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COMPARISON OF A CHLOROPHYLL *a*–PROTEIN COMPLEX ISOLATED FROM A BLUE-GREEN ALGA WITH CHLOROPHYLL–PROTEIN COMPLEXES OBTAINED FROM GREEN BACTERIA AND HIGHER PLANTS

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SUMMARY

1. An improved procedure has been devised for the purification of large quantities of a detergent-soluble chlorophyll *a*–protein complex (λ_{max} 677 nm) from a blue-green alga. The complex, which is pure by the criteria of gel electrophoresis and ultracentrifugation, contains approx. 70% of the chlorophyll *a* of the organism. Studies of the composition of the complex indicate that the protein moiety is probably made up of subunits (mol. wt. 35000), each of which contains five moles of chlorophyll and a trace of β-carotene and echinonone.

2. Comparison of the physical and chemical properties of the algal chlorophyll–protein complex with those of a water-soluble bacteriochlorophyll–protein of green bacteria, and another detergent-soluble chlorophyll *a*–protein complex of higher plants suggests that all photosynthetic organisms might contain an analogous class of chlorophyll–proteins (mol. wt. 150000–160000), which are associated with photochemical system I activity, and which are composed of four identical protein subunits and 20 moles of chlorophyll.

3. The water insolubility of the algal and higher plant chlorophyll–protein complexes is most probably explained by the presence of apolar amino acid residues on the surface of the molecule. *In vivo* the chlorophyll *a*–proteins are postulated to be attached to the lamellar membrane by hydrophobic bonding of the apolar regions to the lamellar lipids.

4. Comparative studies indicate that the algal and higher plant complexes are much more closely related to each other than to the bacterial complex.

INTRODUCTION

Comparison of the composition and function of (bacterio)chlorophyll–protein complexes isolated from photosynthetic green bacteria[1] and higher plants[2,3] has indicated[4] that organisms which carry out photochemical system I activity may contain an analogous class of chlorophyll–protein complexes, whose structure and composition are best typified by those of the water-soluble bacteriochlorophyll–

Abbreviations used: Chl, chlorophyll *a*; Bchl, bacteriochlorophyll *a*; (B)Chl-P, (Bacterio)-chlorophyll–protein.

protein of green bacteria. Thus one of the aims of the present work was to obtain further evidence for this hypothesis by investigating whether another class of photosynthetic organisms, blue-green algae, contains a member of this group of chlorophyll–protein complexes.

All photosynthetic organisms, apart from the green bacteria, appear to require detergent to solubilize their chlorophyll–protein complexes. The methods available[2,5] for the separation of such detergent-soluble complexes from other components of solubilized photosynthetic structures is not satisfactory; it is a laborious, one-step procedure, which is possibly degradative, and which gives small yields. Hence a second aim of the present investigation was to devise a more suitable preparative method. A blue-green alga was chosen as the starting material for these studies, since preliminary observations had indicated that, unlike most other chlorophyll *a*-containing organisms blue-green algae contain only one major chlorophyll–protein complex, and thus purification of the complex should be less complicated; thereafter it should be possible to devise methods to resolve extracts of organisms that contain more than one chlorophyll–protein complex, *e.g.*, green algae and higher plants. The availability of large quantities of such complexes will allow many chemical and physical studies to be carried out, the results of which should lead to a greater knowledge of the mechanism of primary photosynthetic processes, of chlorophyll–protein interactions, of the structure and organization within photosynthetic lamellae, and of the nature of chlorophyll *in vivo*.

METHODS

Material

Batches of the blue-green alga, *Phormidium luridum* var. *olivacea*, were grown in 180-l polyethylene tanks as described by LYMAN AND SIEGELMAN[6]. The cells were harvested by using a continuous flow centrifuge; potassium aluminum sulfate was sometimes added to the growth medium to aggregate the algal cells and thus facilitate their centrifugation.

Isolation of the protein (Fig. 1)

Step 1. 75 g of the alga were mixed with Tris buffer (pH 8.0) and frozen (—17°), and then allowed to thaw at room temperature. Diatomaceous earth (Celite 545) was added to the suspension of broken cells (10 g per 100 ml suspension), and the slurry poured into a column. The packed column was eluted with 50 mM Tris (pH 8.0), until the eluate was colorless, *i. e.*, all the soluble phycocyanin had been eluted. A solution of 50 mM Tris–0.5 % sodium dodecyl sulfate (pH 8.0, 400 ml) was passed through the column, and the chlorophyll-containing eluate collected; elution was continued until all the chlorophyll was removed from the column.

Step 2. Solid ammonium sulfate was added to the detergent extract until precipitation of the chlorophyll was visible (10 g ammonium sulfate per 100 ml solution), and then Celite 545 added until the solution became a slurry (20 g per 100 ml of solution), which was poured into a column (12 cm × 4 cm). The packed column was eluted with 35 % ammonium sulfate (100 ml), and then with ammonium sulfate (35 %)–methanol (100:30, v/v), until the eluate was colorless (300 ml). Next the column was eluted with 50 mM Tris (pH 8.0, 100 ml) and finally with 50 mM Tris–0.1 %

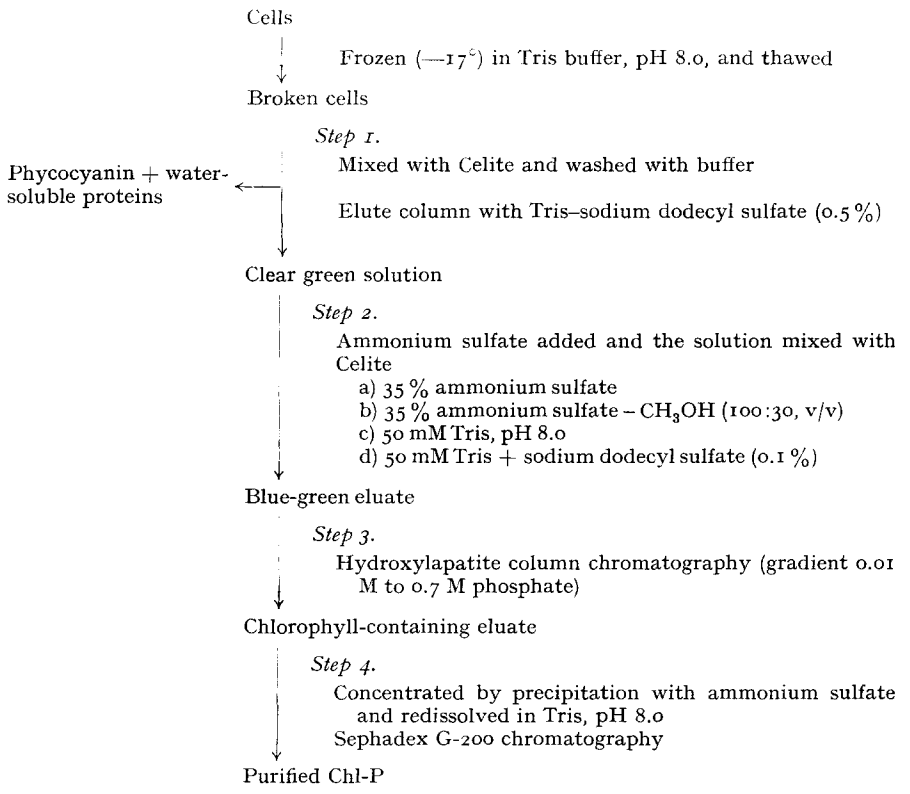

Fig. 1. Schematic diagram of the procedure for the purification of the chlorophyll *a*–protein.

sodium dodecyl sulfate (about 200 ml) until all the chlorophyll-containing components remaining on the column were eluted. This last fraction was used immediately for further purification.

Step 3. A column of hydroxylapatite (5 cm × 10 cm) was equilibrated with 10 mM sodium phosphate, pH 7.0. The detergent eluate from the last step was run into the column and washed with 100 ml of the phosphate buffer. A linear gradient (0.01 to 0.7 M) of sodium phosphate, pH 7.0 (total volume 600 ml), was passed through the column, and that portion of the eluate containing chlorophyll was collected. The solution was concentrated by addition of ammonium sulfate, centrifugation, and dissolution of the precipitate in a minimal volume of 50 mM Tris (pH 8.0). The precipitate was dissolved by gentle homogenization with the buffer in a Potter homogenizer.

Step 4. The concentrated solution was chromatographed on a column of Sephadex G-200 (3 cm × 50 cm) in 50 mM Tris, pH 8.0. The major chlorophyll-containing fraction to be eluted was collected, and used for further studies.

Other methods

Ultracentrifugation was performed in a Spinco Model E ultracentrifuge using the Schlieren optical system and cells of 30 mm path length. Corrections to the *s* values determined were made for the viscosity and density of the solvent used.

A Cary Model 14R spectrophotometer was used for all absorbance measurements

and for obtaining the absorption spectra of solutions; opal glasses were inserted between sample and detector when the spectra of strongly scattering suspensions were recorded.

Polyacrylamide gel electrophoresis was carried out using 8 % acrylamide gels and a buffer of 50 mM Tris–0.25 % sodium dodecyl sulfate, pH 8.0. Gels were stained with a solution of Amido Schwarz (Buffalo Black NBR, Allied Chemical Corp., New York). The electrophoretic mobility of Chl-P complexes was determined from the rate of movement of the complexes through 8 % acrylamide gels in a buffer of 50 mM Tris–0.25 % sodium dodecyl sulfate, pH 8.0.

Chlorophyll *a* was determined by the method of VERNON[7]. Nitrogen was determined by micro-Kjeldahl procedure of MARKHAM[8].

Carotenoids in the purified material were analyzed according to the procedure of DAVIES[9]: To a solution of known Chl concentration, 3 vol. of ethanol and 1 vol. of 10 M NaOH were added, and the mixture stored in the dark at room temperature for 4 h. The solution was diluted 4 times with water and the carotenoids were extracted into ether. In order to estimate the total carotenoid, the ether solution was evaporated to dryness and the residue dissolved in a known convenient volume of ethanol and the absorbance determined at λ_{max}. Total carotenoid was calculated using $E_{1\%} = 2500\ cm^{-1}$. Chromatography of the carotenoid extract was carried out by thin-layer chromatography on Absorbosil 5 in hexane–acetone (80:20, v/v), and components identified by comparison with authentic samples. The identity of the carotenoids was confirmed by elution of each component from the plate with ethanol and examination of the spectrum. The quantity of each component was determined from the spectra as described above.

Amino acid analysis was performed as follows: constant boiling HCl was added to about 1 mg of dried Chl-P samples contained in pyrex tubes. The contents were cooled in a mixture of ethanol and solid CO_2, and the tubes evacuated until the contents ceased to bubble. The tubes were then sealed under vacuum ($< 100\ \mu$) and the complex hydrolyzed at 110° for 24–95 h. An automatic amino acid analyzer of the type described by ALONZO AND HIRS[10] was used to determine the composition of the hydrolyzates. Performic acid-oxidized samples were prepared by the method of MOORE[11] and half-cystine and methionine in their hydrolyzates estimated as cysteic acid and methionine sulfone, respectively; it was assumed that 94 % of the half-cystine was recovered as cysteic acid[11].

The tryptophan content of the complex was obtained from a spectrophotometric determination[12] of the molar ratio of tyrosine: tryptophan in the protein. Samples of Chl-P were adjusted to pH 4.5 and denatured by heating at 100° for 30 sec. The precipitate was washed with 0.05 M ammonium acetate buffer, pH 8.5, and suspended in the same buffer. The protein was then digested with Nagarse (Teikoko Chemical Industry Co. Ltd., Osaka, Japan) (protein–Nagarse, 25:1, w/w) and the molar ratio of tyrosine: tryptophan determined on the resultant supernatant which contained the majority of the protein, but none of the chlorophyll.

RESULTS AND DISCUSSION

Purification of Chl-P (Fig. 1)

Elution with sodium dodecyl sulfate of the broken algae mixed with Celite in a column, compared to the more conventional successive centrifugation method[2], was a

preferable technique for extraction of the Chl-containing substances from the organism, since it was easier to see the complete solubilization of the Chl, and also the presence of excess detergent, which is damaging to the protein (see later), was limited better in this way. For this latter reason the concentration of sodium dodecyl sulfate in buffers used in the preparative procedure is kept as low as is compatible with dissolution of the Chl-containing components, and, if possible, is removed entirely. The Chl-P appears to have bound sufficient detergent to maintain its solubility in water after Step 2. Solutions were buffered by Tris throughout the preparation of the complex; phosphate buffer was not used, since in the presence of sodium dodecyl sulfate it accelerates the rate of pheophytinization of Chl.

Examination of the sodium dodecyl sulfate extract of the cells by electrophoresis on polyacrylamide gels revealed the presence of only one readily visible Chl-containing zone, and after staining the gels, this Chl was observed to be associated with a protein zone; the electrophoretic mobility ($1.60 \cdot 10^{-5}$ $cm^2/V \cdot sec$) of the Chl-P complex was measured; the Chl *a*–protein isolated from spinach[2] was run in the same experiment as a control. Upon spectroscopic examination of the unstained gel, a second minor Chl-containing zone was observed, which was electrophoresed together with carotenoids in a manner analogous to the free pigment zone observed with sodium dodecyl benzene sulfate-dispersed higher plant chloroplasts[2,3].

Certain standard techniques for protein separation (ion-exchange chromatography, solvent precipitation and ammonium sulfate fractionation) proved to be of no use for the resolution of detergent-soluble chlorophyll-containing complexes in the sodium dodecyl sulfate extract; however, other standard methods (calcium phosphate chromatography and gel filtration) were applicable, and gave excellent results provided a preliminary purification was carried out of the Chl-P in the extract. This initial purification was by an unconventional procedure, which was adapted from the method of SIEGELMAN, CHAPMAN AND COLE[13] for the purification of phycocyanin. In purification Step 2, four solutions were used for the elution of the column, the rationale behind which was as follows: 35 % ammonium sulfate solution removed any excess detergent from the column, whilst still allowing the proteins in the precipitated extract to remain on the column. No color was eluted during this wash, whereas the subsequent ammonium sulfate–methanol mixture removed some yellow-brown material (carotenoids and some Chl, probably occurring as free pigments complexed with sodium dodecyl sulfate). The methanol-containing salt solution also removed from the column all the complexed detergent. The elution with 50 mM Tris was used to wash out the ammonium sulfate and remove water-soluble proteins (if any) before sodium dodecyl sulfate was added to the eluting medium to remove all the Chl-P in the column; this demonstrated that detergent is essential for the solubility of the Chl-P. Analytical gel electrophoresis showed that Chl-P was the major component of the Tris–sodium dodecyl sulfate eluate; the other zone containing carotenoids and Chl, which was observed in the original extract, was now virtually absent. The eluate also contained a pink high molecular weight component which did not enter the acrylamide gel column, and a few minor colorless proteins of higher electrophoretic mobility, and thus probably of lower molecular weight than Chl-P[14]. The next two purification steps (3 and 4) removed the impurities. Polyacrylamide gel electrophoresis of the eluate from the Sephadex chromatography showed only a single zone of chlorophyll and protein which were coincident.

Proportion of the alga's Chl contained in Chl-P

The majority of the Chl in the alga is associated with the Chl-P, and a small proportion gives rise to the detergent-complexed free pigment (see above); the ammonium sulfate–methanol wash (Fig. 1) isolated this small proportion of Chl, which represented 30 % of the total Chl in the sodium dodecyl sulfate extract of the cells. Hence, since all the pigment in the only other Chl-containing eluate from Step 2 is present in the Chl-P complex, then the Chl-P must account for 70 % of the organism's Chl. This value may be greater *in vivo* since the action of excess sodium dodecyl sulfate on isolated Chl-P can result in the production of free Chl. This action has been observed previously by OGAWA, OBATA AND SHIBATA[5] and by THORNBER *et al.*[2,3]. In the present work it was also found that addition of excess sodium dodecyl sulfate to the Chl-P eluted from the Celite column (Step 2) and a recycling of the resulting solution through that stage of preparation produced Chl, which was eluted by the methanol-containing ammonium sulfate solution (*i.e.*, free pigment), whereas a recyclization without the addition of excess sodium dodecyl sulfate gave no free Chl. These observations pose the question whether the Chl released from purified Chl-P is derived from that Chl truly complexed with protein, or whether it results from Chl attached to the exterior of the protein. The author favors the former supposition, since an effect of excess detergent on Chl-P is to cause a blue shift in the λ_{max} of the red peak, which indicates that a change has occurred in the environment of some or all of the Chl molecules; such a change would probably not have occurred if the extracted Chls had been on the outside of the complex. In addition, sodium dodecyl sulfate is a known dissociating agent for proteins, and as such might be expected to unfold the protein chain, thereby allowing the detergent to dislodge the Chls, which are predicted to be located inside

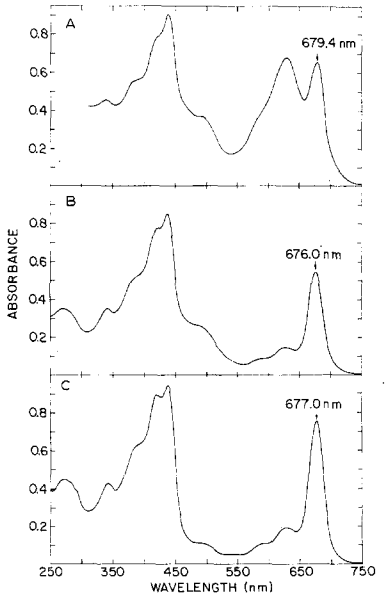

Fig. 2. Absorption spectra of (A) whole *P. luridum* cells (0.05 M Tris, pH 8.0), (B) the detergent extract (50 mM Tris–0.5 % sodium dodecyl sulfate, pH 8.0) of the broken cells, and (C) the isolated chlorophyll–protein complex in 50 mM Tris–0.02 % sodium dodecyl sulfate, pH 8.0.

the protein in the same manner as Bchl-P[15]. Hence, sodium dodecyl sulfate is almost certainly deleterious to the Chl-P, and in the preparative procedure (Fig. 1) the concentration of sodium dodecyl sulfate has been kept minimal to decrease extraction of Chl from Chl-P, and dissociation of the complex. Some of the smaller proportion (30 %) of the Chl eluted by methanol therefore probably originated from the Chl-P, and some must have been derived from other components in the organism, such as the strongly fluorescent species of chlorophyll associated with system II (Chl a_2 of DUYSENS AND SWEERS[16]).

Spectrum of purified Chl-P

The spectra of whole algal cells, the sodium dodecyl sulfate extract of such cells, and the isolated Chl-P are shown in Fig. 2. All peaks in the visible region of the spectrum of the purified Chl-P are due to Chl *a*; the shoulder at about 490 nm is probably contributed by the β-carotene present in the complex (Table III). The molar absorptivities, relative to Chl *a*, at the absorption maximum of the Chl-P are given in Table I. These results show that, although the ratio of the blue:red absorption maxima (1.30) is identical to that reported by STRAIN, THOMAS AND KATZ[17] for Chl *a* in ether, the molar absorptivities at both wavelengths are decreased some 30 %. The most probable explanation of this decrease is that the red absorption peak of the complex is made up of several spectral forms of Chl *a*, each of which has a different λ_{max}. The various forms are a reflection of different environments of the chlorophyll molecules within the complex. Preliminary observations of the low temperature (−196°) absorption spectra of the complex suggest that there are different spectral forms. There are peaks at 674 and 705 nm, and shoulders at ~678 and ~685 nm.

TABLE I

MOLAR ABSORPTIVITY VALUES BASED ON CHLOROPHYLL *a*

Limit of error estimated as ± 3 %.

λ (nm)	275	342	420	437	629	677
ε ($mM^{-1} \cdot cm^{-1}$)	43.9	34.9	75.4	78.0	14.9	59.9

The spectrum of the Chl-P was examined in the "IR1" and "IR2" modes of the Cary 14R spectrophotometer; no reversible bleaching was observed. It appears therefore, that the complex does not contain the photochemical reaction center (P700).

Upon addition of detergent to the broken algal cells, there is a shift in the λ_{max} of the red peak of Chl from 679 nm to about 676 nm (Fig. 2). This shift is probably the result mainly of extraction of the Chl-P from its environment in the lamellar system, and, to a lesser extent, to an effect of sodium dodecyl sulfate upon the Chl molecules in the complex (see above) and upon other Chl-containing moieties. Gel electrophoresis has revealed that there are two separate entities containing Chl in the sodium dodecyl sulfate extract of broken cells: the 677 nm Chl-P, and a lower wavelength form (λ_{max} approx. 670 nm) of sodium dodecyl sulfate-solubilized Chl; the combination of these two forms would thus give the 676 nm peak maximum observed for the extract.

Ultracentrifugation of Chl-P

The purity of the isolated complex was examined in the ultracentrifuge. A single boundary of 9.1 S was observed when the protein (1 mg/ml) was centrifuged in 50 mM Tris–0.2 % sodium dodecyl sulfate, pH 8.0. The chlorophyll sedimented with this boundary, indicating that an homogeneous complex of Chl and protein was being examined.

Amino acid composition and subunit size of Chl-P

The amino acid compositions of Chl-P after different times of hydrolysis are reported in Table II along with those of oxidized protein samples. The molar ratio of tyrosine:tryptophan was determined as 1.64 ± 0.05. This value was used to calculate the tryptophan content given in Table II. From the "best values" the number of residues of each amino acid in the minimum molecular weight unit (subunit) was calculated in the same manner as described previously for Bchl-P (ref. 4). The most likely mol. wt. of the subunit is 35060 (minimum 34970; maximum 35220). There is

TABLE II

AMINO ACID COMPOSITION OF THE ALGAL CHLOROPHYLL–PROTEIN COMPLEX

In order to compare one analysis to another, each set of values was multiplied by a factor so that the sum of the recoveries of lysine, arginine, aspartic and glutamic acids, proline, glycine, alanine and phenylalanine was constant. *Ox*: duplicate samples oxidized with performic acid and hydrolyzed for 48 h. The values obtained were not used for the determination of the best values except those of cysteic acid and methionine, which was determined as methionine sulfone. Averages of the values after 24-, 48-, 72- and 95-h hydrolysis were used for each amino acid except threonine, serine, valine and isoleucine, leucine and phenylalanine. The values for threonine and serine were obtained by extrapolation of their recoveries to zero time, and those for valine, isoleucine, leucine and phenylalanine were the mean of the 72- and 95-h recoveries. The number of μmoles equivalent to one residue of amino acid in a minimum molecular weight subunit was determined by dividing the sum of the best values for cysteic acid, methionine, tyrosine, lysine and arginine by 41; a value of 0.0976 ± 0.0020 was obtained.

Amino acid	*Amount of amino acid recovered (μmoles)*							*Residues/subunit*	
	Hydrolysis time (h)				*Ox*	*Ox*	*Best value*	*Best value/ 0.0976 ± 0.002*	*Nearest integer*
	24	*48*	*72*	*95*					
Lysine	1.100	1.094	1.057	1.068	1.145	1.098	1.080	11.1 ± 0.2	11
Histidine	1.337	1.299	1.304	1.310	0.766	0.940	1.313	13.5 ± 0.3	13–14
Arginine	1.118	1.115	1.066	1.067	1.100	1.070	1.092	11.2 ± 0.2	11
Aspartic acid	2.665	2.601	2.621	2.633	2.816	2.821	2.630	26.9 ± 0.5	27
Threonine	1.754	1.683	1.630	1.572	1.733	1.753	1.813	18.6 ± 0.4	19
Serine	1.711	1.581	1.478	1.387	1.682	1.714	1.812	18.6 ± 0.4	19
Glutamic acid	2.050	2.078	2.081	2.062	2.096	2.115	2.068	21.2 ± 0.4	21
Proline	1.537	1.518	1.572	1.579	1.495	1.484	1.552	15.9 ± 0.3	16
Glycine	3.229	3.220	3.205	3.191	3.228	3.261	3.211	32.9 ± 0.7	33
Alanine	3.179	3.181	3.221	3.192	3.160	3.173	3.193	32.7 ± 0.7	32–33
Valine	1.755	1.932	1.978	1.966	1.863	1.854	1.972	20.2 ± 0.4	20
Methionine	0.533	0.535	—	0.606	0.581	0.592	0.587	6.0 ± 0.1	6
Isoleucine	1.662	1.800	1.913	1.928	1.763	1.755	1.921	19.7 ± 0.4	20
Leucine	3.281	3.345	3.426	3.430	3.319	3.387	3.428	35.1 ± 0.7	35
Tyrosine	0.940	0.962	0.941	0.958	—	—	0.950	9.7 ± 0.2	10
Phenylalanine	1.908	1.976	1.974	1.990	1.746	1.762	1.982	20.4 ± 0.4	20
Cysteic acid	—	—	—	—	0.286	0.299	0.293	3.0 ± 0.1	3
Tryptophan	—	—	—	—	—	—	0.580	5.9 ± 0.1	6

15.9 % N in the protein as calculated from the amino acid composition, and thus a factor of 6.28 was used to convert protein-N into weight of protein. The amino acid composition is discussed later.

Chlorophyll and carotenoid content of Chl-P

The isolated complex contains protein, chlorophyll *a* and a small quantity of carotenoid; no evidence has been obtained so far for the presence of any other component except detergent. The concentrations of Chl and N in three preparations of Chl-P in sodium borate buffer were determined, and a mean value of 66 ± 2 μg Chl/84.6 ± 2.3 μg N obtained. After correction for that N derived from Chl, and assuming that all the other N is derived from the protein (probably a reasonable assumption, *cf.* refs. 3, 4), it can be calculated that there are 5.1 ± 0.3 moles Chl/35 000 g protein (subunit). The carotenoids in the complex were identified as β-carotene and echinonone. Their proportion to Chl was determined for 4 different preparations of Chl-P and the following results obtained: 20 moles Chl; 0.7 ± 0.1 mole β-carotene; 0.1 ± 0.05 mole echinonone. Using these values, and assuming that the pigments are evenly distributed between the protein subunits, the molecular weight of the pigmented subunit is 39 600 (range 39 500–39 800).

Comparison with chlorophyll–protein complexes of other photosynthetic organisms

A Bchl-P complex has been isolated from green bacteria by OLSON AND ROMANO[18] and its composition and nature studied[1,4,15,19,20]. A similar component in higher plants has been investigated by THORNBER *et al.*[2,3]. Both complexes are associated with photochemical system I. The properties described above for the algal Chl-P are compared with those of the other two complexes in Tables III and IV. The comparison strongly suggests that all three complexes belong to a class of analogous proteins, thus providing further support for the hypothesis[4] that there is a class of system I

TABLE III

COMPARISON OF SYSTEM I CHLOROPHYLL–PROTEINS

Sedimentation coefficients of chlorophyll *a*–proteins and electrophoretic mobilities of each complex were determined in the presence of sodium dodecyl sulfate. The carotenoid content is calculated in moles/4 subunits (see text).

	Green bacterium Cps. ethylicum[4]	*Blue-green alga P. luridum*	*Higher plant B. vulgaris*[3]
Determined mol. wt.	152 000 ± 4000	—	—
$s_{20,w}$	7.3 S	9.1 S	8–9 S
Electrophoretic mobility (molecular size)[14] (cm²/V·sec)	$1.52 \cdot 10^{-5}$	$1.60 \cdot 10^{-5}$	$1.60 \cdot 10^{-5}$
Minimum mol. wt. of protein (subunit size) (Table IV)	33 400	34 900	33 300
Chlorophyll content (moles/subunit)	5.1 ± 0.1	5.1 ± 0.3	4–4.5
Carotenoid content (moles/4 subunits)	0	0.7 β-carotene 0.1 echinonone	1.2 β-carotene 0.2 lutein
Other lipids	0	—	0
Carbohydrate content	Tr unknown	—	Tr Gal Glu Man Ara Xyl
Red absorption band	809 nm	677 nm	671–672 nm

TABLE IV

AMINO ACID COMPOSITION OF SYSTEM I CHLOROPHYLL–PROTEINS

	Number of residues		
	Green bacterium Cps. ethylicum[4]	*Blue-green alga P. luridum*	*Higher plant B. vulgaris*[3]
Polar residues	(138)	(121–122)	(118)
Acidic residues			
Asp	33	27	26
Glu	28	21	24
Basic residues			
Lys	16	11	9
His	7	13–14	15
Arg	18	11	11
Hydroxyl residues			
Thr	13	19	16
Ser	23	19	17
Nonpolar residues	(167)	(201–202)	(190)
Aliphatic residues			
Gly	34	33	34
Ala	18	32–33	30
Val	29	20	19
Ile	20	20	20
Leu	18	35	35
Pro	15	16	15
Met	3	6	4
Cys	2	3	1
Aromatic residues			
Tyr	8	10	8
Phe	15	20	21
Trp	5	6	3
Total residues	305	322–324	308
Minimum mol. wt. of subunit	33 400	35 000	33 300

chlorophyll–protein complexes in photosynthetic organisms. Each component has almost the same electrophoretic mobility and their *s* values are also approximately identical (the *s* value of Bchl-P is probably smaller since it does not contain bound detergent); thus each component has nearly the same molecular size[14]. The only easily experimentally determinable molecular weight is that of Bchl-P (mol. wt. = $1.52 \cdot 10^5$ g/mole)[4], however, since the other two Chl-Ps are of almost the same molecular size and composition, it is postulated that they also have a mol. wt. of about 150000–160000. Further similarities in the three complexes are observed in the size of their subunits (Table IV). There is strong evidence[4,15] that there are four identical subunits (mol. wt. 37900) per molecule of Bchl-P. Whether the subunits of the Chl-Ps are identical has not been examined; however, the size of the subunits, calculated from the amino acid composition of the proteins, when related to the probable molecular weight of the whole complex, indicates that 4 identical subunits per molecule is also a probable ratio for the Chl-Ps. Another convincing indication that the three complexes are analogous is that they each contain the same ratio of moles Chl/subunit (Table III).

Some differences between the three complexes are observed in their amino acid composition and carotenoid content. These variations make it apparent that the algal

Chl-P is much more closely related to the higher plant component than to the bacterial one (*cf.* the sequence studies of MATSUBARA, CANTOR AND JUKES[21] on ferrodoxins). Bchl-P is unique since it does not contain any carotenoids. Although the function of carotenoids in photosynthesis is not yet known, a possible function *in vivo* is hydrophobic bonding of the Chl-P complexes to the lamellae (*cf.* JI AND BENSON[22]). In which case carotenoids might occur in the isolated Chl-Ps if they have not been fully displaced from the surface of the Chl-P by sodium dodecyl sulfate during purification.

There are some differences between the Chl-*a* proteins, but these are so small for components of organisms so widely separated phylogenetically that there can be little doubt that an analogous component of each organism is being studied. It is therefore reasonable to presume that the algal Chl-P is involved in photochemical system I activity (*cf.* THORNBER *et al.*[2]), and since the proportion of the organisms' Chl associated with the Chl-P is so high, then the Chl-P complex must function as a light-absorbing antennae for system I, and in the transfer of absorbed energy to the reaction center.

The Bchl-P contains just sufficient polar amino acid residues to fully occupy the outer surface of the molecule[19,20], and thus the complex is soluble in water. The Chl-Ps have a much more apolar nature (Table IV), and hence, since they are of approximately the same molecular size as Bchl-P (Table III), the Chl-P molecules will have many apolar residues on their surface; thus the Chl-Ps will be insoluble in water. Ionic detergents make the complexes water-soluble by protecting these apolar areas from the solvent. *In vivo* the Chl-Ps are most probably attached to the lamellar membrane by hydrophobic interactions of these nonpolar regions with the hydrophobic portions of the lamellar lipids, and/or by interaction with other protein molecules (*cf.* JI AND BENSON[22]); the lack of such possible interactions for the Bchl-P probably explains why this component can be readily extracted from the bacterial vesicles by alkaline solutions without any detergent.

The nature of the binding of Chl within the complex has been discussed elsewhere[4,15]. It was suggested that the Chl:Ile:Pro:Gly (1:4:3:7) ratio may indicate how the Chls are held within the Bchl-P and the Chl-*a*-P of higher plants (*i.e.*, 4 isoleucines constitute a suitable hydrophobic region for the binding of the phytyl chain). This stoichiometric ratio also occurs in the algal complex.

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