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Bacteriochlorophyll *g* epimer as a possible reaction center component of heliobacteria

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Normal-phase HPLC analysis of acetone extracts of cells, membranes and antenna-reaction center complexes of *Heliobacterium chlorum* and *Heliobacillus mobilis* showed the presence of bacteriochlorophyll (BChl) *g′*, the 13²-epimer of BChl *g*. The molar ratio of BChl *g*:BChl *g′* in these preparations was approx. 18 in cells and membranes of both species. This value, when combined with a molar ratio of BChl *g* to the primary electron donor, P-798, of 35-40, yields a BChl *g′*:P-798 ratio of 2, suggesting that P-798 may be a dimer of BChl *g′*. The amount of BChl *g′* in the antenna-reaction center complex of *H. chlorum* was slightly higher, suggesting that some epimerization may have occurred during the isolation of the complex. In contrast, bacteriopheophytins (BPhe) *g* and *g′* were present in too small amouts to be essential components of the photosynthetic apparatus. This confirms the idea that the reaction center of heliobacteria is basically different from those of purple bacteria and Photosystem II. A detailed interpretation, based on Correlated Spectroscopy and Double Resonance experiments is given of the ^{1}H-NMR spectra of BChl *g* and BChl *g′*, confirming the structure and identity of both pigments. Absorption and circular dichroism spectra of BChl *g* and BChl *g′* are also presented.

Introduction

The heliobacteria, *Heliobacterium chlorum* [1] and the more recently discovered species, *Heliobacillus mobilis* [2], *Heliobacterium gestii* and *Heliobacterium fasciculum* [3] have a 'new' type of bacteriochlorophyll, BChl *g* (Fig. 1) as their main photosynthetic pigment. The structure of BChl *g* resembles that of BChl *b*, both pigments containing the unusual ethylidene group at ring II [4]. Photoisomerization of BChl *g* at ring II yields Chl *a*, the main chlorophyll of oxygenic photosynthesis.

Abbreviations: A-RC, antenna-reaction center; BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; CD, circular dichroism; Chl, chlorophyll; HPLC, high-performance liquid chromatography; P-798, primary electron donor of heliobacteria; PS, Photosystem.

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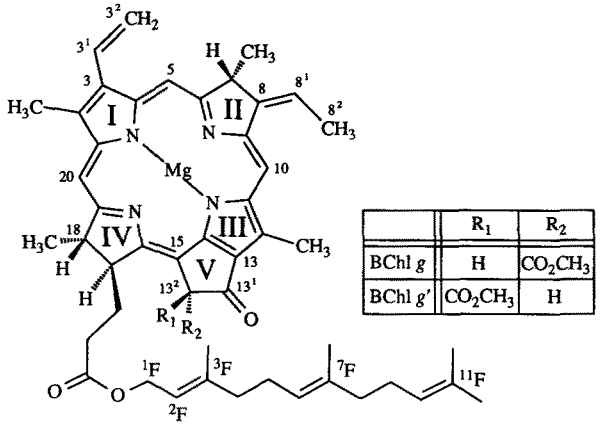
The primary electron donor of heliobacteria, P-798, has been proposed to be a dimer of BChl *g* on the basis of optical and EPR measurements [5,6]. Flash-spectroscopic evidence suggests that the primary electron acceptor is a BChl *c* or Chl *a*-like pigment [7,8]. Little is known about the electron acceptor chain. EPR studies suggest that a quinone, like A_1 in PS I of higher plants, may be present [9,10]. EPR and optical measurements have shown the light-induced reduction of an iron-sulfur center, but direct evidence for its presence in the main electron transport pathway is lacking [10,11].

Recently it was demonstrated that one molecule of the 13²-epimer of Chl *a*, Chl *a′*, is present in the PS I core [12] and evidence was obtained that it is associated with the reaction center [13]. Epimers, however, are absent from PS II, purple bacteria and green sulfur bacteria [14], although the reaction center of green sulfur bacteria is thought to be structurally and functionally related to PS I [15,16]. In view of the above results and the functional similarities between the reac-



	R_1	R_2
BChl *g*	H	CO_2CH_3
BChl *g'*	CO_2CH_3	H

Fig. 1. Structure and partial carbon numbering of BChl *g* and BChl *g'*. Replacement of Mg^{2+} by two protons gives the corresponding BPhes.

tion centers of PS I and heliobacteria, it was therefore of interest to examine the pigment composition of heliobacteria also.

In the present communication we report the results of a pigment analysis of *H. chlorum* and *Hb. mobilis* by means of silica normal-phase HPLC. In both species the 13^2-epimer of BChl *g*, BChl *g'* (Fig. 1) was found to be present at a molar ratio BChl *g*:BChl *g'* of 18. This indicates the presence of two BChls *g'* per reaction center, suggesting that P-798 may be a dimer of BChl *g'*.

Materials and Methods

Culturing. Heliobacterium chlorum and *Heliobacillus mobilis* were grown in medium No. 1552 of the American Type Culture Collection, containing 2.5 mM sodium ascorbate, as described in Ref. 17. *H. chlorum* was also grown in medium No. 112 for comparison. The cells were harvested by centrifugation near the end of the exponential growth phase and washed once with 10 mM Tris-HCl buffer (pH 8) containing 10 mM sodium ascorbate.

Isolation of membranes. Membrane fragments were prepared by sonication of washed cells followed by one centrifugation step of 20 min at 20000 × *g* at 4° C to remove unbroken cells and large cell fragments. The membranes were further concentrated by centrifugation of the supernatant at 265000 × *g* at 4° C for 1 h.

Preparation of the antenna-reaction center complex. The A-RC complex of *H. chlorum* was prepared as described before using sulfobetaine-12 as detergent [17], except that for preparative size-exclusion HPLC chromatography an XK 26/70 column was used (700 × 26 mm i.d.), packed with Sephacryl S-300 HR gel, both obtained from Pharmacia/LKB. The column was operated at a flow rate of 2 ml min^{-1} with an eluent of pH 8.0 containing 20 mM Tris-HCl, 10 mM sodium ascorbate, 0.1 M NaCl and 0.1% (w/v) sulfobetaine-12 at room temperature in the dark.

Pigment extraction. 2 ml of acetone was added to 10 µl of sonicated cells ($A_{788} \approx 70$ cm^{-1}), or membrane fragments ($A_{788} \approx 200$ cm^{-1}); 8 ml of acetone to 80 µl of the A-RC complex ($A_{788} \approx 2.5$ cm^{-1}). Pigments were extracted by sonication at 4° C for 2 min. The solution was then filtered through a Teflon filter (Tosoh, Tokyo, H-13-5, prewashed with acetone) and immediately dried in a rotary evaporator at about 10^{-2} Torr. These operations were conducted in dim light.

HPLC analysis. The brownish-green solid material obtained by the above procedure was immediately dissolved in about 20 µl of chloroform. Approx. 3 µl of the solution was injected into a silica HPLC column (Senshupak 1151 N, 150 × 4.6 mm i.d.) cooled to 4° C in an ice-water bath. The pigments were eluted isocratically with degassed *n*-hexane/2-propanol/methanol (100:0.8:0.4, v/v) at a flow rate of 0.7 ml min^{-1}. Pigment elution was monitored by means of an Applied Biosystems Spectroflow 757 UV-detector or a JASCO 875 UV-detector and a JASCO Multiwavelength 340 detector operating in series.

Sufficiently pure pigments for the measurement of absorption, CD and ^{1}H-NMR spectra were prepared by means of preparative-scale HPLC (Senshupak 5251-N, 250 × 20 mm i.d.) with *n*-hexane/2-propanol/methanol (100:1.5:0.2, v/v) as an eluent at a flow rate of 4.9 ml min^{-1}. The solvents used for analytical and preparative HPLC were analytical and reagent grade, respectively.

UV-visible spectral measurements. A JASCO Uvidec 660 or Shimadzu UV-360 spectrophotometer and the multiwavelength HPLC detector mentioned above were used for measurement of absorption spectra of the separated compounds in acetone, diethyl ether, benzene and eluent. JASCO J-20 (300-700 nm) and JASCO J-500A (700–900 nm) spectropolarimeters were used for CD measurements of BChl *g* and BChl *g'* in benzene. All solvents were degassed before use. Chemically induced oxidized-minus-reduced difference spectra of cells, membranes and the A-RC complex were measured as described in Ref. 18. Oxidation of P-798 in these experiments was brought about by the addition of ferricyanide (0.3 mM), reduction by ascorbate (5 mM) in the presence of 2,6-dichlorophenolindophenol (3 µM).

^{1}H-NMR measurements. ^{1}H-NMR spectra were recorded on a Bruker WM-300 spectrometer. Correlated Spectroscopy (COSY) and Double Resonance experiments for additional structure elucidation were also conducted on this instrument. The samples were measured in [$^2H_6$]acetone (Janssen Chimica, Belgium) at low temperature to reduce degradation reactions and to enhance spectral resolution.

Epimerization. Approx. 1 mg of purified BChl *g* or BChl *g'* was dissolved in 20 ml of diethyl ether containing 0.1 M triethylamine. The solution was then im-

mediately frozen in liquid N_2 and degassed at 10^{-3} Torr. The solution was then allowed to stand overnight in the dark at room temperature. This treatment promoted the epimerization of BChl *g* or BChl *g'*, respectively, without significant side reactions. Epimerization was reversible.

Results

Extraction procedure

Since BChl *g* is reported to be quite unstable [4,19] it was important to devise a rapid procedure for the complete extraction of bacteriochlorophyll(s) and carotenoid(s) from heliobacteria.

Preliminary experiments with methanol, acetone, chloroform and diethyl ether to extract pigments from suspended or lyophilized cells were unsuccessful, yielding only small amounts of extracted material. Lyophilized membranes did not yield satisfactory results either, although the yield of extraction was higher. Rapid extraction, however, with high yield, was obtained by sonication of aqueous samples in the presence of acetone (see Material and Methods). Methanol was somewhat less efficient under these conditions, and chloroform and diethyl ether were completely unsuited with aqueous samples as expected.

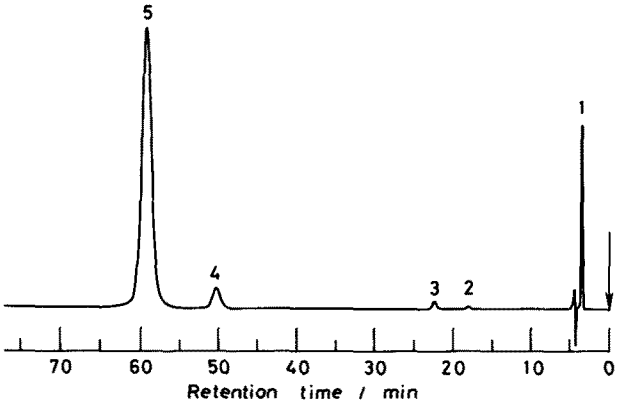


Fig. 2. HPLC tracing of an acetone extract of sonicated cells of *H. chlorum*. Detection wavelength, 400 nm.

The extraction efficiency was examined by measurement of the absorption and HPLC-analysis of the remaining material. A single extraction removed more

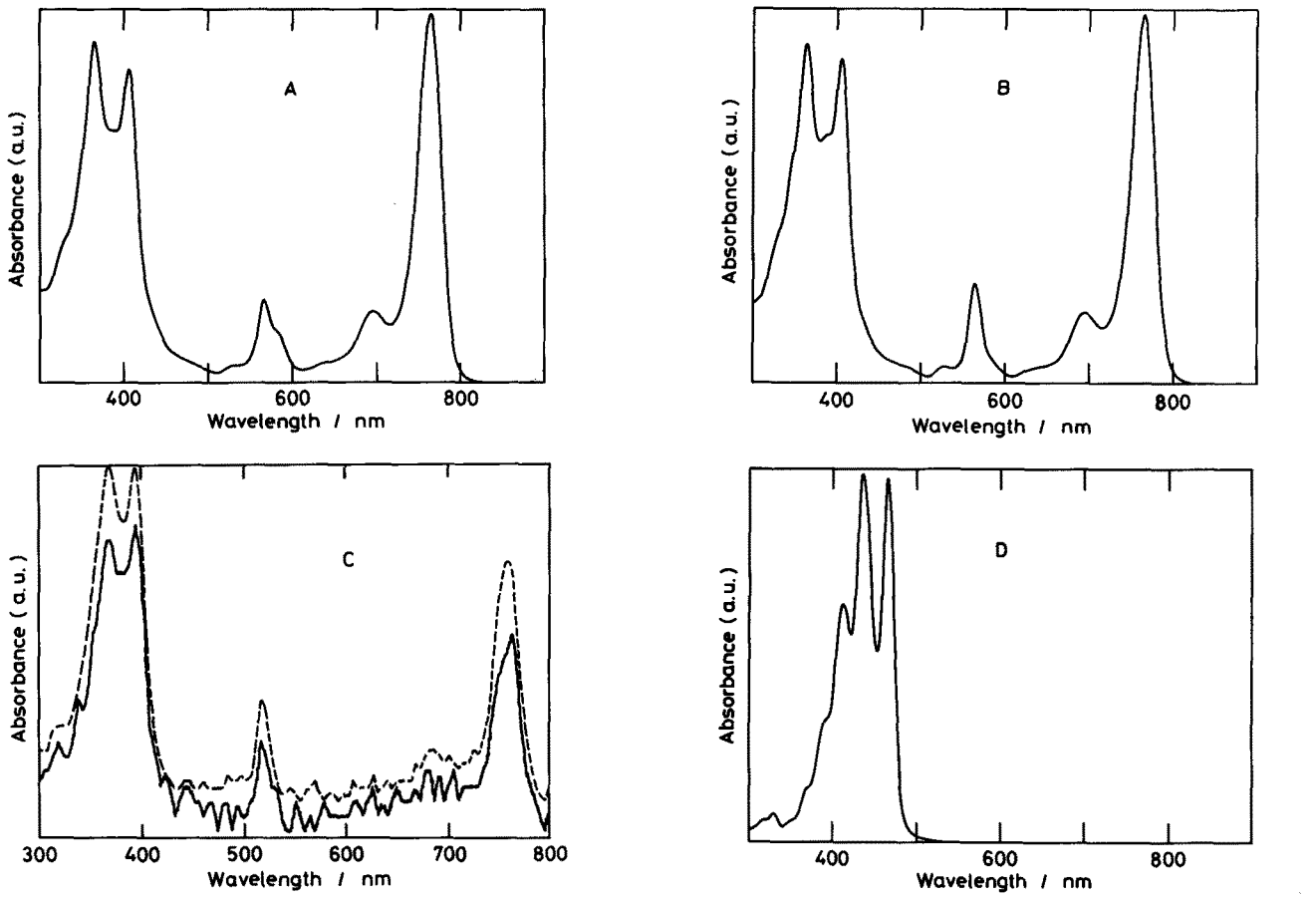


Fig. 3. Absorption spectra of pigments found in extracts of *H. chlorum* and *Hb. mobilis*. (A) BChl *g* (peak 5); (B) BChl *g'* (peak 4); (C) broken line: BPhe *g* (peak 3), solid line: BPhe *g'* (peak 2); (D) neurosporene (peak 1). Solvent, *n*-hexane/2-propanol/methanol (100:0.8:0.4, v/v). Temperature approx. 4 °C.

than 99% of BChl *g* and no significant improvement was obtained by a second extraction. Therefore a single extraction with acetone was adopted as standard method for the experiments to be described below.

Pigment analysis

Fig. 2 shows a typical HPLC tracing obtained with an acetone extract of sonicated cells of *H. chlorum*. Five peaks are observed in the chromatogram. Peak No. 5, with the longest retention time, showed absorption bands at 365.6, 406.8, 567.2 and 765.6 nm (Fig. 3A) and was obviously due to BChl *g*, the major bacteriochlorophyll of heliobacteria [4]. A pigment with similar absorption spectrum (peak No. 4; Fig. 3B) eluted somewhat faster and was present in much smaller amount. Evidence will be given below that this pigment is the 13^2-epimer of BChl *g*, BChl *g'*. It is of interest to note here that, in contrast to the corresponding Chl *a*/*a'* epimer pair [20], the absorption spectra of BChl *g* and BChl *g'* showed slight but significant differences in the Soret and Q_x-band regions. Especially in eluent (but less so in other solvents) the Q_x-bands showed markedly different shapes. Table I summarizes the peak wavelengths and their relative intensities for BChl *g* and BChl *g'* in acetone, diethyl ether and benzene. The CD spectra of BChl *g* and BChl *g'* (Fig. 4) were distinctly different, like those of Chl *a* and Chl *a'*, as reported earlier [20,21].

Evidence to support the hypothesis that peak No. 4 is due to the epimer of BChl *g* was obtained by epimerization treatment. Epimerization of a BChl *g* solution yielded a pigment that showed the same retention time and was spectroscopically indistinguishable from the 'natural' BChl *g'*. The same applied to BChl *g* derived by epimerization of BChl *g'*. The equilibrium BChl *g*: BChl *g'* ratio was approx. 3.

Absorption spectra of the minor pigments of peaks 2 and 3 are shown in Fig. 3C. The two spectra are virtually identical; comparison with the spectrum published by Michalski et al. [19] indicates that they are both due to BPhe *g*, probably present in the two epimeric forms, peak 2 being BPhe *g'* (see below).

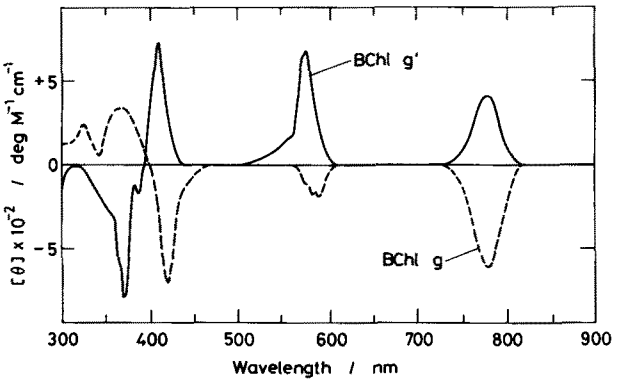


Fig. 4. CD spectra of BChl *g* and BChl *g'* in benzene at room temperature (approx. 20 °C). For the calculation of Θ, an extinction coefficient of 100 $mM^{-1} cm^{-1}$ at the Q_y maximum was used.

Peak No. 1 showed an absorption spectrum (Fig. 3D) with maxima at 412.8, 436.4 and 466.4 nm, similar to that obtained for all-*trans*-neurosporene from various sources in *n*-hexane [22,23]. The spectrum and retention time were the same as of the main carotenoid extracted from sonicated cells of the neurosporene-containing mutant [24] G1C of *Rhodobacter sphaeroides*. This confirms the conclusion that *H. chlorum* contains neurosporene [1,25]; no other carotenoid could be distinuished in *H. chlorum* or *Hb. mobilis* by HPLC analysis.

In addition to the pigments mentioned above small amounts of at least two more polar chlorophyllous pigments with elution times of 5–8 h were observed. The nature of these pigments will be discussed in a subsequent paper.

^{1}H-NMR measurements

In order to obtain final confirmation of the identity of the pigment of peaks 4 and 5, ^{1}H-NMR experiments

TABLE I

Spectroscopic parameters for BChl g, BChl g' and neurosporene at room temperature (20 °C)

Compound	Peak wavelengths (nm) and relative intensities (in parentheses)											
	acetone				diethyl ether				benzene			
Band type [a]	B_1	B_2	Q_x	Q_y	B_1	B_2	Q_x	Q_y	B_1	B_2	Q_x	Q_y
BChl *g*	364.8	404.8	566.4	761.6	364.0	404.0	564.8	767.2	366.4	409.6	569.6	776.0
	(100)	(89)	(30)	(75)	(94)	(88)	(31)	(100)	(98)	(95)	(32)	(100)
BChl *g'*	364.4	404.8	566.8	761.6	363.6	404.0	564.0	767.2	365.6	408.4	566.8	775.2
	(100)	(91)	(30)	(75)	(97)	(95)	(33)	(100)	(100)	(99)	(35)	(99)
Neurosporene	416.0	439.2	468.8		412.8	436.0	465.6		424.0	449.6	480.0	
	(66)	(100)	(97)		(66)	(100)	(98)		(66)	(100)	(97)	

[a] Assignment holds for the BChls only.

were performed. NMR and mass spectroscopy of BPhe *g* by Brockmann and Lipinski [4] and Michalski et al. [19] resulted in the structure shown in Fig. 1. We used their results as starting point for the interpretation of our spectra.

Initial measurements in $CDCl_3$, performed as in Ref. 4, resulted in almost total degradation of the pigments. Therefore we developed other experimenting conditions, using [$^2H_6$]acetone as solvent and lowering the temperature to 192 K. Under these conditions more than 5 h of experimenting was possible, without degradation of the pigments investigated. This allowed us to perform detailed COSY and Double Resonance experiments (not shown), resulting in the assignment of all significant NMR-lines shown in the spectra (Fig. 5 and Table II). Temperature changes to 203 and 213 K, respectively, were conducted to reveal lines hidden under the H_2O lines, which lines shift strongly upon a change in temperature.

Shifts of the proton lines on ring V were anticipated to occur in BChl *g'* with respect to BChl *g* and such shifts were indeed observed experimentally. In addition

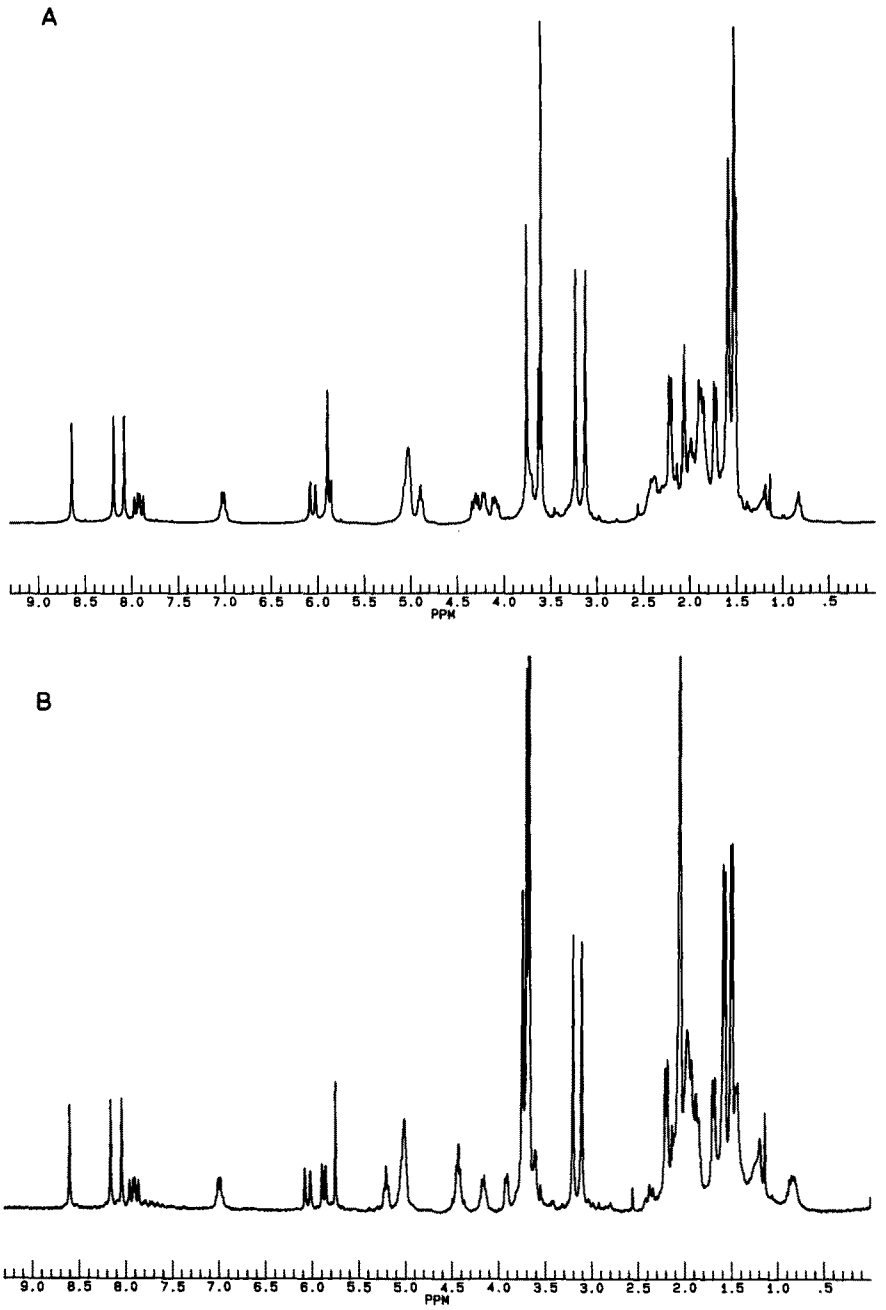


Fig. 5. ^{1}H-NMR spectra of BChl *g* (A), and BChl *g'* (B) in [$^2H_6$]acetone at 192 K. The lines at 2.05 ppm and at 3.62 and 3.64 ppm are due to acetone and water, respectively. The assignment of the other lines is shown in Table II.

TABLE II

Assignment of the ^{1}H-NMR lines of BChl g and BChl g′

The spectra were measured in [$^2H_6$]acetone at 192 K, using acetone as internal standard (2.05 ppm). The assignment is also based on COSY and Double Resonance spectra; the numbering of the protons is given in Fig. 1.

Protons	δ values in ppm and multiplicity [a]	
	BChl *g*	BChl *g′*
10-H	8.63 (s)	8.60 (s)
5-H	8.18 (s)	8.16 (s)
20-H	8.08 (s)	8.04 (s)
3^1-H	7.91 (dd)	7.91 (dd)
8^1-H	7.01 (q)	6.99 (q)
3^2-H_B	6.05 (dd)	6.04 (dd)
3^2-H_A	5.87 (dd)	5.87 (dd)
13^2-H	5.89 (s)	5.75 (s)
7-H	5.04 (o)	5.01 (o)
^{6,10}F-H	5.04 (o)	5.01 (o)
^{2}F-H	4.89 (t)	5.20 (t)
^{1}F-H_A	4.31 (dd)	4.43 (m)
^{1}F-H_B	4.09 (dd)	4.43 (m)
18-H	4.21 (q)	4.15 (m)
13^2-CO_2CH_3	3.75 (s)	3.74 (s)
17-H	3.71 (o)	3.91 (m)
12-CH_3	3.23 (s)	3.20 (s)
2-CH_3	3.12 (s)	3.10 (s)
17^1, 17^2-H	2.1–2.5 (m)	2.0–2.4 (m)
8^1-CH_3	2.20 (d)	2.19 (d)
^{4,5,8,9}F-H	1.8–2.0 (m)	1.8–2.0 (m)
7-CH_3	1.72 (d)	1.69 (d)
18-CH_3	1.58 (o)	1.44 (d)
^{3}F-CH_3	1.58 (s)	1.48–1.61 (s)
^{7,11}F-CH_3	1.48–1.55 (s)	1.48–1.61 (s)

[a] s-singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, o = overlapping with other signals.

to this, significant shifts were observed for the protons on ring IV and on farnesol. Both can be explained by changes of interaction with the ring V proton; with respect to the farnesol protons, this interaction suggests that the alcohol tail is folded over the BChl *g* macrocycle. Although some of our assigmnents are different from those of Brockmann and Lipinski [4], notably those for the esterifying alcohol and ring IV protons, our measurements confirm the structure proposal for BChl *g* as given before [4,19], and the identity of farnesol as esterifying alcohol as earlier concluded from mass spectroscopy [19]. In accordance with our earlier conclusions, peak 4 and 5 thus were identified as BChl *g′* and BChl *g*, respectively, both esterified with farnesol.

Pigment integrity during extraction and chromatography

The amount of BChl *g′* relative to BChl *g* in extracts of sonicated cells of *H. chlorum*, measured from the HPLC peak areas was 5.6%. The two epimers of BPhe *g* were present at concentrations of 0.3 and 1.1%, respectively. In order to examine whether these levels reflected the in vivo pigment distribution or if they were affected by epimerization and pheophytinization reactions during extraction and chromatography we carried out the experiment illustrated in Fig. 6.

We first prepared 99.8% pure BChl *g*, containing 0.2% of BChl *g′*, as determined by HPLC analysis. Known amounts of an acetone solution of BChl *g* were added to a suspension of sonicated *H. chlorum* cells, and the resulting suspension was submitted to the normal extraction and HPLC procedure. Fig. 6 shows the amounts of BChl *g′* and of the BPhes relative to BChl *g*, as determined by HPLC, in the extracts as a function of the amount of added BChl *g* (plotted as carotenoid : BChl *g* ratio). For each of the pigments straight lines were obtained. For the two BPhes these lines converged to 0% for a carotenoid : BChl *g* ratio of 0; for BChl *g′* the point of intersection with the ordinate was 0.2%, corresponding to its relative concentration in the BChl *g* preparation. We thus conclude that all three pigments are cellular components, rather than artifacts produced during extraction or chromatography.

Pigment composition of cells and of subcellular fractions

Table III summarizes the pigment composition of the various preparations examined. Results obtained with *H. chlorum* and *Hb. mobilis* were remarkably similar. For both species a BChl *g* : BChl *g′* ratio of 18 was obtained for cells grown in medium No. 1552. Approximately the same ratio was obtained upon extraction of intact cells of *H. chlorum* and of cells broken by French press treatment, although a less complete extraction was obtained (data not shown). This shows that BChl *g′* is not an artifact produced during the sonica-

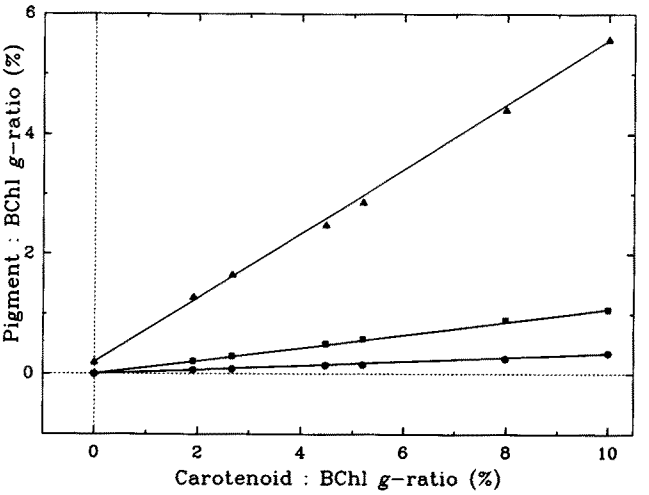


Fig. 6. Pigment content relative to BChl *g* measured by HPLC analysis after the admixing of increasing amounts of 99.8% pure BChl *g* to sonicated *H. chlorum* before extraction. Triangles: BChl *g′*; squares: BPhe *g*; circles: BPhe *g′*. The abscissa gives the carotenoid : BChl *g* ratio of the solution after extraction; 10.0% corresponds to the ratio in sonicated cells (see text).

TABLE III

Pigment composition of H. chlorum and Hb. mobilis

For each pigment the molar ratio of BChl *g* to the pigment indicated is given. It was assumed that the molar extinction coefficients at 400 nm are the same for BChl *g* and both BPhes *g*; for the calculation of the molar ratios BChl *g*:neurosporene and BChl *g*:BChl *g'* an extinction coefficient in eluent of 150 $mM^{-1}\,cm^{-1}$ in the middle absorption maximum (436 nm) was used for neurosporene [22] and of 106 $mM^{-1}\,cm^{-1}$ for BChl *g'* and BChl *g* in the Q_y-band maximum, equal to that of BChl *b* in diethyl ether [26]. Except for the bottom line, all experiments were done with material derived from the same batch of cells for each species. Unless otherwise indicated, the numbers are the average of 5–8 separate determinations. Numbers without standard deviation are the average of two separate measurements only. The last two columns give the amplitude of the bleaching at 798 nm relative to the absorbance in the Q_y maximum near 787 nm, induced by a saturating laser flash or by chemical oxidation.

Material	Molar ratio of BChl *g* to:					
	BChl *g'* (peak 4)	BPhe *g* (peak 3)	BPhe *g'* (peak 2)	neurosporene	$A_{max}/\Delta A_{798}$	
					Flash	Chem.
H. chlorum cells	18.0±0.6 [b]	92± 4	311±33	10.0±0.4	–	20
Membranes	17.3±0.5	85± 7	405±83	10.4±0.7	21 [d]	21
A-RC complex	14.1±0.5	123±19 [c]	825	7.1	21 [d]	22
Hb. mobilis cells	17.7±0.4	40± 1	139± 54	9.1±0.2	–	20
Membranes	15.4	37	126	10.5	22 [d]	20
H. chlorum cells [a]	9.6±0.4 [c]	12.4	35	11.9±0.4 [c]	–	–

[a] Grown in medium No. 112.
[b] Ten measurements.
[c] Three measurements.
[d] From Ref. 17.

tion of the cells. Also the amount of neurosporene was very similar for the two species, being about 10% of the BChl *g* content. When combined with a BChl *g*: P-798 ratio of 35–40 [17], our data suggest that P-798 may be a dimer of BChl *g'*. BPhes were present at a much lower level; in *H. chlorum* at too low a level to be essential components of the reaction center.

The pigment composition of *H. chlorum* membranes was essentially the same as for sonicated cells, but a somewhat higher BChl *g'* content was observed in membranes of *Hb. mobilis*, suggesting that some epimerization may have occurred in this case.

H. chlorum cells grown in medium No. 112 provided a quite different picture. The amount of carotenoid relative to BChl *g* was somewhat lower, whereas the BChl *g'* content was almost twice as high. The amount of BPhes *g* was about 8-times higher relative to BChl *g*. Since *H. chlorum* grows less rapidly in this medium [17] and tends to lyse [1], we conclude that these high levels are due to epimerization and pheophytinization occurring in decaying cells. This illustrates the importance of optimal growth conditions for a representative pigment analysis.

Results obtained with the isolated A-RC complex of *H. chlorum* showed an approx. 2-fold reduction in the BPhe *g* content. This shows that a significant 'purification' of the photosynthetic apparatus was obtained upon isolation of the complex, and confirms the notion that these components are degradation products formed in vivo which are not essential for photosynthesis. On the other hand, a significant enrichment was obtained for BChl *g'*, suggesting some epimerization during the isolation procedure. In agreement with the absorption spectrum [17], there was no evidence for a decrease in neurosporene content as compared to cells and isolated membranes. This stands in contrast to results of Trost and Blankenship [27] with their A-RC preparation of *Hb. mobilis*, who reported a loss of about 75% upon isolation.

Table III also lists relative amplitudes of the bleaching due to oxidation of P-798, either induced by a saturating flash or brought about chemically. It can be seen that the results were the same for various preparations, independent of the method used.

Discussion

The results reported in this paper provide strong evidence that the epimer of BChl *g*, BChl *g'* is an essential constituent of the photosynthetic apparatus and possibly of the reaction center of heliobacteria. In this respect, the reaction center of heliobacteria thus appears to be more similar to that of PS I than to the functionally and perhaps structurally related reaction center of green sulfur bacteria. The identification of BChl *g'* in extracts of various preparations from heliobacteria is based on three lines of evidence: (1) an identical pigment is reversibly produced by epimerization of BChl *g* in vitro; (2) analysis of the ^{1}H-NMR spectra and (3) the CD spectra, which show opposite chiralities for BChl *g* and BChl *g'*. A BChl *g*:BChl *g'* ratio of 18 was observed in *H. chlorum* and in *Hb.*

mobilis and in isolated membranes of *H. chlorum*; as noted already, the amount of BChl *g'* in membranes of *Hb. mobilis* appeared to be slightly higher, suggesting that some epimerization may have occurred in preparing these membranes. The same may be true for the isolated antenna-reaction center complex of *H. chlorum*.

In contrast to the relative stability of the BChl *g'* content in the various preparations examined, the bacteriopheophytin levels were highly variable. They were quite low in the A-RC complex of *H. chlorum* (1% of BChl *g*), about 3.5-times higher in cells and membranes of *Hb. mobilis* and as high as 11% in *H. chlorum* grown in No. 112 medium. These results clearly show that BPhe *g* is not an intrinsic component of the antenna or reaction center of heliobacteria but an artifact produced in small amounts in vivo, even in rapidly growing cultures. The approximately constant ratio of about 4 for the two BPhe *g* peaks in cells and membranes supports the hypothesis that they represent the two epimeric forms, which are apparently present in near-equilibrium concentrations. In view of the equilibrium ratio of 5 for pheophytin *a* and its epimer in diethyl ether [28], we tentatively attribute peaks 2 and 3 to BPhe *g'* and BPhe *g*, respectively.

It is of interest to compare the BChl *g*: BChl *g'* ratio to the size of the photosynthetic unit of heliobacteria. Unfortunately, the molar extinction coefficient of antenna BChl *g* and the differential extinction coefficient of P-798 are not known. Moreover, somewhat varying numbers have been reported for the maximum bleaching either obtained photochemically [17,27] or chemically [27] in cells, membranes or A-RC complexes of *H. chlorum* and *Hb. mobilis*. Since we consistently obtained an $A_{max}: \Delta A_{798}$ ratio of 20–22 by both methods for all these preparations, we assume that higher numbers reported in the literature are due either to incomplete photooxidation or to incomplete reduction between flashes. Combined with the assumption that $P\text{-}798^+$ has no significant absorbance in the region around 800 nm and that P-798 and antenna BChl *g* have the same oscillator strength, we arrive, by comparing the integrated Q_y absorbances [29] at a BChl *g*: P-798 ratio of about 50, which is in fair agreement with the estimated number (35–40) of BChl *g* molecules in the A-RC complex [17]. This suggests that there are two molecules of BChl *g'* per reaction center. Since there is no evidence that BChl *g* is part of the electron acceptor chain [7,8], we conclude as a working hypothesis that P-798 may be a dimer of BChl *g'*.

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