The Amino Acid Sequences of the Cytochromes *c*-555 from Two Green Sulphur Bacteria of the Genus *Chlorobium*

By J. VAN BEEUMEN* and R. P. AMBLER
Department of Molecular Biology, University of Edinburgh,
Edinburgh EH9 3JR, Scotland, U.K.

and T. E. MEYER and M. D. KAMEN†
Department of Chemistry, University of California at San Diego,
La Jolla, CA 92037, U.S.A.

and J. M. OLSON and E. K. SHAW
Department of Biology, Brookhaven National Laboratory,
Upton, NY 11973, U.S.A.

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Amino acid sequences are proposed for the cytochromes *c*-555 from *Chlorobium thiosulphatophilum* and from the *Chlorobium limicola* component of '*Chloropseudomonas ethylica* 2K'. Each is a single polypeptide chain, the former of 86, the latter of 99 residues, and, when aligned so as to give the best match, 47 residues are common to the two sequences. The sequences show some resemblance to those of cytochromes *c*₅ and *f*. The bacteriochlorophyll *a*-proteins were also isolated and purified, and their amino acid compositions compared (see the Appendix). There are significant differences in the compositions, but not as great as those found for the cytochromes *c*-555. The significance of these observations for the taxonomy of the Chlorobiaceae and for the further development of the comparative biochemistry of cytochrome *c* is discussed. Detailed evidence for the sequences of the cytochromes *c*-555 has been deposited as Supplementary Publication SUP 50073 (36 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies may be obtained on the terms given in *Biochem. J.* (1976) 153, 5.

The green sulphur bacteria (Chlorobiaceae) form, with the purple non-sulphur bacteria (Rhodospirillaceae), the purple sulphur bacteria (Chromatiaceae) and the blue-green bacteria (Cyanophyceae), one of the four families of photosynthetic prokaryotes, distinguished from each other by metabolism, pigments and morphology. The principal genus of the family is *Chlorobium* (Pfennig & Trüper, 1974).

Cytochromes were first reported in *Chlorobium* by Kamen & Vernon (1954), and the main basic cytochrome *c* (at present called *Chlorobium* cytochrome *c*-555) was purified by Gibson (1961). This protein has been further purified and characterized by Yamanaka & Okunuki (1968) and by Meyer *et al.* (1968). A cytochrome of similar properties was isolated from '*Chloropseudomonas ethylica* 2K' (Olson & Shaw, 1969; Shioi *et al.*, 1972), which was then thought to be

a representative of a new genus of green sulphur bacteria, but which has now been shown to be an association of a strain of *Chlorobium limicola* (now called strain 2K) with a new species of *Desulfovibrio* (Gray *et al.*, 1972, 1973; Olson, 1973).

Yamanaka & Okunuki (1968) compared the properties of *Chlorobium* cytochrome *c*-555 with those of algal cytochromes *f*. The optical-absorption spectra were found to be markedly similar, particularly in the red-shifted and asymmetric α -peak of lowered extinction. Both algal cytochromes *f* and green-sulphur-bacterial cytochromes *c*-555 are soluble monomeric proteins containing a single haem group and a polypeptide chain of mol.wt. 10000–12000. This contrasts with the cytochrome *f* of plants, which is particulate and has a much larger polypeptide subunit (Nelson & Racker, 1972). The oxidation-reduction properties of cytochromes *c*-555 are very different from those of cytochromes *f*. *Chlorobium thiosulphatophilum* cytochrome *c*-555 has an E_m^7 (midpoint reduction potential at pH7) of 145 mV (Gibson, 1961) and *C. limicola* cytochrome *c*-555 one of 103 mV (Shioi *et al.*, 1972), as opposed to typical

* Present address: Laboratory of Microbiology and Microbial Genetics, Faculty of Sciences, State University, Gent B-9000, Belgium.

† Present address: Chemical Biology Development Laboratory, University of Southern California, Los Angeles, CA 90007, U.S.A.

values for algal cytochrome *f* of around 370 mV (e.g. for *Euglena gracilis*; Perini *et al.*, 1964).

In the present investigation, which is part of a comparative survey of the electron-transport components of photosynthetic bacteria, we have examined the amino acid sequences of the cytochromes *c*-555 from two strains of *C. thiosulphatophilum* and from the '*Chloropseudomonas ethylica* 2K' strain of *C. limicola*. A preliminary account of part of this work has already been published (Van Beeumen & Ambler, 1973).

The bacteriochlorophyll *a*-proteins were also isolated and purified, and their amino acid compositions compared (see the Appendix). There are significant differences in their compositions, but not as great as those found for the cytochromes *c*-555, suggesting that the latter proteins are evolving at a more rapid rate.

In an authoritative account of the Chlorobiaceae (Pfennig & Trüper, 1974), the *thiosulphatophilum* strains are considered to be only forms of *C. limicola*. As discussed below, we consider that they should still be regarded as belonging to a distinct species, and refer to them as such throughout this paper.

Experimental

Preparation of cytochromes *c*-555

The cytochromes were prepared from *C. thiosulphatophilum* PM (N.C.I.B. 8346) and L (N.C.I.B. 8327) by the method of Meyer *et al.* (1968). The protein from the '*Chloropseudomonas ethylica* 2K' *C. limicola* was prepared as described by Olson & Shaw (1969). As a final purification, the protein was adsorbed on CM-cellulose (Whatman CM-52) from 0.05 M-ammonium acetate, pH 4.5, and eluted with 0.05 M-ammonium acetate, pH 5.1. After concentration with CM-cellulose, the protein was subjected to gel filtration through Sephadex G-75 (superfine grade) in 0.05 M-ammonium acetate, pH 5.1, and then freeze-dried. The detailed conditions for these steps were as described by Ambler & Wynn (1973).

The purities of the protein preparations were assessed by the ratio of E_{280} to that of the Soret maximum (Fe^{3+} form) at 412 nm, by homogeneity on gel filtration through Sephadex G-75, and (for the *C. thiosulphatophilum* proteins) by amino acid analysis for phenylalanine and arginine (see Table 1). The final preparations were homogeneous by *N*-terminal-group analysis, and the amino acid compositions agreed adequately with the subsequently determined sequences (see Figs. 1 and 2).

Amino acid sequence determination

The amino acid sequences were determined by the general methods used for other bacterial cytochromes *c* (Ambler & Wynn, 1973; Ambler, 1973*a*), and to

similar standards. Protein (2–3 μmol) was treated with HgCl_2 in 8 M-urea/0.1 M-HCl at 37°C for 16 h to remove the haem moiety, and, after gel filtration and freeze-drying, the sample was digested with a protease or treated with CNBr. The peptides were fractionated by gel filtration followed by high-voltage paper electrophoresis and chromatography, and then analysed quantitatively for amino acid composition and purity. Peptide sequences were investigated by the dansyl-phenyl isothiocyanate method, and by exo- and endo-peptidase digestion. Amide groups were assigned from peptide electrophoretic mobilities and exopeptidase analysis. The *N*-terminal sequences of the *C. thiosulphatophilum* strain L and the *C. limicola* 2K proteins were also investigated with an automatic sequenator (Beckman model 890A; Edman & Begg, 1967), by using a Quadrol programme.

Results

The amino acid compositions of the proteins are shown in Table 1. The evidence for the amino acid sequences of the cytochromes *c*-555 from *C. thiosulphatophilum* strain PM and *C. limicola* strain 2K are summarized in Figs. 1 and 2 respectively. Details of the purification, analysis and sequence-determination experiments on all the peptides shown in Figs. 1 and 2 are given in the Supplementary Publication (SUP 50073). The criteria for satisfactory results, and the nature and format of the Supplementary Publication, are given in previous papers (Ambler & Wynn, 1973; Ambler, 1973*a*, 1975).

The amino acid sequence of the protein from *C. thiosulphatophilum* strain PM (Fig. 1) was determined by characterization of peptides from two tryptic, one chymotryptic and one CNBr experiment. The second tryptic digestion (under mild conditions at pH 4) was unsuccessful in its aim to strengthen the main region of weak evidence, residues 42–46. The low-pH digest was an attempt to minimize the action of pseudotrypsin (Keil-Dlouhá *et al.*, 1971), as it was hoped that this trypsin degradation product would be less stable than the parent enzyme under these conditions. A normal enzyme/substrate ratio (1:40, w/w) was used, and after 4 h incubation at 37°C there was very little undigested protein. The activity of trypsin at pH 4 has been confirmed by experiments on peptide substrates (R. P. Ambler, unpublished observations). The predicted tryptic peptide corresponding to residues 41–51 was not found in either digest, but in the first digest peptide T22c (residues 49–51) was found in the adequate yield of 11%, together with peptide T27d (with analysis corresponding to residues 41–48) in the low yield of 1%. Determination of the *N*-terminal group of the latter peptide by dansylation failed. Hydrolysis of the asparagine-serine peptide bond at residues 48–49 is completely compatible with pseudotrypsin activity (Keil-Dlouhá *et al.*, 1971).

Table 1. Amino acid compositions of *Chlorobium cytochromes c-555*

Results are shown as residues/molecule. Samples were hydrolysed at 105°C for 24h (samples 1, 3, 4, 6, 8) or 96h (samples 2, 5, 7, 9). Samples (5) and (9) were hydrolysed with 3M-mercaptoethanesulphonic acid (Penke *et al.*, 1974), the remainder with 6M-HCl. Samples were of native protein except for (3) and (8), which had been treated to remove the haem moiety and then oxidized with performic acid before hydrolysis. The *C. thiosulphatophilum* results were calculated on the basis that (Leu+Ile+Glu+His) = 7 residues, and the *C. limicola* results on the basis that (Leu+Phe+Pro+His+Arg) = 14 residues.

	<i>Chlorobium thiosulphatophilum</i>					<i>Chlorobium limicola</i>				
	Strain PM			Com-position	Strain L		Strain 2K			
	(1)	(2)	(3)		(4)	(5)	(6)	(7)	(8)	(9)
Glycine	12.0	12.0	12.4	12	12.1	11.1	10.4	10.5	10.3	10.5
Alanine	16.3	16.7	16.0	16	15.4	16.3	13.0	13.4	13.1	12.9
Valine	5.6	5.6	5.9	6	5.7	5.0	7.4	7.4	7.5	7.2
Leucine	1.1	1.1	1.1	1	1.1	1.1	5.0	5.0	4.9	4.9
Isoleucine	2.0	2.0	2.0	2	1.9	1.4	2.1	2.1	2.1	2.0
Serine	3.0	2.4	3.1	3	3.2	3.0	3.6	2.4	3.7	3.7
Threonine	3.8	3.3	3.9	4	3.9	3.8	6.2	5.3	6.3	6.2
Aspartic acid	8.2	7.8	7.6	4	7.9	8.6	10.8	11.3	10.9	10.8
Asparagine				4						
Glutamic acid	2.1	2.1	2.1	0	2.3	2.3	8.4	8.5	8.4	8.2
Glutamine				2						
Phenylalanine	<0.05	<0.05	<0.05	0	(0.13)		1.2	1.2	1.2	1.1
Tyrosine	3.8	3.8	3.6	4	3.6	4.0	4.6	4.6	3.8	4.6
Tryptophan				1		1.0				1.0
Cysteine			2.0*	2					1.6*	
Methionine	7.6	8.0	7.7*	8	7.4	7.0	3.6	3.7	3.7*	3.5
Proline	3.9	3.8	4.2	4	3.5	4.5	3.6	3.8	4.0	3.8
Lysine	10.6	10.5	11.3	11	9.7	10.4	8.0	7.9	7.7	8.0
Histidine	1.9	1.8	1.7	2	1.8	1.9	1.1	0.9	1.0	1.0
Arginine	<0.05	<0.05	<0.05		(0.13)		3.1	3.1	2.9	3.1
Total				86						99

* As cysteic acid and methionine sulphone.

The low-yield peptide T27d is the only evidence for the overlap at the bond at residues 45–46. The bond was completely cleaved by chymotrypsin and CNBr, and is likely to be equally susceptible to thermolysin. A peptide with properties like those expected for Gly-Tyr-Lys (residues 52–54) was recognized in the early stages of the fractionation of the first tryptic digest, but was lost before final purification. In the second tryptic digest, only the larger peptide T31 (residues 52–57) was isolated, but this region was well characterized in the other digests.

The protein from *C. thiosulphatophilum* strain L seems to be identical with that from strain PM (Fig. 1). Tryptic peptides corresponding to the whole of the sequence shown in Fig. 1 (including residues 52–54) were isolated and analysed, and are listed in the Supplementary Publication. As with the cytochrome from strain PM, peptide T27d was isolated in very low yield. Although the analysis was poor, the

sequence of the first five residues was determined by the dansyl-phenyl isothiocyanate method, and agreed with expectation. In an attempt to get definitive evidence for the overlap at the bond at residues 45–46, a chymotryptic digest of performic acid-oxidized protein was examined, as it was hoped that chymotryptic action adjacent to the methionine sulphone residues (particularly residues 42 and 45) would be much decreased. This hope was not realized, and only the peptides shown for this region in Fig. 1 were found. An automatic sequencer run on the protein enabled 14 of the first 18 residues to be positively identified. The residues missed were 13 (serine), 14 and 17 (haem-bound cysteine) and 16 (methionine).

The *C. limicola* strain 2K protein was investigated with tryptic, chymotryptic and thermolysin digests (Fig. 2). The weakest point in the sequence evidence was the overlap at the bond at residues 93–94. No tryptic peptide isolated bridged this gap, as the tyro-

Fig. 2. Amino acid sequence of *Chlorobium limicola* strain 2K cytochrome c-555

The symbols used are as in Fig. 1. Peptides derived by digestion with thermolysin (H) are shown above the sequence. Under the sequence, → indicates residues identified after degradation in a sequenator, only tentatively identified if marked -→. C-Terminal residues of peptides identified as free amino acids after removal of the remainder by phenyl isothiocyanate degradation are shown □.

sine-methionine peptide bond seemed to be very sensitive to the chymotrypsin-like activity in pseudotrypsin. The minor thermolysin peptide H57aii [Val-Ala(Tyr, Met), 2% yield] provided some evidence, and more was given by the action of carboxypeptidase A on the whole haem-free protein. With intense exopeptidase treatment (100 µg of enzyme/30 nmol of protein, 12 h, 37°C, pH 6), amino acids were released in the following molar ratios: leucine, 0.97; valine, 1.81; glutamic acid, 0.44; asparagine, serine and glutamine, 0.33; methionine, 0.38; tyrosine, 0.49; alanine, 0.49; aspartic acid, 0.11; glycine, 0.11. An automatic sequenator run on the protein enabled 18 of the first 24 residues to be positively identified. Residues 12 (leucine) and 17 (threonine) were tentatively identified, but residues 3 (threonine), 19 (tyrosine), 22 (asparagine) and 23 (haem-bound cysteine) were missed.

The sequences shown in Figs. 1 and 2 are as reported in the preliminary publication (Van Beeumen & Ambler, 1973) except that for the *C. limicola* strain 2K protein the original assignments for residues 22 and 25 are now transposed, the tentative sequence for residues 28–31 is confirmed, and residues 79 and 81 are now recognized as being asparagine and aspartic acid respectively.

Discussion

Accuracy of proposed sequences

The weakest part of the proposed sequences is the poor overlap in the region of residues 42–46 of the *C. thiosulphatophilum* strain PM (Fig. 1) and strain L sequences. Concern is magnified by the presence of four extra residues in this region in the *C. limicola* strain 2K sequence (Fig. 3). However, we maintain that an error here is unlikely, in view of the detection of the low-yield peptide T27d and the adequate agreement of the whole-protein amino acid analyses with the postulated sequence (Table 1). Further, a missing fragment would need to contain a C-terminal methionine residue to account for the observed CNBr fragments.

Status of C. thiosulphatophilum

Pfennig & Trüper (1974) have lowered the status of the previously recognized species *C. thiosulphatophilum* to the subspecies level (*C. limicola* forma *thiosulphatophilum*) because the only character separating it from *C. limicola* is utilization of thiosulphate. Studies of cytochromes *c* from other bacterial genera (Ambler, 1973b, 1974) have suggested sequence criteria for placing strains within or outwith a single species. The extent of sequence similarity for the *Chlorobium* cytochromes *c*-555 (only 55%; Fig. 3) would firmly place these organisms in separate species. Further, three strains of *C. thiosulphatophilum* (PM, L and a third strain, Tassajara)

have identical cytochrome complements (R. G. Bartsch, T. E. Meyer, J. M. Olson & E. Shaw, unpublished work), and the cytochromes *c*-555 from strains PM and L are apparently identical in sequence, even though the organisms are distinguished by possessing different bacteriochlorophylls *c* and *d* (Clayton, 1963). By contrast, *C. limicola* 2K lacks both the flavocytochrome *c*-553 and the cytochrome *c*-551 found in the *C. thiosulphatophilum* strains (Olson & Shaw, 1969; Meyer, 1970).

A taxonomic problem is thus created by the finding that *Chlorobium* strains PM and L (and Tassajara) are likely to represent a species different from that of strain 2K. We have arbitrarily chosen the designations *thiosulphatophilum* and *limicola* given in an earlier edition of *Bergey's Manual* (Van Niel, 1957), which implies that thiosulphate utilization is an important taxonomic character in the green sulphur bacteria. Mandel *et al.* (1971) examined the DNA base composition of 17 strains of the *C. limicola/thiosulphatophilum* group. The compositions fell into two groups: 12 of the thiosulphate-utilizing strains had compositions between 56.1 and 58.1% G+C, whereas two of the thiosulphate-utilizers and all the five strains that could not use it had compositions between 51.0 and 52.5% G+C. These observations are consistent with the hypothesis that two distinct species are represented in the *C. limicola/thiosulphatophilum* group, and that thiosulphate utilization is largely but not exclusively confined to one of the species.

The adaptation of green sulphur bacteria to symbiosis with colourless bacteria may require more modification of the genome than does the acquisition of the ability to use thiosulphate, and so be a better taxonomic character, although little is known about the processes. The *Desulfovibrio* component of the '*Chloropseudomonas ethylica* 2K' consortium has a cytochrome *c* that contains three haem groups (Ambler, 1971) instead of the four groups usual in the *Desulfovibrio* cytochromes *c*₃, an indication that this strain is representative of a hitherto unrecognized species of sulphate reducer.

It is believed that adaptation to a halophilic environment (and perhaps also, though to a lesser extent, to a marine habitat) is a long and gradual process (Larsen, 1967), which involves among other things the lowering of the isoelectric points of the cellular proteins. The cytochrome *c*-555 of the marine *C. limicola* strain 2K is more acidic (pI = 4.65; Shioi *et al.*, 1972) than the cytochromes from the freshwater *C. thiosulphatophilum* strains PM, L and Tassajara which are all basic (pI = 10.5). In the same way, the bacteriochlorophyll *a*-protein from *C. limicola* strain 2K is more acidic (pI = 6.0) than the protein from strain Tassajara (pI = 7.0; see the Appendix).

Which if any of the three characters discussed above (thiosulphate utilization, symbiotic association or marine habitat) may be the best practical guide to

Fig. 3. Comparison of the amino acid sequences of *Chlorobium* cytochromes c-555 and other bacterial cytochromes

The sequences are: (1) *Spirulina maxima* cytochrome *f* (Ambler & Bartsch, 1975); (2) *Chlorobium thiosulphatophilum* strain PM cytochrome c-555; (3) *Chlorobium limicola* strain 2K cytochrome c-555, from the '*Chloropseudomonas ethylica* 2K' consortium; (4) *Pseudomonas mendocina* strain CH110 cytochrome *c*, (Ambler & Taylor, 1973). The sequences are aligned so as to give good matching. Vertical bars between sequences (2) and (3) show residues common to the two proteins. Residues that are the same in sequences (1) or (4) and the *Chlorobium* proteins are arrowed, and double-arrowed if common to both of the cytochromes c-555. The one-letter notation used is that recommended by the IUPAC-IUB Commission on Biochemical Nomenclature [*Biochem. J.* (1969) 113, 1-4], namely: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.

speciation in the green sulphur bacteria can only be determined by further study. Unfortunately, no other *C. limicola* strains, nor any consortia other than '*Chloropseudomonas ethylica* 2K', have been examined for cytochrome content. Eventually enough information may become available for a taxonomy based only on protein and nucleic acid sequences to be built, but we realize that gene transfer between species may be a complicating factor.

Comparison with other cytochrome *c* sequences

The sequences of cytochromes *c*-555 from *C. thiosulphatophilum* and the *C. limicola* strain 2K can be aligned so that long stretches of the two sequences match well (Fig. 3). The *C. limicola* protein is nine residues longer at the *N*-terminus than the *C. thiosulphatophilum* protein. It has a longer sequence before the haem-attachment site than any of the normal low-spin cytochromes *c* so far fully characterized (Ambler, 1973b), although the intact *Azotobacter/Pseudomonas* cytochrome *c*₅ (Ambler & Taylor, 1973) seems likely to have an even longer pre-haem sequence. To get matching at both the *N*- and *C*-termini, a four-residue deletion in the *C. thiosulphatophilum* protein needs to be postulated. Equally good matching is obtained if this gap is supposed to be between residues 45 and 46 or between residues 53 and 54 (Fig. 3).

The *Chlorobium* cytochromes *c*-555, although not very similar in overall sequence to any of the other defined types of cytochrome *c* (Ambler, 1973b), do share a few features. The cytochromes *f* of algae and blue-green bacteria (Ambler & Bartsch, 1975) are similar to the *Chlorobium* proteins in size, and like them [and *Pseudomonas* cytochromes *c*-551 and *c*₅ (Ambler, 1973b)] have a proline residue immediately after the methionine residue that is presumed to be the sixth iron ligand. Apart from this, sequence similarity is confined to small regions near the *N*- and *C*-termini (residues 2–10 and 77–82 in the *C. thiosulphatophilum* sequence), regions that also seem similar to the *N*- and *C*-terminal α -helices of *Rhodospirillum rubrum* cytochrome *c*₂ (Salemme *et al.*, 1973). The cytochromes *c*₅ of *Azotobacter* and *Pseudomonas* (Swank & Burris, 1969; Ambler & Taylor, 1973) closely resemble the *Chlorobium* proteins in the sequence of the segment immediately after the haem-attachment site (residues 20–35 in the *C. thiosulphatophilum* sequence, Fig. 3), but there is very little similarity elsewhere in the sequences.

The spectral characteristic of a red-shifted asymmetric α -peak of lowered extinction is shared by the *Chlorobium* cytochromes *c*-555, algal cytochromes *f* and the *Azotobacter/Pseudomonas* cytochromes *c*₅. A cytochrome *c*-553(550) isolated from the purple sulphur bacterium *Chromatium vinosum* (Cusanovich & Bartsch, 1969) has a similar optical spectrum, but no sequence information about it is yet available. By

analogy with algal cytochrome *f*, *Chlorobium* cytochrome *c*-555 has been supposed to have a primary role in photosynthesis, although the light-reactive *Chlorobium* cytochrome *c*-553 in bacteriochlorophyll *a* reaction-centre complexes (Fowler *et al.*, 1971; Olson *et al.*, 1973; Prince & Olson, 1976) does not seem to be a complexed form of the soluble cytochrome *c*-555. The evidence for the two cytochromes being distinct molecular entities is that the α -peak wavelength maxima are 2 nm apart, the redox potentials are 67 mV different (in *C. limicola* 2K; Fowler *et al.*, 1971; Shioi *et al.*, 1972), and incubation of reaction-centre complex I from *C. thiosulphatophilum* with 0.4 M-Na₂CO₃, pH 10.3, does not convert any bound cytochrome *c*-553 into soluble cytochrome *c*-555 (J. M. Olson, unpublished work). Further evidence is provided by the bacterium *Chloroflexus*, which contains chlorobium vesicles and a particulate cytochrome *c*-554, but contains no soluble cytochrome whatsoever (Bartsch, 1976).

The *C. thiosulphatophilum* cytochrome *c*-555 is peculiar in that it exhibits a relatively high degree of activity with ox mitochondrial cytochrome *c* oxidase (Yamanaka & Okunuki, 1968; Davis *et al.*, 1972; B. J. Errede & M. D. Kamen, unpublished work). The characteristic is not shared by the apparent homologue *C. limicola* 2K cytochrome *c*-555 nor by any other prokaryote cytochromes *c*, except *Thiobacillus novellus* cytochrome *c*-550 (Yamanaka *et al.*, 1971). Algal cytochromes *f* are unreactive with the oxidase. Both *Chlorobium* cytochromes *c*-555 and algal cytochromes *f* are unreactive with mitochondrial cytochrome *c* reductase (Davis *et al.*, 1972; B. J. Errede & M. D. Kamen, unpublished work), behaviour which is in contrast with the cytochromes *c*₂, proteins that are found mainly in the purple non-sulphur photosynthetic bacteria. These results suggest that a cytochrome oxidase reactive site has arisen independently by parallel evolution in *C. thiosulphatophilum* cytochrome *c*-555. A three-dimensional structure determination of the *Chlorobium* protein is in progress (F. R. Salemme, personal communication), and when it is completed may help towards the elucidation of the mechanism of action of mitochondrial cytochrome oxidase and reductase.

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APPENDIX

Comparison of Bacteriochlorophyll *a*-Proteins from Two Green Bacteria

By JOHN M. OLSON, ELIZABETH K. SHAW and FRIEDA M. ENGLBERGER

Department of Biology, Brookhaven National Laboratory, Upton, NY 11973, U.S.A.

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Bacteriochlorophyll *a*-proteins have been obtained from three strains of green bacteria, namely *Chlorobium limicola* 2K, *Chlorobium thiosulphatophilum* L and *C. thiosulphatophilum* 6230 (Tassajara) (Olson & Romano, 1962; Olson *et al.*, 1973). The best characterized bacteriochlorophyll *a*-protein is that from *C. limicola* 2K, which when grown in mixed culture with one or more non-photosynthetic sulphate-reducing bacteria has been known as '*Chloropseudomonas ethylica* 2K' (Gray *et al.*, 1973; Olson, 1973). The bacteriochlorophyll *a*-protein from strain 2K is a trimer (Fenna *et al.*, 1974), composed solely of bacteriochlorophyll *a* and protein of known composition (Thorner & Olson, 1968). Each subunit con-

sists of a protein bag (354 residues) with seven chlorophyll molecules inside the bag (Fenna & Matthews, 1975, 1976). Since the absorption and circular-dichroism spectra at 77K are distinctly different for bacteriochlorophyll *a*-proteins from strains 2K and Tassajara (Olson *et al.*, 1973, 1976), we wished to find out whether these spectral differences might be correlated with biochemical differences between the proteins.

Experimental*Materials*

Chlorobium limicola 2K was grown in pure culture (Gray *et al.*, 1973) or in mixed culture as