of particulate and soluble fractions from dark aerobically grown R. spheroides

The dithionite-reduced *minus* oxidised spectra of the particulate fraction at room temperature and at 77°K (Fig. 1) suggest that the cytochromes of aerobically grown *R. spheroides* are a mixture of *a*-, *b*- and *c*-type. The absorption maxima at about 607 and 445 nm indicate the presence of an *a*-type cytochrome and the room

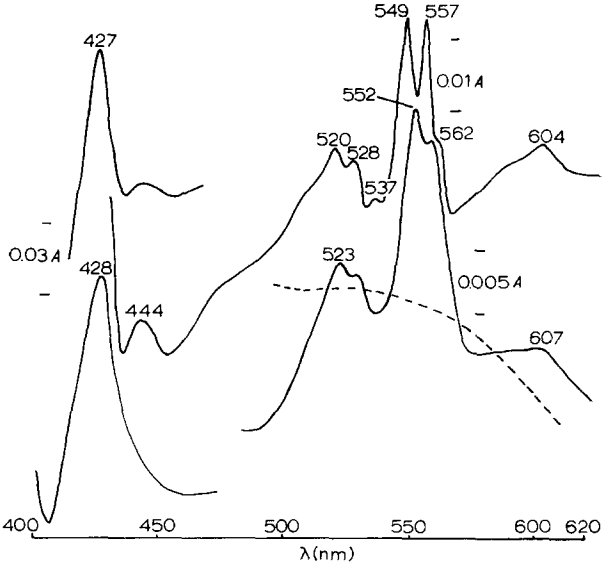


Fig. 1. Reduced *minus* oxidised difference spectra of particles prepared from aerobically grown *R. spheroides*. The particles were suspended at a concentration of 1 mg protein/ml in 100 mM KCl, 20 mM MES (pH 6.8). Lower trace; dithionite added to test, no addition to reference cuvette, recorded at room temperature. Upper trace; dithionite to test, 40 μM potassium ferricyanide to reference cuvette, recorded at 77°K. -------, baseline.

temperature maxima at 560 and 552 nm (with associated β peaks at 531 and 523 nm) suggest the presence of *b*- and *c*-type cytochromes. These assignations are supported by the spectra at 77°K, characteristically intensified and moved to slightly shorter

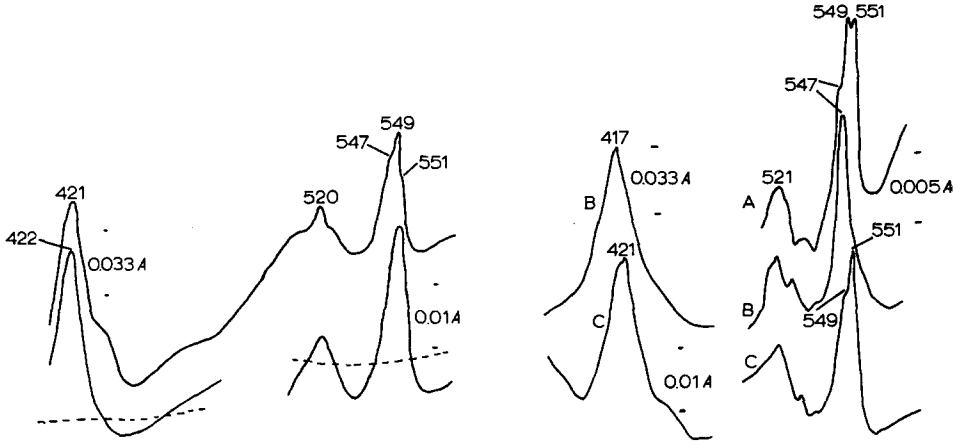


Fig. 2. Reduced *minus* oxidised difference spectra of the soluble fraction prepared from aerobically grown *R. spheroides*. Cells were disrupted in a French pressure cell at 10 tons/inch^2 in 100 mM KCl, 20 mM MES (pH 6.8). The soluble fraction (30 mg protein/ml) was the supernatant obtained after prolonged high-speed centrifugation (see MATERIALS AND METHODS). Dithionite was added to the test cuvette, potassium ferricyanide (40 μM) to reference cuvette. Upper trace, 77°K; lower trace, room temperature. -------, baseline.

Fig. 3. Spectroscopic separation of the mixture of cytochromes present in the soluble fraction from aerobically grown *R. spheroides*. The soluble fraction was prepared as described in Fig. 2. All spectra at 77°K. A, ascorbate (1 mM) + DCIP (15 μM) to test, ferricyanide (40 μM) to reference. B, dithionite to test, ascorbate (1 mM) + TMPD (25 μM) to reference. C, ascorbate (1 mM) + TMPD (25 μM) to test, no addition to reference.

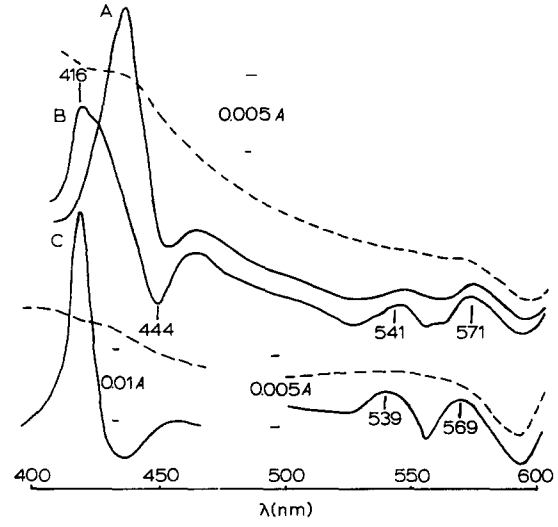


Fig. 4. CO difference spectra of particulate and soluble fractions from aerobically grown *R. spheroides*. A and B, particles (1 mg protein/ml) in 100 mM KCl, 20 mM MES (pH 6.8) reduced with dithionite, CO bubbled into test cuvette; A, 1 min after CO addition; B, 10 min after CO addition. C, soluble supernatant (12 mg protein/ml) reduced with dithionite, CO bubbled into test cuvette, spectrum recorded after 10 min. -------, baseline before CO addition.

wavelengths, with α peaks at 557 and 549 nm and β peaks at 528 and 520 nm[13]. Close examination of the low-temperature spectrum suggested the presence of an additional pigment, with a shoulder at 562 nm and a β peak at 537 nm. Later work (*cf.* Fig. 8) confirmed the presence of this pigment. The reduced *minus* oxidised difference spectrum of the high-speed supernatant at room temperature was very like that of cytochrome *c* with maxima at 552, 523 and 421 nm (Fig. 2). At 77°K there was an indication that other materials might be present: shoulders were visible on both the long and short wavelength side of the 549-nm maximum. By the use of suitable difference spectra it proved possible to resolve what appear, from the positions of their absorption maxima, to be three cytochromes of the *c*-type in this high-speed supernatant (Fig. 3). One component with absorption maxima at 551, 521 and 421 nm at 77°K is apparently autoxidisable and is reduced by ascorbate/TMPD. The second minor component has absorption maxima at 547, 520 and 417 nm at 77°K; it appears to be of lower potential since it is not well reduced by ascorbate/TMPD and appears in dithionite *minus* ascorbate/TMPD reduced spectra. The 547- and 549-nm pigments were also found in the particulate fraction. The absorption maxima for the "551" component obtained in room-temperature difference spectra were at 554, 526 and 422 nm.

CO difference spectra

CO difference spectra of the particulate and soluble fractions are shown in Fig. 4. The soluble fraction shows broad maxima at 569 and 539 nm with a trough at 554 nm and an intense band at 416 nm. These agree well with those published for CO difference spectra of cytochromoid *c* from a variety of photosynthetic organisms[14]. Results with the particles tend to be in agreement with the recently published suggestion[5] that both an *a*-type and an *o*-type of oxidase may be present in *R. spheroides*. The trough at 445 nm, which is invariably present, develops very rapidly and is characteristic of an *a*-type pigment.

The more slowly developing bands in the region of 540 and 575 nm may be due to components from both cytochrome *o* and cytochromoid *c*. As shown in Fig. 4 these bands develop concurrently with a shift of the absorption maximum in the Soret region to a shorter wavelength around 416 nm, while in some samples after a similar time interval a trough at 428 nm was seen. A CO difference spectrum of the soluble fraction showed absorption maxima in the 540- and 570-nm region, and a well-defined peak at 416 nm. The complex nature of the changes seen in the particulate fraction could thus well be due to the presence of three CO-binding components, cytochromes *o* and *a* and cytochromoid *c*.

Rates of oxidation of NADH were significantly lower than rates of succinate oxidation by aerobic particles (Table I); differences in the rates of reduction of cytochrome *c* by either substrate were less marked. Determinations of respiratory activity did not always give consistent results from batch to batch, but the general pattern of low NADH oxidation rates remained the same, and contrasts with results obtained by us with particles from photosynthetically grown particles[15]. The soluble diaphorase activity was high in the supernatant fraction suggesting that this is not a function associated with photosynthesis.

The effect of various inhibitors upon respiratory enzymes of *R. spheroides* are summarized in Table II and some differences from their effects upon mitochondrial

electron transport are apparent. There is a relatively large proportion of rotenone- or piericidin A-insensitive NADH oxidation, but NADH oxidation is more sensitive to HQNO inhibition than is succinate oxidase. In contrast, succinate oxidase is more sensitive than NADH oxidase to antimycin A. DTFB and TTFB both inhibit succinate or NADH oxidation. These effects of substrates and inhibitors were then examined spectroscopically.

The addition of succinate (or NADH) causes a steady-state reduction of cytochromes *c*552 and *b*560 (Fig. 5); with the onset of anaerobiosis a new increased level of reduction of cytochromes *b* and *c* is attained together with the reduction of the *a*-type pigment, as indicated by increased absorption at 607 and 445 nm.

TABLE I

RESPIRATORY ACTIVITIES OF PARTICLES AND SOLUBLE FRACTIONS PREPARED FROM AEROBICALLY GROWN *R. spheroides*

Rates of O_2 uptake were measured, using an oxygen electrode, in 50 mM Tris–HCl (pH 8.0) at 25° with 2 mM succinate or 0.8 mM NADH as substrates added to start reaction. Cytochrome *c* was measured at 25° in a dual-wavelength spectrophotometer (551 nm *minus* 540 nm) in 50 mM Tris–HCl (pH 8.0) containing 1.6 mM KCN; horse heart cytochrome *c* (33 μM) was used as electron acceptor and the substrates succinate (3.3 mM) or NADH (133 μM) were added to start the reactions.

Fraction	*Reaction measured*	*Reaction rate (μatoms O/min per mg protein for oxidases, μmoles cytochrome c reduced/min per mg protein for reductases)*
Particulate	Succinate oxidation	0.39
Particulate	NADH oxidation	0.05
Particulate	Succinate–cytochrome *c* reductase	0.352
Particulate	NADH–cytochrome *c* reductase	0.156
Soluble	NADH–cytochrome *c* reductase	0.010

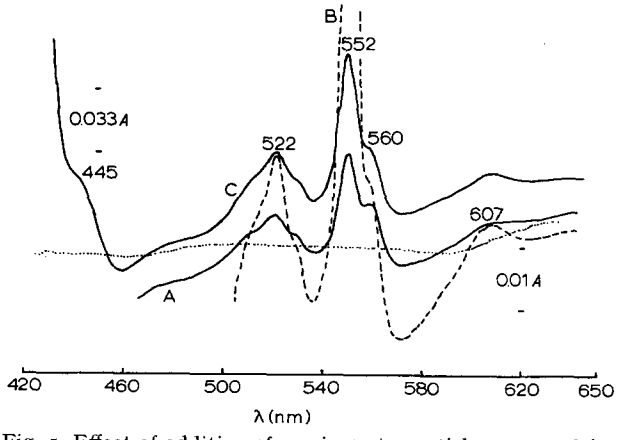


Fig. 5. Effect of addition of succinate to particles prepared from aerobically grown *R. spheroides*. Particles were suspended in KCl–MES buffer (pH 6.8) at a concentration of 3.0 mg protein/ml; succinate (4 mM) was added to the test cuvette. Trace A was recorded immediately after adding succinate; Trace B, 5 min later. Trace C is the same as Trace B, recorded at lower sensitivity. ....., baseline.

The effect of antimycin A upon the aerobic steady-state levels of $c552$ and $b560$ are shown in Fig. 6. Decreased reduction of c and increased reduction of b can be seen, showing an antimycin A-sensitive crossover point between these two cytochromes, as is found in mitochondria[16]. Essentially the same results were obtained with either NADH or succinate as substrate. Anaerobic conditions were eventually achieved as a

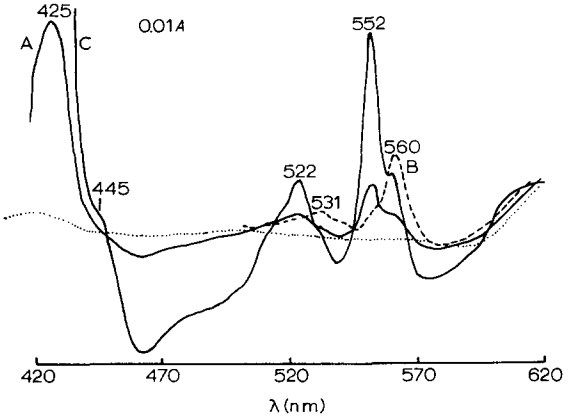


Fig. 6. Effect of addition of succinate and antimycin A to particles prepared from aerobically grown *R. spheroides*. Particles (1.5 mg protein/ml) were suspended in KCl–MES buffer (pH 6.8). Trace A was recorded after succinate (4 mM) was added to the test cuvette, followed by, Trace B, antimycin A (2 μM). Trace C was recorded 6 min after the last addition, when anaerobic conditions had been attained. ·····, baseline.

TABLE II

EFFECT OF INHIBITORS ON OXIDASE AND CYTOCHROME *c* REDUCTASE ACTIVITIES OF PARTICLES PREPARED FROM AEROBICALLY GROWN *R. spheroides*

Inhibitor	*Concn.*	*% Inhibition*	
1. Oxidases	*(μM)*	*Succinate oxidase*	*NADH oxidase*
Antimycin A	1.5	74	45
HQNO	32	9	40
Rotenone	3.0	0	48
DTFB	89	32	16
	160	45	48
TTFB	80	89	22
	160	—	48
KCN	200	88	89
NaN_3	300	65	—
2. Cytochrome c reductases	*Concn. (μM)*	*Succinate–cytochrome c*	*NADH–cytochrome c*
Antimycin A	1.5	80	50
HQNO	70	18	25
Rotenone	2.5	0	55
Piericidin A	1.2	0	70
DTFB	50	40	35
TTFB	50	84	30

TABLE III

SUMMARY OF PROPERTIES OF HAEMPROTEINS DETECTED IN SOLUBLE AND PARTICULATE FRACTIONS PREPARED FROM AEROBICALLY GROWN *R. spheroides*

Cytochrome type*	*Reduced minus oxidised spectra*						*Properties* (*P* = *Particulate*; *S* = *Soluble*)
	Absorption maxima at room temp. (*nm*)			*Absorption maxima at 77 °K* (*nm*)			
c	552	523	421	549	520	420	P and S; substrate-reducible in particles, ascorbate/DPIP-reducible
c	N.R.**			547	520	417	P and S; substrate-reducible in particles; not readily reduced by ascorbate/DPIP
c	554	526	423	551	521	421	S only; reduced by ascorbate/DCIP, autoxidizable
b	560	531	430	557	528	429	P; reduced by substrates; a component not reduced by substrates
b	565	538	NR	562	536	N.R.	P; not substrate-reduced
a	607		445	604		444	P; substrate-reduced; CO binding
Cytochromoid *c*	± CO spectrum						S; CO binding; broad absorption band between 560 and 570 nm in reduced *minus* oxidised spectrum
	569	538	416				
o							P; 428 trough in ± CO difference spectra

* Cytochromes classified only on basis of band positions.
** N.R. = not resolved.

result of the incomplete inhibition of respiration by antimycin A (Table II) and the extent of reduction of cytochromes was then the same as in uninhibited anaerobic conditions.

It can be shown by spectroscopy at 77 °K that under aerobic steady-state conditions NADH and succinate appear to reduce identical *b*- and *c*-type cytochromes with maxima at 547, 549 and 557 nm (Fig. 7a). Cytochromes *c*547 and *c*549 appear about equally reduced in the aerobic steady state, but with the onset of anaerobiosis, *c*549 becomes the major reduced component (Fig. 7b).

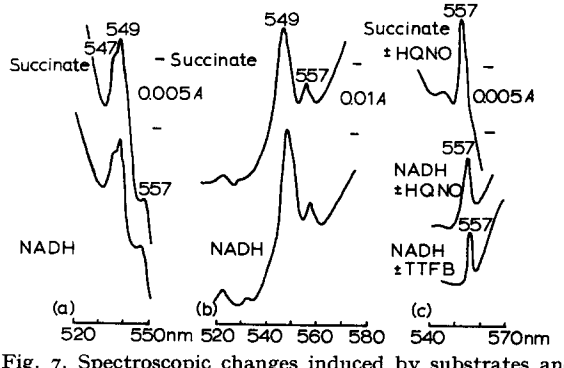


Fig. 7. Spectroscopic changes induced by substrates and inhibitors in particles prepared from aerobically grown *R. spheroides*. Particles (3.0 mg protein/ml) were suspended in KCl–MES (pH 6.8), succinate (2.5 mM), NADH (0.2 mM), HQNO (40 μM) or TTFB (133 μM) were added, as indicated, and spectra recorded at 77 °K. (a), substrates added to test cuvette and spectra recorded before the onset of anaerobiosis; b, substrates added to both test and reference cuvettes, test allowed to become anaerobic before spectra recorded; c, substrates added to test cuvette followed by inhibitors, spectra recorded before onset of anaerobiosis.

Surprisingly HQNO at concentrations that had little effect upon NADH oxidation or succinate–cytochrome *c* reduction caused an increased reduction of cytochrome *b* though oxidation of cytochromes *c* was less obvious (Fig. 7c). Similarly, TTFB caused the reduction of *b* when either NADH or succinate was the substrate. This substance thus seems capable of inhibiting electron flow between cytochromes *b* and *c*; care should obviously be taken in its use as an uncoupler.

Non-substrate-reducible haemproteins

Additional *b*-type components became apparent in a further series of difference spectra. These do not appear to be components of the substrate-linked electron transport chain since they are not readily reduced by ascorbate/TMPD, NADH or succinate and appear in difference spectra of dithionite-reduced particles *minus* ascorbate/TMPD-reduced particles or between dithionite-reduced particles and substrate-reduced anaerobic particles (Fig. 8). One pigment appears to have the same spectroscopic properties at room temperature and 77°K as the "bulk" cytochrome *b*, the other pigment has absorption maxima at 565 and 538 nm at room temperature and at 562 and 536 nm at 77°K. No clear indication of the Soret band could be obtained. As far as can be tested by spectroscopy it is not substrate-reducible; the joint appearance of α and β bands suggests that it is a cytochrome of the *b*-type and not cytochromoid *c*.

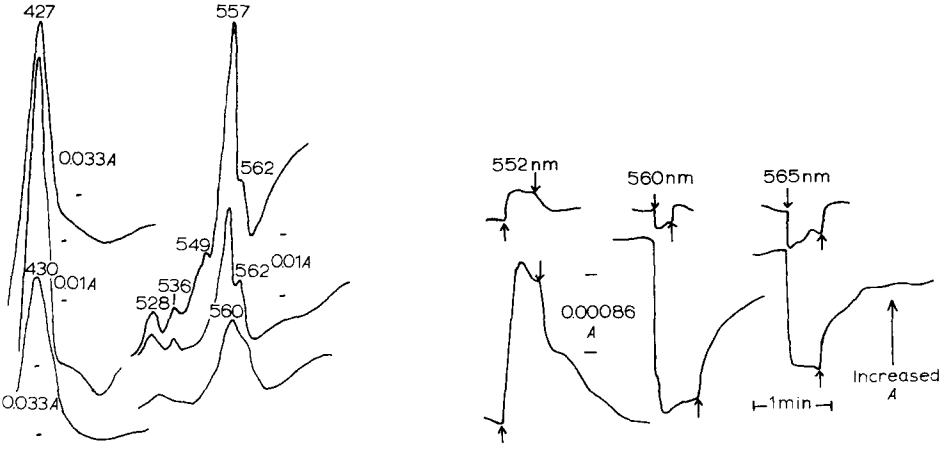


Fig. 8. Non-substrate-reduced pigments in particles prepared from aerobically grown *R. spheroides*. Particles (1.5 mg protein/ml) were suspended in KCl–MES buffer (pH 6.8) in cuvettes. Upper trace; 0.2 mM NADH + 2.5 mM succinate added to both reference and test cuvette, followed, after anaerobiosis, by 1 mM KCN, difference spectra recorded at 77°K after the addition of dithionite to the test cuvette. Middle trace; 1 mM ascorbate and 25 μM DCIP added to both cuvettes and samples allowed to become anaerobic; difference spectra recorded at 77°K after the addition of dithionite to test cuvette. Lower trace, as for middle trace, recorded at room temperature.

Fig. 9. Light-induced changes in particles from aerobically grown *R. spheroides*. Particles were suspended (3.5 mg protein/ml) in KCl–MES buffer (pH 6.8) in a cuvette placed in the dual-wavelength spectrophotometer, succinate (2 mM) was added and particles allowed to become anaerobic. Upper traces; particles illuminated with far-red light and cytochrome changes monitored at the wavelengths shown. Lower traces; 2 μM antimycin A was added to the cuvette and the experiment repeated. The reference wavelength used was 585 nm. ↑, light on; ↓, light off.

Photosynthetic reactions of particles from aerobically grown R. spheroides

These aerobically grown cells contain a small amount of bacterial chlorophyll and it can be seen from Fig. 10 that these particles behaved on illumination like particles from light-grown cells of *R. spheroides*[12] although the extent of the changes was less. Illumination of particles made anaerobic by respiratory activity caused some oxidation of cytochrome *c* with little change in the steady-state levels at 560 or 565 nm. The addition of antimycin A increased the light-induced oxidation at 552 nm and reduction at 560 and 565 nm (Fig. 9). The reoxidation at 565 nm was faster than at 560 nm, as had been found previously in the blue-green mutant of *R. spheroides* by JONES[12]. The extent of light-induced reduction at 560 nm was not as great as that caused by the addition of dithionite to the anaerobic chromatophores.

DISCUSSION

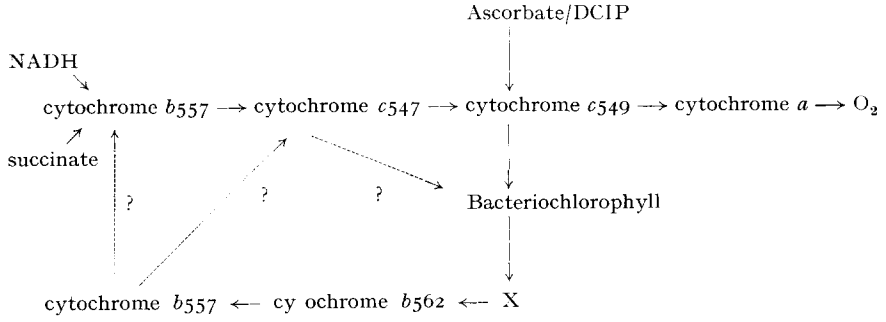
The membrane fraction from dark, aerobically grown *R. spheroides* contains both succinate and NADH oxidase activity that differs in some respects from the corresponding fraction of aerobically grown *Rhodospirillum rubrum* that was studied by TANIGUCHI AND KAMEN[17]. In this latter organism a mixture of *b*-type components was present with a substantial amount of a *b*, absorbing at 564 nm, that was reduced by ascorbate/2,6-dichlorophenolindophenol (DCIP), that would bind CO and had the characteristics of a cytochrome *o*. The *b*-type component absorbing at 565 nm that we have found in *R. spheroides* differs in several important respects from that of *R. rubrum*. It is not reduced by ascorbate/DCIP nor by NADH or succinate even under anaerobic conditions and so cannot be a terminal oxidase. It appears likely that it functions as an electron carrier in the photosynthetic reactions of this organism; an apparently identical material was observed in light *minus* dark difference spectra of *R. spheroides*[12] and our dual-wavelength traces (Fig. 9) of the effect of illumination on the aerobic particles show that here, too, this component is light-reduced when antimycin is present. There may be a *b*-type component acting as a cytochrome *o* in our sample of aerobically grown cells; CO difference spectra of dithionite-reduced particles (Fig. 4) showed a trough at 428 nm that could be due to cytochrome *o*. However, the absorption bands in the visible region were too similar to those of cytochromoid *c* for a definite identification of these CO-binding pigments to be made and the slight shoulders in the trough between 570 and 540 nm suggested the presence of several components. Our spectra do however confirm previous observations[2–4] that *R. spheroides* contains an oxidase of the *a*-type. Both reduced *minus* oxidised spectra and CO difference spectra show the characteristic absorption at about 445 nm and the respiration is sensitive to KCN and NaN_3. The terminal oxidase of *R. spheroides* grown aerobically thus differs from that of two closely related purple non-sulphur bacteria, *R. rubrum* and *Rhodopseudomonas capsulata*[18] both of which lack *a*-type cytochromes even when grown aerobically.

The addition of succinate or NADH to particles from *R. spheroides* leads to the reduction of a cytochrome of the *b*-type and of two cytochromes of the *c*-type (Fig. 7a) and there is an antimycin-sensitive site between the *b* and the *c* pigments that probably explains the effect of antimycin in inhibiting respiration. (The effect of HQNO is more complex: it causes increased reduction of cytochrome *b* in the aerobic steady state, yet has only a slight effect upon succinate respiration although it is as effective

as antimycin in blocking NADH oxidation.) Since the *c*547 is not readily reduced by ascorbate/TMPD it is probably of lower oxidation–reduction potential than the *c*549 and for this reason we propose a sequence of electron carriers with *c*547 between *b*557 and *c*549. Cytochrome *c*549 is the main *c* component of particles and probably corresponds with the cytochrome c_2 of the photosynthetically grown form (it has an approximate mid-point potential, determined by titration with ferri-/ferrocyanide of +0.3 V); cytochrome *c*547 is not resolved at room temperature and its mid-point potential has not been measured but it may be the same cytochrome that was observed in light *minus* dark difference spectra of photosynthetically grown *R. spheroides*[12]. The respiratory role of the third *c*-type cytochrome (*c*551) is uncertain, since it was detected in the soluble but not in the particulate fraction and so its interaction with substrates could not be measured. It is autoxidisable and is absent from the photosynthetically grown cells (F. R. WHALE AND O. T. G. JONES, unpublished work) and is therefore likely to be implicated in the aerobic electron flow system.

There is a considerable amount of cytochrome *b* absorbing at 560 nm at room temperature (557 nm at 77°K) that is not substrate-reducible and its function is uncertain. It appears to be partly reduced by illumination when antimycin A is present (Fig. 9) and may represent a separate pool of cytochrome *b* associated with light reactions and not involved in respiration.

Combining the information discussed above a scheme of electron transfer may be proposed (Scheme 1). The interactions of carriers in photosynthetic flow are uncertain and are the subject of further work.



Scheme 1. Electron transfer pathways in particles from aerobically grown *R. spheroides*. Cytochromes are identified from the positions of the α band at 77°K.

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