Cytochromes and Anaerobic Sulfide Oxidation in the Purple Sulfur Bacterium *Chromatium warmingii*

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Z. Naturforsch. **38 c,** 960–967 (1983); received August 8, 1983

Chromatium warmingii, Cytochrome *c'*, Cytochrome *c*-552, Anaerobic Sulfide Oxidation, Phototrophic Bacteria

Two soluble acidic *c*-type cytochromes – *c'* and *c*-552 – were isolated by ion exchange chromatography, gel filtration and ammonium sulfate fractionation. Cytochrome *c'* is a high-spin cytochrome with maxima at 399 nm, 490 nm, and 634 nm in the oxidized form and at 550 nm, 425 nm and a characteristic shoulder at 434 nm in the reduced state. The best purity index obtained (A_{280}/A_{399}) was 0.35. Cytochrome *c'* is autoxidizable, has a molecular weight of 12000 (estimated by sodium dodecylsulfate electrophoresis), a midpoint redoxpotential of + 10 mV and an isoelectric point at pH 4.0. The reduced cytochrome *c'* reacts with carbon monoxide. The reaction is reversible. Cytochrome *c*-552 shows maxima at 552 nm, 523 nm and 417 nm in the reduced form and at 408 nm in the oxidized state. The best purity index obtained (A_{280}/A_{408}) was 0.94. Cytochrome *c*-552 has a molecular weight of 30000 and an isoelectric point between pH 4.3 and 5.0.

Chromatium warmingii also contains a membrane-bound cytochrome *c*-552. During anaerobic sulfide oxidation, elemental sulfur and sulfate were formed at the same time. When all sulfide was consumed by the cells, the remaining intracellular elemental sulfur was further oxidized to sulfate.

Introduction

Reduced sulfur compounds, like sulfide or thiosulfate, are used as electron donors for anoxygenic photosynthesis by phototrophic sulfur bacteria [1, 2]. Electron transfer proteins, like cytochromes or high potential iron sulfur proteins, are generally involved in sulfur metabolism in purple and green sulfur bacteria [2, 3]. The molecular properties of cytochromes, their distribution and their function in sulfide and thiosulfate oxidation in green sulfur bacteria (Chlorobiaceae) have been intensively studied [4–13]. In these organisms, flavocytochromes act as sulfide: cytochrome *c* reductases [7, 9–11]. Cytochrome *c*-551, only present in the thiosulfate utilizing *Chlorobium limicola f. thiosulfatophilum* [6, 9] and *Chlorobium vibrioforme f. thiosulfatophilum* [11] is the only endogenous electron acceptor in thiosulfate oxidation. A small cytochrome *c*-555, comparable to plant *f*-type cytochromes, was found in all green sulfur bacteria and functions as an electron mediator. It accepts the electrons from both cytochromes mentioned above and carries them to bacteriochlorophyll [8, 9, 13].

Cytochromes of the purple sulfur bacteria (Chromatiaceae) have been isolated so far only from small-cell species, like *Chromatium vinosum* [14–17], *Thiocapsa pfennigii* [18] *Thiocapsa roseopersicina* [19–21], and from some species of the genus *Ectothiorhodospira* [22]. Flavocytochrome of *Chr. vinosum* acts not only as a sulfide:cytochrome *c* reductase, but also catalyses the reverse reaction from elemental sulfur to sulfide [21, 23, 24]. On the other hand, flavocytochrome of *Thiocapsa roseopersicina* shows adenylylsulfate reductase activity, forming APS from AMP and sulfite [19]. A membrane-bound cytochrome *c*-552 [25], a soluble cytochrome *c*-551 [26] and a high potential iron sulfur protein [27] of *Chr. vinosum* have been described to be effective electron acceptors of thiosulfate oxidation. So far known, none of the large-cell *Chromatium* species, *e.g. Chr. okenii* or *Chr. warmingii* were examined with respect to their

Abbreviations: Chr. Chromatium; APS, adenylylsulfate; SDS, sodium dodecylsulfate.

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cytochrome content. The aim of this study was, to isolate and to characterize the cytochromes of *Chromatium warmingii* and to compare the results with those of the small-cell *Chromatium* species.

Materials and Methods

Chromatium warmingii strain 6512 (DSM Number 173) was kindly provided by Prof. Dr. N. Pfennig, University of Konstanz, West Germany. and was grown photolithoautotrophically at 26 °C and 1000 lux in Pfennig's medium [28, 29], using the trace element solution of Pfennig and Lippert [30]. To get a better cell yield, a 11 culture was fed 4–5 times with 10–15 ml of a sulfide solution, containing 30 g $Na_2S \times 8H_2O$ in 360 ml water, partially neutralized with 7.5 ml of a sterile 2 M H_2SO_4 solution. After feeding, the pH of the culture medium was checked and adjusted to pH 7.0–7.3 with sterile 2 M H_2SO_4.

Cells were harvested by centrifugation for 10 min at 7000 rpm and washed twice with 100 mM potassium phosphate buffer, pH 7.5. The wet cell material obtained was suspended in the same buffer (w/v 1:1) and broken up by sonification (1 min/ml cell suspension) in a Schoellerschall TG 250 or Branson Sonifier Cell Disruptor B 15 at 4 °C. The disrupted cell suspension was first centrifuged for 20 min at 15000 rpm and the supernatant was then centrifuged for 5 h at 45000 rpm. The supernatant of this ultracentrifugation contained all soluble cytochromes, which were further purified according to the method of Bartsch [31, 32].

Spectrophotometric determination of cytochromes, estimation of protein, molecular weight and isoelectric point, and analytical determination of sulfur compounds (sulfide, sulfate and elemental sulfur) were carried out as described by Steinmetz and Fischer [10–12].

Polyacrylamide gel electrophoresis (7.5% gel) was performed as described by Maurer [33].

Alkaline pyridine spectra of cytochromes were prepared following the method of Bartsch [31].

Redox potential of cytochrome *c'* was determined anaerobically at pH 7.0 and 23 °C by the stepwise addition of 50 mM $K_3(Fe(CN)_6)$ solution and a 100 mM $Na_2S_2O_4$ solution. To ensure anaerobic conditions all suspensions and reaction vessels were flushed with oxygenfree nitrogen. Oxygen was removed from nitrogen gas by the method described by Nickerson and Strauss [34]. Cytochrome *c'* was suspended in 50 mM potassium phosphate buffer, pH 7.0 and the redox potential was determined as described by Dutton [35] and Steinmetz and Fischer [11].

Chemicals were of analytical grade and obtained from Merck, Darmstadt, West Germany; biochemicals, test combinations and combitheks from Boehringer, Mannheim, West Germany; DEAE- and CM cellulose from Whatman Biochemicals Ltd., Maidstone, England; Sephadex G-25, G-75 and Sephacryl S-200 from Pharmacia, Uppsala, Sweden; Ampholine and PAG-plates from LKB, Stockholm, Sweden; phenazine ethosulfate from ICN Pharmaceuticals Inc., Plainview, New York, USA and all other redox mediators from EGA Chemie, Steinheim, West Germany.

Results

Two soluble acidic *c*-type cytochromes (*c*-552 and *c'*) and a membrane-bound cytochrome *c* were found in the purple sulfur bacterium *Chromatium warmingii*. Cytochrome *c*-552 was a labile protein which denaturated during purification procedure and therefore could only be partially purified. The native spectrum of the enriched cytochrome showed maxima at 552 nm (α-band), 523 nm (β-band), and 417 nm (γ-band) in the reduced form, while the γ-band of the oxidized form was at 408 nm (Fig. 1). The α-band absorption maximum of an alkaline pyridine spectrum of this cytochrome was at 551 nm, characteristic for a *c*-type cytochrome [31]. After the addition of dithionite to the oxidized form of cytochrome *c*-552, full reduction was obtained after half an hour. Such a behaviour was also observed for cytochrome *c*-552 (550) of *Thiocapsa pfennigii* [18]. The best purity index obtained for cytochrome *c*-552 of *Chr. warmingii* (A_{280}/A_{408}) was 0.94. The molecular weight of cytochrome *c*-552 – estimated by comparative gel filtration through Sephadex G-75, with three marker proteins of known molecular weight – was about 30000 (Fig. 2). The isoelectric point was in the range of pH 4.3–5.0, determined by flat bed electrofocusing.

The other soluble cytochrome found in *Chr. warmingii* was a high-spin cytochrome *c'* and could be purified to homogeneity. Electrophoresis in 7.5% polyacrylamide gel of cytochrome *c'* revealed a single band with an R_f-value of 0.84 (Fig. 3). The

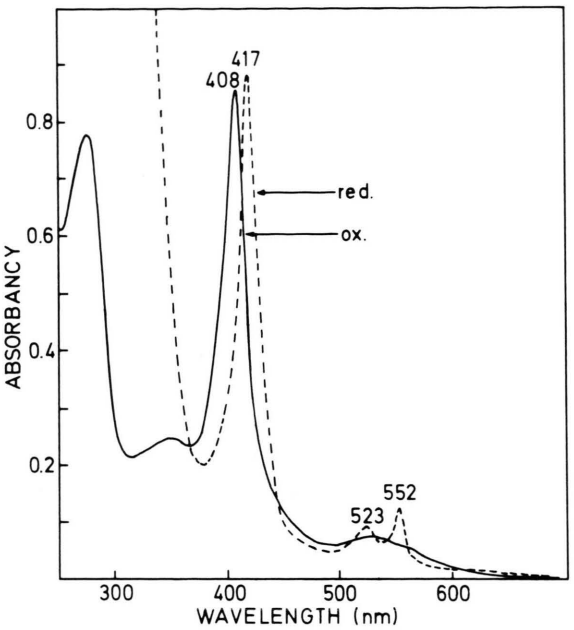

Fig. 1. Absorption spectra of oxidized and reduced (plus a few crystals of sodium dithionite) cytochrome *c*-552 of *Chromatium warmingii*. 1 cm cells contained 0.95 mg protein in 1 ml of 100 mM potassium phosphate buffer, pH 7.5.

best purity index obtained (A_{280}/A_{399}) was 0.35. We could isolate 4.44 µmol of cytochrome *c′* out of 1000 g wet cell paste. Cytochrome *c′* was isolated in its oxidized form and showed absorption maxima at 399 nm, 490 nm and 634 nm, characteristic for high-spin cytochromes. After the addition of a few crystals of solid sodium dithionite, a broad α-band appeared at around 550 nm, no β-band was detectable and the γ-band shifted towards 425 nm with a typical shoulder at 434 nm. The maximum at 634 nm was bleached out (Fig. 4). At pH values ≧ 11, the spectrum of cytochrome *c′* showed low-spin character with a γ-band at 408 nm and a broad maximum around 550 nm in the oxidized form and 3 distinguishable maxima at 552 nm (α-band), 522 nm (β-band) and 417 nm (γ-band) in the reduced form. Changing back to neutral pH values, the cytochrome *c′* spectra shown in Fig. 4 reappeared.

Reduced cytochromes *c′* typically bind reversibly to CO, forming a cytochrome *c′*-CO-complex. When cytochrome *c′* of *Chr. warmingii* (suspended in 50 mM potassium phosphate buffer, pH 7.5) was treated for 5 min with carbon monoxide, a typical absorption spectrum of a CO-binding cytochrome was obtained with a γ-band at 418 nm. When the so treated cytochrome *c′* was allowed to stand in the dark for 14 h, no significant absorbance changes could be observed. When the sample was then exposed to light, a rapid absorption change back to the oxidized form occurred within several hours

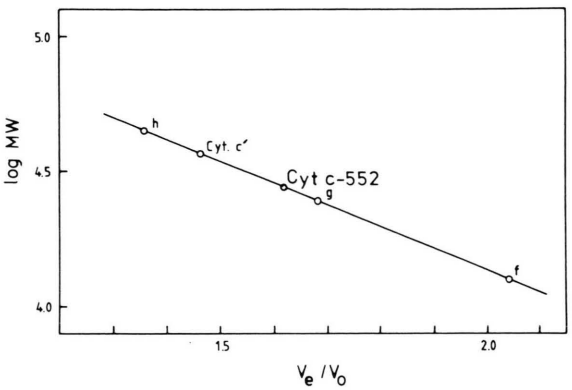

Fig. 2. Estimation of molecular weight of *Chromatium warmingii* cytochromes *c*-552 and *c′* by gel filtration through Sephadex G-75. The elution volumes (V_e) of both cytochromes (determined by separate runs) were compared with those of three calibration proteins with known molecular weights. h, hen egg albumin (MW: 45000); g, chymotrypsinogen A (MW: 25000); f, horse heart cytochrome *c* (MW: 12500). The void volume (V_0) was determined with dextran blue.

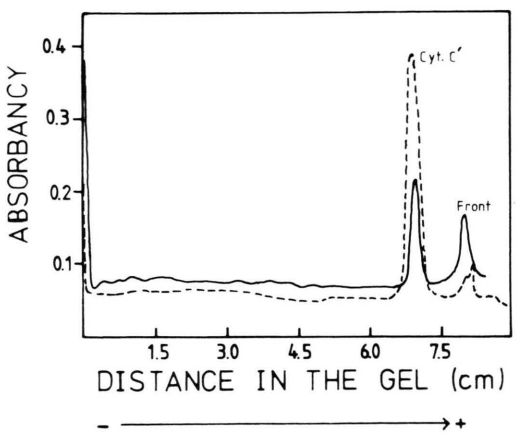

Fig. 3. Densitogram of purified cytochrome *c′* of *Chromatium warmingii* obtained by gel electrophoresis in 7.5% polyacrylamide gel with bromophenol blue as marker. Dotted line: after staining with Coomassie blue R-150 (0.25%) dissolved in a mixture of acetic acid:methanol:water = 1:5:5. Dashed line: unstained gel. Gel scanning was carried out in an Acta M VI double beam spectrophotometer at 580 nm (---) and 410 nm (——).

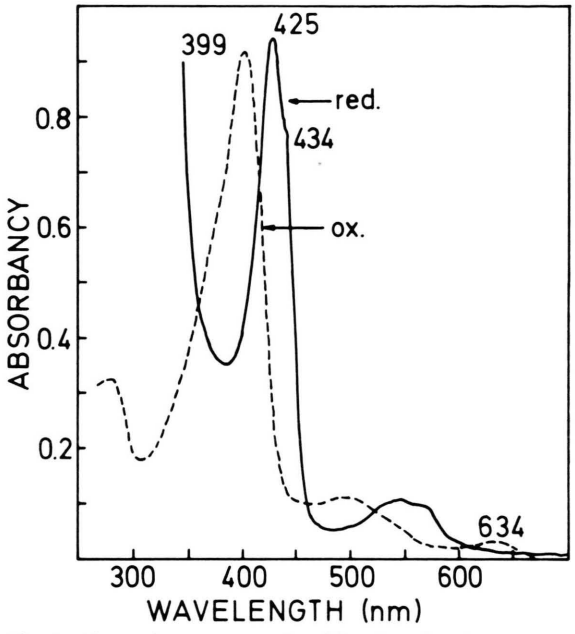

Fig. 4. Absorption spectra of oxidized and reduced cytochrome *c'* (plus a few crystals of dithionite) of *Chromatium warmingii*. 1 cm cells contained 0.27 mg protein in 1 ml of 100 mM potassium phosphate buffer, pH 7.5.

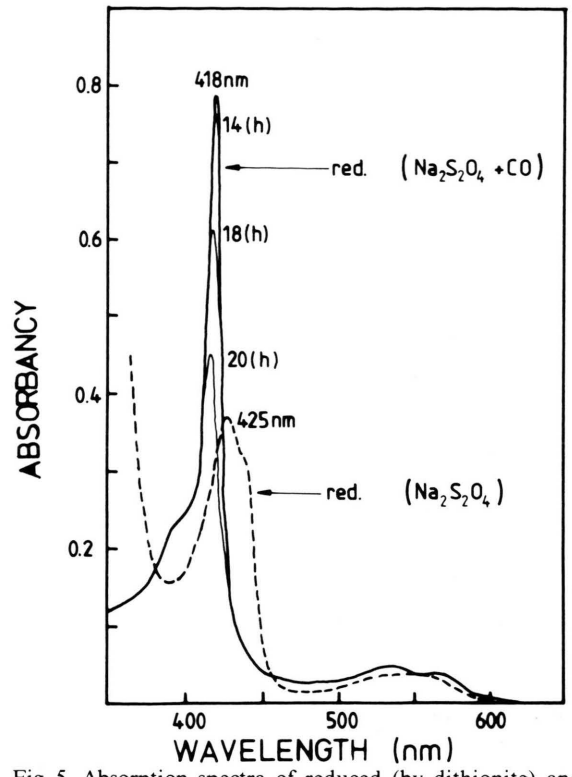

Fig. 5. Absorption spectra of reduced (by dithionite) and reduced carbon monoxide treated cytochrome *c'* of *Chromatium warmingii*. Carbon monoxide was bubbled through the sample for 5 min in the cells sealed with a serum stopper. Absorption changes of the carbon monoxide treated cytochrome *c'* were followed with time. After 14 h the sample was exposed to light. 1 cm cells contained 0.2 mg protein in 1 ml of 100 mM potassium phosphate buffer, pH 7.5.

(Fig. 5). This behaviour indicates that cytochrome *c'* of *Chr. warmingii* is also autoxidizable. This has already been described for other *c'*-cytochromes [32]. Oxidized cytochrome *c'* did not react with CO, and no absorbance changes could be observed.

Cytochromes *c'* have been normally isolated in their dimeric form with molecular weights in the range of 28000–30000 [32] and which were able to dissociate under denaturating conditions [36]. Therefore, the molecular weight of cytochrome *c'* of *Chr. warmingii* was estimated not only by comparative gel filtration through Sephadex G 75, but also by sodium dodecylsulfate polyacrylamide gel electrophoresis (7.5% gel). With the former procedure we obtained a molecular weight of 37100 (Fig. 2), indicating a polymer of the cytochrome. With the latter procedure we determined a molecular weight of 12100 for the monomeric form (Fig. 6).

The isoelectric point of oxidized cytochrome *c'* was found at pH 4.0 by flat bed electrofocusing on Ampholine PAG-plates, pH range 3.5–9.5.

At pH 7.0, midpoint oxidation-reduction potential of cytochrome *c'* was +10 mV. The redox

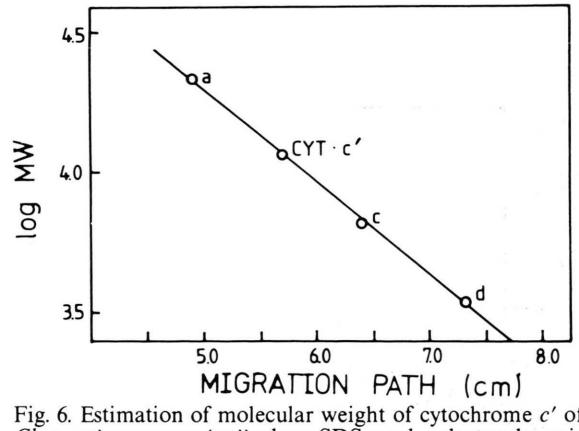

Fig. 6. Estimation of molecular weight of cytochrome *c'* of *Chromatium warmingii* by SDS gel electrophoresis (7.5% gel). The migration path of cytochrome *c'* was compared with that of several marker proteins of the Boehringer Combithek No. 236292. a, trypsin inhibitor from soy bean (MW: 21500); c, aprotinin (MW: 6500); d, insulin chain B (MW: 3400).

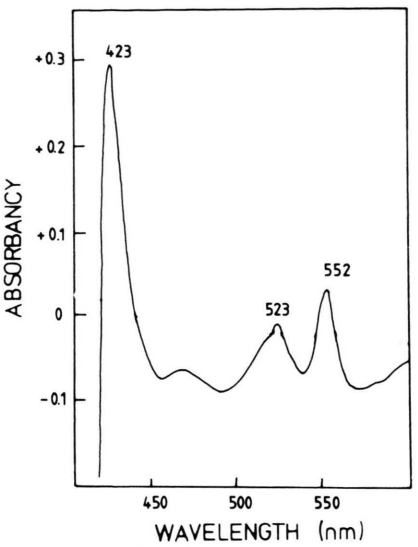

Fig. 7. Difference spectrum of a membrane-bound cytochrome *c*-552 of *Chromatium warmingii*, reduced (by dithionite) minus oxidized.

potential was measured with a PHM 64 research pH Meter Radiometer Kopenhagen and a platinum and a calomel electrode under stirring in the reaction cell. The relative absorption changes at 490 nm and 634 nm, respectively, of cytochrome *c'* were followed with time in a Beckman Acta M VI recording double beam spectrophotometer until equilibrium conditions were reached.

Chr. warmingii contained also a membrane-bound cytochrome which could be identified by a reduced-minus-oxidized difference spectrum (Fig. 7). This was obtained from the sediment of the 45000 rpm ultracentrifugation (see Materials and Methods) by the following procedure: 5 g of this sediment was treated seven times with a mixture of acetone and methanol (7:2) in an ice bath and centrifuged at 15000 rpm for 10 min at 4 °C. After the last treatment, the remaining sediment was dissolved in 50 mM potassium phosphate buffer, pH 7.5 containing 0.1%. Triton X-100. The mixture was centrifuged again and the red supernatant was fractionated with ammonium sulfate. The cytochrome fraction precipitating between 25 and 50% saturation was redissolved in the buffer described above. This solution was used for the registration of the difference spectrum shown in Fig. 7.

All species of Chromatiaceae examined so far, *e.g. Chr. okenii*, *Chr. vinosum* or *Thiocapsa roseopersicina*, began to oxidize the formed elemental sulfur to sulfate even before sulfide was completely consumed by the cells [37, 38]. Thiosulfate was not utilized by the large-cell Chromatiaceae [37].

During anaerobic sulfide oxidation of a growing culture of *Chr. warmingii* we could show that sulfate appeared in the medium in addition to intracellular elemental sulfur, before all sulfide was oxidized to elemental sulfur (Fig. 8). These findings are in good accordance with the results given above.

The experiment with *Chr. warmingii* was carried out at 25 °C and 1000 lux in a 10 l carboy. The medium was inoculated with 1 l of a washed preculture (1.15 mg protein/ml). In this case, the pH of the medium was adjusted with a steril 1 N HCl

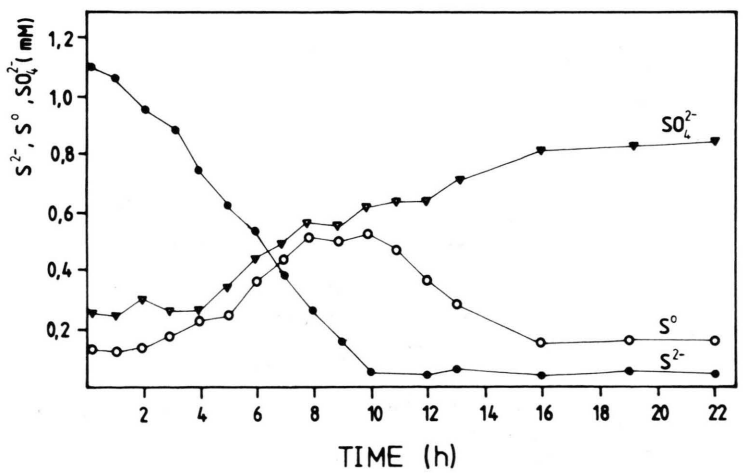

Fig. 8. Anaerobic sulfide oxidation by a growing culture of *Chromatium warmingii* in a 10 l carboy followed with time.

solution to pH 7.0–7.3. Samples of 10–15 ml were removed from the growing culture at different intervals of time for the determination of sulfur compounds.

Discussion

In *Chromatium warmingii* strain 6512 we have found two soluble acidic *c*-type cytochromes – *c*-552 and *c'* – and a membrane-bound cytochrome. We have had no success in isolating a flavocytochrome, like the one found in *Chr. vinosum* [39] or green sulfur bacteria [6, 10, 11]. In addition to our findings, *Thiocapsa pfennigii* does not possess a flavocytochrome, either [18]. Because soluble cytochrome *c*-552 denaturated during purification procedure, only a few molecular data (spectral properties, molecular weight and isoelectric point) were obtained. The molecular weight of 30000 for cytochrome *c*-552 of *Chr. warmingii* is comparable to cytochromes *c*-552 (550) and *c*-552 (545) of *Thiocapsa pfennigii* which have the same molecular size [18]. The minimum size of cytochrome *c*-552 (545) was 12000, calculated from amino acid composition. This indicates that this protein has a multiheme character [18]. Meyer and coworkers [18] also reported that full reduction of cytochrome *c*-552 (545) of *Thiocapsa pfennigii* by dithionite was obtained after 30 min. This finding agrees very well with our result obtained for cytochrome *c*-552. Normally, most cytochromes are reduced by dithionite in less than 10 s, even the low redox potential cytochromes c_3 of sulfate reducing bacteria [18]. The function of *Thiocapsa pfennigii* cytochrome *c*-552 (545) is still unclear. Although the spectrum resembles that of cytochrome *c* peroxidase of *Pseudomonas aeruginosa*, cytochrome *c*-552 (545) of *Thiocapsa pfennigii* shows no peroxidase activity [32]. On the other hand, cytochrome *c*-550 (molecular weight of 34000) of *Thiocapsa roseopersicina* is involved in the oxidation process of sulfide to elemental sulfur [20]. Whether cytochrome *c*-552 of *Chr. warmingii* has a similar function, must be clarified.

Among the phototrophic bacteria, cytochrome *c'* was found in Chromatiaceae and Rhodospirillaceae but seems to be absent in Chlorobiaceae [32]. The molecular properties obtained for the high-spin cytochrome *c'* of *Chr. warmingii* are summarized in Table I and are compared to those of other *c'*-cytochromes from different phototrophic bacteria. Two main characteristics of cytochromes *c'* are that the absorption spectra undergo reversible changes as a function of pH and that the reduced cytochromes *c'* react with carbon monoxide [32]. The isolated hemoprotein of *Chr. warmingii* satisfies these criteria and can therefore be designated as cytochrome *c'*.

The main properties of cytochrome *c'* of *Chr. warmingii* resemble those of the corresponding hemoproteins of *Chr. vinosum* [40] and *Thiocapsa pfennigii* [18]. As can be seen in Table I, all listed cytochromes *c'* from different phototrophic bacteria have similar absorption spectra. Molecular weights of cytochromes *c'* – determined by gel filtration – range from 25000 to 37000 and indicate dimeric forms. When cytochromes *c'* are applied to SDS-polyacrylamide gel electrophoresis, the dimers dissociate into their monomers which have molecular weights between 12000 and 14000. Cytochrome *c'* of *Chr. warmingii* showed the same behaviour when both methods were used to determine the molecular weights.

Isoelectric point and redox potential of cytochrome *c'* of *Chr. warmingii* are also in good accordance with the data of the other microorganisms listed in Table I. But one should point out that there are also basic cytochromes *c'* with a more positive redox potential. Cytochrome *c'* of *Rhodopseudomonas palustris*, for example, has an isoelectric point at pH 9.4 and a midpoint redox potential of +102 mV [32]. The amounts of cytochrome *c'* isolated from *Thiocapsa pfennigii* [18] and *Chr. warmingii* are nearly identical. 4.6 µmol/kg wet cell material was isolated from the former organism and 4.44 µmol/kg from the latter.

A unique function of cytochrome *c'* has not been found so far. The distribution of the protein contradicts a direct participation in the photosynthetic electron transport pathway, because cytochrome *c'* is also present in nonphototrophic bacteria (see 32). In addition, cytochrome *c'* was absent in one of three strains of *Rhodopseudomonas palustris* examined, although no anomalies in photosynthesis or respiration could be observed in this strain [41].

The function of cytochrome *c'* in denitrifying bacteria can perhaps be seen in its ability to bind NO during the reduction of nitrite to nitrogen [42]. In phototrophic bacteria cytochrome *c'* might be involved not only in nitrogen metabolism but also in

Table I. Comparison of some molecular properties of *Chromatium warmingii* cytochrome *c'* with those of other phototrophic bacteria.

Organism Properties	*Chromatium vinosum*[a]	*Thiocapsa pfennigii*[a]	*Rhodopseudomonas sphaeroides*[a]	*Rhodopseudomonas capsulata*[a]	*Chromatium warmingii*[b]
Molecular weight[c]	14000 (A) 37000 (S)	14000 (A) 11000 (P)	25000 (S)	28000 (S)	12100 (P) 37100 (S)
Redox potential (E_{m7})	−5 mV	n. d.	+30 mV	0.0 mV	+10 mV
Isoelectric point	4.6	n. d.	4.9	4.7	4.0
Purity index ($A_{280}/A_{\gamma\text{-band ox.}}$) (ox. = oxidized)	0.33	0.37	0.29	0.23	0.35
Maxima (nm) Oxidized form	399, 495, 634	399, 490, 632	400, 496, 633	399, 495, 635	399, 498, 634
Reduced form	426, 547	426.5, 546	425, 546	425, 550	425, 547

[a] Data taken from Bartsch [32].
[b] Own results.
[c] Molecular weights were determined by: amino acid composition (A); gel filtration (S); polyacrylamide gel electrophoresis with sodium dodecylsulfate (P).
n. d. = not determined.

sulfur metabolism. In *Chr. vinosum* cytochrome *c'* showed weak sulfite reductase activity but strong hydroxlamine reductase activity (Kobayashi, pers. communication). The latter activity was also described for cytochrome *c'* of a halophilic *Micrococcus* [41]. On the other hand, Brückenhaus [43] found sulfite oxidoreductase activity for cytochrome *c'* of *Chr. vinosum*, and Fischer and Trüper [21] reported that cytochrome *c'* of *Thiocapsa roseopersicina* might be involved in the thermostable sulfide oxidizing system. Further studies must clarify the real function of cytochrome *c'* in sulfur metabolism of phototrophic bacteria.

Chr. warmingii also contains a membrane-bound cytochrome with an α-band at 552 nm, observed in a reduced-minus-oxidized difference spectrum. The membrane-bound cytochrome *c*-552 is not identical to the soluble cytochrome *c*-552, because the former was immediately reduced by dithionite. Membrane-bound cytochromes or even cytochrome complexes have also been described for *Chr. vinosum* [16, 17], *Thiocapsa pfennigii* [18] and green sulfur bacteria [10, 32]. The membrane-bound cytochrome *c*-complex of *Chr. vinosum* was solubilized with potassium cholate and consists of a low potential cytochrome *c*-552 and a high potential cytochrome *c*-556 [16, 17]. Using the same extraction method, Meyer *et al.* [18] could isolate a high potential *c*-557 and a low potential *c*-552 cytochrome complex from the chromatophore fraction of *Thiocapsa pfennigii.* When we tried to solubilize the chromatophore fraction of *Chr. warmingii* with Triton X-100 to extract bound cytochromes, the reduced-minus-oxidized difference spectrum showed only one absorbancy band at 552 nm. Because of the different method used to extract bound cytochromes, we will not exclude the possibility that perhaps a second cytochrome, like *c*-556 or *c*-557, will also be located in the membranes. Further studies of more chemical properties of membrane-bound cytochromes of *Chr. warmingii* will allow a better comparison to the above described cytochrome complexes.

The anaerobic sulfide oxidation by whole cells of *Chr. warmingii* shows no unusual behaviour (see Results). The observation that sulfate appears in the medium simultaneous with intracellular elemental sulfur agrees very well with the results found for sulfide oxidation of other Chromatiaceae [37, 38]. The results obtained with a growing culture of *Chr. warmingii* during sulfur turnover are therefore a good supplement to same experiments done with other *Chromatium* species.

Acknowledgement

This study was supported by a grant from the Deutsche Forschungsgemeinschaft (Fi 295/2-1).

[1] H. G. Trüper, Pl. Soil **43,** 29 (1977).
[2] H. G. Trüper and U. Fischer, Phil. Trans. R. Soc. Lond. B **298,** 529 (1982).
[3] U. Fischer, Sulfur – a unique element (A. Müller and B. Krebs, eds.), in press, Elsevier Scientific Publishing Company, Amsterdam 1983.
[4] J. Gibson, Biochem. J. **79,** 151 (1961).
[5] J. H. Mathewson, L. J. Burger, and H. G. Millstone, Fed. Proc. **27,** 774 (1968).
[6] T. E. Meyer, R. G. Bartsch, M. A. Cusanovich, and J. H. Mathewson, Biochim. Biophys. Acta **153,** 854 (1968).
[7] A. Kusai and T. Yamanaka, FEBS Lett. **34,** 235 (1973).
[8] A. Kusai and T. Yamanaka, Biochim. Biophys. Res. Comm. **51,** 107 (1973).
[9] A. Kusai and T. Yamanaka, Biochim. Biophys. Acta **325,** 304 (1973).
[10] M. A. Steinmetz and U. Fischer, Arch. Microbiol. **130,** 31 (1981).
[11] M. A. Steinmetz and U. Fischer, Arch. Microbiol. **131,** 19 (1982).
[12] M. A. Steinmetz and U. Fischer, Arch. Microbiol. **132,** 204 (1982).
[13] M. A. Steinmetz, H. G. Trüper, and U. Fischer, Arch. Microbiol. **135,** 186 (1983).
[14] R. G. Bartsch and M. D. Kamen, J. Biol. Chem. **235,** 825 (1960).
[15] M. A. Cusanovich and R. G. Bartsch, Biochim. Biophys. Acta **189,** 245 (1969).
[16] S. J. Kennel and M. D. Kamen, Biochim. Biophys. Acta **234,** 458 (1971).
[17] S. J. Kennel and M. D. Kamen, Biochim. Biophys. Acta **253,** 153 (1971).
[18] T. E. Meyer, S. J. Kennel, S. M. Tedro, and M. D. Kamen, Biochim. Biophys. Acta **292,** 634 (1973).
[19] H. G. Trüper and L. A. Rogers, J. Bacteriol. **108,** 1112 (1971).
[20] U. Fischer and H. G. Trüper, FEMS Microbiol. Lett. **1,** 87 (1977).
[21] U. Fischer and H. G. Trüper, Curr. Microbiol. **3,** 41 (1979).
[22] T. E. Meyer, Abstracts of the IV International Symposium on Photosynthetic Prokaryotes (G. Stanier, ed.), p. A 46, Bombannes, France 1982.
[23] T. Yamanaka and A. Kusai, Flavin and Flavoproteins (T. P. Singer, ed.), pp. 292–301, Elsevier Scientific Publishing Company, Amsterdam 1976.
[24] Y. Fukumori and T. Yamanaka, J. Biochem. **85,** 1405 (1979).
[25] W. Schmitt, G. Schleifer, and K. Knobloch, Arch. Microbiol. **130,** 334 (1981).
[26] R. van Grondelle, L. N. M. Duysens, J. A. van der Wel, and H. N. van der Wal, Biochim. Biophys. Acta **461,** 188 (1977).
[27] Y. Fukumori and T. Yamanaka, Curr. Microbiol. **3,** 117 (1979).
[28] N. Pfennig, Anreicherungskulturen und Mutantenauslese (H. G. Schlegel and E. Kröger, eds.), pp. 179–189, 503–504, Fischer Verlag, Stuttgart 1965.
[29] N. Pfennig and H. G. Trüper, The Prokaryotes (M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel, eds.), pp. 279–289, Springer Verlag, Berlin-Heidelberg 1982.
[30] N. Pfennig and K. D. Lippert, Arch. Mikrobiol. **55,** 245 (1966).
[31] R. G. Bartsch, Methods in Enzymology (S. P. Collowick and N. O. Kaplan, eds.), **Vol. XXIII,** pp. 344–363, Academic Press, New York 1971.
[32] R. G. Bartsch, The Photosynthetic Bacteria (R. K. Clayton and W. R. Sistrom, eds.), pp. 249–279, Plenum Press, New York and London 1978.
[33] H. R. Maurer, Disc Electrophoresis and Related Techniques of Polyacrylamide Gel Electrophoresis. 2. Edition, W. de Gruyter Verlag, Berlin and New York 1971.
[34] W. J. Nickerson and P. W. Strauss, J. Amer. Chem. Soc. **82,** 5007 (1960).
[35] L. Dutton, Methods in Enzymology (S. P. Collowick and N. O. Kaplan, eds.), **Vol. 53,** pp. 411–435, Academic Press, New York 1978.
[36] M. A. Cusanovich, Biochim. Biophys. Acta **236,** 238 (1971).
[37] H. G. Trüper and H. G. Schlegel, Antonie van Leeuwenhoek, J. Microbiol. Serol. **30,** 225 (1964).
[38] H. van Gemerden, Arch. Microbiol. **64,** 111 (1968).
[39] R. G. Bartsch, T. E. Meyer, and A. B. Robinson, Structure and Function of Cytochromes (K. Okunuki, M. D. Kamen, and I. Sekuzu, eds.), pp. 443–451, University of Tokyo Press 1968.
[40] R. G. Bartsch and M. D. Kamen, J. Biol. Chem. **235,** 825 (1960).
[41] R. P. Ambler, R. G. Bartsch, M. Daniel, M. D. Kamen, L. McLellan, T. E. Meyer, and J. van Beeumen, Proc. Natl. Acad. Sci. USA **78,** 6854 (1981).
[42] S. Taniguchi and M. D. Kamen, Biochim. Biophys. Acta **74,** 438 (1963).
[43] I. Brückenhaus, Diploma Thesis, University Bonn, West Germany 1977.