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Pigment composition of the photosynthetic membrane and reaction center of the green bacterium *Prosthecochloris aestuarii*

Thomas Braumann [a], Henk Vasmel [b,*], L. Horst Grimme [a] and Jan Amesz [b]

[a] *Department of Biology / Chemistry, University of Bremen, 28 Bremen 33 (F.R.G.) and* [b] *Department of Biophysics, Huygens Laboratory of the State University, P.O. Box 9504, 2300 RA Leiden (The Netherlands)*

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We have performed a quantitative analysis of the pigment composition of different pigment-protein complexes present in the membrane of the green sulfur bacterium *Prosthecochloris aestuarii*, using the resolving power of reversed-phase high-performance liquid chromatography. The most purified photochemically active complexes contained only carotenoids (OH-chlorobactene and rhodopin), bacteriochlorophyll *a* and a chlorophyllous pigment with absorption maxima at 663 and 433 nm, like bacteriochlorophyll *c*. However, the lipophilicity of this pigment, labeled BChl 663, is quite high and indicates that it contains 5–6 additional methylene groups compared to the BChl *c* homologue known as most lipophilic. Comparison of the BChl 663 content of various pigment-protein complexes indicates that BChl 663 is present in an amount of 10–15 molecules per reaction center. BChl 663 absorbs at 670 nm in vivo, with a specific extinction coefficient of 85 (± 10) $mM^{-1} \cdot cm^{-1}$. In view of the evidence that the primary electron acceptor in *P. aestuarii* is a pigment with absorption maximum at 670 nm (Nuijs, A.M., Vasmel, H., Joppe, H.L.P., Duysens, L.N.M. and Amesz, J. (1985) Biochim. Biophys. Acta 807, 24–34) a direct consequence of these experiments is the fact that only BChl 663 can be a likely candidate for the role of primary electron acceptor as no other pigments absorbing around 670 nm (e.g., bacteriopheophytin *c*) are present in a photochemically active pigment-protein complex derived from the membrane of this green bacterium.

Introduction

A combination of several optical and EPR techniques has led to a general insight in the architecture of the photosynthetic membrane of the green sulfur bacterium *Prosthecochloris aestuarii*. The extra-membraneous chlorosome, containing BChl *c*, serves as the main light-harvesting structure, with some 1000–2000 BChl *c* molecules per reaction center [1]. The chlorosome is thought to transfer its excitation energy to the membrane-bound pigment-protein complexes via a regular array ('baseplate') of so-called water-soluble light-harvesting BChl *a* complexes [2,3], which consist of identical subunits of seven BChl *a* molecules each, bound to a 40 kDa polypeptide [4]. We have recently shown that some of these subunits are strongly associated with a so-called core complex [5], that is thought to incorporate the reaction center [6]. In addition to the light-harvesting BChl *a* complex and the core complex several photochemically active fractions have been isolated from

* To whom correspondence should be addressed.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; Cyt, cytochrome; Chl, chlorophyll; HPLC, high-performance liquid chromatography.

P. aestuarii. The largest of these is the membrane preparation Complex I [7], that contains light-harvesting BChl *a* complexes from the crystalline baseplate together with membrane-bound pigment-protein complexes; it contains about 100 BChl *a* molecules per reaction center [7,8]. Triton incubation of Complex I yields the so-called pigment-protein complex, with a slightly lower BChl *a*/reaction center ratio [8]. Most of the bound light-harvesting BChl *a* complexes can be removed from the pigment-protein complex by guanidine-HCl treatment to give the reaction center pigment-protein complex, which comprises about 35 BChl *a* molecules per primary donor [8].

Relatively little is known about the structure and the polypeptide composition of the reaction center of *P. aestuarii*, as no isolated reaction centers have been obtained yet. However, the optical properties of the reaction center components that are involved in primary photochemistry have been elucidated by various spectroscopic techniques (for recent reviews, see Refs. 9 and 10). The reaction center components include BChl *a*, which in a dimeric form makes up the primary donor [11,12] and a pigment absorbing at 670 nm, that serves as primary acceptor [13,14]. This situation is clearly different from that in purple bacteria, where BPh *a* functions as primary electron acceptor [15].

Thus, an accurate analysis of the pigment composition of the photochemically active complexes of *P. aestuarii* is not only of interest for the determination of the antenna structure, but might also shed more light on the nature of the primary charge separation in green photosynthetic bacteria. Earlier investigations on the pigment composition of membranes of *P. aestuarii* by means of thin layer chromatography [16] provided qualitative information on the pigment distribution: BChl *a* and carotenoid (OH-chlorobactene and rhodopin) were found to be present as the main light-harvesting pigments, but in addition small amounts of BPh *c* and of an unidentified pigment (labeled P-665), with an absorption spectrum similar to that of BPh *c*, were detected.

The present study reports the results of a quantitative analysis of the pigment composition of various pigment-protein complexes present in the membrane of *P. aestuarii*, using the resolving power of reversed-phase high-performance liquid chromatography. It will be shown that the membrane contains only two different chlorophyllous pigments, BChl *a* and a pigment related to BChl *c*. We will present evidence that it is this latter pigment that functions as primary electron acceptor in this green bacterium.

Materials and Methods

Isolation of pigment-protein complexes

Prosthecochloris aestuarii, strain 2K, was grown anaerobically in a mixed culture originally known as *Chloropseudomonas ethylica* [17], as described by Holt et al. [18]. Complex I, photosystem-pigment complex and reaction center pigment-protein complex were prepared following the method of Swarthoff and Amesz [8], using either 10 mM phosphate and 10 mM sodium ascorbate (pH 7.4) (phosphate ascorbate buffer) or 10 mM Tris-HCl (pH 7.4) (Tris buffer) as isolation buffer. Light-harvesting BChl *a* complex was prepared according to ref. [19]. Reaction centers of *Rhodopseudomonas sphaeroides* (wild type) were isolated as described by Vadeboncoeur et al. [20]. All samples were stored in liquid nitrogen before use.

Chromatography

Reversed-phase HPLC was performed as described by Braumann and Grimme [21]. A stainless steel column (30 cm × 4.6 mm internal diameter) packed with a 5 μm RP-18 stationary phase (Lichrospher, Merck, Darmstadt) was used for the experiments. Solvents were degassed before use in an ultrasonic bath under low pressure. Mobile phase composition and other chromatographic details are indicated in the figure legends.

Pigment extraction

We found the following method to be most effective in rapidly extracting all pigments (all extraction steps were performed at 4°C in the dark): 10 ml diethyl ether/methanol (7:2, v/v) was added to 1 ml sample. The extract was centrifuged for 45 s in a bench centrifuge (5000 rpm) and the pellet was resuspended in 10 ml methanol (96%) and again centrifuged for 45 s. To the combined supernatants 5 ml diethyl ether and 25 ml 10% KCl were added to separate the phases, which were subsequently washed three times with

distilled water. The ether layer was removed and concentrated by blowing pure nitrogen over the surface of the extract; its final volume was determined by weighing. The whole extraction procedure was performed in less than 10 min.

Pigment identification and quantification

Pigments were identified by their absorption spectra and by comparison of their retention times with those of known compounds from an extract of spinach thylakoids. Up to three different extractions and two injections were performed to determine the standard deviation in the data. The molar extinction coefficients at 410 nm, used to calculate the amount of pigment present are listed in Table I. With the exception of BChl *a*, they were determined from the absorption spectra and from the extinction coefficients in the absorption maxima given in Table I. The extinction coefficient of BChl *a* at 410 nm was determined experimentally by injection (six experiments) of a diethyl ether extract of chromatophores from *Rps. sphaeroides* (wild type) followed by comparison of the peak areas of BChl *a* and spheroidene thus obtained, assuming $\varepsilon_{410} = 52.6\ mM^{-1} \cdot cm^{-1}$ for the latter. For the original ether extract $\varepsilon_{770} = 101.4\ mM^{-1} \cdot cm^{-1}$ was taken for BChl *a* [22]. The value of ε_{410} ($4.6\ mM^{-1} \cdot cm^{-1}$) reported here for BChl *a* is significantly lower than that observed before on the basis of the absorption spectrum of BChl *a* (Ref. 23, see also Fig. 2D). This is probably due to the complete absence of trace amounts of the oxidation product 2-desvinyl-2-aceteyl Chl *a* in our determination; as can be seen in Table I this compound would seriously interfere with the determination of ε_{410} for BChl *a*, due to its relatively high extinction coefficient at this wavelength.

Calibration of the integrating unit, that was used for the analysis of the chromatograms, was performed by injecting known amounts of β-carotene (highest purity available, Sigma, Munich), using $E^{1\%}_{1\,cm} = 2212$ at 453 nm, as determined for the specific lot by the supplier.

Results and Discussion

Relative pigment composition

We have analyzed all pigment-protein complexes prepared both with phosphate ascorbate buffer as well as with Tris buffer to ascertain the absence of artefacts due to the use of the reducing agent sodium ascorbate in the first buffer. The chromatograms of Complex I prepared in Tris buffer and of pigment-protein and reaction center pigment-protein complex, both prepared in phosphate ascorbate buffer, are shown in Figs. 1A–C, respectively. Representative absorption spectra of the resolved peaks, labeled as in Fig. 1, are shown in Fig. 2 for the different compounds and were used for the identification of the pigments, together with their retention times, that are given in Table II.

TABLE I

SPECIFIC EXTINCTION COEFFICIENTS USED FOR THE PIGMENTS FROM *P. AESTUARII*

Compound	ε_{410} ($mM^{-1} \cdot cm^{-1}$)	ε_{max} ($mM^{-1} \cdot cm^{-1}$)	λ_{max} (nm)	Solvent	Ref.
BChl *a*	4.6	61.0	769	acetonitrile	22
2-Desvinyl-2-acetyl Chl *a*	52.8	65.2	677	acetone	25
BChl *c*	64.9	74.0	663	acetone/methanol (7:2)	26
BPh *c*	140.0	60.0	666	diethyl ether	23
BChl 663	66.5	74.0 [a]	663	acetone/methanol (7:2)	
OH-chlorobactene	52.9	135.0	460	light petroleum ether	27
Rhodopin	41.6	166.5	473	hexane	27

[a] ε_{max} was assumed to be equal to that of BChl *c*.

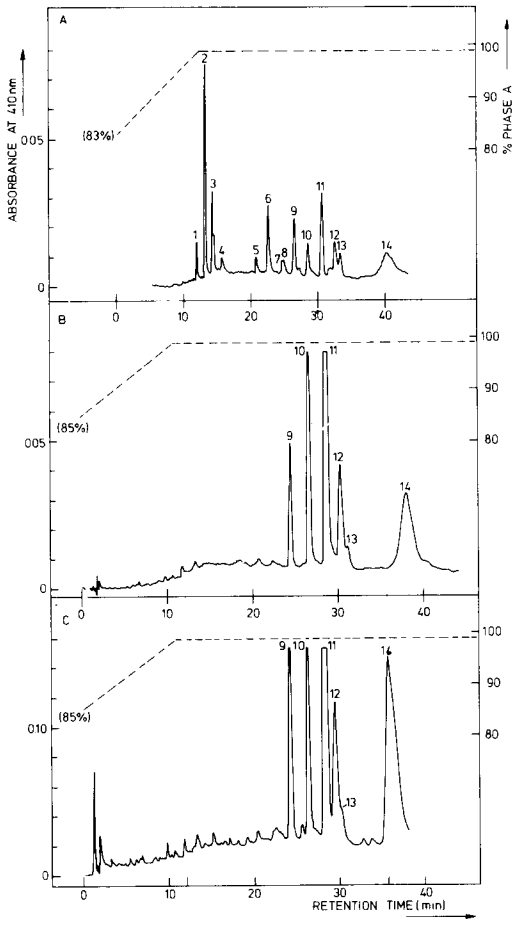

Fig. 1. Chromatograms of total pigment extracts of pigment-protein complexes of *P. aestuarii*. Mobile phase gradient is indicated on the right hand scale. Phase A: 96.5% (v/v) acetonitrile, 3.5% tetrahydrofuran. Phase B: distilled H_2O. Flow: 1.6 ml/min, gradient slope: 1.3%/min. Note the different gradient in (A), causing slightly different retention times. (A) Complex I (prepared with Tris-buffer), (B) pigment-protein complex (phosphate-ascorbate buffer), (C) reaction center pigment-protein complex (phosphate-ascorbate buffer). Peak numbers as in Table II.

The chromatogram of the reaction center pigment-protein complex (Fig. 1C) shows the presence of a large number of minor components, due to the large amount of material injected. However, the total area of the peaks with retention times between 2 and 20 min amounts to less than 2% of the total peak areas. The slightly shorter retention times (especially for rhodopin) are due to the high amount of material loaded on the column. Peaks

TABLE II

RETENTION TIMES AND NUMBER SCHEME FOR PIGMENTS FROM *P. AESTUARII* AND REFERENCES

(1) Peak number scheme and chromatographic condition is as in Figs. 1B and C. (2) Extract from spinach thylakoids.

Peak	Compound (1)	Reference (2)	Retention time (min)
		neoxanthin	6.44
		violaxanthin	8.89
1	BChl *c*		10.89
2	BChl *c*		11.94
		lutein 5–6 epoxide	12.49
3	BChl *c*		13.00
4	BChl *c*		14.11
		lutein	15.49
5	BPh *c*		19.09
6	BPh *c*		20.84
7	BPh *c*		22.80
8	BPh *c*		23.01
9	2-Desvinyl-2-acetyl Chl *a*		24.96
		Chl *b*	25.99
10	BChl *a*		26.91
11	BChl 663		29.11
12	OH-Chlorobactene		30.16
13	*cis*-OH-Chlorobactene		31.01
		Chl *a*	36.29
14	Rhodopin		37.31
		β-carotene	56.94

1–4 were identified as BChl *c* (Fig. 2A). They were present in rather large amounts in the Tris-prepared Complex I (Fig. 1A) and, as to be expected, absent in the purified light-harvesting BChl *a* complex (data not shown). Five of the six known homologues of BChl *c* are separated in the case of Complex I (peak 3 consists clearly of two components) and their relative quantities, as well as the sequence of elution agree well with those reported by Smith et al. [24] for the bulk BChl *c* originating from the chlorosomes in six different strains of *P. aestuarii*. The relatively most abundant form (peak 2) is a compound with two ethyl and one methyl substituent. The amount of BChl *c* present varied from preparation to preparation and was almost reduced to zero in the most purified pigment-protein and reaction center pigment-protein complexes (see Table III, below). We therefore conclude that BChl *c* is a contaminating pigment in these membrane preparations that originates completely from the chlorosomes.

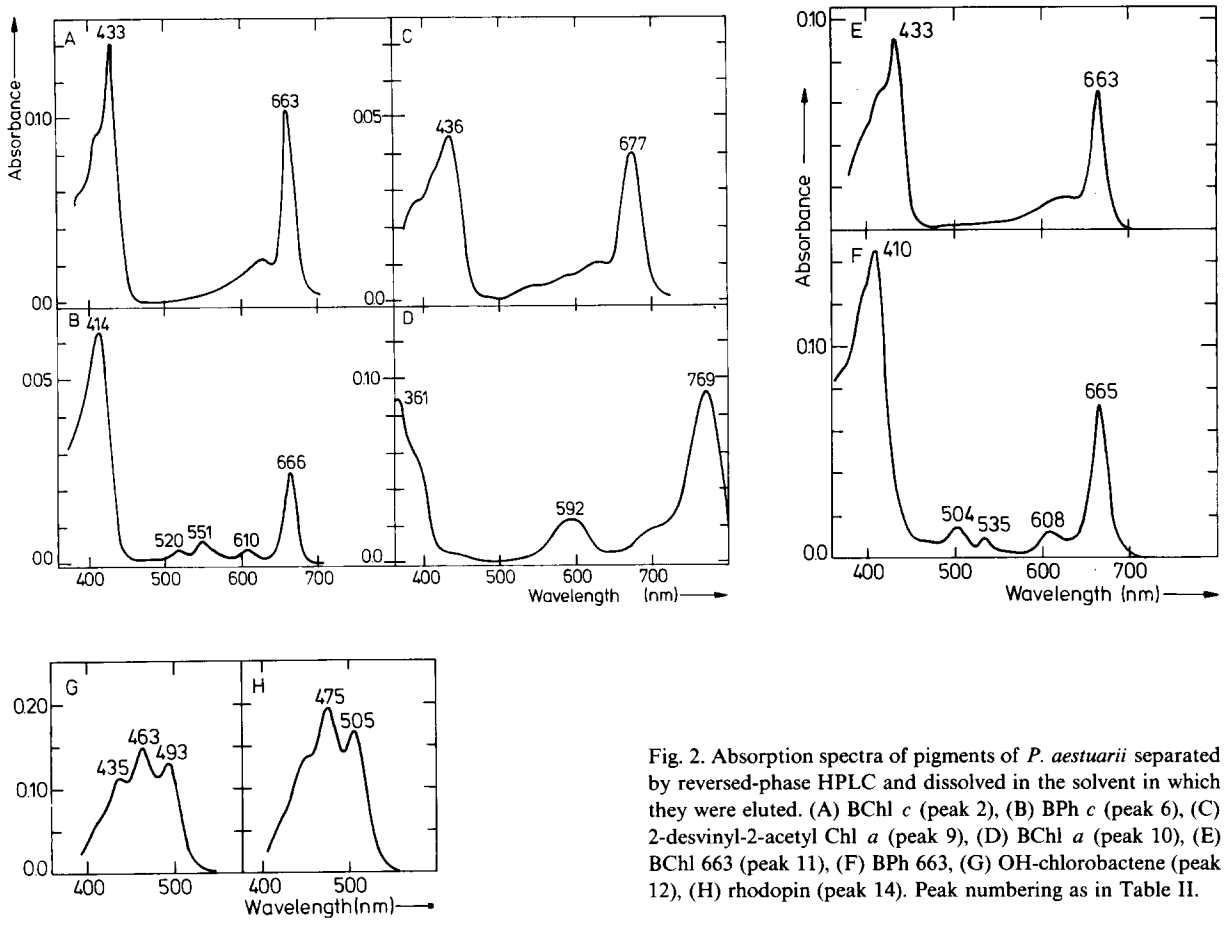

Fig. 2. Absorption spectra of pigments of *P. aestuarii* separated by reversed-phase HPLC and dissolved in the solvent in which they were eluted. (A) BChl *c* (peak 2), (B) BPh *c* (peak 6), (C) 2-desvinyl-2-acetyl Chl *a* (peak 9), (D) BChl *a* (peak 10), (E) BChl 663 (peak 11), (F) BPh 663, (G) OH-chlorobactene (peak 12), (H) rhodopin (peak 14). Peak numbering as in Table II.

Peaks 5–8 in Fig. 1A were identified as different homologues of BPh *c* with identical absorption spectra (Fig. 2B), but slightly different retention times. They were virtually absent from the most purified pigment-protein and reaction-center pigment-protein complexes (Fig. 1B and C). Peak 9 was identified as 2-desvinyl-2-acetyl Chl *a* on the basis of its absorption spectrum (Fig. 2C) and its slightly higher polarity than BChl *a* (peak 10, Fig. 2D); this compound has been shown to be the main oxidation product of BChl *a* [24]. Most interesting was the presence of a compound (peak 11) with an absorption maximum at 663 nm (Fig. 2E) and an absorption spectrum closely resembling that of BChl *c*, but with a much higher liophilicity. As can be seen upon inspection of Fig. 1 this compound was present in all pigment-protein complexes (except for the light-harvesting BChl *a* complex, data not shown). We have labeled this pigment BChl 663 and will discuss its possible nature and function below. Peaks 12 and 13 can be attributed to OH-chlorobactene and probably to its *cis*-isomer, respectively, while peak 14 is clearly rhodopin, the main carotenoid of the membrane-bound photosystem (Fig. 2G and H, respectively).

Table III summarizes the pigment composition of the three different preparations, using the extinction coefficients given in Table I. The BChl *a* content has been normalized to 100.0, assuming that all 2-desvinyl-2-acetyl Chl *a* (about 15% in Complex I, 6% in pigment protein and 10% in reaction center pigment-protein complex, respectively) arose from the oxidation of BChl *a*. The standard deviation for these data, obtained from six measurements (three for reaction center pigment-protein complex) was less than 10%.

From inspection of Table III it appears that the

BPh *c* present in Complex I originates from BChl *c*, derived from the chlorosome. The most purified pigment-protein and reaction center pigment-protein preparations contained virtually no BPh *c*, as can be seen immediately upon inspection of their chromatograms (Figs. 1B and C, respectively) for peaks around 20 min retention time. The maximum limit for the amount of BPh *c* present could be determined with quite high precision, due to the high extinction coefficient of BPh *c* compared to that of BChl *a* at 410 nm (see Table I). As the pigment-protein complex was photochemically fully active, this rules out the possibility that BPh *c* functions as primary electron acceptor in *P. aestuarii*, as has been suggested before [28,13,14]. We will return to this point later. The BChl 663 content of Complex I is comparable to that of the pigment-protein complex but the pigment is enriched about two-fold in the reaction center pigment-protein complex, relative to BChl *a*. The carotenoid content of Complex I, especially that of rhodopin, varied greatly from preparation to preparation, but was drastically reduced in the pigment-protein and reaction center pigment-protein complexes.

Pigment stability

In order to clarify the interrelationship between the different pigments and to investigate the presence of degradation products we have performed two oxidation tests on freeze-thawed Complex I. (This particular sample contained initially already a larger amount of degradation products than the samples used in the experiments described above). To test the stability of the pigments against photochemical oxidation 1 ml of Complex I was illuminated with white light in the presence of oxygen and then extracted and analyzed as described in the experimental section. Table IV lists the percentages of pigments with regard to the original pigment content, after 90 and 250 min of illumination, respectively. The most significant result is the relative stability of BChl *a*. Apparently, an effective protection mechanism against photochemical oxidation is operating in intact membranes at the expense of mainly the carotenoids. The photo-oxidation product 2-desvinyl-2-acetyl Chl *a* (peak 9) is not accumulated during illumination, but it is, as an intermediate, also subject to degradation. Similar effects have been observed during the bleaching of thylakoid membranes of

TABLE III

RELATIVE PIGMENT COMPOSITION OF PHOTOSYNTHETIC MEMBRANES AND PIGMENT-PROTEIN COMPLEXES OF *P. AESTUARII*

The total amount of BChl *a* and 2-desvinyl-2-acetyl Chl *a* was arbitrarily set to 100. Complex I (Tris) and Complex I (Asc) are two different preparations, prepared with Tris-buffer and phosphate-ascorbate buffer, respectively. The pigment-protein (PP) and reaction-center pigment-protein (RCPP) complex were both derived from the Complex I (Asc) sample.

Peak (No.)	Compound	Complex I (Tris)	Complex I (Asc)	PP (Asc)	RCPP (Asc)
1	BChl *c*	3	< 0.4	< 0.3	< 0.1
2	BChl *c*	31	9	1	0.2
3	BChl *c*	11	4	< 0.3	< 0.1
4	BChl *c*	2	1	< 0.3	< 0.1
5	BPh *c*	1	< 0.4	0.0	0.0
6	BPh *c*	7	6	0.1	0.1
7	BPh *c*	3	2	0.0	0.0
8	BPh *c*				
9	2-Desvinyl-2-acetyl Chl *a*	100	100	100	100
10	BChl *a*				
11	BChl 663	19	26	20	36
12	OH-Chlorobactene	12	11	4	8
13	*cis*-OH-Chlorobactene	6	7	1	–
14	Rhodopin	45	94	12	23

higher plants [29]. BPh *c* is seen to be more labile than BChl *c*, while BChl 663 has about the same stability as BChl *c*.

In a second test Complex I was incubated with ferricyanide in the dark. The results indicate (right hand column, Table IV) that both BChl *c* and BChl 663, as well as the carotenoids are quite stable against chemical oxidation, but that BChl *a* and its photochemical oxidation product, and, to a lesser extent, also BPh *c* are much more sensitive.

Nature of BChl 663

As stated above, an unexpected finding was the presence of a pigment with an absorption spectrum resembling that of BChl *c*, but with a much higher lipophilicity (peak 11, Fig. 1), which we called BChl 663, according to its absorption maximum in the elution solvent. Fig. 2E shows the absorption spectrum of this compound and Fig. 2F that of its pheophytin, obtained by addition of 0.1 M citric acid. From this experiment it is clear that BChl 663 is indeed a BChl-like compound and not a BPh. The absorption spectrum of BPh 663 resembles closely that of BPh *c*, apart from small shifts of the minor peaks in the region 500–550 nm.

Earlier experiments to elucidate the pigment composition of membranes of *P. aestuarii* by Swarthoff et al. [16], who used thin layer chromatography, have also resulted in the isolation of an 'unidentified pigment' (labeled P-665 at the time), which was shown to be a pheophytinized dihydroporphyrin. The absorption spectrum of this pigment was practically identical to that of BPh 663, so we assume that P-665 was in fact pheophytinized BChl 663. Inspection of Table III shows that the amount of BChl 663 present in the different complexes does not at all run in parallel with the BChl *c* content, excluding the possibility that BChl 663 is yet another homologue of BChl *c*, derived from the chlorosome.

Although the degradation experiments discussed before already indicated that it is highly unlikely that BChl 663 is a degradation product of BChl *a*, we performed a final check by applying our extraction and reversed-phase HPLC method to purified reaction centers of *Rps. sphaeroides* (wild type) that contain only Bchl *a*, BPh *a* and spheroidene [30]. Fig. 3 shows the chromatogram thus obtained. Detection was at 665 nm, at the maximum absorption of BChl 663. Major peaks are due to 2-desvinyl-2-acetyl Chl *a* (1), BChl *a* (2) and BPh *a* [3]. The arrow indicates the expected retention time for BChl 663, which pigment is seen to be completely absent. This is clear proof that the BChl 663 present in the membrane of *P. aestuarii* does certainly not derive from BChl *a*. On the basis of all available data (absorption spectrum, chemical stability, retention behavior and distribution) we assume that BChl 663 is an, as yet unknown, homologue of BChl *c*, with more lipophilic substituents or esterifying alcohol than those reported for the known compounds. By extrapolation of the methylene group contribution to the log *k* values [31] for the six known BChl *c*'s present in Complex I (that each differ in the

TABLE IV

PIGMENT STABILITY AGAINST PHOTO-OXIDATION

(1) White light, 0.12 W/cm^2 at 25°C. Oxygen present. Results are given as a percentage of the amount originally present. (2) Incubation for 90 min at 4°C in the dark.

Peak (No.)	Compound	Illumination (1)		5 μg/ml ferricyanide (2)
		90 min	250 min	
1–2	BChl *c*	87	45	99
5–8	BPh *c*	86	29	77
9	2-Desvinyl-2-acetyl Chl *a*	78	30	47
10	BChl *a*	104	76	33
11	BChl 663	88	46	108
12–14	Carotenoids	93	35	106

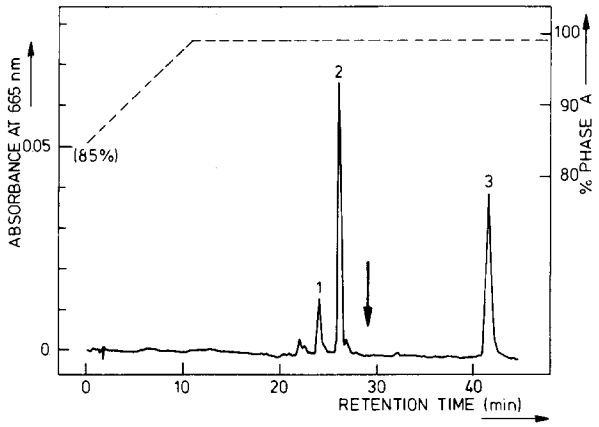

Fig. 3. Chromatogram of total pigment extract of reaction centers of *Rhodopseudomonas sphaeroides* (wild type). Chromatographic conditions as in Fig. 1. Detection wavelength, 665 nm. The arrow indicates the expected retention time of BChl 663.

addition of one methylene group) we estimated that BChl 663 should contain 5–6 additional methylene groups, compared to the most lipophilic known BChl *c*, which is BChl c_1 (according to the numbering scheme of Ref. 24). In accordance with this, preliminary results of gas chromatography-mass spectroscopy indicate that the esterifying alcohol of BChl 663 is phytol. In this respect it is interesting to note that Caple et al. [32] reported the presence of minor amounts of BChl *c* in *Chlorobium limicola* that were esterified with phytol instead of the major esterifying alcohol *trans, trans*-farnesol, which is one iso-prenoid group smaller than phytol. The lipophilicity of this minor compound was also slightly higher than that of BChl *a*, as is that of BChl 663. However, the spectral differences between the known BPh *c*'s and BPh 663 in the region 500–550 nm give an indication that also different ring substituents may be present in BChl 663. In fact the spectrum of BPh 663 in this region bears more resemblance to that of pheophytin *a* than to BPh *c* [33]. Mass spectroscopy experiments are in progress to check these hypotheses.

Composition of pigment-protein complexes from P. aestuarii

Table V gives the absolute pigment composition of the several pigment-protein complexes of *P. aestuarii*, that we have studied with reversed-phase

TABLE V

PIGMENT COMPOSITION OF MEMBRANE PREPARATIONS AND PIGMENT-PROTEIN COMPLEXES OF *P. AESTUARII* EXPRESSED AS THE NUMBER OF MOLECULES PER REACTION CENTER

	Complex I	Pigment protein	Reaction center pigment protein
BChl *a*	100	75	35
BChl 663	26	15	13
BChl *c*	< 14	< 1	< 0.2
BPh *c*	< 8	0.1	< 0.1
OH-Chlorobactene + *cis*-isomer	18	4	3
Rhodopin	50–100	9	8

HPLC. On the basis of the particle weight of the pigment-protein complex (600 ± 50 kDa) and the reaction center pigment-protein complex (350 ± 50 kDa) [8] it is reasonable to assume that only one reaction center is present per pigment-protein complex. The pigment/reaction center ratio was calculated from the maximum extent of photobleaching at 840 nm, due to photo-oxidation of the primary donor P-840, and the total absorbance at 810 nm; the assumption was made that the extinction coefficients for P-840 at 840 nm and for the antenna BChl *a* at 810 nm are identical. For the latter we obtained $\varepsilon_{810} = 113 \pm 3\ mM^{-1} \cdot cm^{-1}$ for both Complex I and pigment-protein complex (Vasmel, H. and Zonneveld, F.T.M., unpublished results), while preliminary experiments, relating the extent of bleaching of the primary donor at 840 nm to the amount of cyt c_{553} oxidation yielded values for ε_{840} in the range 100–120 $mM^{-1} \cdot cm^{-1}$. This justifies the assumption that both extinction coefficients are approximately the same. As can be clearly seen from Table V the conclusion of Swarthoff and Amesz [8] that the reaction center pigment-protein complex is formed by removal of two light-harvesting BChl *a* proteins (that contain in total 42 BChl *a* molecules and no carotenoid) from the pigment-protein complex, is confirmed by our data. Both the carotenoid and BChl 663 content of the pigment-protein and reaction center pigment-protein complexes are the same, while only removal of BChl *a* is observed. Our data also show, that the 670 nm peak in the in vivo absorption spectrum of pigment-protein and reaction

center pigment-protein complexes (and probably also in the cytoplasmic membrane) must be attributed solely to the newly found BChl 663 and not partly to BPh *c*, as has been assumed until now [28,13,14]. The in vivo extinction coefficient of BChl 663 would then be 85 ± 10 $mM^{-1} \cdot cm^{-1}$, which seems a reasonable value. As it is now clear that the primary electron acceptor in *P. aestuarii* is a pigment with absorption maximum at 670 nm [13,14], an interesting consequence of our experiments is the conclusion that only BChl 663 can be a likely candidate for the role as electron acceptor as no other pigments absorbing around 670 nm are present in preparations that retain full photochemical activity. If BChl 663 has the same redox properties as BChl *c*, which has a midpoint potential of -1.03 V [28], its redox potential is indeed low enough for the reduction of the iron-sulfur center ($E_m = -560$ mV [11]), that functions as secondary electron acceptor.

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