Biochimica et Biophysica Acta, 292 (1973) 634–643

BBA 46504

IRON PROTEIN CONTENT OF *THIOCAPSA PFENNIGII*, A PURPLE SULFUR BACTERIUM OF ATYPICAL CHLOROPHYLL COMPOSITION

T. E. MEYER, S. J. KENNEL*, S. M. TEDRO and M. D. KAMEN

Department of Chemistry, University of California, San Diego, La Jolla, Calif. 92037 (U.S.A.)

(Received October 23rd, 1972)

SUMMARY

Four out of five soluble electron transport iron proteins of *Thiocapsa pfennigii* plus the particulate cytochromes have been found to be analogous to those of *Chromatium vinosum* strain D. In addition to ferredoxin, high potential iron–sulfur protein. cytochrome *c'*, and cytochrome *c*-552(550), *T. pfennigii* contains a cytochrome *c*-552(545) not previously isolated from photosynthetic bacteria. It is concluded that *T. pfennigii* is more closely related to *C. vinosum* than to *Rhodopseudomonas viridis*, the only other known bacterial species having bacteriochlorophyll *b*.

INTRODUCTION

Two species of photosynthetic bacteria have been isolated relatively recently and shown to contain chlorophyll component(s) — bacteriochlorophylls "*b*" — different from those of bacteria previously studied. These species, *Rhodopseudomonas viridis*[1] and *Thiocapsa pfennigii*[2], have been classified with the *Athiorhodaceae* and *Thiorhodaceae*, respectively, on the basis of classic microbiological criteria[3]. Light-induced absorbance changes, however, suggest that there may be some similarity between *R. viridis*[4] and *Chromatium vinosum* strain D[5,6], the most extensively characterized organism of the *Thiorhodaceae*. Unlike other photosynthetic bacteria, *R. viridis* is reported to have only one soluble cytochrome[4]. The active center chlorophyll species has been isolated from *R. viridis* particles[7] and shown to be similar to that in the purple and purple sulfur bacteria.

In this study we compare *T. pfennigii* electron transport iron proteins, particularly with those reported in *R. viridis* and *C. vinosum*, as well as with those of the other purple and non-sulfur purple bacteria.

METHODS AND RESULTS

A culture of Eimhjellen's *T. pfennigii* strain KIMG 8816 was grown in the medium of Pfennig[8] using 0.2 g $Na_2S \cdot 9H_2O$ and 1.0 g sodium acetate per l at pH 6.6, in 20-l carboys at 25 °C, illuminated with 40-W showcase lamps (approx. 25 ft-

* Present address: Department of Experimental Pathology, Scripps Clinic and Research Foundation, La Jolla, Calif. 92037, U.S.A.

candles). Additions of 0.2 g $Na_2S \cdot 9H_2O$/l of culture were made at 2-day intervals after inoculation. Cells were harvested after 6 days' growth to yield 2.5 g wet wt/l. Cells were suspended 20% (w/v) in 0.1 M Tris (pH 7.3), stirred overnight, a few mg crystalline deoxyribonuclease added, and then disruption completed in the Ribi Cell Fractionator operated at 20000 lb/inch^2. Large-cell fragments were removed by centrifugation in the Servall SS-1 rotor (30000 $\times g$, 15 min) and smaller particles in the Spinco 42 rotor operated at 35000 rev./min (145000 $\times g$, 2 h). The Spinco pellet was resuspended and reduced-*minus*-oxidized difference spectra were traced (Fig. 1). The supernatant solution was desalted on Sephadex G-25-C with buffer change to 1 mM Tris (pH 7.3) and adsorbed on DEAE-cellulose (Brown Co., Type 20). Proteins were eluted with a linear gradient from 0 to 0.25 M NaCl in 20 mM Tris (pH 7.3), which was followed by a stepwise increase to 0.5 M NaCl.

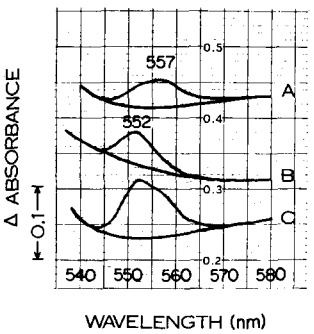

Fig. 1. Reduced-*minus*-oxidized difference spectra of "Spinco pellet" resuspended in 0.01 M Tris (pH 7.3) to give $A_{1010\ nm,\ cm^{-1}} \sim 4.5$. (A) 2-Mercaptoethanol reduced *minus* ferricyanide oxidized. (B) Dithionite reduced *minus* 2-mercaptoethanol reduced. (C) Dithionite reduced *minus* ferricyanide oxidized. All three baselines have been drawn arbitrarily.

Minor components

The first iron proteins to be eluted from the DEAE-cellulose column (at 0.03–0.06 M NaCl) were two minor cytochrome *c*-551 components, but they were present in concentrations too low (<0.5 μmole/kg) to attempt purification. The first component eluted was partially reduced, and precipitated at 40–60% satd $(NH_4)_2SO_4$. The second component was isolated completely oxidized, and precipitated at slightly higher $(NH_4)_2SO_4$ concentrations.

High-potential iron–sulfur protein

A high-potential iron–sulfur protein[9,10] was eluted in very large quantity from DEAE-cellulose at 0.08–0.12 M NaCl. Small amounts of the purple oxidized form preceded the bulk of the protein, which was present as the green reduced form. The concentration, while difficult to determine in crude eluates, was estimated at 50–100 μmoles/kg wet wt cells. The protein was desalted, adsorbed on a DEAE-cellulose column, eluted with 0.5 M NaCl solution, precipitated at 60–70% satd $(NH_4)_2SO_4$, re-dissolved in a minimal volume of water, chromatographed on Sephadex G-100 (in which the protein size appeared to be approx. 10000 daltons), and further purified by DEAE-cellulose chromatography. The protein crystallized as fine needles from

65% satd $(NH_4)_2SO_4$ in 0.1 M potassium phosphate, pH 7.0. The best ratio of 280 nm to 375 nm (reduced form) absorbance was 2.8 as shown in Fig. 2.

Iron content[11], absorption spectra, and amino acid analyses (Table I) were determined on aliquots of purified protein assuming 4 Fe/protein, in analogy with *Chromatium* high-potential iron–sulfur protein[10]. The millimolar absorptivity at 375 nm was found to be 15.3 cm^{-1}. Sodium dodecyl sulfate polyacrylamide gel electrophoresis[12,13] revealed two bands: a major band at 20000 and a minor band at 31000 daltons rather than the expected band at 10000 based on Sephadex chromatography. This anomaly is probably due to aggregation through disulfide interchange under denaturing conditions although 2-mercaptoethanol was added to prevent just such an occurrence. Perhaps a better approach in the future would be oxidation of cysteine residues with performic acid prior to sodium dodecyl sulfate polyacrylamide gel electrophoresis. The protein was better than 95% pure, as shown by amino-terminal[14–18] and carboxyl-terminal[19] analyses. The partial sequence was compared with the published sequences[10] of *C. vinosum* and *Rhodopseudomonas gelatinosa* high-potential iron–sulfur protein, *viz.*

TABLE I

AMINO ACID COMPOSITIONS OF *THIOCAPSA PFENNIGII* CYTOCHROMES AND IRON PROTEINS

Amino acid	*Cytochrome c-552(550)*		*Cytochrome c'*		*Cytochrome c-552(545)*		*High-potential iron–sulfur protein*	
	Av./ heme	*Tentative composition*	*Av./ heme*	*Tentative composition*	*Av./ heme*	*Tentative composition*	*Av./ 4 Fe*	*Tentative composition*
Asp	9.03	9	15.80	16	14.25	14	8.53	9
Thr	5.93	6	2.80	3	4.30	5	5.98	6
Ser	4.11	4	3.56	4	4.44	5	6.04	6
Glu	11.90	12	12.99	13	12.3	12	8.92	9
Pro	5.83	6	4.01	4	7.04	7	5.40	5
Gly	9.00	9	9.09	9	12.12	12	3.98	4
Ala	14.68	15	18.9	19	9.82	10	8,64	9
Cys	2.27	(3–4)	–	(2)	1.21	(2)	3.07*	(4)
Val	4.16	4	8.00	8	6.18	6	2.86	3
Met	3.46	4	2.75	3	1.67	2	–	0
Ile	2.58	3	5.05	5	3.82	4	2.88	3
Leu	6.65	7	6.28	6	7.08	7	4.93	5
Tyr	2.40	3	4.27	4	4.72	5	2.72	3
Phe	2.21	2	5.24	5	2.17	2	1.84	2
His	2.37	2	1.79	2	3.05	3	3.72	4
Lys	4.39	5	8.82	9	3.38	3	1.88	2
Arg	2.27	2	4.68	5	6.06	6	1.80	2
Trp	–	(1)	–	(1)	–	(0)	–	(2–4)
Total		97–98		118		105		78–80

* Determined as cysteic acid after performic acid oxidation.

R. gelatinosa[10] strain 1	Ala–Pro–Val–Asp–Glu–Lys–Asn . . . . . Ala
C. vinosum strain D[10, 20]	Ser–Ala–Pro–Ala–Asn–Ala–Val–Ala . . . Lys–Ala–Gly
T. pfennigii strain KIMG 8816	Glx–Asx–Leu–Pro–His–Val–Asx–Ala–Ala . . . Lys–Thr–Ala

Cytochrome c-552(550)

Following very closely behind the high-potential iron–sulfur protein on DEAE-cellulose chromatography was cytochrome *c*-552(550)[21], which was eluted at 0.10–0.13 M NaCl in the reduced form. Following concentration on DEAE-cellulose and elution with 0.5 M NaCl, it was partially purified by $(NH_4)_2SO_4$ precipitation at 30–60% satn (the same $(NH_4)_2SO_4$ fractions from the high-potential iron–sulfur protein purification steps were added to increase the yield to approx. 2.5 μmoles/kg wet wt cells). It was then fractionated on Sephadex G-100 and subsequently chromatographed twice in succession on DEAE-cellulose, once in the reduced form and then in the oxidized form. To obtain the oxidized form, the cytochrome was passed through a small column of Amberlite IRA-400 anion-exchange resin half-saturated with ferricyanide. The best ratio of 280 nm to 417 nm absorbance was 0.16 (Fig. 3). Sodium dodecyl sulfate polyacrylamide gel electrophoresis indicated a size of 30000 daltons. The tentative amino acid composition is given in Table I. Unlike the analogous *C. vinosum* cytochrome *c*-553(550)[21], the *T. pfennigii* cytochrome *c*-552(550) could not be extracted from chromatophores with 50% (v/v) acetone.

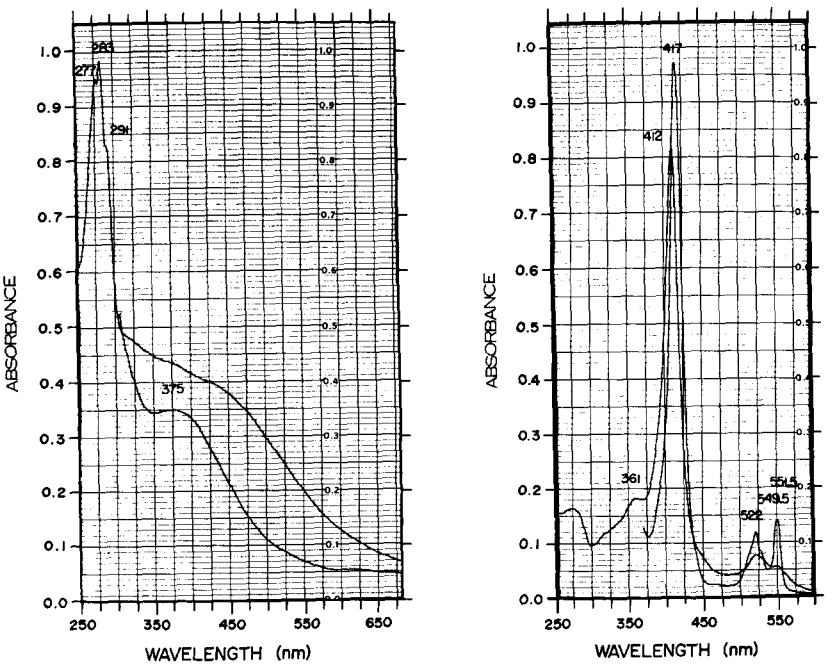

Fig. 2. Absorption spectra of purified high-potential iron–sulfur protein. The protein was initially passed through Amberlite IRA 400, half-saturated with ferricyanide, then diluted in 0.1 M phosphate buffer (pH 7.0), and the oxidized spectrum recorded. The reduced form was obtained by addition of 2-mercaptoethanol.

Fig. 3. Absorption spectra of cytochrome *c*-552(550) in 0.1 M phosphate buffer (pH 7.0). Reduced form was obtained by adding solid sodium dithionite.

Cytochrome c'

Next to be eluted from the DEAE-cellulose column was a mixture of cytochrome *c'* and another minor cytochrome *c*-551 component at 0.14–0.18 M NaCl. Approximately 4.5 μmoles cytochrome *c'* per kg wet wt cells was isolated, assuming ε_{mM} (426 nm) = 100 cm^{-1}. The cytochrome *c'* was purified by $(NH_4)_2SO_4$ precipitation at 50–90% saturation, Sephadex G-100 chromatography, and two more passages through DEAE-cellulose. The minor cytochrome *c*-551 precipitated at 20–40% satn and its purification was not pursued. The best ratio of 280 nm to 426 nm absorbance for cytochrome *c'* was 0.30 (Fig. 4). Sodium dodecyl sulfate polyacrylamide gel electrophoresis indicated a size of approx. 11000 daltons. The tentative amino acid composition is given in Table I.

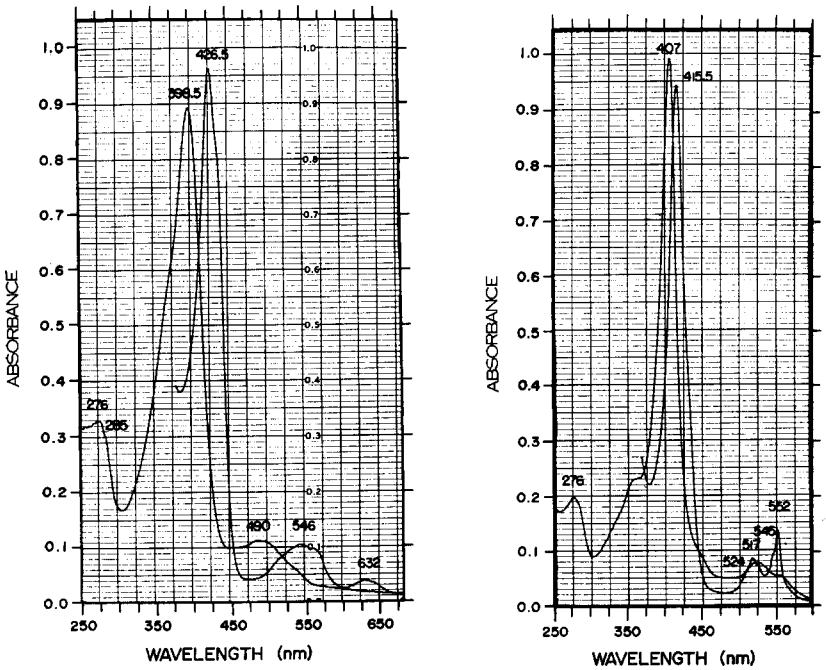

Fig. 4. Absorption spectra of cytochrome *c'* in 0.1 M phosphate buffer (pH 7.0). Reduced with dithionite.

Fig. 5. Absorption spectra of cytochrome *c*-552(545) in 0.1 M phosphate buffer (pH 7.0). Reduced spectrum traced 0.5 h after adding sodium dithionite.

Cytochrome c-552(545)

The last cytochrome eluted from the DEAE-cellulose at 0.20–0.25 M NaCl was cytochrome *c*-552(545). Approximately 10.5 μmoles/kg wet wt cells was isolated assuming ε_{mM} (407 nm) = 125 cm^{-1}. Cytochrome *c*-552(545) was purified by $(NH_4)_2SO_4$ precipitation at 50–70% satn, Sephadex G-100 fractionation, and DEAE-cellulose chromatography. The best ratio of 280 nm to 407 nm absorbance was 0.20 (Fig. 5). The size was 30000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, although the minimal formula weight calculated from amino acid composition, Table I, was roughly 12000, demonstrating the multiheme character

of this protein. It was noted that apparently complete reduction by sodium dithionite took about 30 min while most cytochromes (even the low redox potential cytochromes c_3) can be reduced in less than the mixing time (approx. 10 s). Eventual measurement of the redox potential of this protein will require use of an efficient redox mediator.

Ferredoxin

At the completion of the linear gradient elution of proteins from the initial DEAE-cellulose column, bacterial ferredoxin was eluted with 0.5 M NaCl. The yield was approximately 7 μmoles/kg wet wt cells, assuming ε_{mM} (390 nm) = 20 cm^{-1}. Owing to inherent instability of this class of proteins, further purification was not attempted.

Protoheme protein

By using an alternate isolation procedure, in which the proteins in the supernatant solution obtained by 145000 $\times g$ Spinco centrifugation were precipitated with $(NH_4)_2SO_4$ and chromatographed on Sephadex G-100, still another minor heme protein component was recognized. This apparently high-spin protoheme protein has also been observed in other bacteria, *viz. Azotobacter vinelandii*, *Rhodopseudomonas palustris*, *C. vinosum* (Meyer, T. E., unpublished observations). It was eluted with $V/V_0 = 1.48$ on the Sephadex G-100 column and precipitated at 30–50% satd $(NH_4)_2SO_4$. The quantity was too small to continue purification. This protein was slowly reduced by sodium dithionite at pH 7.

Solubilization of particulate proteins

The isolation and characterization of a complex cytochrome *c*-552–*c*-556 by extraction of lipid-depleted *Chromatium* chromatophore preparations with 2% potassium cholate *plus* 0.5 M NaCl has been described in detail[22]. As indicated by the reduced-*minus*-oxidized difference spectra (Fig. 1), the chromatophore fraction of *T. pfennigii* contains a high potential *c*-557, low potential *c*-552 cytochrome pair analogous to that of *Chromatium* chromatophores. These heme components constitute a large percentage of the cell cytochrome, in contrast to other photosynthetic bacteria[23]. About 39% of the *T. pfennigii* particulate cytochrome was solubilized by treating the chromatophores with potassium cholate in the manner previously used in extracting *Chromatium* chromatophores. Unlike the *Chromatium* extract, the solubilized cytochrome from *T. pfennigii* was not appreciably purified by hydroxylapatite chromatography, but was purified slightly by successive desalting (Sephadex G-25), passage over DEAE-cellulose (which adsorbed little of the cytochrome but removed colorless protein), and finally by precipitation with 60–100% satd $(NH_4)_2SO_4$. The cytochrome formed a floating mass on the surface of the $(NH_4)_2SO_4$, possibly owing to associated detergent or lipid. The cytochrome solubilized by detergent appeared to be only the *c*-552 component, although the cytochrome remaining in particles after extraction displayed the same ratio of *c*-552 to *c*-557 as was observed before extraction. If any of the *c*-557 component was solubilized, the absorption spectra must have been altered in the process. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the partially purified preparation indicated a size of approx. 70000 daltons.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis of whole chromatophores or of chromatophores extracted with acetone–methanol (7:2, v/v) resulted

only in a small number of protein bands, when stained with amido black, in contrast to the protein pattern of *Chromatium* chromatophores[23]. In addition, sodium dodecyl sulfate polyacrylamide gel electrophoresis showed the presence of a substance, which precipitated as a streak throughout the gel when the proteins were fixed in trichloroacetic acid. This substance was not stained by amido black and could possibly have masked staining of proteins in the gel.

When the initial DEAE-cellulose column used for isolation of cytochromes was eluted with 0.5 M NaCl instead of by the usual linear gradient chromatographic procedure, the eluate was highly viscous and gave a voluminous colorless precipitate at 20–40% satd $(NH_4)_2SO_4$, with considerably reduced viscosity of the remaining supernatant solution. This precipitated material may be the same substance which precipitated upon addition of trichloroacetic acid to the sodium dodecyl sulfate polyacrylamide gels of chromatophores. It was not further characterized.

DISCUSSION

The comparison of electron transport proteins of *T. pfennigii* with those of other photosynthetic bacteria has revealed that of five major soluble components, four have also been found in *C. vinosum* (Table II). Bacterial ferredoxin is common to all photosynthetic bacteria with the exception of the blue-green algae or bacteria (Bartsch, R. G. and Meyer, T. E., unpublished). Cytochrome *c'* is found in nearly all purple non-sulfur and purple sulfur bacteria, and in a few non-photosynthetic bacteria, but has never been isolated from the green sulfur, or from the blue-green bacteria[24]. High-potential iron–sulfur protein and cytochrome *c*-552(550) have only been found previously in *C. vinosum* and *R. gelatinosa*. In contrast, flavocytochrome *c* (ref. 24), so typical of *C. vinosum* and a green sulfur bacterium, *Chlorobium thiosulfatophilum*, is not found in *T. pfennigii*, in which the fifth soluble component is a cytochrome *c*-552(545) not found in other photosynthetic bacteria.

Of the electron transport proteins of *T. pfennigii*, high-potential iron–sulfur protein is isolated in the greatest quantity. It is also the major soluble component in *C. vinosum* and *R. gelatinosa*. The minimum size (10000) and iron content (4 Fe per 10000 size) of the high-potential iron–sulfur proteins are similar, although

TABLE II

IRON PROTEIN DISTRIBUTION IN SOME PHOTOSYNTHETIC BACTERIA

	T. pfennigii	*C. vinosum*	*R. gelatinosa*	*R. viridis*
Soluble proteins				
Ferredoxin	+	+	+	+
Cytochrome *c'*	+	+	+	−
High-potential iron–sulfur protein	+	+	+	−
Cytochrome *c*-552(550)	+	+	+	−
Flavocytochrome *c*	−	+	−	−
Cytochrome *c*-552(545)	+	−	−	−
Cytochrome c_2	−	−	±	±
Particle bound proteins				
Cytochrome *c*-557	+	+	+	+
Cytochrome *c*-552	+	+	+	+

the amino acid compositions and amino-terminal sequences are considerably different. The amino acid composition of *T. pfennigii* high-potential iron–sulfur protein is more like that of *C. vinosum* than that of *R. gelatinosa*.

The concentration of cytochrome *c'* is lower in *T. pfennigii* (5–10% concn of high-potential iron–sulfur protein) than it is in other photosynthetic bacteria, with the exception of *R. palustris* strain 37 (ref. 25). It is usually isolated in high yields, roughly equivalent to the high-potential iron–sulfur protein or to the cytochrome c_2 content, although it is presently impossible to determine whether there is any correlation between the concentrations of these proteins because of the difficulty in growing cells under strictly defined conditions. However, cytochrome *c'* is most likely not directly involved in photosynthesis since it is not found in all strains of closely related purple bacteria. It is notably absent in *R. viridis*[4], *R. palustris* strain 6 (ref. 25), and in *Rhodomicrobium vannielii*[26].

Most cytochromes *c'* contain roughly 125 amino acid residues, although *C. vinosum* cytochrome *c'* is an exception in being slightly larger (approx. 136 residues)[23]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis indicates a size of 11000 daltons for *T. pfennigii* cytochrome *c'* as contrasted with *C. vinosum* cytochrome *c'*, which is about 14000 daltons[23]. The smaller number of amino acid residues recovered from *T. pfennigii* cytochrome *c'* (approx. 118) is consistent with a smaller size. The amino acid composition is most similar to "*Pseudomonas denitrificans*" cytochrome *c'* (ref. 27).

T. pfennigii cytochrome *c*-552(550) is isolated in small quantity, as is the analogous cytochrome from the other two known sources, *C. vinosum*[21] and *R. gelatinosa*[26]. It has been pointed out[21] that the absorption spectra of this protein are similar to those of green sulfur bacterial cytochrome *c*-555 and those of blue-green bacterial and algal cytochrome *f*. A further similarity may be noted to cytochrome c_5, a dimeric protein most extensively characterized in *Azotobacter vinelandii*[28], but also found in most *Pseudomonads*[29]. The amino acid sequence of *Pseudomonas mendocina* cytochrome c_5 (ref. 29) shows four cysteines, only two of which are required to bind the heme. The amino acid composition of *T. pfennigii* cytochrome *c*-552(550) indicates slightly more cysteine than required to bind the heme if one assumes the usual recovery of cysteine from heme proteins is approximately 60%. Only two cysteines were reported[21] for the *C. vinosum* cytochrome *c*-553(550), a result which perhaps should be reevaluated. The size of cytochrome *c*-552(550) is 30000 daltons as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, which is somewhat greater than twice the minimal formula weight derived from amino acid analyses. It is possible that it is dimerized through interchain disulfide formation. The *C. vinosum* cytochrome is 19000 daltons and the *R. gelatinosa* cytochrome is 26000 daltons in size when measured by Sephadex G-75 gel filtration[26]. The *C. vinosum*[21] and *T. pfennigii* cytochromes are very similar to each other in amino acid composition, but cytochrome c_5 (ref. 29) is notably different in that it lacks phenylalanine and tyrosine residues.

The *T. pfennigii* cytochrome *c*-552(545) is not similar to any other protein previously found in photosynthetic bacteria but could be related to the *Pseudomonas stutzeri* cytochrome *c*-558(552)[30], *Alcaligenes faecalis* cytochrome *c*-557(552)[31], and to the *Pseudomonas aeruginosa* cytochrome *c* peroxidase[32]. The *T. pfennigii* protein has qualitatively similar absorption spectra although the wavelength maxima are

shifted to the blue. The *T. pfennigii* cytochrome is 30000 daltons in size as compared to 70000 daltons[30] and 58000 daltons[33] for the *Pseudomonas* proteins. There appear to be at least two hemes per single peptide chain in the *T. pfennigii* cytochrome, whereas the *Pseudomonas* proteins, reported to have two hemes, could be dimeric. The *P. aeruginosa* cytochrome *c*-558(552) has peroxidase activity which is specific for *Pseudomonas* cytochrome *c*-551 or azurin as substrate[34].

In particles from *T. pfennigii*[35], *C. vinosum*[5,6,22], *R. viridis*[4,7] and *R. gelatinosa*[36], both light-induced absorbance changes and chemical difference spectra suggest the presence of a high redox potential cytochrome *c*-557 and low redox potential cytochrome *c*-552 closely connected to photosynthetic pathways. Nearly all of this cytochrome can be extracted from *C. vinosum* with cholate detergent, whereas only a portion of the *T. pfennigii* and *R. gelatinosa* protein has been solubilized[23] and shown to be different from the buffer-soluble components. A contrasting situation occurs in most non-sulfur purple bacteria, in which prominent light-induced absorbance changes and chemical difference spectra are associated with the predominantly buffer-soluble cytochrome c_2. In *Rhodospirillum rubrum*, that cytochrome which remains bound to particles on disruption of cells, can be solubilized and shown to be identical to cytochrome c_2 (ref. 37).

Neither *T. pfennigii* nor *C. vinosum* have cytochromes analogous to cytochrome c_2, although both *R. gelatinosa* and *R. viridis* have a soluble cytochrome *c* which is indistinguishable from cytochrome c_2 on superficial examination. Amino acid sequence determinations and enzyme assays might be used to reveal differences, since cytochrome c_2 reacts slowly with cytochrome oxidase[38] and the sequence[39] reveals several gaps when compared to the homologous mitochondrial cytochrome c_0. If the soluble cytochrome is found to be identical to cytochrome c_2, then one might entertain the possibility of more than one functional role for cytochrome c_2, *i.e.* that in *R. viridis* and *R. gelatinosa* and that in the other non-sulfur purple bacteria.

It should be noted that each of the three major groups of photosynthetic bacteria is characterized by the presence of unique chlorophyll species; in the green sulfur bacteria they are bacteriochlorophylls *a*, *c*, *d*; in the blue–green bacteria it is chlorophyll *a*; and in the purple sulfur and non-sulfur bacteria it is the sole presence of bacteriochlorophyll *a*. Consequently, one would expect the bacteria having bacteriochlorophyll *b* to have numerous other distinguishing characteristics, *e.g.* iron protein content, which would establish them in a separate major group. This, however, is experimentally untrue. *T. pfennigii* strongly resembles the purple sulfur group, and *C. vinosum* in particular, while *R. viridis* has characteristics intermediate between the sulfur and non-sulfur purple bacterial sub-groups. The fact that in addition *R. viridis* and *T. pfennigii* are more similar to specific purple bacteria than they are to each other suggests that bacteriochlorophyll *b* may have independently arisen in these two species through a simple mutation in bacteriochlorophyll *a*. The tentative structure of bacteriochlorophyll *b*[40] differs from bacteriochlorophyll *a* only in *cis*- as opposed to *trans*-configuration of hydrogens at positions 3 and 4, and is consistent with the above argument for parallel evolution.

ACKNOWLEDGEMENTS

We thank Dr J. Oelze for a culture of *T. pfennigii* and gratefully acknowledge

helpful discussions with Dr R. G. Bartsch. This work was supported by grants-in-aid from the National Institutes of Health (HD-01262-10) and the National Science Foundation (GB-25050). S.J.K. and T.E.M. were supported by a U.S.P.H.S. training grant (2-T01-01045).

REFERENCES

1 Eimhjellen, K. E. Aasmundrud, O. and Jensen, A. (1965) *Biochem. Biophys. Res. Commun.* 10, 232–236
2 Eimhjellen, K. E., Steensland, H. and Traetteberg, J. (1967) *Arch. Mikrobiol.* 59, 82–92
3 Pfennig, N., in *Bergey's Manual of Determinative Bacteriology*, 8th edn, Williams and Wilkins, Baltimore, Md., to be published
4 Olson, J. M. and Nadler, K. D. (1965) *Photochem. Photobiol.* 4, 783–791
5 Olson, J. M. and Chance, B. (1960) *Arch. Biochem. Biophys.* 88, 26–39
6 Olson, J. M. and Chance, B. (1960) *Arch. Biochem. Biophys.* 88, 40–53
7 Thornber, J. P., Olson, J. M., Williams, D. M. and Clayton, M. L. (1969) *Biochim. Biophys. Acta* 172, 351–354
8 Pfennig, N. (1965) *Zentr. Bakteriol., I. Abt. Suppl.* 1, 179–189
9 Bartsch, R. G. (1963) in *Bacterial Photosynthesis* (Gest, H., San Pietro, A. and Vernon, L. P., eds), pp. 315–326, Antioch Press, Yellow Springs, Ohio
10 Dus, K., DeKlerk, H., Sletten, K. and Bartsch, R. G. (1967) *Biochim. Biophys. Acta* 140, 291–311
11 Cameron, B. F. (1965) *Anal. Biochem.* 11, 164–169
12 Weber, K. and Osborn, M. (1969) *J. Biol. Chem.* 244, 4406–4412
13 Dunkert, A. K. and Ruckert, R. R. (1969) *J. Biol. Chem.* 244, 5074–5080
14 Edman, P. (1960) *Ann. N. Y. Acad. Sci.* 88, 602–610
15 Doolittle, R. F. (1965) *Biochem. J.* 94, 742–750
16 Edman, P. and Begg, G. (1967) *Eur. J. Biochem.* 1, 80–91
17 Edman, P. and Sjöquist, J. (1965) *Acta Chem. Scand.* 10, 1507–1509
18 Van Orden, H. O. and Carpenter, F. H. (1963) *Biochem. Biophys. Res. Commun.* 14, 399–403
19 Tschesche, H. and Kupfer, S. (1972) *Eur. J. Biochem.* 26, 33–36
20 Dus, K., Tedro, S., Bartsch, R. G. and Kamen, M. D. (1971) *Biochem. Biophys. Res. Commun.* 43, 1239–1245
21 Cusanovich, M. A. and Bartsch, R. G. (1969) *Biochim. Biophys. Acta* 189, 245–255
22 Kennel, S. J. and Kamen, M. D. (1971) *Biochim. Biophys. Acta* 253, 153–166
23 Kennel, S. J. (1972) Doctoral dissertation, University of California, San Diego, Calif.
24 Kamen, M. D. and Horio, T. (1970) *Annu. Rev. Biochem.* 39, 673–700
25 DeKlerk, H. and Kamen, M. D. (1966) *Biochim. Biophys. Acta* 112, 175–178
26 Meyer, T. E. (1970) Doctoral dissertation, University of California, San Diego, Calif.
27 Cusanovich, M. A., Tedro, S. M. and Kamen, M. D. (1970) *Arch. Biochem. Biophys.* 141, 557–570
28 Swank, R. T. and Burris, R. H. (1969) *Biochim. Biophys. Acta* 180, 473–489
29 Ambler, R. P. and Taylor, E. (1973) *Trans. Biochem. Soc.* 1, 111–113
30 Kodama, T. and Mori, T. (1969) *J. Biochem. Tokyo* 65, 621–628
31 Iwasaki, H. and Matsubara, T. (1971) *J. Biochem. Tokyo* 69, 847–857
32 Ellfolk, N. and Soininen, R. (1970) *Acta Chem. Scand.* 24, 2126–2136
33 Ellfolk, N. and Soininen, R. (1971) *Acta Chem. Scand.* 25, 1535–1540
34 Soininen, R. and Ellfolk, N. (1972) *Acta Chem. Scand.* 26, 861–872
35 Olson, J. M., Carroll, J. W., Clayton, M. L., Gardner, G. M., Linkins, A. E., III and Moreth, C. M. C. (1969) *Biochim. Biophys. Acta* 172, 338–339
36 Dutton, P. L. (1971) *Biochim. Biophys. Acta* 226, 63–80
37 Kakuno, T., Bartsch, R. G., Nishikawa, K. and Horio, T. (1971) *J. Biochem. Tokyo* 70, 79–94
38 Yamanaka, T. and Okunuki, K. (1968) in *Structure and Function of Cytochromes* (Okunuki, K., Kamen, M. D. and Sekuzu, I., eds), pp. 390–403, University of Tokyo Press, Tokyo
39 Dus, K., Sletten, K. and Kamen, M. D. (1968) *J. Biol. Chem.* 243, 5507–5518
40 Brockman, H., Jr and Kleber, I. (1970) *Tetrahedron Lett.* 25, 2195–2197