Biochimica et Biophysica Acta, 722 (1983) 310–319
Elsevier Biomedical Press

BBA 41245

ISOLATION AND CHARACTERIZATION OF A MAJOR CHLOROPHYLL a/c_2 LIGHT-HARVESTING PROTEIN FROM A *CHROOMONAS* SPECIES (CRYPTOPHYCEAE)

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(Received July 19th, 1982)
(Revised manuscript received October 22nd, 1982)

Key words: Chlorophyll-protein; Light-harvesting complex; Chlorophyll c_2; Fluorescence; (Cryptophyceae)

Three chlorophyll-protein complexes of a *Chroomonas* species (Cryptophyceae) have been separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis. The two bands at 100 and 42 kDa are Complex I (CP I) and Complex IV (CP IV), the ubiquitous chlorophyll *a*-proteins associated with Photosystems I and II, respectively. The third 55 kDa band, which had two peptide subunits (24 and 20 kDa), contained both chlorophyll *a* and chlorophyll c_2 in a molar ratio of 1.4 chlorophyll *a* to 1 chlorophyll c_2 (chlorophyll *a*/chlorophyll c_2 ratio in whole cells = 4). A chlorophyll a/c_2 fraction with similar spectral and electrophoretic properties was isolated by digitonin-sucrose density gradient centrifugation. This fraction had no photochemical activity and contained only a single carotenoid species with absorbance maxima in methanol at 424, 448 and 476 nm. Efficient energy transfer from chlorophyll c_2 to chlorophyll *a* occurred in the complex.

Introduction

In oxygen-evolving organisms the accessory photosynthetic pigments harvest light energy and transfer it to the photochemical reaction centres of PS I and PS II. These accessory pigments include Chl *b* and *c*, carotenoids and phycobiliproteins. Both the light-harvesting pigments and the reaction centre Chl *a* exist in distinct pigment-protein complexes [1].

Three main chlorophyll-protein complexes have been isolated from higher plants and green algae by SDS-polyacrylamide gel electrophoresis. Two of these appear ubiquitous to organisms containing Chl *a*. These are the 100 kDa chlorophyll-protein complex 1 (CP I), which contains P-700 and CP IV, a complex of approx. 45 kDa for which there is circumstantial evidence of the presence of the PS II reaction centre. The third complex which has been isolated is the light-harvesting Chl *a*/*b*-protein complex (LHCP). It is photochemically inactive, transfers light energy to PS I and PS II and accounts for 45% of the total Chl *a* and most, if not all of the Chl *b* [2].

The light-harvesting systems of other organisms, especially those of the brown algae, diatoms, chrysophytes and dinoflagellates which contain Chl *c*, have received much less attention. Barrett and Anderson [3,4] have used Triton X-100 sucrose density gradient centrifugation to isolate several different carotenoid-Chl *a*/*c*-protein complexes from brown algae. A complex of fucoxanthin, Chl *a*, c_1 and c_2 with some photochemical activity has

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Abbreviations: Chl, chlorophyll; CP, chlorophyll-protein complex; LHCP, light-harvesting chlorophyll-protein complex; PS, photosystem; P-700, PS I reaction centre; Tricine, *N*-tris(hydroxymethyl)methylglycine; DCMU, 3-(3,4 dichlorophenyl)-1,1-dimethylurea; TEMED, *N,N,N',N'*-tetraethylmethylenediamine.

also been described from brown algae [5]. Boczar et al. [6] have described a Chl *c*-enriched band separated on Deriphat-polyacrylamide gel electrophoresis from dinoflagellate thylakoid membranes solubilized in SDS. SDS-polyacrylamide gel electrophoresis has been used by Alberte et al. [7] to obtain Chl *a*/*c*-protein complexes in diatoms and brown algae. There is, however, as yet insufficient data available to know if the light-harvesting complexes containing Chl *c* are as uniform as those which contain Chl *b*.

The light-harvesting system of the cryptomonads is unusual in that it has phycobiliproteins located in the intrathylakoid space [8] as well as accessory Chl c_2. This study reports the isolation and partial characterization of a new major Chl a/c_2-protein complex in a phycoerythrin-containing *Chroomonas* species (Cryptophyceae) as an initial approach to the investigation of energy transfer between phycoerythrin, Chl c_2 and Chl *a* at the molecular level.

Methods and Materials

Algal strain and culture methods. Cells of an unidentified *Chroomonas* species (CSIRO Division of Fisheries No. CS 24; Cronulla Australia 2230) [9] were grown in axenic culture at 18°C in medium 'Fe' as described by Guillard and Ryther [10]. Cultures were illuminated continuously with fluorescent white light (Mazda universal white) at an intensity of 20 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Harvesting of cells and isolation of thylakoids. Cells in the log phase of growth were harvested by centrifugation ($3000 \times g$ for 10 min) and resuspended in 40 ml of 0.5 M KH_2PO_4, 0.3 M potassium citrate (pH 7.2). The resuspended cells were broken by a single passage through a French pressure cell at 12000 lb/inch^2. The resultant brown homogenate was centrifuged at $27000 \times g$ for 30 min giving a green pellet containing the thylakoids and cell debris, and a pink supernatant containing phycoerythrin. The supernatant was stored at −22°C and the thylakoids were either used immediately or stored in 40% sucrose buffer solution at 77 K.

Preparation and solubilization of thylakoid membranes for digitonin-sucrose density gradient centrifugation. Thylakoid membranes were washed twice in 15 ml of 20 mM Tricine, 10 mM KCl (pH 7.5). Resuspended thylakoids were centrifuged first at $3000 \times g$ for 5 min to remove whole cells and debris, and then at $27000 \times g$ for 1 h at 4°C. The washed thylakoid membranes were resuspended in the Tricine-KCl buffer at a concentration of 350 μg Chl/ml and solubilized in 2% digitonin. Incubation was carried out, with stirring at 4°C, for 4 h. The digitonin-solubilized thylakoid membranes were centrifuged at $27000 \times g$ for 30 min and the brown pellet discarded.

The clear green supernatant from the digitonin solubilization was loaded onto a 10–40% (w/v) linear sucrose gradient containing 20 mM Tricine, 10 mM KCl (pH 7.5) and 0.1% (w/v) digitonin. The gradient was centrifuged at $238000 \times g$ for 16 h in a Beckman SW-41 rotor at 4°C, and analyzed at 670 and 638 nm, for Chl *a* and Chl *a* plus Chl c_2, respectively, by displacement with 60% sucrose through a 3 mm path length flow cell in a Gilford 2400 spectrophotometer. The peaks from the gradient scans were cut out and weighed for determination of the relative Chl *a* distribution.

Preparation and solubilization of thylakoid membranes for SDS-polyacrylamide gel electrophoresis. Thylakoid membranes were washed twice in 15 ml of 0.1 M Tris-acetate (pH 9.2). Resuspended thylakoids were centrifuged first at $1500 \times g$ for 5 min, to remove whole cells and debris, and then at $27000 \times g$ for 1 h at 4°C. The washed membranes were resuspended in the Tris-acetate buffer at a concentration of 250 μg Chl/ml and solubilized in SDS at an SDS/Chl ratio (w/w) of 12–30. In each preparation the minimum SDS concentration required to give complete chlorophyll solubilization was used. The SDS-solubilized thylakoids were centrifuged first at $27000 \times g$ for 15 min and then at $100000 \times g$ for 5 min in a Beckman Airfuge at 4°C. The supernatant was used immediately for polyacrylamide gel electrophoresis if separation of chlorophyll-protein complexes was desired. For separation of polypeptides an SDS/Chl ratio (w/w) of 30 was used, and samples were boiled for 2 min.

Polyacrylamide gel electrophoresis. Two systems were used: tube gels were used to separate and isolate chlorophyll-protein complexes and gradient slab gels based on the procedure of Chua and

Bennoun [11] were used to separate thylakoid membrane polypeptides.

Tube gels (0.6 × 9 cm) contained 6% (w/v) acrylamide, 0.21% (w/v) *N,N'*-methylenebisacrylamide, 0.04% (w/v) TEMED, 0.01% (w/v) EDTA and 0.083% (w/v) ammonium persulphate in 0.1 M Tris-acetate (pH 9.2). Both upper and lower reservoir buffers were 0.1 M Tris-acetate (pH 9.2) and the upper reservoir buffer contained 0.1% (w/v) SDS. Electrophoresis was performed at 4°C at a constant current of 2 mA/tube until the samples (5–30 μl; 1.25–7.5 μg Chl) entered the gel and then at 6 mA/tube until the desired band separation was achieved (20–30 min). Gels were scanned at 670 and 638 nm for Chl *a* and Chl *a* plus c_2, respectively, in a Gilford 2400 spectrophotometer with a linear-transport gel scanner. The relative distribution of Chl *a* in the various bands was estimated as previously described [12]. Gels were fixed with methanol/acetic acid/H_2O (5 : 1 : 5, v/v), stained for protein with 0.25% (w/v) Coomassie brilliant blue R in fixative, and excess dye was removed with 7% (v/v) acetic acid.

Molecular weights were obtained by co-electrophoresis of the following proteins: phosphorylase *a* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and lactalbumin (14.4 kDa).

Determination of pigments. Concentrations of Chl *a* and Chl c_2 were calculated from the absorbance, at 663 and 630 nm, of chloroplast extracts in acetone/H_2O (4 : 1, v/v), using the equations of Jeffrey and Humphrey [13]. Chlorophyll and carotenoids were analyzed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Samples were adjusted to methanol/H_2O (7 : 3, v/v) for application to a Sep-paK (Waters associates, reverse phase C_{18} - BondapaK) and the pigments were successively eluted using the method of Eskins and Dutton [14]. Chl c_2 was eluted with methanol/H_2O (4 : 1, v/v), carotenoids with methanol/H_2O (9 : 1, v/v) and Chl *a* with methanol. These fractions were then evaporated under nitrogen and reconstituted in a small volume of methanol. Samples (10 μl) were applied to an HPLC column and eluted with methanol, to determine the purity of the pigments which were identified by their absorbance spectra. These fragments were again evaporated under nitrogen, reconstituted with 100 μl methanol and applied to a Silica gel HR plate (Merck) with acetone/benzene/H_2O (93 : 30 : 8, v/v) as the developing solvent [15].

P-700 concentration in fractions was assayed, using a millimolar extinction coefficient of 64 [16], from the light-induced absorbance change at 700 nm (730 nm reference wavelength) measured on an Aminco dual-wavelength spectrophotometer. Samples contained 1 mM sodium ascorbate and 1 mM DCMU. Using the same sample, molar ratios of Chl to P-700 were measured as an estimate of photosynthetic unit size [17,18].

Absorption and fluorescence (emission and excitation) spectra. Absorption spectra were recorded at room temperature using a Pye Unicam SP8-200 spectrophotometer. Fluorescence emission and excitation spectra were recorded on samples either at room temperature diluted in buffer, or at 77 K in glycerol (2 glycerol : 1 sample, v/v) on a Perkin-Elmer MPF-44B fluorescence spectrophotometer in the ratio mode but not further corrected. All fluorescence measurements were made at less than 0.05*A* at the red absorbance maximum of Chl *a*.

Results

Chroomonas CS24 cells grew readily in culture. Alterations in light intensity or nutrient composition led to a marked change in pigment ratios as described by Lichtlé [19,20] for *Chroomonas rufescens*.

The typical absorption spectrum of *Chroomonas* CS24 whole cells is shown in Fig. 1. The peak at 676 nm represents mainly Chl *a*, the peak at 636 nm Chl c_2 and some satellite bands of Chl *a*, and the peak at 544 nm and shoulder at 564 nm represent phycoerythrin. The peak at 436 nm and shoulder at 454 nm are the Soret bands of Chl *a* and Chl c_2, respectively, and the other shoulders at 416 and 494 nm represent the carotenoids. In contrast, the absorption spectrum of washed thylakoids shows no peaks at 544 and 564 nm.

SDS-polyacrylamide gel electrophoresis of SDS-solubilized thylakoids, on 6% acrylamide tube gels, yielded four main zones containing chloro-

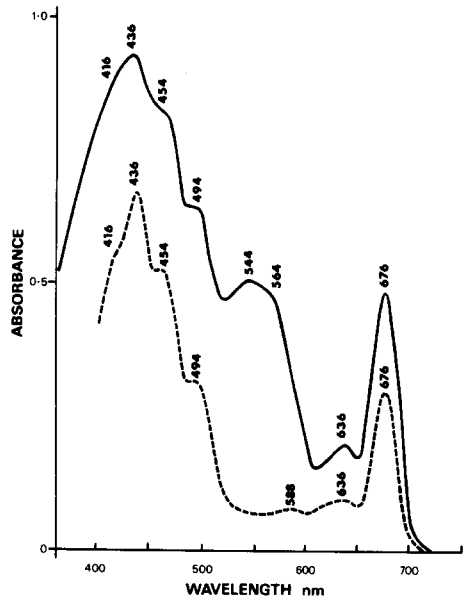

Fig. 1. Absorbance spectrum at 25°C of *Chroomonas* CS24. (———) Whole cells in medium 'Fe'. (— — —) Washed thylakoid membranes in 0.1 M Tris-acetate, pH 9.2.

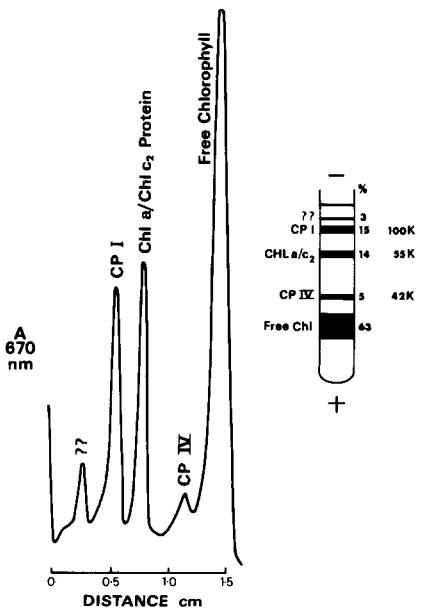

Fig. 2. A densitometer tracing at 670 nm of the chlorophyll-protein complexes separated by SDS-polyacrylamide gel electrophoresis. A schematic representation of the gel is also shown together with the relative distribution of Chl *a* among the complexes and their apparent molecular mass. K, kDa.

phyll, three of which were chlorophyll-protein complexes. Fig. 2 shows densitometer scans of a gel at 670 nm (Chl *a*) and a schematic representation of the gel column. The slowest migrating band was blue-green with an apparent molecular mass of 100 kDa. It contained 15% of the total Chl *a* on the gel together with a small amount of Chl c_2. It comigrated with CP I from SDS-polyacrylamide gel electrophoresis of higher plant thylakoids, contained P-700 and had an absorbance maximum of 676 nm (results not shown). Fluorescence emission spectra at 77 K of this band showed the presence of Chl c_2. When the complex was excited with light of 435 nm the predominant emission was at 682 nm with a minor shoulder at 720 nm. However, when the excitation wavelength was 460 nm the principle emission was 640 nm due to Chl c_2 with a secondary peak at 682 nm (Fig. 3C). Absence of a predominant emission from this complex at 730 nm when Chl *a* is excited might be ascribed to its denaturation on SDS polyacrylamide gel electrophoresis but whole cells of *Chroomonas* CS24, when energy transfer from all pigments to Chl *a* remains (Fig. 3A and B), also lack a major fluorescence emission at 730 nm at 77 K.

The second band is a Chl a/c_2-protein complex which contained 14% of the total Chl *a* together with most of the Chl c_2 not present as free chlorophyll. This complex had an apparent molecular mass of 55 kDa. When heated it lost its chlorophyll and dissociated into two peptides of 20 and 24 kDa (Fig. 4).

The third band did not often retain its chlorophyll under our electrophoretic conditions but stained for protein and had an apparent molecular mass of 42 kDa. It is probably the chlorophyl-protein (CP IV) associated with PS II of higher plants [1], but it was a more yellow-green colour indicating the additional presence of Chl c_2 or a carotenoid.

Studies on the Chl a / c_2-protein complex

The absorption spectrum (Fig. 5A) shows maxima at 672 nm (Chl *a*), 640 and 586 nm (principally Chl c_2), and in the Soret region bands at 458 and 436 nm for Chl c_2 and Chl *a*, respectively. The fluorescence emission spectra (Fig. 6A) show that when the Chl c_2 Soret band was excited at 460 nm, an emission band at 682 nm, due to Chl *a*, was

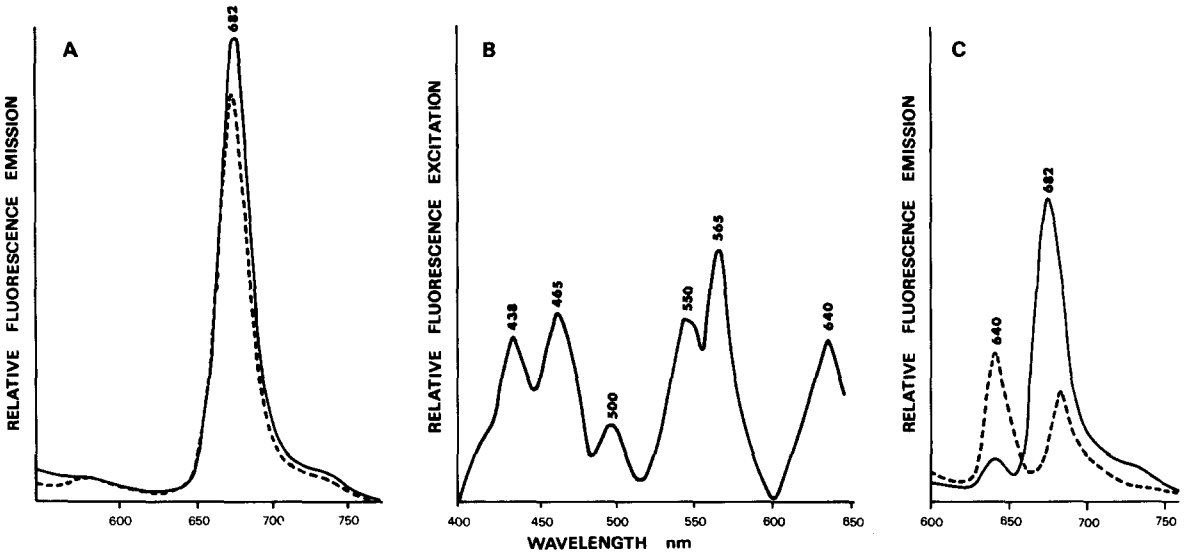

Fig. 3. Fluorescence spectra at 77 K of whole cells and CP I of *Chroomonas* CS24. (A) Fluorescence emission spectra of *Chroomonas* CS24 cells. (B) Fluorescence excitation spectra of *Chroomonas* CS24 cells. (C) Fluorescence emission of CP I recorded from an SDS-polyacrylamide gel electrophoresis slice. (———) Excitation with 435 nm light, (— — —) excitation with 460 nm light.

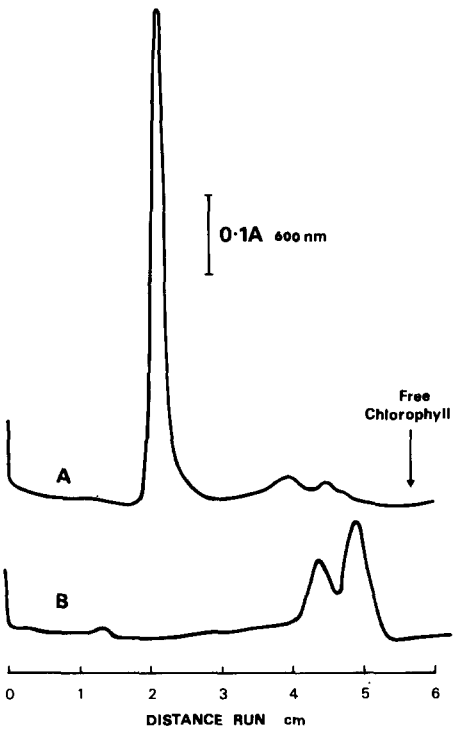

Fig. 4. Densitometer tracing at 600 nm of the Chl a/c_2-protein complex rerun on an 8% SDS gel being cut from an original 6% SDS gel. The gels were stained with Coomassie blue. (A) Unboiled. (B) Boiled.

observed. A small peak at 640 nm indicates that transfer of energy from Chl c_2 to Chl *a* was less than 100% efficient in the isolated complex. The excitation spectrum for the 682 nm emission (Fig. 6B) shows the Soret bands of Chl *a* (435 nm) and Chl c_2 (460 nm) and contributions from satellite Chl *a*, and Chl c_2 (590 and 640 nm) in the red region. A shoulder at 500 nm and a small peak at 550 nm may be contributions from energy absorbed by carotenoids.

Variation in thylakoid solubilization techniques and SDS-polyacrylamide gel electrophoresis conditions did not lead to a reduction in the large percentage of free chlorophyll generated. For this reason further characterization of the complex has been carried out on fractions separated by digitonin-sucrose density gradient centrifugation based on the methods of Newman and Sherman [21]. Digitonin-solubilized thylakoids were separated, on a 10–40% linear sucrose gradient, into five distinct fractions: a pale-yellow fraction in the 10% sucrose layer (lipids) and a pink fraction in the 15% sucrose layer (phycoerythrin) neither of which contained Chl *a* or Chl c_2. A yellow-green fraction (zone 1) immediately below the pink layer and a dark-green fraction (zone 2) were contiguous

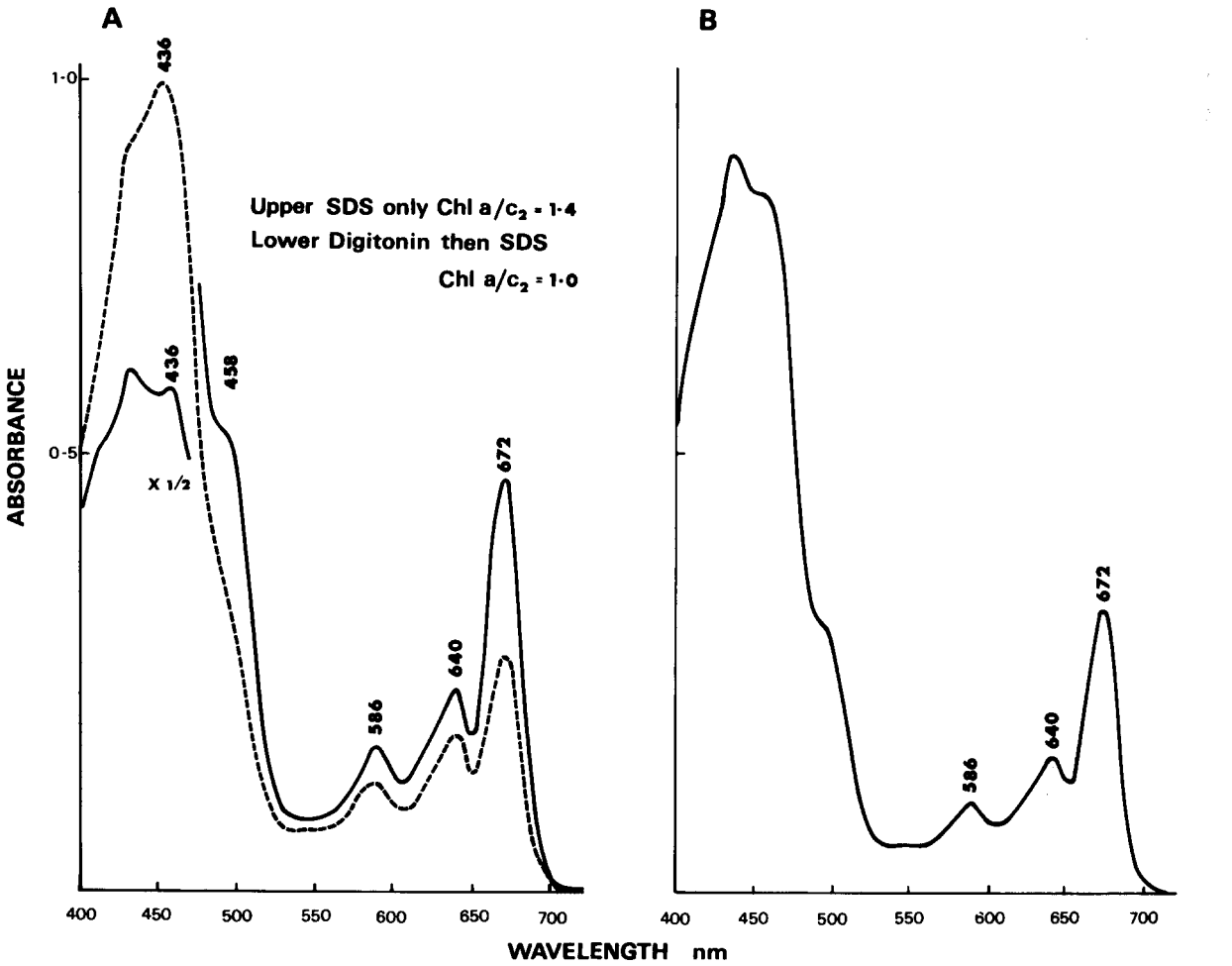

Fig. 5. Absorbance spectra of Chl a/c_2-protein complexes. (A) Absorbance spectra of the Chl a/c_2-protein complexes recorded in a gel slice at room temperature after separation by SDS-polyacrylamide gel electrophoresis: (———) SDS solubilization then SDS-polyacrylamide gel electrophoresis. (- - - - -) Digitonin solubilization (zone 2) then SDS-polyacrylamide gel electrophoresis. (B) Absorbance spectrum of Chl a/c_2-protein complex (zone 2) from digitonin-sucrose gradient.

(Fig. 7A). Near the bottom of the gradient a bright-green fraction (zone 3) was surrounded by chlorophyll pigment. When a zone 2 fraction, diluted to 10% sucrose, was re-run on an identical gradient, a single diffuse band was obtained, corresponding to zone 2 of the original gradient (results not shown). A densitometer scan for Chl *a* at 670 nm (Fig. 7B) showed a major peak (zone 2) with a shoulder (zone 1) and a minor peak (zone 3). Zone 1 contained 19% of the total Chl *a*, showed no photochemical P-700 activity and migrated on 6% acrylamide gels as a yellow-green band in the position of free chlorophyll and carotenoid (Fig. 7C). When the gel was stained for protein it contained a principle peptide with an apparent molecular mass of 45 kDa, suggesting that this fraction contained PS II particles from which the chlorophyll and carotenoid pigments had been removed by subsequent exposure to SDS.

Zone 3 contained 22% of the total Chl *a*, showed P-700 activity but migrated on 6% acrylamide gels (Fig. 7C) at a higher molecular mass (approx. 140 kDa) and was more yellow-green in colour than CP I separated on SDS-polyacrylamide gel electrophoresis after SDS solubilization.

Zone 2 contained 57% of total Chl *a*, showed

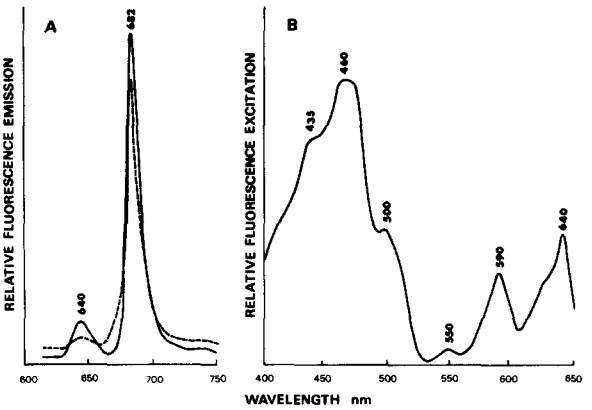

Fig. 6. Fluorescence emission and excitation spectra of *Chroomonas* CS24 Chl a/c_2-protein complex recorded in gel slice at 77 K. (A) Fluorescence emission spectra. (- - - - - -) excited with 435 nm light. (———) Excited with 460 nm light. (B) Fluorescence excitation spectrum for the 682 nm emission.

no P-700 activity and migrated on 6% acrylamide gels in the same position as the Chl a/c_2-protein complex and free pigment of SDS-solubilized thylakoids (Fig. 7C). Its Chl a/Chl c_2 ratio was 1.54 compared with a ratio of 4 for whole cells and thylakoid membranes. A densitometer scan at 638 nm of a similar gradient showed that most of the

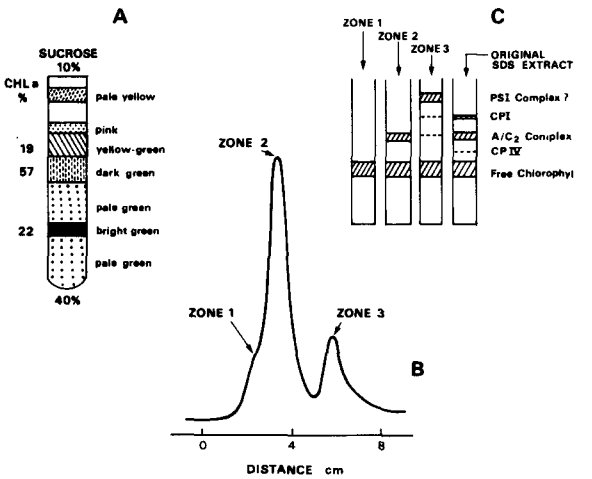

Fig. 7. Separation of chlorophyll-protein complexes by digitonin-sucrose density gradient centrifugation. (A) Schematic representation of the digitonin sucrose density gradient. (B) Absorbance of the gradient recorded at 670 nm. (C) Diagram of the SDS-polyacrylamide gel electrophoresis analysis of the zones from the gradient. Only bands containing chlorophyll are recorded.

Chl c_2 was in zone 2 (results not shown).

The molecular mass of the Chl a/c_2-protein complex obtained from zone 2 differed depending on the electrophoretic conditions used: 55 kDa on 6% acrylamide tube gels, 74 kDa on 15–20% gradient slab gels run at room temperature and 80 kDa on 15–20% gradient slab gels run at 4°C. The 20 and 24 kDa peptides of the Chl a/c_2-protein obtained after boiling with SDS were not resolved further by the gradient slab gel technique.

TLC of the separated pigments of zone 2 fractions showed that this Chl a/c_2-protein complex contains one yellow carotenoid, a xanthophyll with absorbance maxima in methanol at 424, 448 and 476 nm which is similar to the absorption spectrum [22] of diadinoxanthin (results not shown).

The Chl a/c_2-protein complex isolated on digitonin-sucrose density gradients had the same absorbance and fluorescence properties as the Chl a/c_2-protein complex separated from SDS-solubilized thylakoids electrophoresed on 6% acrylamide gels. However, when the digitonin-sucrose gradient fraction (Zone 2) was re-run on SDS-polyacryla-

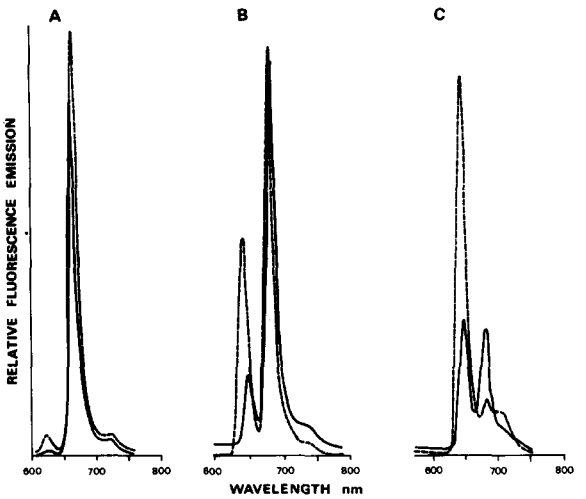

Fig. 8. Fluorescence emission spectra of the Chl a/c_2-protein fraction (zone 2) of the digitonin-sucrose density gradient, in glycerol at 77 K, under three different conditions. (A) Fluorescence emission spectra of untreated fractions, containing only digitonin, which have been stored at −22°C. (———) Excited with 435 nm light. (- - - - - -) Excited with 460 nm light. (B) Fluorescence emission spectra of the zone 2 fraction with SDS added (final concentration = 10% w/v). (C) Fluorescence emission spectra of the zone 2 fraction as treated in B but boiled for 2 min.

mide gel electrophoresis, the Chl a/Chl c_2 ratio decreased from 1.4 to 1.0 (Fig. 5A) and there was also some loss of carotenoid absorbing at 490 nm.

Energy transfer from Chl c_2 to Chl a in the Chl a/c_2-protein complex from zone 2 of the digitonin sucrose gradient was partially uncoupled after addition of SDS (Fig. 8B) and completely uncoupled after heating (Fig. 8C). However, digitonin at the concentration reached in the digitonin-sucrose gradient fractions had no effect on energy transfer from Chl c_2 to Chl a even after samples had been stored for some time (Fig. 8A).

Discussion

This is the first time that an attempt has been made to separate the chlorophyll-protein complexes of a cryptomonad. We encountered the same problems as have other workers, when fractionating brown algae [3–5,7], diatoms [7] and dinoflagellates [6], in that the detergent/chlorophyll ratios used successfully in higher plants resulted in incomplete solubilization of thylakoid membranes [1]. Ratios as high as 30 SDS/1 chlorophyll (w/w), were required for solubilization of *Chroomonas* CS24 thylakoid membranes, but this ratio generated a high percentage of free chlorophyll (approx. 60%). This free chlorophyll could originate from the bands already defined or from other bands not stable in this SDS-polyacrylamide gel electrophoresis system. From Chl a/Chl c_2 ratios of 1.4 for the Chl a/c_2-protein complex isolated by SDS-polyacrylamide gel electrophoresis compared to that of 4 for the whole chloroplasts, it is estimated that the Chl a/c_2-protein complex contains approx. 40% of the total Chl a.

Digitonin solubilization removed less chlorophyll from the protein complexes and the percentage of total Chl a attributable to each chlorophyll-protein complex was congruent with the hypothetical percentages estimated for the polyacrylamide gel bands. The 57% of total Chl a calculated to be attached to the Chl a/c_2-protein complex in the zone 2 fraction is probably an overestimation as there was some unresolved chlorophyll on each side of zone 2. The P-700-Chl a-protein complex was more resistant to solubilization, by either SDS or digitonin, than the Chl a/c_2-protein complex. A similar phenomenon has been reported for *Ecklonia radiata* [4] and is the reverse of what happens in higher plants [23].

The chlorophyll-protein present in zone 3 of the digitonin-sucrose gradient contained P-700 and had similar spectra properties to CP I but migrated on SDS-polyacrylamide gel electrophoresis at a lower rate. This may reflect retention of some low molecular mass proteins associated with Chl c_2. In higher plants, CP Ia, resolved under mild SDS-polyacrylamide gel electrophoresis [24], contains both Chl b (Anderson, J.M., perconal communication) and low molecular mass unpigmented polypeptides [25].

The Chl a/c_2-protein band resolved on SDS-polyacrylamide gel electrophoresis and that isolated on the digitonin-sucrose gradient had similar spectral and electrophoretic properties but different Chl a/Chl c_2 ratios (1.4 and 1.54, respectively). When the zone 2 fraction was electrophoresed on SDS-polyacrylamide gel electrophoresis (Fig. 5) an even lower ratio of 1.0 was obtained. A possible explanation is suggested by the result from re-electrophoresis of the Chl a/c_2-protein gel band, which after heating yields two low molecular mass peptides of 20 and 24 kDa. These peptides may represent separate Chl a- and c_2-binding proteins with very different structures.

The Chl a/c_2-protein complex had no photochemical activity and had fluorescence emission spectra which overlap with the absorption of the reaction centre complexes of PS I and PS II. It also showed efficient light-energy transfer from Chl c_2 to Chl a. For these reasons this Chl a/c_2-protein complex, containing at least 40% of the total Chl a, most of the total Chl c_2 and a xanthophyll (in roughly equal molar amount to Chl c_2), can be designated a light-harvesting complex similar to LHCP of higher plants. It is suggested that this Chl a/c_2-protein complex is the major light-harvesting 'A_0' thylakoid component proposed by Lichtlé et al. [26] for the cryptomonad *Cryptomonas rufescens*. There is, however, a marked difference between the fluorescence emission spectrum at 77 K of the *Chroomonas* CS24 (dominant emission peak 682 nm) and that of *Cr. rufescens* with its dominant emission peak at 730 nm and a shoulder at 690 nm, attributed to PS I and PS II, respectively [26]. This has made it impossible to demonstrate differential transfer of

TABLE I

PROPERTIES OF CHLOROPHYLL *a*/*c*-PROTEIN COMPLEXES ISOLATED FROM CRYPTOMONADS, DINOFLAGELLATES, DIATOMS AND BROWN ALGAE

Molecular mass of complex is the relative molecular mass of complex + chlorophyll on SDS-polyacrylamide gel electrophoresis. Molecular mass of peptide is the relative molecular mass of peptide(s) without chlorophyll on SDS-polyacrylamide gel electrophoresis.

Group	Species	Reference	Complex molecular mass (kDa)	Type of Chl *c*	Associated carotenoid	Chl *a*/*c* ratio	Peptides molecular mass (kDa)	Absorbance maxima (nm)	Dominant fluorescence emission peak (nm)
Cryptophyceae	*Chroomonas* sp.	This work	55	Chl c_2	Diadinoxanthin?	1.54	24 20	672, 640, 588 488, 456, 436	682
Dinoflagellates	*Glenodium* sp.	[6]	50	Chl c_2	Dinoxanthin + diadinoxanthin	0.21	20	672, 636, 585 485, 453, 432	–
Brown algae Diatoms	Several species	[7]	40	–	–	0.5	–	–	680
Brown algae	*Acrocarpia paniculata*	[3]	–	(1) Chl c_2 (2) Chl $c_1 + c_2$	Fucoxanthin Violaxanthin	2 0.25	20	671, 634, 460, 438 670, 630, 460, 438	680
Brown algae	*Phaedactylum tricornatum*	[5]	–	Chl $c_1 + c_2$	Fucoxanthin	2	–	–	–

energy by accessory pigments to the two photosystems in the cryptomonad under study here.

The absorption spectrum and apparent molecular mass of the dinoflagellate Chl a/c_2-protein complex isolated by Boczar et al. [6] most closely resemble those of this *Chroomonas* CS24 (Table I). However, the Chl a/Chl c_2 ratio of 0.25 for the *Glenodinium* species complex is very different from the Chl a/Chl c_2 ratio of 1.54 for the *Chroomonas* CS24 complex. This is clearly not an effect of isolation techniques as the *Glenodinium* species whole cell ratio (0.71 Chl a/Chl c_2) is also different from the *Chroomonas* CS24 whole cell ratio (4 Chl a/1 Chl c_2). A Chl a/c_2-protein complex isolated from the brown alga *Acrocarpia paniculata* [3] has a Chl a/Chl c_2 ratio of 2 which is similar to the ratio of the *Chroomonas* CS24 complex, but the associated carotenoid is fucoxanthin in the former organism.

Although Chl c is as common to cryptomonads, diatoms, dinoflagellates, brown algae and chrysophytes as Chl b is to higher plants and green algae, it can be observed from Table I that the structure and pigment composition of protein complexes containing Chl c are diverse. Chl c_1 is not always present and a variety of carotenoids is associated with these light-harvesting complexes. There may be some subunit homology between organisms containing Chl c but the characterization of their Chl c-enriched light-harvesting complexes is as yet too incomplete to draw conclusions as to their evolutionary relationships.

Acknowledgements

We thank Dr. S.W. Jeffrey for the original culture of *Chroomonas* CS24 and advice on its growth. The work was supported by the Australian Research Grants Scheme (D27915708).

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