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Chlorophyll-protein complexes of a marine green alga, *Codium* species (Siphonales)

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The main pigment-protein complexes of a *Codium* sp. (Siphonales), a marine green alga, have been isolated and characterized and shown to retain their in vivo spectral characteristics. The isolation procedure involves fragmentation of *Codium* thylakoids with Triton X-100 and sucrose gradient centrifugation. A PS I complex with a Chl *a*/Chl *b* ratio of 2.34 and a Chl/P-700 ratio of 156 contains also siphonaxanthin. A reaction centre PS II complex with some associated Chl *a*/*b*-proteins has a Chl-*a*/Chl-*b* ratio of 2.68 and no P-700; it has the characteristic fluorescence emission bands of PS II at 685 and 695 nm at 77 K. The novel light-harvesting Chl *a*/*b*-protein complex of PS II has a Chl *a*/Chl *b* ratio of approx. 0.7 and no P-700; it also contains siphonaxanthin and siphonein and 35.5, 34, 29.1, 28.5 and 27 kDa polypeptides. Analyses of the fluorescence properties of *Codium* light-harvesting protein complex demonstrates that chlorophyll b, siphonaxanthin and siphonein are integral components of these proteins. It is proposed that the role of these siphonaxanthin-siphonein-Chl *a*/*b*-proteins is to extend the light-harvesting capacity of PS II into the blue-green and green regions of the spectrum, where chlorophyll *a* absorbance is minimal and transmission of light in the turbid marine habitat is maximal.

Introduction

Marine benthic green algae have a much greater content of chlorophyll *b* compared to higher plants or freshwater algae [1–4]. Moreover, members of the Siphonales have an unusual carotenoid content. Generally, they contain more α-carotene than β-carotene which is the almost universally distributed carotene amongst all other algae [5]; indeed *Codium fragile* has only α- and ϵ-carotene, but no β-carotene [6]. The siphonaceous algae also have siphonaxanthin, and its esterified form, siphonein instead of or as well as lutein, the main xanthophyll of higher plants and most green algae [7]. In vivo, the presence of siphonaxanthin or siphonein or both in marine green algae allows enhanced absorption in the blue-green to green region of the visible spectrum (500–550 nm) [8]. Increased amounts of these xanthophylls in siphonaceous algae correlate with their location in deeper waters or shaded environments [9–11]. The light available in oceans and coastal waters not only rapidly diminishes with increasing depth of water, but also is spectrally altered with the attenuation of far-red, red, yellow and then blue light [12,13]. Hence, the siphonaceous algae with siphonaxanthin, siphonein and enhanced amounts of chlorophyll *b* relative to

Abbreviations: Chl, chlorophyll; LHC, light-harvesting protein complex; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; PS, Photosystem; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

chlorophyll *a* are adapted for deeper water or more shaded habitats than other orders of the Chlorophyta.

The chlorophyll-protein complexes of higher plants and green algae have been isolated by either detergent and sucrose density gradient centrifugation or mild SDS polyacrylamide gel electrophoresis [14]. The reaction centre Chl *a*-proteins of PS II (47 and 41 kDa apoproteins) and PS I (68 kDa apoprotein), appear to be ubiquitous to all photosynthetic organisms that contain chlorophyll *a* [14]. In addition, there are the light-harvesting chlorophyll *a*/*b*-proteins of PS II and PS I, termed LHC II and LHC I, respectively. In higher plants and most green algae, these are lutein-Chl *a*/*b*-proteins with LHC II having a Chl *a*/Chl *b* ratio of approx. 1.2 and LHC I having a higher ratio of approx. 3.5. Recently, the chlorophyll-proteins of siphonaceous algae have been resolved by mild SDS polyacrylamide gel electrophoresis [8,15]. The light-harvesting role of siphonaxanthin has been established, and most of the chlorophyll *b*, siphonaxanthin and siphonein are located in novel siphonaxanthin-Chl *a*/*b*-proteins of the main light-harvesting complex of PS II which has a Chl *a*/Chl *b* ratio of approx. 0.7 [8]. However, the PS I complexes also contain chlorophyll *b* and siphonaxanthin [8]. Apart from mild polyacrylamide gel electrophoresis, no other methods for isolation of the pigment-protein complexes of siphonaceous algae have been reported, except for a preliminary report on Triton X-100 fractionation [16].

This study reports on the isolation and characterization of the chlorophyll-protein complexes of PS II and PS I and the main Chl *a*/*b*-proteins associated with PS II (LHC II) from *Codium* thylakoids with unaltered characteristics from their in vivo state. There are significant differences between the *Codium* LHC II and the LHC II_s of higher plants and most green algae.

Methods

Intact *Codium* plants were collected at low tide (0–1 m below ocean surface) from Guerrilla Bay or Cronulla, New South Wales. They were transported to Canberra in chilled, aerated seawater, which was changed at intervals until the chloroplasts were isolated from *Codium* thalli. Very well-washed thylakoid membranes were then isolated from chloroplasts as previously described [8] and either used immediately or stored for short periods in liquid N_2.

Triton X-100 fragmentation of Codium thylakoids

A single-step Triton X-100 fractionation method was modified from the procedure of Mullett et al. [17] previously used for the isolation of a 'native' PS I complex from pea thylakoids. The well-washed *Codium* thylakoids were resuspended in 50 mM Sorbitol/0.75 mM EDTA (pH 7.8) and pelleted by centrifugation at $10000 \times g$ for 20 min at least twice. The pellet was resuspended in glass-distilled water to which 20% Triton X-100 was added so that the final Chl concentration was 0.8 mg Chl/ml and the Triton X-100 concentration was 0.7%. The pH of the incubation mixture was checked and adjusted to pH 7.8 with $(CH_3)_4NOH$ if necessary. The mixture was stirred gently at 4°C for 30 min, and centrifuged at $40000 \times g$ for 30 min. Aliquots (0.8–1.0 ml) of the Triton X-100 supernatant (approx. 86% total Chl) were immediately loaded onto SW 41 tubes containing a linear gradient of 0.1–0.8 M sucrose with 0.02 or 0.04% Triton X-100, placed over a 2 M sucrose cushion (1.5–2.0 ml). The gradients were centrifuged in a Beckman SW 41 rotor at $150000 \times g$ for 16–20 h.

Following sucrose density gradient centrifugation, zones 2 and 3 were diluted with 2 to 3 volumes of 10 mM Tricine (pH 7.8) and centrifuged in a Ti 70 Rotor at $360000 \times g$ for 1.5 h to remove the residual Triton X-100. The pellets were resuspended in 50 mM Tricine (pH 7.8) at 1 mg Chl/ml. The upper deep red fluorescent band was collected and 1 M $MgCl_2$ added to give 10 mM $MgCl_2$; the solution was stirred gently at 25°C for 15 min and loaded above an equal volume of 50 mM Tricine (pH 7.8), 0.5 M sucrose in Corex tubes and centrifuged at $10000 \times g$ for 10 min [18]. The pellets were resuspended in 50 mM Tricine (pH 7.8) at high chlorophyll concentrations. For comparison, the spinach chlorophyll-protein complexes were also isolated by the same procedure used for *Codium* thylakoids.

Spectral measurements

Chlorophyll concentrations and Chl *a*/Chl *b*

ratios were determined in 80% acetone [19], protein concentrations were determined by the Lowry method [20], and P-700 concentrations were determined by the chemical method as in [8]. Absorption spectra were recorded on an Hitachi-Perkin Elmer 557 spectrophotometer linked to a computer. Fluorescence spectra at 77 K were recorded with a Perkin Elmer MPF-44B fluorescence spectrophotometer incorporating automatic correction. The fractions in 20 mM Tricine buffer (pH 7.8) contained 70% glycerol and were all less than 0.1 absorbance at their red absorption maxima.

Gel electrophoresis

SDS discontinuous polyacrylamide gel electrophoresis on gradient slab gels (20 × 20 × 1.5 mm) followed the method of O'Farrell [21]. The separating gel (170 mm) contained 0.375 M Tris-HCl (pH 8.8), 0.1% SDS with an acrylamide gradient of 10–16%, 0–50% glycerol, 0.02–0.03% ammonium persulphate and 0.04–0.05% TEMED. The stacking gel (25 mm) had 0.125 M Tris-HCl (pH 6.8), 0.1% SDS, 3.4% acrylamide, 0.03% ammonium persulphate, 0.1% TEMED. The acrylamide to *N,N'*-methylenebisacrylamide weight ratio was 30 : 0.8. Gels were run at 4°C in 0.625 M Tris, 0.192 M glycine, 0.1% SDS either overnight at 10 mA or for 4 h at 40 mA per gel.

Samples were incubated in 65 mM Tris-HCl (pH 6.8), 2% SDS, 15% glycerol, 5% dithiothreitol for 15 min at 25°C, and the protein standards (Pharmacia, low molecular weight calibration kit) were boiled for 2 min in the above medium. After electrophoresis, the gels were photographed to record green bands, then soaked for 30 min in ethanol : acetic acid : water (25 : 7 : 68, v/v/v), stained with Coomassie brilliant blue R-250 (0.25% w/v in the same mixture) for 3 h and destained in the same mixture with gentle shaking.

Results

The Chl *a/b*-proteins of the main light-harvesting complex associated with PS II (LHC II) (zone 1) were separated from PS I complex (zone 3) and a PS II reaction-centre complex with some LHC II still attached (zone 2) bu sucrose density gradient centrifugation, following fragmentation of *Codium* thylakoids with Triton X-100. When the Triton X-100 incubation and sucrose density gradient centrifugation are performed in the absence of cations, LHC-II is non-aggregated and located at the top of the gradients, whereas the undissociated PS I complex is located further down the gradient with both higher plant [17,18] and *Codium* thylakoids. In the presence of cations, the respective positions of these pigment-protein complexes is reversed as *Codium* LHC-II avidly aggregates in 150 mM NaCl or 5–10 mM $MgCl_2$, while the *Codium* PS I complex is non-aggregated (data not shown). In contrast to Ref. 16 where only two zones corresponding to LHC-II and PS I complex were resolved, three distinct zones were resolved from *Codium* thylakoids.

Several factors are essential for the success of this isolation procedure. First, it is vital to have very well washed thylakoids which have been freed of mucilage liberated by the initial homogenization of *Codium* thalli. Second, it is necessary to use large quantities of the 50 mM sorbitol, 7.5 mM EDTA (pH 7.8), to ensure the removal of cations from the outer surface of *Codium* thylakoids. Third, the pH of the mixture of *Codium* thylakoids and Triton X-100 must be checked and increased to pH 7.8 if required. Fourth, the amount of Triton X-100 required in the sucrose density gradients varied with different thylakoid preparations, so an initial trial run using either 0.02 or 0.04% Triton X-100 in the gradients was tested prior to large scale density gradient centrifugation to ensure that the best separation of the three zones was obtained.

TABLE I

CHLOROPHYLL COMPOSITION OF THE *CODIUM* PIGMENT-PROTEIN COMPLEXES RESOLVED BY TRITON X-100 FRAGMENTATION AND SUCROSE GRADIENT CENTRIFUGATION

Fraction	Chlorophyll distribution (%)	Chl *a*/Chl *b*	Chl/P-700
Thylakoids	100	1.45	551
Zone 1	59	0.76	0
Zone 2	14	2.68	0
Zone 3	27	2.34	156

Pigment composition of the chlorophyll-protein complexes of Codium

Codium thylakoids have a Chl *a*/Chl *b* ratio of 1.45. They have more chlorophyll *b* relative to chlorophyll *a* than any higher plants studied so far, even shade plants [14]. The three major zones separated by sucrose density gradient centrifugation of Triton X-100-fragmented thylakoids were characterised with respect to intact *Codium* thylakoids (Table I). Zone 1 had a very low Chl *a*/Chl *b* ratio of 0.7, while the Chl *a*/Chl *b* ratios of zones 2 and 3 were greater than those of the unfractionated thylakoids (Table I). Only zone 3 contained P-700 as expected for a PS I complex. The Chl/P-700 ratios of 156 for *Codium* PS I complex and 551 for *Codium* thylakoids were higher than values reported for spinach thylakoids.

Chlorophyll-protein complexes

The chlorophyll-protein complexes present in each of the zones were compared with those of *Codium* thylakoids by mildy denaturating SDS polyacrylamide gel electrophoresis [8]. Prior to electrophoresis, only *Codium* thylakoids were solubilized with SDS (Chl/SDS wt. ratio of 15), as it was unnecessary to add any extra detergent to the Triton X-100 sucrose gradient bands. *Codium* thylakoids are resolved into eight bands (Fig. 1A) which have been previously characterised [8]. CP1 is the P-700-Chl *a*-protein of the PS I reaction centre complex and $CP1a^1$ and $CP1a^2$ are PS I complexes [8]. CPa is the reaction-centre complex of PS II, while $LHCP^1$ and $LHCP^3$ are siphonaxanthin-siphonein-Chl *a*/*b*-proteins of the main light-harvesting antenna of PS II (LHC II) [8]. Since the Mg^{2+}-aggregated zone 1 from the sucrose gradients contained only $LHCP^1$ and $LHCP^3$ (Fig. 1B), it clearly represents the supramolecular com-

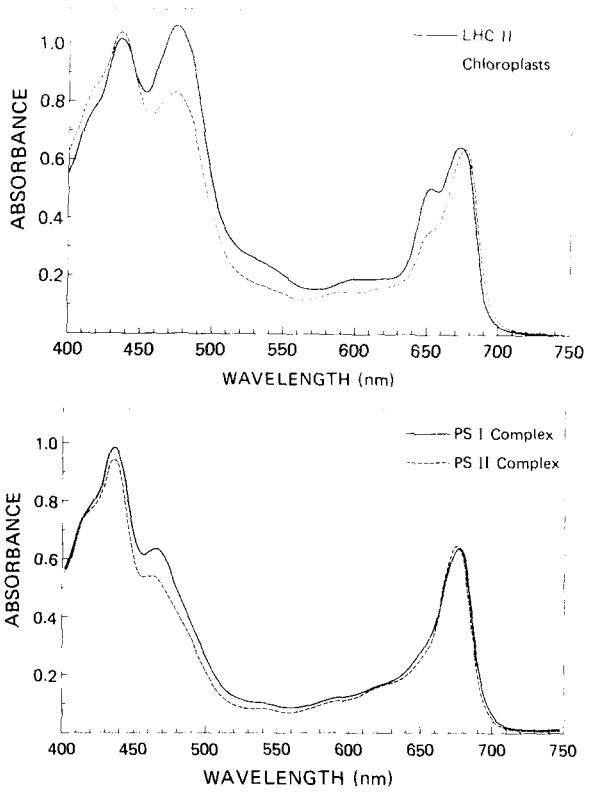

Fig. 2. Absorption spectra at 25°C of (a) *Codium* thylakoids and LHC II and (b) PS I and PS II complexes.

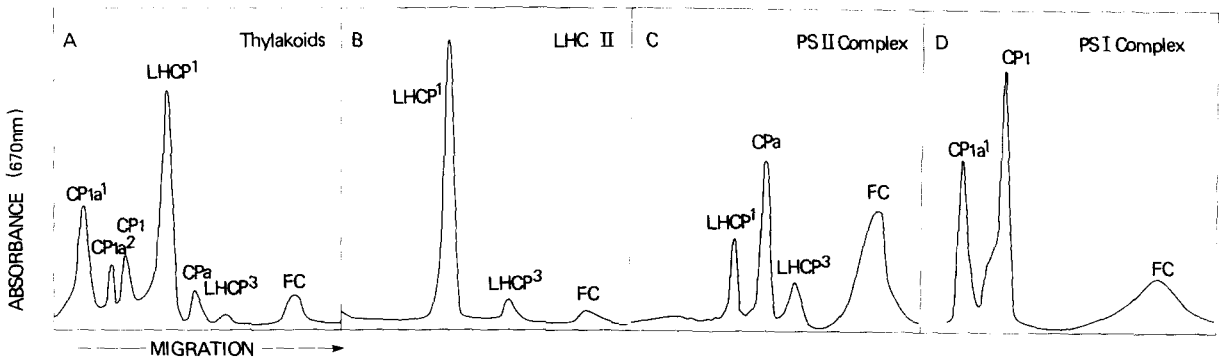

Fig. 1. Resolution of the isolated chlorophyll-protein complexes of *Codium* by mild SDS polyacrylamide gel electrophoresis according to Ref. 8. Densitometer tracings at 672 nm of (A) *Codium* thylakoids; (B) LHC II; (C) PS II reaction centre complex with some LHC II; (D) PS I complex.

plex LHC II. Zone 2 contained mainly CPa and some LHC-II (Fig. 1C); it is a PS II reaction-centre complex with some LHC II still attached. Zone 3 contained only CP1a[1], CP1a[2] and CP1; it corresponds to an undissociated PS I complex (Fig. 1D). This identification of the three main chlorophyll-protein complexes resolved by Triton X-100 sucrose density gradients is confirmed by their spectral properties and polypeptide composition described below.

Absorption and fluorescence spectra of Codium chlorophyll-protein complexes

The absorption spectrum of unfractionated *Codium* thylakoids is compared with those of the isolated chlorophyll-protein complexes in Fig. 2a and b. The enhanced absorption in the 500–540 nm region of *Codium* thylakoids is due to siphonaxanthin and siphonein [8]. The absorption spectrum of LHC II (Fig. 2a) shows maxima at 672 nm (Chl *a*), 650 nm (Chl *b*), 540 and 508 nm (siphonaxanthin and siphonein), 470 nm (Chl *b*) and 438 nm (Chl *a*). This spectrum of *Codium* LHC-II is very similar to those of *Codium* LHCP[1] and LHCP[3] previously resolved by mild SDS polyacrylamide gel electrophoresis [8]. Comparison of the LHC II spectrum with those of PS I and PS II complexes (Fig. 2a and b) clearly shows that most of the chlorophyll *b* and the main xanthophylls, siphonaxanthin and siphonein are located in LHC II.

The PS II complex has main absorption peaks at 675 and 438 nm. The presence of some LHC II still associated with the reaction centre Chl *a*-proteins of PS II can be seen by the minor shoulders at 652, 550–500 and 470 nm (Fig. 2b). The PS I complex has a red absorption maximum at 679 nm (Fig. 2b) and its absorption spectrum is similar to those of CP1a[1] and CP1a[2], the PS I complexes resolved by mild SDS polyacrylamide gel electrophoresis [8]. The *Codium* PS I complex clearly has some chlorophyll *b* and siphonaxanthin present (Fig. 2b), as was the case for CP1a[1] and CP1a[2] [8]. Recently, PS I complex isolated from higher plants has been shown to include a specific Chl *a/b* protein antenna complex [22–24]. As reported in the following paper [25] we have also been able to isolate a specific siphonaxanthin-Chl a/b-protein complex of PS I using the Triton X-100-PS I

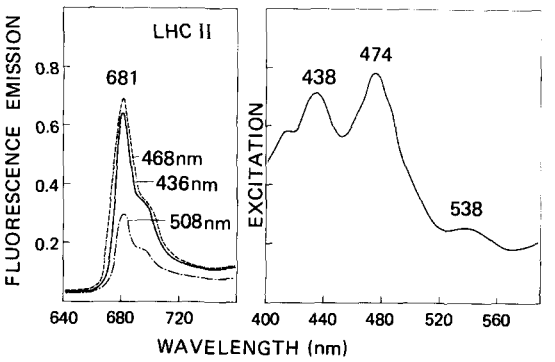

Fig. 3. Fluorescence emission and excitation spectra at 77 K of the main Chl *a/b*-proteins of PS II (LHC II). The excitation wavelength for emission was 438, 468 or 508 nm, and the emission wavelenght for excitation was at 681 nm.

complex as the starting material.

The fluorescence emission spectrum at 77 K of *Codium* thylakoids had maxima at 685, 696 and 710–718 nm [8]; the 685 and 696 nm bands are associated with PS II, and the far-red band with PS I [26]. In most higher plants and green algae this far-red band occurs at 735 nm; however, in some green algae and in chlorophyll c_2-containing algae it occurs at lower wavelenghts of 710–720 nm [7]. As shown in Fig. 3, *Codium* LHC II has a fluorescence emission maximum at 681 nm whether excitation is at 438 nm (Chl *a*), 468 nm (Chl *b*) or 508 nm (siphonaxanthin) with a narrow half bandwidth. The fluorescence emission spectrum of LHC-II is identical to those of *Codium* LHCP[1] and LHCP[3] [8], and resembles those of LHC-II isolated from higher plant thylakoids. There is a dramatic difference, however, in the excitation spectrum of *C*odium LHC II (Fig. 3) compared to that of spinach LHC II, with enhanced absorption at 470 nm (Chl *b*) and 500–550 nm (siphonaxanthin and siphonein). The similarity of the excitation (Fig. 3) and absorption (Fig. 2a) spectra of *Codium* LHC II indicates that the accessory pigments of this chlorophyll-protein complex are in a highly ordered arrangement that permits efficient transfer of light excitation energy from siphonaxanthin, siphonein and chlorophyll *b* to chlorophyll *a*. There is no chlorophyll *b* fluorescence at 660 nm. These points taken together demonstrate that siphonaxanthin and siphonein are integral components of the *Codium* LHC II.

The fluorescence emission spectrum at 77 K of

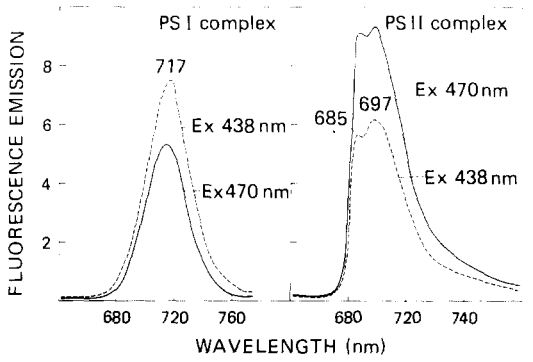

Fig. 4. Fluorescence emission spectra at 77 K of the PS II complex (zone 2) and PS I complex (zone 3) with an excitation wavelength of 438 or 470 nm.

Codium PS II complex has maxima at 697 and 685 nm (Fig. 4), the two characteristic bands of PS II [26]. The *Codium* PS I complex has a very different spectrum (Fig. 4) with an emission at 717 nm when excited either at 438 nm (Chl *a*), 470 nm (Chl *b*) or 540 nm (siphonaxanthin) (not shown) demonstrating that chlorophyll *b* is an integral component of this PS I complex.

Polypeptide composition of Codium chlorophyll-protein complexes

The polypeptide composition of the chlorophyll-protein complexes isolated from *Codium* and spinach thylakoids are compared in Fig. 5. Since the SDS polyacrylamide electrophoresis gel was run at 4°C, many of the upper bands retain chlorophyll. *Codium* PS I complex has a green band at an apparent molecular weight of 103 kDa which is resolved at about the same position as the P-700-Chl *a*-protein of spinach PS I. Both bands, if cut out and dissociated with solubilization buffer and reelectrophoresed, yield a single polypeptide of 67 kDa (not shown). Hence, the reaction centre polypeptides of *Codium* and spinach PS I complexes are probably similar. In addition, *Codium* PS I complex has two unkown proteins at 50.5 and

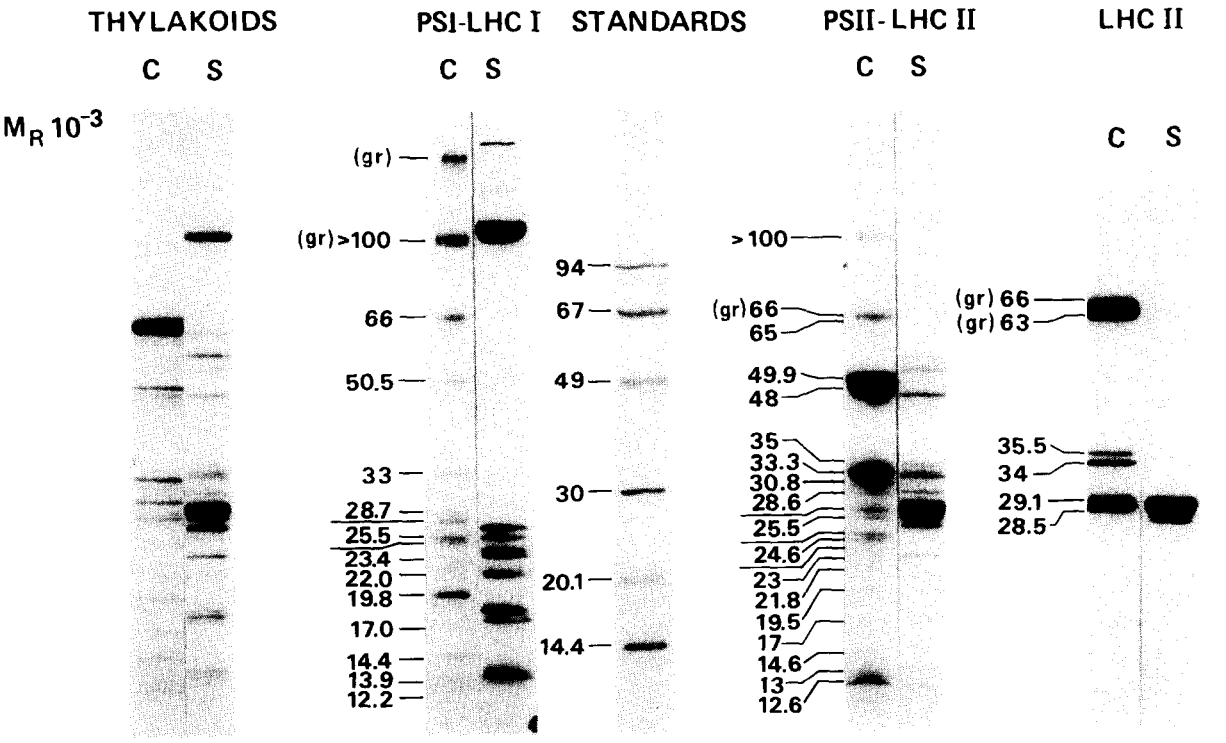

Fig. 5. Polypeptide composition of the chlorophyll-protein complexes of *Codium* (C) or spinach (S) separated by SDS polyacrylamide gel electrophoresis at 4°C. Protein standards are phosphorylase *a* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.5 kDa).

33 kDa which are not present in spinach PS I complex, and a number of low molecular polypeptides which are resolved at rather different positions to those in spinach PS I complex. The *Codium* proteins in the 26–20 kDa range belong to the specific Chl *a/b*-protein complex of PS I [24] as is found for the 25–20 kDa peptides in spinach LHC-I [22–24].

The *Codium* PS II complex has several green bands: those at 66 and 63 kDa are due to aggregated LHC II, and those at 49.9 and 48 kDa are the green Chl *a*-proteins of PS II which have not been further characterised. The 35, 34, 30.8 and 28.6 bands belong to LHC II, although the 33.3 kDa band may also include the extrinsic O_2-evolving peptide and the 32 kDa herbicide-binding protein . *Codium* LHC II was mainly present as green bands at apparent molecular weights of 66000 and 63000. The striking aspect of the *Codium* LHC II proteins, however, is the presence of two 'extra bands' at 35.5 and 34 kDa. These 35.5 and 34 kDa bands have never been observed in the light-harvesting complexes II of higher plants or most green algae; they may be a characteristic for siphonaxanthin-Chl *a/b*-proteins. Further characterization will be needed to determine which of these proteins bind pigments.

Discussion

This paper reports the first isolation of the major pigment-protein complexes of a siphonaceous alga, *Codium*, by means of Triton X-100 fragmention and sucrose gradient centrifugation. This isolation procedure offers certain advantages over the mild SDS polyacrylamide gel electrophoresis procedures used previously for *Codium* [8,15], because less pigment is likely to be removed from the proteins, and larger scale isolation is feasible. Triton X-100 has been a useful detergent with higher plants and green algae, since, in the absence of cations, LHC II can be separated from an undissociated PS I complex [17,18]. This simple one-step method was also successful with *Codium* thylakoids; indeed, it has an additional bonus in that the PS II reaction centre complex with some associated LHC II is also resolvable. Strangely, the useful Triton X-100 method of Berthold et al. [27] which allows the isolation of an undissociated PS II with LHC II from the grana stacks of higher plant thylakoids did not work with *Codium* thylakoids (Anderson, J.M., unpublished results).

The main core Chl *a*-proteins of *Codium* sp. appear to be identical with those of other algal groups. *Codium* PS I complex has an apoprotein of 66 kDa and PS II complex has polypeptides in 50–40 kDa range characteristic of the two Chl *a*-proteins of higher plants and green algae, viz., the P-680-Chl *a*-protein that contains a 47–51 kDa protein, and the additional Chl *a*-protein that contains a 39–44 kDa protein [28,29]. The fluorescence emission of PS II at 695 nm is associated with the P-680-Chl *a*-protein and that at 685 nm with the lower molecular mass Chl-*a*-protein [28,29]. The fluorescence emission of isolated *Codium* PS II complex (Fig. 4) contains both 685 and 695 nm emission, and is similar to that of the spinach PS II complex [14]. Interestingly, some of the Chl *a/b*-proteins of LHC II remain tightly associated with the *Codium* PS II complex. This *Codium* PS II complex must have a greater stability in Triton X-100 than is the case with higher plants, since a zone corresponding to spinach or pea PS II complex has not been isolated in the sucrose gradients [17,18]. *Codium* PS I complex has a roughly comparable polypeptide composition to that of spinach PS I complex. As shown in the following paper [25] PS I complex contains a specific siphonaxanthin-Chl *a/b*-protein complex (20–25 kDa apoproteins) as a peripheral light-harvesting antenna for PS I. The fluorescence emission of *Codium* PS I complex is at 717 nm and is rather similar to that of unfractionated thylakoids [8]. As is the case with some other Chlorophyta and the Chromophyta, *Codium* does not have the characteristic 735 nm fluorescence emission observed for most green algae and higher plants [7]; perhaps the 735 nm fluorescence is attributable only to the Chl *a/b*-proteins of higher plants [22], which are lutein-Chl *a/b*-proteins [14].

Codium LHC II is enriched in chlorophyll *b* and contains siphonaxanthin and siphonein instead of lutein as found in most green algal and higher plant LHC II. The similarity of the absorption and fluorescence excitation spectra of *Codium* LHC II (Figs. 2 and 3), together with an identical emission band at 681 nm when excited at 436 nm (Chl *a*), 470 nm (Chl *b*), or 508 or 540 nm (siphona-

xanthin), indicate a highly ordered molecular arrangement of these pigments in *Codium* LHC II. Further, the emission spectrum of *Codium* LHC II is very similar to that of spinach LHC II; this indicates that the accessory pigments of *Codium* LHC II are all transferring light-excitation energy to chlorophyll *a*, probably with 100 % efficiency. Sieffermann-Harms and Ninnemann [30] demonstrated that the plant xanthophylls, which comprised 43% of the total absorption in the blue-green region of lettuce LHC II, transferred their energy to chlorophyll a with 100% efficiency. These authors [30] also demonstrated that the pigments of lettuce LHC II were resistant to strong acid attack, and they suggested a highly ordered packing of pigments within hydrophobic crevices buried in the interior of their apoproteins. This may also be the case fo the siphonaxanthin-Chl *a*/*b*-proteins of *Codium* LHC II.

Despite the similarity of the fluorescence emission of *Codium* LHC II with that of higher plant LHC II, there are significant differences not only in their pigment composition, but also in their protein composition. Significantly, *Codium* LHC II has two 'extra' proteins of 35.5 and 34 kDa (Fig. 6) never found in higher plant or other Chlorophyta LHC II, as well as the polypeptides of apparently similar molecular weights to those of higher plant LHC II. Interestingly, the isolated Chl *a*/*b*-protein complex of a *Prochloron* sp., a prokaryotic organism, also has a polypeptide of 34 kDa, and none of the lower molecular weight proteins usually associated with higher plant or green algal LHC II [31]. It will be interesting to compare the primary amino acid sequence and secondary structural homology of the proteins of these different LHC II. The presence of both siphonaxanthin and siphonein in scaly monads [32,33], which are considered to be the nearest extant relatives of ancestral green algae [34], suggests these pigments, and hence the Chl *a*/*b*-proteins with which they are associated (as in *Codium* LHC II), evolved before the lutein-Chl *a*/*b*-proteins of the more recent green algae and higher plants. The evolutionary relationship between these light-harvesting proteins encoded by the nuclear genome [35–37] should be solved when their nucleotide sequences are determined.

Clearly that there is a marked structural heterogeneity in the proteins of *Codium* LHC II. This is likely to be even greater than the structural heterogeneity of the proteins of higher plant LHC II, in that *Codium* LHC II contains at least six proteins, two of these being distinctly different from those of higher plant LHC II. The structural heterogeneity of higher plant LHC II is evident because of the various green light-harvesting complexes II isolated by different electrophoretic and isoelectric focusing procedures [cf. Refs. 14 and 35]. Further, higher plant LHC II is encoded by multiple gene families [36,37]. This marked structural heterogeneity of *Codium* LHC II may also imply functional heterogeneity, since only part of the higher plant LHC II is reversibly phosphorylated, and this appears to regulate the distribution of excitation energy between the photosystems in response to changes in the spectral quality of light [35].

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