Arch Microbiol (1985) 143:260–265

Archives of
Microbiology



# Cytochromes in *Chloroflexus aurantiacus* grown with and without oxygen

**Beverly K. Pierson**

Biology Department, University of Puget Sound, Tacoma, WA 98416, USA

**Abstract.** The major types of cytochromes present in *Chloroflexus aurantiacus* strain J-10-fl were determined by analysis of cell-free preparations and membranes from cells grown phototrophically and chemotrophically. Reduced minus oxidized difference spectroscopy as well as quantitative analysis of the pyridine ferrohemochromogens in alkaline solution were used to determine the classes of cytochromes present.

Evidence for membrane-bound cytochromes of the *b* and *c*-type was found in cell-free preparations of phototrophically grown cells. Difference spectra revealed absorption maxima at 553 nm, 523 nm, and 418 nm and a shoulder at 562 nm. Protoheme and heme *c* were identified as alkaline pyridine ferrohemochromogens with absorption maxima at 556 and 550 nm respectively. The ratio of heme *c* to protoheme in the cell-free preparation was about 10 to 1 with 0.65 μmol of heme *c* and 0.067 μmol of protoheme per gram of total cell protein. Dithionite plus carbon monoxide minus dithionite reduced difference spectra of membranes revealed a peak at 414 nm and troughs at 552, 523, and 423 nm consistent with a CO-binding cytochrome *c*.

Similar analyses of chemotrophically grown cells revealed evidence for the presence of membrane-bound cytochromes of the *c*, *b*, and *a* classes with absorption maxima at 552–557 and 593–596 nm. Protoheme, heme *c* and heme *a* were detected as pyridine ferrohemochromogens with maxima at 556, 550, and 588 nm respectively. The ratio of heme *c* to protoheme was about 3 to 1 with 0.44 μmol of heme *c* and 0.16 μmol of protoheme per gram of total cell protein.

**Key words:** *Chloroflexus aurantiacus* – Photosynthetic bacteria – Thermophile – Cytochromes – Hemes – Aerobic respiration – Electron transfer

*Chloroflexus aurantiacus*, a thermophilic photosynthetic bacterium containing bacteriochlorophylls *a* and *c*, is capable of phototrophic growth under anaerobic conditions and chemoheterotrophic growth under aerobic conditions (Pierson and Castenholz 1974a). The presence of both bacteriochlorophylls *a* and *c* and chlorosomes in phototrophically grown *Chloroflexus* is a characteristic shared with the green sulfur bacteria which are obligate photoautotrophs. The metabolic versatility of *Chloroflexus*, however, in possessing both phototrophic and chemotrophic metabolism is similar to that of the purple non-sulfur bacteria.

The cytochrome composition of the purple non-sulfur bacteria varies with the conditions of growth (Bartsch 1978). While some of the same cytochromes appear to function in both respiratory and photosynthetic electron transfer reactions (Vermeglio and Carrier 1984), the presence of *a*-type cytochromes is associated only with respiratory metabolism in some of the bacteria, while others contain an *o*-type cytochrome oxidase (Bartsch 1978). A light-dependent inhibition of respiratory activity has been shown for *C. aurantiacus* (Pierson and Castenholz 1974b) suggesting some shared components in the photosynthetic and respiratory electron transfer chains.

It was the objective of this study to determine what major classes of cytochromes were present in *C. aurantiacus* grown anaerobically in the light and aerobically in the dark and to quantify the hemes of the *b* and *c*-type cytochromes in cells grown under both conditions.

## Materials and methods

Bacterial strain and growth conditions: *Chloroflexus aurantiacus* culture J-10-fl was used in all experiments. All cells were grown in medium D (Castenholz and Pierson 1981) containing yeast extract (Difco) 1.0 g/l, vitamin-free casein hydrolysate (Difco) 2.0 g/l, and glycylglycine (Sigma, free-base) 1.0 g/l adjusted to pH 8.2. The cultures were grown in 700 ml batches in Roux culture bottles suspended in an aquarium water bath maintained at 55° C with a Haake ED Unitherm heater circulator. Phototrophic cultures were continuously sparged with a humidified gas mixture of 99.5% $N_2$ and 0.5% $CO_2$ (Airco industrial gases) passed through 10 cm of sterile cotton. The rate of gas flow through the 700 ml cultures was 400 $cm^3$/min. Light was provided by 75 W reflector flood lamps. Incident radiation was 60–150 $W/m^2$ measured with a Radiometer Model 65A (Yellow-Springs Instrument Co.). Chemotrophic cultures were grown in the dark with constant sparging with a gas mixture of 99.5% air and 0.5% $CO_2$. Growth was measured by increase in optical density at 650 nm (Turner 330 spectrophotometer). Phototrophic cultures were inoculated with cells grown phototrophically in screw cap tubes for 3–5 days. Aerobic cultures were inoculated with cells grown aerobically in flasks shaken vigorously for 3–5 days. Cells were harvested in mid to late exponential growth by centrifugation.

Preparation of cell-free extracts and membranes: Harvested cells were washed twice in Tris-Sodium-Magnesium (TSM) buffer pH 7.8 (Sistrom and Clayton 1964). The bacteriochlorophylls and carotenoids were

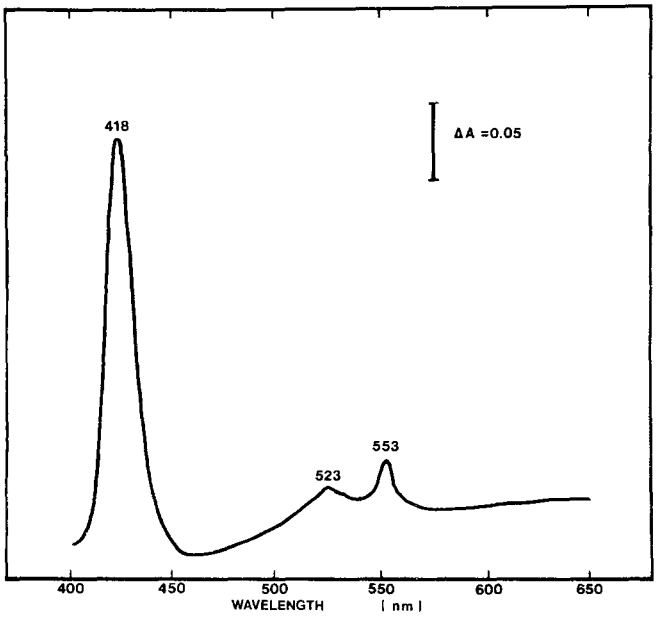


**Fig. 1.** Dithionite-reduced minus $H_2O_2$-oxidized difference spectrum of a crude cell-free extract of *Chloroflexus aurantiacus* grown anaerobically. Chlorophylls and carotenoids were extracted with methanol and acetone before ultrasonic disruption of the cells

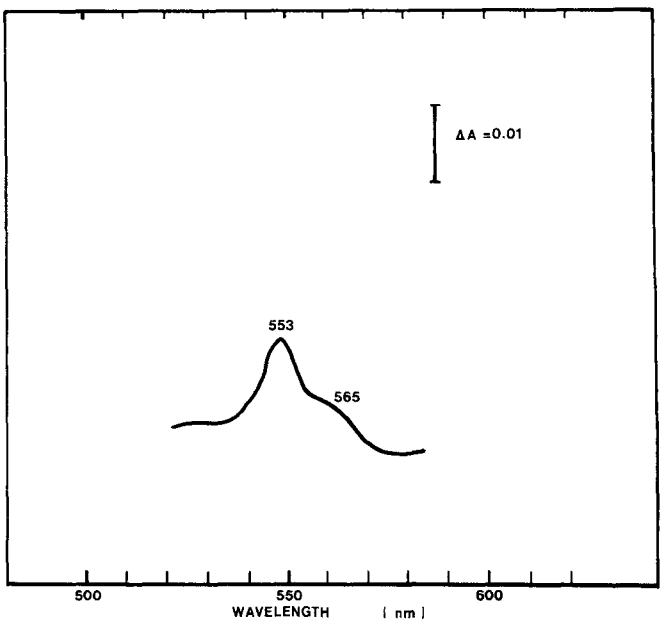


**Fig. 2.** Dithionite-reduced minus ascorbate-reduced difference spectrum of a crude cell-free extract of *Chloroflexus aurantiacus* grown anaerobically. Chlorophylls and carotenoids were extracted with methanol and acetone before ultrasonic disruption of the cells

extracted with acetone:methanol, 7:2 (v/v) at 4° C for 1–6 h. Cells were extracted until the pellets were white. The extracted cells were disrupted ultrasonically (Bronwill Biosonik IV). Ultrasonically disrupted cells were centrifuged (12,100 × *g* for 5 min) to remove whole cells and debris. The cell-free extract was centrifuged at 100,000 × *g* for 30 min (Beckman L5-50, 50-TI rotor) to prepare a membrane fraction which was then washed twice with TSM Buffer.

Preparation of ferrohemochromogens: The pyridine ferrohemochromogens were prepared as described by Sperry and Wilkins (1976).

Difference spectroscopy: All spectra were recorded with a double beam recording spectrophotometer (Varian Techtron UV/VIS Model 635). Samples were reduced with a few crystals of sodium dithionite (J. T. Baker Chemical Co., USA) and oxidized with air, or the addition of 2–3 drops of 3% $H_2O_2$ or potassium ferricyanide.

Quantification of hemes as pyridine hemochromes: The bound heme of cytochrome *c* and the extracted protoheme were quantified as pyridine hemochromes from reduced minus oxidized difference spectra using the extinction coefficients of 19.1 at 550 nm and 30 at 555–556 nm respectively (Bartsch 1971). The hemes were quantified relative to total cell protein determined by the Folin-phenol method (Lowry et al. 1951).

CO spectra: Ultrasonically disrupted preparations of cells or membrane fractions were reduced with dithionite and bubbled for 4 min with CO (Matheson Gas Products, USA). Spectra were then recorded at 10-min intervals for 30 min.

## Results

### *Cell grown phototrophically*

The reduced minus oxidized (dithionite minus $H_2O_2$) difference spectrum of a cell-free extract of phototrophically grown *Chloroflexus aurantiacus* from which carotenoids and chlorophylls had been extracted had absorption maxima at 553, 523, and 418 nm typical of a *c*-type cytochrome (Fig. 1). No evidence for other cytochromes was present in such spectra of either whole cells or separated membrane and soluble fractions. The difference spectrum of a dithionite reduced minus ascorbate reduced cell-free preparation revealed a shoulder from 560 to 570 nm indicating the presence of a *b*-type cytochrome in phototrophically grown cells (Fig. 2). These cytochrome absorbance characteristics were located in the membrane fraction only and not in the soluble fraction. When CO was bubbled through a dithionite-reduced membrane fraction and the spectrum recorded with the dithionite-reduced absorbance substracted, the results showed the presence of a CO-binding cytochrome *c* with troughs at 552, 523, and 423 nm and a peak at 414 nm (Fig. 3). No evidence for a CO-binding cytochrome *b* was obtained.

Membrane fractions from which the chlorophylls and carotenoids had not been extracted with methanol and acetone also revealed a single cytochrome peak at 553 nm (data not shown) in dithionite minus air, $H_2O_2$ or ferricyanide difference spectra.

The pyridine ferrohemochromes were examined in whole cells that had been previously extracted with acetone/methanol to remove all chlorophylls and carotenoids. An acidic acetone extract from whole cells was obtained and transferred to ethyl ether which was dried. The dried residue was dissolved in alkaline pyridine and the reduced minus oxidized spectrum recorded. The spectrum of the extractable hemes in alkaline pyridine (Fig. 4) clearly revealed the presence of protoheme, the heme of cytochrome *b* with maxima at 556, 522, and 417 nm. The unextractable hemes remaining after the acidic acetone extraction were also examined in alkaline pyridine (Fig. 5). A characteristic spectrum for the mesoheme of cytochrome *c* with absorption maxima at 550, 521, and 415 nm was obtained.

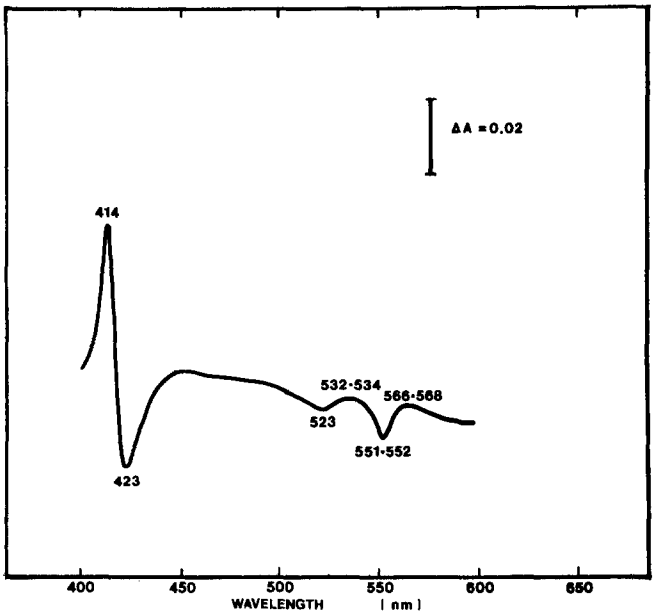


**Fig. 3.** Dithionite-reduced plus carbon monoxide minus dithionite-reduced difference spectrum of the membrane fraction from phototrophically grown cells. The reduced preparation was bubbled with CO for 4 min before the spectrum was recorded

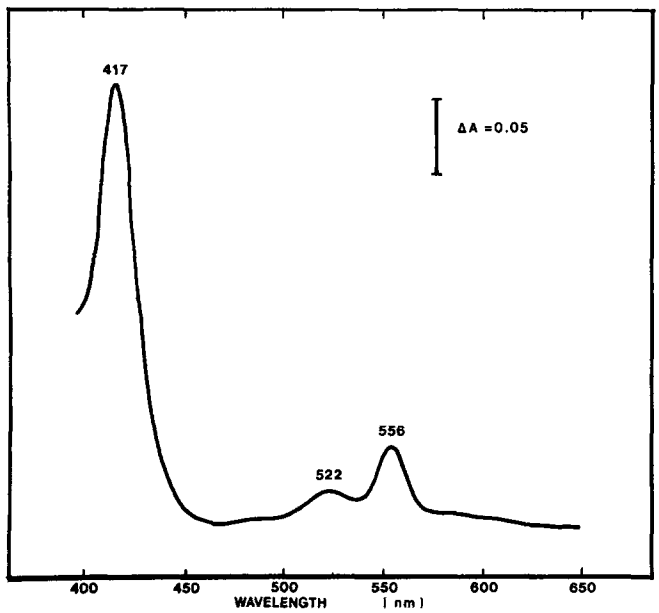


**Fig. 4.** Dithionite-reduced minus $H_2O_2$-oxidized difference spectrum of hemochromes in alkaline pyridine obtained by extraction with acidic acetone of cells of *Chloroflexus aurantiacus* grown anaerobically. Chlorophylls and carotenoids were extracted with acetone and methanol prior to extraction of hemes with acidic acetone

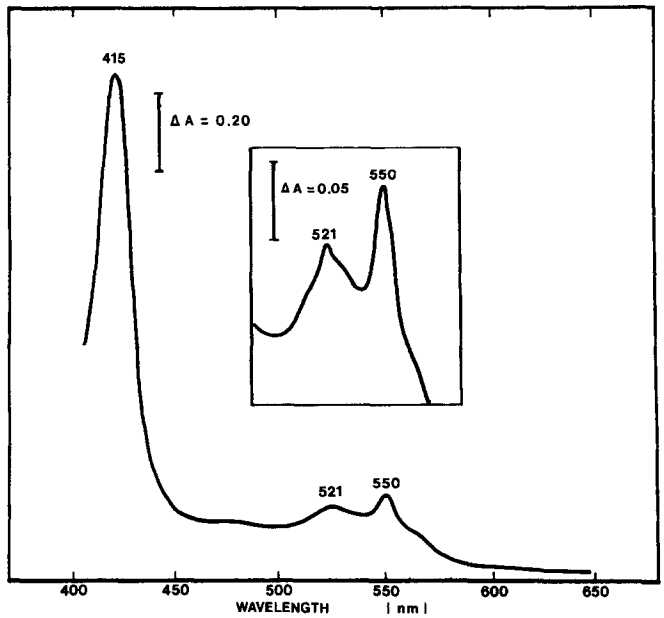


**Fig. 5.** Dithionite-reduced minus $H_2O_2$-oxidized difference spectrum of a cell-free preparation in alkaline pyridine of *Chloroflexus aurantiacus* grown anaerobically. Chlorophylls and carotenoids were extracted with methanol and acetone, and dissociable hemes were extracted with acidic acetone. The remaining cell fraction was ultrasonically disrupted in alkaline pyridine. (Insert) Same preparation on an expanded scale

**Table 1.** Quantification of hemes in *Chloroflexus aurantiacus*

| | Cells grown anaerobically | Cells grown aerobically |
|---|---|---|
| Heme *c* | 0.50 | 0.54 |
| | 0.42 | 0.38 |
| | 0.74 | 0.36 |
| | 0.93 | 0.47 |
| | mean 0.65 | mean 0.44 |
| Protoheme | 0.055 | 0.14 |
| | 0.075 | 0.08 |
| | 0.072 | 0.19 |
| | | 0.21 |
| | mean 0.067 | mean 0.155 |
| Heme *c*/protoheme | 9.7 | 2.8 |

Data are given in μmol/g total cell protein. Protoheme was extracted from cells with acidic acetone and transferred to ethyl ether which was dried over $Na_2SO_4$ and then taken to dryness under $N_2$. The dried extract was redissolved in alkaline aqueous pyridine and the protoheme determined from the absorbance at 556 nm in reduced minus oxidized spectra using $\Delta\varepsilon = 30$ (Bartsch 1971). Heme *c* was determined from the non-extractable residue dissolved in aqueous alkaline pyridine. The concentration was calculated from the absorption maximum at 550 nm in reduced minus oxidized spectra using $\Delta\varepsilon = 19.1$ (Bartsch 1971)

The heme *c* and protoheme were quantitatively estimated relative to total cell protein using extinction coefficients for reduced minus oxidized difference spectra of the alkaline pyridine hemochromes (Bartsch 1971). The data for several different determinations (Table 1) revealed that there was about 10 times as much heme *c* as protoheme which explains why it was difficult to see the α-peak for the *b*-type cytochrome in phototrophically grown cells.

*Cells grown chemotrophically*

Cells grown aerobically in the dark had a very high carotenoid content that interfered with spectroscopy of cytochromes. With the carotenoids present, the absorption maxima for cytochromes were observed at 525, 555–557, and 596 nm. These peaks were found associated with the membrane fraction (Fig. 6). The spectra indicated the pres-

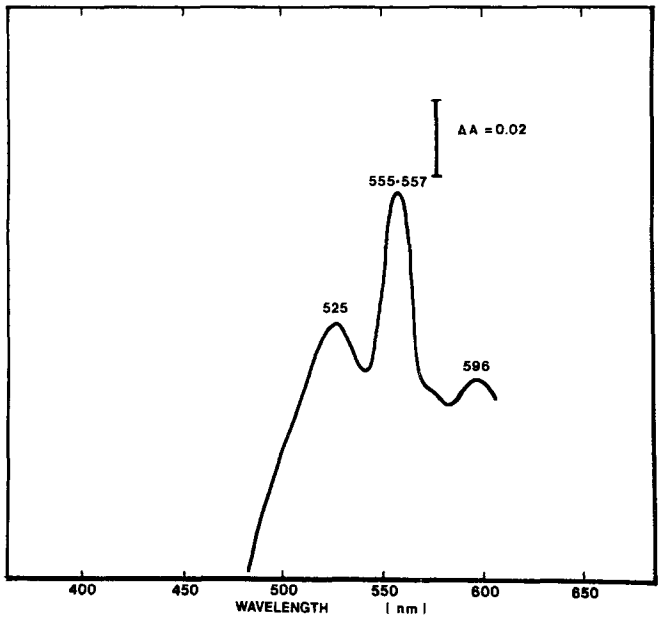


**Fig. 6.** Dithionite-reduced minus air-oxidized difference spectrum of a membrane preparation of *Chloroflexus aurantiacus* grown aerobically. Carotenoids were not extracted. Cells were disrupted ultrasonically and centrifuged at 100,000 × *g* to obtain the membrane fraction used in this spectrum. An additional peak was at 429 nm

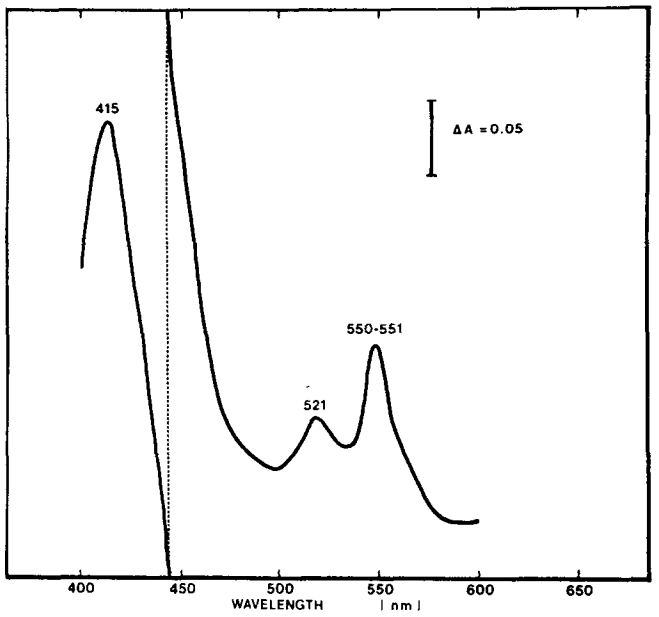


**Fig. 7.** Dithionite-reduced minus $H_2O_2$-oxidized difference spectrum of a cell-free preparation in alkaline pyridine of *Chloroflexus aurantiacus* grown aerobically. Carotenoids were extracted with methanol and acetone, and dissociable hemes were extracted with acidic acetone. The remaining cell fraction was ultrasonically disrupted in alkaline pyridine

ence of cytochromes of the *c* and *a*-types although a *b*-type cytochrome could be included in the 555 – 557 nm peak. Very small absorbancies at the same positions were seen in the soluble fraction. It appeared that the cytochromes were not as tightly associated with the membrane in aerobically grown cells as in anaerobically grown cells or that small amounts of membrane remained in the "soluble" fraction. When carotenoids were extracted with methanol and acetone prior to recording difference spectra of whole cells or membranes, the cytochrome absorption maxima were shifted to 552 – 553 nm and 593 nm, and the 525 nm peak was depressed (data not shown).

The reduced minus oxidized difference spectrum in alkaline pyridine of the bound hemes remaining after acidic acetone extraction revealed peaks at 550, 521, and 415 nm typical of the mesoheme of cytochrome *c* (Fig. 7). The reduced minus oxidized difference spectrum of acid/acetone extracted hemes after transfer to alkaline pyridine had absorption maxima at 556, 525, and 418 nm typical of protoheme, as well as an additional maximum at 587 – 589 nm typical of heme *a* (Fig. 8).

No CO-binding activity was detected in the aerobically grown cells.

Quantitative determination of hemes in aerobically grown cells (Table 1) revealed approximately three times as much heme *c* as protoheme. The lower ratio of heme *c* to protoheme in aerobically grown cells compared with anaerobically grown cells was due to both a lower specific content of cytochrome *c* and a higher specific content of cytochrome *b* in aerobically grown cells.

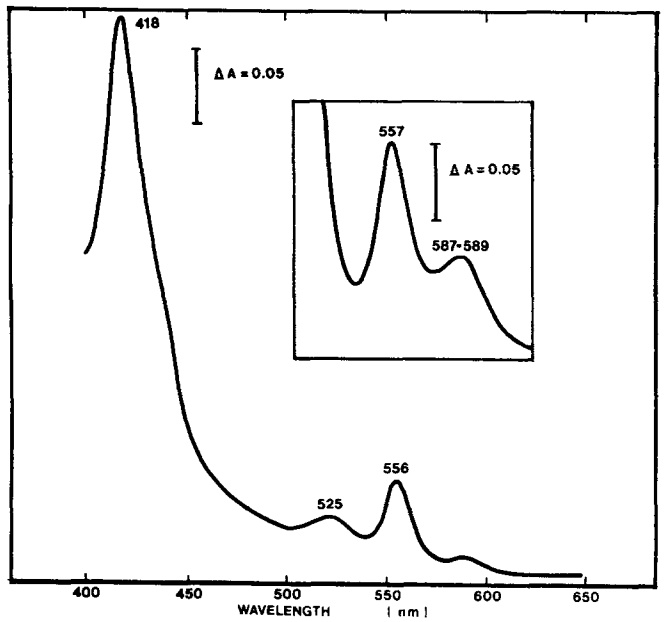


**Fig. 8.** Dithionite-reduced minus $H_2O_2$-oxidized difference spectrum of hemochromes in alkaline pyridine obtained by extraction with acidic acetone of cells of *Chloroflexus aurantiacus* grown aerobically. Carotenoids were extracted with acetone and methanol prior to extraction of hemes with acidic acetone. (Insert) Dithionite-reduced minus air oxidized difference spectrum of a similar preparation in alkaline pyridine from which carotenoids were not extracted with acetone and methanol prior to extraction of the hemes into acidic acetone

## Discussion

*Chloroflexus aurantiacus* appeared to be similar to all other photosynthetic bacteria in having cytochromes of both the *b* and *c*-types when grown photosynthetically. It was previously reported that *C. aurantiacus* had only a *c*-type cytochrome (Bartsch 1978; Bruce et al. 1982). However, *b*-cytochromes are often difficult to demonstrate in the

presence of relatively large amounts of *c* cytochromes necessitating the use of altered potentials, low temperature spectroscopy, or the use of heme extraction to verify their presence (Bowyer and Crofts 1980; Knaff and Kraichoke 1983; Takamiya and Hanada 1980; Knaff and Buchanan 1975). In this study the presence of cytochrome *b* was indicated by the shoulder at 560 – 565 nm in dithionite reduced minus ascorbate reduced difference spectra of cell-free preparations. Its presence was confirmed with the demonstration of protoheme. The low amount of protoheme relative to heme *c* (0.067 μmol protoheme/g protein and 0.65 μmol heme *c*/g protein) accounted for its lack of detection in dithionite-reduced minus oxidized difference spectra when air, $H_2O_2$, or ferricyanide were used as oxidants.

Both the *b* and *c*-type cytochromes were membrane bound in photosynthetic cells. No evidence was found for any of the soluble *c*-type cytochromes such as are found in the green sulfur bacteria: cytochrome *c*-555 and cytochrome *c*-553 used in sulfide oxidation and cytochrome *c*-551 used in thiosulfate oxidation (Steinmetz and Fischer 1982; Steinmetz et al. 1983). It should be noted, however, that strain J-10-fl used in this study was grown photoheterotrophically in the absence of sulfide and thiosulfate. It may well be that the cytochrome composition could be altered in cells grown photoautotrophically with reduced sulfur compounds. It appears that *C. aurantiacus* has only the membrane-bound cytochrome *c*-553 to reduce the photochemically oxidized reaction center (Bruce et al. 1982) whereas all the green sulfur bacteria have a cytochrome *c*-555. *C. aurantiacus* also differs from the purple non-sulfur bacteria in lacking a soluble *c*-type cytochrome. The membrane-bound cytochrome *c*-553 in *Chloroflexus* was spectroscopically similar to the $c_1$-type of cytochrome found in the membrane bound *b*-$c_1$ complex of mitochondria and the similar complex found in purple non-sulfur bacteria (Yu et al. 1984; Takamiya et al. 1982) and in *Chromatium vinosum* (Gaul and Knaff 1983).

The membrane bound cytochrome *c*-553 of phototrophically grown *C. aurantiacus* is unique in binding CO. The absorption spectrum of the CO plus dithionite minus dithionite reduced membrane fraction from these cells (Fig. 3) had a typical pattern of peaks and troughs for a cytochrome *c*-CO complex. In particular the troughs at 552 and 423 nm and the peak at 414 nm are typical of cytochrome *c* binding to CO. A membrane bound cytochrome *c* which binds CO has been reported for chemotrophically grown *R. capsulata* (Hüdig and Drews 1983) but not for phototrophically grown cells. While membrane bound CO-binding cytochrome *c*'s have been reported for various chemotrophic bacteria (Matsushita et al. 1982) including the extreme thermophile PS3 (Poole et al. 1983) their role is not known. The presence of the CO-binding cytochrome *c* in membranes of phototrophically grown *C. aurantiacus* is particularly interesting because there is a lack of any evidence for cytochrome *o* activity. None of the *b*-type cytochrome appears to bind CO as evidenced by the lack of troughs near 430 and 560 nm in the CO spectra. The absence of cytochrome *o* or *b*-binding CO activity in *C. aurantiacus* is different from the situation observed in phototrophically grown purple non-sulfur bacteria which show such CO binding (Takamiya 1983; Takamiya and Tanaka 1983).

The cytochrome composition of chemotrophically grown cells differed significantly from that of phototrophically grown cells. Technical difficulties were also encountered in obtaining spectra of the membranes from aerobically grown cells. It was necessary to extract the cells with acetone and methanol to remove the intensely absorbing carotenoids (derivatives of β and γ-carotenes) which severely interfered with spectroscopy of cytochromes in the region of the β peaks and in the Soret region. There was no indication of any adverse effect of these extractions on membranes from phototrophic cells, and the procedure has been used with similar satisfactory results by others (Kienzl and Peschek 1982). However, the extraction process proved detrimental to the *b* and *a*-type cytochromes of aerobically grown cells. While evidence for *b* and *a* cytochromes was consistently found in cells that had not been previously extracted with acetone and methanol (Fig. 6), the *b* and *a* hemes were occasionally lost from cells that were extracted and the peak positions were shifted a few nm (data not shown). The broad peak at 555 – 557 nm in membranes of chemotrophically grown cells was due to absorption by both *c* and *b* cytochromes, verified by subsequent analysis of the pyridine ferrohemochromogens. The presence of a cytochrome *a* was indicated by the maximum at 596 nm (Fig. 6). Absorbance in the region from 585 to 598 nm is usually interpreted to be due to an $a_1$-type of cytochrome rather than the $aa_3$ complex which absorbs at 602 nm (Baines et al. 1984). The identity of the *a*-heme was confirmed with the pyridine ferrohemochromogen maximum at 587 nm (Fig. 8). While the membrane fraction clearly contained all three classes of cytochromes some absorbance from all three classes was also detected in the soluble fraction (data not shown). Since the absorption maxima were identical to those of the membrane fraction and the absorbance of the soluble fraction accounted for less than 10% of the total cytochrome in the membrane fraction, it is thought that some of the membranes were disrupted sufficiently to release cytochrome components or that small amounts of membrane remained in the $100{,}000 \times g$ supernatant. This was not the case in phototrophically grown cells.

Despite the presence of both *a* and *b*-type cytochromes in the membranes of chemotrophically grown cells no CO-binding activity was observed. We are unable at this time, therefore, to identify the cytochrome oxidase in aerobically grown cells. Either *a* or *b*-type cytochromes could function as terminal oxidases for aerobic respiration. The membrane bound cytochrome *c* oxidase is of the cytochrome $aa_3$ class in both cyanobacteria (Kienzl and Peschek 1982) and in *R. sphaeroides* (Gennis et al. 1982). *R. palustris* has a cytochrome oxidase of the *o*-type (King and Drews 1975) and even *R. sphaeroides* has an *o*-type cytochrome oxidase which functions at lower oxygen tensions than the cytochrome $aa_3$ (Sasaki et al. 1970).

The recent isolation of a carotenoid-minus mutant that can grow phototrophically (Pierson et al. 1984) should permit complete analysis of cytochromes without prior extraction of carotenoids with acetone and methanol. Isolation of the cytochrome complexes will be necessary to determine if these membrane-bound proteins have intrinsic thermal stability characteristics similar to those of the isolated membrane-bound reaction center protein (Pierson et al. 1983).

*Acknowledgements.* This work was supported by funds from an M. J. Murdock Trust Grant of Research Corporation.

The work of the following people in growing cells, preparing membranes, and recording spectra is gratefully acknowledged: K.

Lindgren, J. Tamashiro, D. Wasson, J. McGourty, J. Stern, K. McCoy, K. Anderson.

## References


Baines BS, Williams HD, Hubbard JAM, Poole RK (1984) Partial purification and characterization of a soluble haemoprotein, having spectral properties similar to cytochrome $a_1$, from anaerobically grown *Escherichia coli*. FEBS Lett 171:309–314

Bartsch RG (1971) Cytochromes: Bacterial. In: San Pietro A (ed) Methods in enzymology, vol XXIII, part A. Academic Press, New York London, pp 344–363

Bartsch RG (1978) Cytochromes. In: Clayton RK, Sistrom WR (eds) The photosynthetic bacteria. Plenum, New York London, pp 249–279

Bowyer JR, Crofts AR (1980) The photosynthetic electron transfer chain of *Chromatium vinosum* chromatophores: Flash-induced cytochrome *b* reduction. Biochim Biophys Acta 591:298–311

Bruce BD, Fuller RC, Blankenship RE (1982) Primary photochemistry in the facultatively aerobic green photosynthetic bacterium *Chloroflexus aurantiacus*. Proc Natl Acad Sci USA 79:6532–6536

Castenholz RW, Pierson BK (1981) Isolation of members of the family Chloroflexaceae. In: Starr MP, Stolz H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes: a handbook on habitats, isolation, and identification of bacteria, vol I. Springer, Berlin Heidelberg New York, pp 290–298

Gaul DF, Knaff DB (1983) The presence of cytochrome $c_1$ in the purple sulfur bacterium *Chromatium vinosum*. FEBS Lett 162:69–74

Gennis RB, Casey RP, Azzi A, Ludwig B (1982) Purification and characterization of the cytochrome *c* oxidase from *Rhodopseudomonas sphaeroides*. Eur J Biochem 125:189–195

Hüdig H, Drews G (1983) Characterization of a new membrane-bound cytochrome *c* of *Rhodopseudomonas capsulata*. FEBS Lett 152:251–255

Kienzl PF, Peschek GA (1982) Oxidation of *c*-type cytochromes by the membrane-bound cytochrome oxidase (cytochrome $aa_3$) of blue-green algae. Plant Physiol 69:580–584

King MT, Drews G (1975) The respiratory electron transport system of heterotrophically grown *Rhodopseudomonas palustris*. Arch Microbiol 102:219–231

Knaff DB, Buchanan BB (1975) Cytochrome *b* and photosynthetic sulfur bacteria. Biochim Biophys Acta 376:549–560

Knaff DB, Kraichoke S (1983) Oxidation-reduction and EPR properties of a cytochrome complex from *Chromatium vinosum*. Photochem Photobiol 37:243–246

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Matsushita K, Shinagawa E, Adachi O, Ameyama M (1982) *o*-Type cytochrome oxidase in the membrane of aerobically grown *Pseudomonas aeruginosa*. FEBS Lett 139:255–258

Pierson BK, Castenholz RW (1974a) A phototrophic gliding filamentous bacterium of hot springs, *Chloroflexus aurantiacus*, gen. and sp. nov. Arch Microbiol 100:5–24

Pierson BK, Castenholz RW (1974b) Studies of pigments and growth in *Chloroflexus aurantiacus*, a phototrophic filamentous bacterium. Arch Microbiol 100:283–305

Pierson BK, Keith LM, Leovy JG (1984) Isolation of pigmentation mutants of the green filamentous photosynthetic bacterium *Chloroflexus aurantiacus*. J Bacteriol 159:222–227

Pierson BK, Thornber JP, Seftor REB (1983) Partial purification, subunit structure and thermal stability of the photochemical reaction center of the thermophilic green bacterium *Chloroflexus aurantiacus*. Biochim Biophys Acta 723:322–326

Poole RK, Van Wielink JE, Baines BS, Reijnders WNM, Salmon I, Oltmann LF (1983) The membrane bound cytochromes of an aerobically grown, extremely thermophilic bacterium, PS3: characterization by spectral deconvolution coupled with potentiometric analysis. J Gen Microbiol 129:2163–2173

Sasaki T, Motokawa Y, Kikuchi G (1970) Occurrence of both *a*-type and *o*-type cytochromes as the functional terminal oxidases in *Rhodopseudomonas spheroides*. Biochim Biophys Acta 197:284–291

Sistrom WR, Clayton RK (1964) Studies on a mutant of *Rhodopseudomonas spheroides* unable to grow photosynthetically. Biochim Biophys Acta 88:61–73

Sperry JF, Wilkins TD (1976) Cytochrome spectrum of an obligate anaerobe, *Eubacterium lentum*. J Bacteriol 125:905–909

Steinmetz MA, Fischer U (1982) Cytochromes, rubredoxin, and sulfur metabolism of the non-thiosulfate-utilizing green sulfur bacterium *Pelodictyon luteolum*. Arch Microbiol 132:204–210

Steinmetz MA, Trüper HG, Fischer U (1983) Cytochrome *c*-555 and iron-sulfur-proteins of the non-thiosulfate-utilizing green sulfur bacterium *Chlorobium vibrioforme*. Arch Microbiol 135:186–190

Takamiya K (1983) Properties of the cytochrome *c* oxidase activity of cytochrome $b_{561}$ from photoanaerobically grown *Rhodopseudomonas sphaeroides*. Plant Cell Physiol 24:1457–1462

Takamiya K, Hanada H (1980) Cytochrome $b_{560}$ in chromatophores from *Chromatium vinosum*. Plant Cell Physiol 21:979–988

Takamiya K, Tanaka H (1983) Isolation and purification of cytochrome $b_{561}$ from a photosynthetic bacterium, *Rhodopseudomonas sphaeroides*. Plant Cell Physiol 24:1449–1455

Takamiya K, Doi M, Okimatsu H (1982) Isolation and purification of a ubiquinone-cytochrome $b$-$c_1$ complex from a photosynthetic bacterium, *Rhodopseudomonas sphaeroides*. Plant Cell Physiol 23:987–997

Vermeglio A, Carrier JM (1984) Photoinhibition by flash and continuous light of oxygen uptake by intact photosynthetic bacteria. Biochim Biophys Acta 764:233–238

Yu, CA, Mei Q-C, Yu L (1984) Isolation and characterization of cytochrome $c_1$ from photosynthetic bacterium *Rhodopseudomonas sphaeroides* R-26. Biochem Biophys Res Comm 118:964–969


Received March 5, 1985/Accepted July 19, 1985