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Isolation and characterization of a siphonaxanthin-chlorophyll *a*/*b*-protein complex of Photosystem I from a *Codium* species (Siphonales)

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A novel siphonaxanthin-chlorophyll *a*/*b*-protein complex associated with Photosystem I (PS I), termed *Codium* light-harvesting complex of Photosystem I (LHC I), has been isolated from a *Codium* sp. (Siphonales). The isolation procedure involves fragmentation of a purified Triton X-100 PS I complex with a zwittergent-316/dodecyl-maltoside mixture followed by dodecyl-maltoside sucrose gradient centrifugation. LHC I has a Chl *a*/Chl *b* ratio of 1.7 and contains siphonaxanthin and no P-700. Fluorescence analyses demonstrates that Chl *b* (chlorophyll *b*) and siphonaxanthin are integral components of *Codium* LHC I. The polypeptide composition of *Codium* LHC I (24.5, 23, 22.5, 20 and 19 kDa) is distinctly different from that of the main light-harvesting chlorophyll *a*/*b*-protein complex of PS II (LHC II) which has proteins of 35.5, 34, 28 and 27 kDa. Compared to the LHC I of higher plants, *Codium* LHC I has more chlorophyll *b* and an unusual xanthophyll, siphonaxanthin. We conclude that siphonaceous algae have a specific light-harvesting antenna for PS I. The role of *Codium* LHC I will be to enhance the capacity for PS I to absorb blue-green and green light, the predominant light available in deep oceanic waters and shaded marine habitats.

Introduction

The high efficiency of solar energy capture by the many chlorophyll and carotenoid molecules and the subsequent transfer of this excitation energy to the photochemical reaction centres of PS II and PS I depends on their intricate organization in discrete pigment-protein complex [1]. Two of these are the reaction centre Chl *a*-protein complexes of PS II and PS I; they appear to be universally distributed throughout the plant kingdom. In higher plants and green algae the accessory pigments, chlorophyll *b* and xanthophylls, are located together with chlorophyll *a* in additional light-harvesting complexes. It was generally thought that chlorophyll *b* was in a common pool of light-harvesting complex connected to both photosystems, though more to PS II than PS I [2]. Now it has been demonstrated that PS I has specific Chl *a*/*b*-proteins which are distinct from the Chl *a*/*b*-proteins associated with the main light-harvesting complex of PS II, LHC II [3–6]. Recently, Haworth et al. [6] used charged detergents and sucrose density gradient centrifugation to isolate the peripheral light-harvesting complex associated with PS I (LHC I) from pea thylakoids. LHC I (Chl *a*/Chl *b* ratio of approx. 3.6) contains three or four polypeptides of 19–25 kDa [6]. Lam et al. [7,8] have shown that chlorophyll *b* is associated with at least two of these polypeptides.

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Abbreviations: Chl, chlorophyll; LHC, light-harvesting complex; PS, Photosystem.

Codium sp. (Siphonales) has more chlorophyll *b* than found in either higher plants or most green algae [9]; they have siphonaxanthin and siphonein as their major xanthophylls, instead of lutein [9]. *Codium* LHC II has been isolated by mild SDS polyacrylamide gel electrophoresis [9] or Triton X-100 fractionation [10] and shown to be a siphonaxanthin-siphonein-Chl *a*/*b*-protein complex (Chl *a*/Chl *b* ratio of approx. 0.7) [9,10]. The *Codium* Triton X-100 PS I complex or the PS I complexes resolved by mild SDS polyacrylamide gel electrophoresis also contain chlorophyll *b* and siphonaxanthin, suggesting that specific Chl *a*/*b*-proteins are also associated with PS I in *Codium* thylakoids [9,10]. Our aim has been to isolate and characterize the siphonaxanthin-Chl *a*/*b*-protein complex of PS I from *Codium* thylakoids. We conclude that siphonaceous algae have a specific peripheral light-harvesting assembly for PS I as is true also for higher plants [5–7] and *Chlamydomonas* [3,4].

Methods

A *Codium* sp. was collected from Cronulla, New South Wales and transported to Canberra in chilled, aerated sea water changed at frequent intervals.

Isolation of Codium thylakoids and Triton X-100 PS I complex

Chloroplasts were isolated from *Codium* thalli as described previously [9], and the thylakoid membranes were then washed several times with 50 mM sorbitol, 7.5 mM EDTA (pH 7.8) until the supernatant had no opaque yellow colour. The Triton X-100 PS I complex was then isolated by sucrose density gradient centrifugation of Triton X-100-treated *Codium* thylakoids [10]. *Codium* PS I complex (the bottom zone in the sucrose gradients) was immediately diluted with 40 mM Tricine buffer (pH 7.8) and centrifuged at $360\,000 \times g$ for 2 h to remove residual Triton X-100. The purity of the *Codium* PS I complex was tested by its Chl *a*/Chl *b* ratio and analysis of the chlorophyll-protein content by mild denaturing SDS polyacrylamide gel electrophoresis [9]. Pure *Codium* PS I complex has a Chl *a*/Chl *b* ratio of 2.2–2.4 and no LHCP[1] or LHCP[3] bands when *Codium* PS I complex is re-electrophoresed on mild SDS polyacrylamide gel electrophoresis [9]. It was important to check the purity of PS I complex which sometimes has trace amounts of LHC II; if present, LHC II would contaminate the zone of LHC I obtained in the isolation procedure outlined next.

Isolation of the Codium Chl a/b-proteins of PS I

A supramolecular Chl *a*/*b*-protein complex was isolated from *Codium* PS I complex by slight modifications of the method of Haworth et al. [6] for the isolation of LHC I from higher plant thylakoids. A detergent solution of zwittergent-316 (Sigma) and dodecyl-β-D-maltoside (Sigma) (2 mg zwittergent and 1.5 mg of dodecyl-β-D-maltoside per ml of 20 mM Tricine buffer (pH 7.8)) was added to an equal volume of PS I complex in 20 mM Tricine (pH 7.8) (0.5 mg Chl/ml) and the mixture stirred in the dark at 4°C for 1 h. The detergent-solubilized PS I complex extract was loaded onto linear sucrose gradients of 0.1–0.8 M sucrose containing 20 mM Tricine (pH 7.8) and 1% dodecyl-β-D-maltoside. The gradients were centrifuged at $238\,000 \times g$ for 16–20 h in a Beckman SW 41 rotor at 2°C. For comparative purposes, LHC I was also isolated from a PS I complex prepared from spinach thylakoids [6,11].

Chl concentrations and Chl *a*/Chl *b* ratios were determined in 80% acetone [12], protein concentrations were determined by the Lowry method [13] and P-700 concentrations were determined in 50 mM Tricine, pH 7.8 by (ferricyanide-oxidised) minus (ascorbate-reduced) difference spectra according to the method of Markwell et al. [14].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Mildly denaturing tube gels were used for the resolution of chlorophyll-protein complexes according to the procedures described in Ref. 9. Discontinuous gradient slab gels based on the procedure of Laemmli [15] were used to separate polypeptides of thylakoid membranes and isolated chlorophyll-protein complexes [10].

Absorption and fluorescence spectra

Absorption spectra were recorded at 25°C on a Hitachi-Perkin Elmer 557 spectrophotometer linked to a computer. Fluorescence emission and exci-

tation spectra were recorded at 77 K on gel slices incubated in 50 mM Tricine buffer (pH 7.8)/glycerol (3:1, v/v) or samples diluted in 50 mM Tricine buffer (pH 7.8) with 70% glycerol on a Perkin-Elmer MPF-44B fluorescence spectrophotometer with automatic correction.

Results

Our previous studies demonstrated that *Codium* PS I complex, isolated by mildly denaturing SDS polyacrylamide gel electrophoresis [9] or by Triton X-100 sucrose gradient centrifugation [10] contain chlorophyll *a*, chlorophyll *b* and siphonaxanthin. Recently, Haworth et al. [6] isolated a specific Chl *a*/*b*-protein complex of the peripheral light-harvesting antenna of PS I using a mixture of charged detergents, zwittergent-316 and dodecyl-β-D-maltoside to dissociate LHC I from PS I complex, followed by sucrose gradient centrifugation. We therefore applied this procedure [6] to purified *Codium* PS I complex in order to attempt to separate the Chl *a*/*b*-protein complex of PS I from the P-700-Chl *a*-protein complex. Following incubation of zwittergent-316 and dodecyl-β-D-maltoside with *Codium* PS I complex and centrifugation, three distinct fractions were resolved on the 0.1–0.8 M sucrose gradients.

The chlorophyll composition, P-700 content, chlorophyll-protein content and polypeptide composition of the three fractions were analysed, and the chlorophyll-protein complexes were characterized as outlined below. Zone 1 (the top of the tube) contained the specific Chl *a*/*b*-protein complex of PS I and zones 2 (middle) and 3 (bottom) contained partly dissociated PS I complexes depleted of some of the peripheral Chl *a*/*b*-proteins.

Analyses of pigments and chlorophyll-protein complexes

We have compared the pigment composition of the fractions from the sucrose density gradients in Table I. The Chl *a*/Chl *b*-ratio of *Codium* LHC I (zone 1) was approx. 1.7 ± 0.1 (7 expts.), a value lower than that of the isolated Triton X-100 PS I complex (Table I). LHC I had no P-700. Zones 2 and 3 had higher Chl *a*/Chl *b* ratios than that of unfractionated PS I complex suggesting they were depleted in Chl *a*/*b*-proteins. This was confirmed by the increased amounts of P-700 per unit chlorophyll in these fractions. *Codium* thylakoids have rather variable Chl/P-700 ratios as determined by chemical methods which range from 450–560 [9,15].

TABLE I

CHLOROPHYLL COMPOSITION OF *CODIUM* PIGMENT-PROTEIN COMPLEXES SEPARATED BY FRACTIONATION OF PS I COMPLEX WITH ZWITTERGENT-316:DODECYL-MALTOSIDE AND SUCROSE GRADIENT CENTRIFUGATION

Fraction	Chlorophyll distribution (%)	Chl *a*/Chl *b*	Chl/P-700
PS I complex	100	2.28	138
Zone 1	54	1.73	0
Zone 2	18	2.90	116
Zone 3	28	3.32	87

The fractions obtained from the sucrose density gradients were also analysed on mildly denaturing SDS polyacrylamide gel electrophoresis tube gels at 4°C [9]. Unfractionated PS I complex gave the previously characterized green bands known as CP1a[1], CP1a[2] and CP1 and some free chlorophyll. LHC I from zone 1 gave only free Chl showing that it has no CP1 or CP1a present. The fractions of zones 2 and 3 yielded less of the CP1a complexes of low mobility and relatively more CP1 (the P-700-Chl *a*-protein complex of PS I) than was resolved from the unfractionated PS I complex itself (results not shown). This latter result confirmed that part of the peripheral Chl *a*/*b*-proteins had been removed from PS I complex.

Polypeptide composition

The polypeptide composition of the chlorophyll-protein complexes is shown in Fig. 1. *Codium* PS I complex has a green band of apparent molecular mass of 103 kDa (which corresponds to undissociated PS I complex), a dominant polypeptide of 67 kDa which is the apoprotein of the P-700-Chl *a*-protein complex of PS I, and several proteins of 25–19 kDa and 18–8 kDa (Fig. 1, lane 1). Zone 1 (Fig. 1, lane 2) had only proteins of 25–19 kDa, while zones 2 and 3 (Fig. 1, lanes 3 and 4) were depleted in these polypeptides, and enriched in the

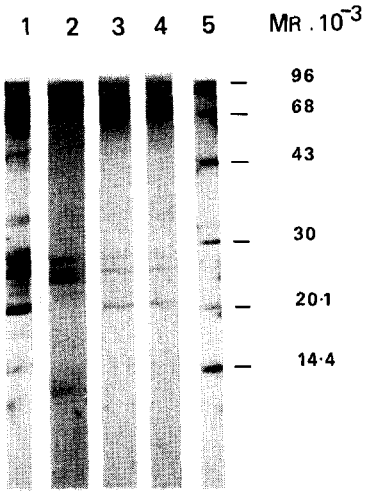

Fig. 1. SDS polyacrylamide gel electrophoresis of the chlorophyll-protein complexes of *Codium* thylakoids. Unfractionated PS I complex (lane 1); LHC I (lane 2); PS I complex, zone 2 (lane 3); PS I complex, zone 3 (lane 4) and the protein standards (lane 5) are phosphorylase *b* (94 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

67 kDa apoprotein of the β-carotene-P-700-Chl *a*-protein, and the lower molecular-weight range compared to PS I complex. This is consistent with the detergent dissociating part of the peripheral Chl *a*/*b*-proteins of LHC I from PS I complex.

Spectral analysis of the Codium chlorophyll-proteins

The absorption spectrum of purified *Codium* LHC I recorded at 25°C (Fig. 2) has a maximum at 671 nm with prominent bands at 650 and 470 nm due to chlorophyll *b*, and some absorption in the 500–550 nm region due to siphonaxanthin [9]. The partly dissociated PS I complexes (Zones 2 and 3) (Fig. 2) have higher red absorption maxima at 675 and 677 nm, respectively, and less chlorophyll *b* and siphonaxanthin than found in the unfractionated PS I complex. The absorption spectrum of the main Chl *a*/*b*-protein complex of PS II (LHC II) isolated as described in the preceding paper [9] is compared with that of LHC I in Fig. 3a. As is clearly seen in the difference spectrum (Fig. 3b), *Codium* LHC II has more chlorophyll *b*, siphonaxanthin and siphonein than *Codium* LHC I.

The fluorescence emission spectrum at 77 K of *Codium* LHC I has a maximum at 686 nm (Fig. 4) whether the excitation wavelength is at 438 nm (Chl *a*), 470 nm (Chl *b*) (not shown) or 540 nm (siphonaxanthin) (not shown). This demonstrates that both chlorophyll *b* and siphonaxanthin are integral components of *Codium* LHC I. Further, there is no separate fluorescence band at 650–660

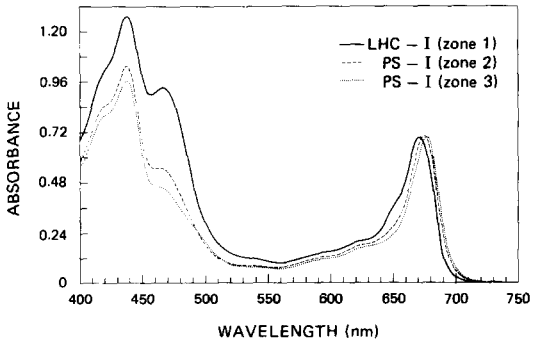

Fig. 2. Absorption spectra recorded at 25°C of LHC I (zone 1) and the partly dissociated PS I complexes (zones 2 and 3).

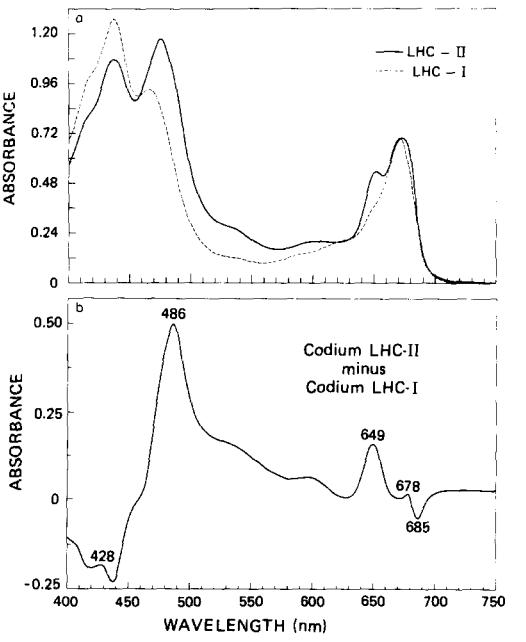

Fig. 3. Comparison of (a) the absorption spectra at 25°C of *Codium* LHC I and purified *Codium* LHC II isolated as in Ref. 9, and (b) a difference spectrum of *Codium* LHC II minus *Codium* LHC I.

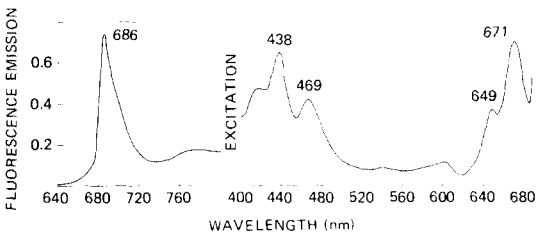

Fig. 4. Fluorescence emission and excitation spectra at 77 K of purified *Codium* LHC I. The excitation wavelength was 438 nm for emission, and the emission wavelength was 681 nm for excitation.

nm as would have been the case if the chlorophyll *b* present were not an integral part of *Codium* LHC I. The excitation spectrum at 77 K for emission at 686 nm of *Codium* LHC I (Fig. 4) is similar to its absorption spectrum and shows that a major form of Chl *a* at 671 nm is contributing to emission at 686 nm.

The fluorescence emission spectra of zones 2 and 3 have maxima further to the red with the PS I complex of zone 2 (Chl/P-700 ratio of 116) having a broad maximum at 708 nm and that of zone 3 (Chl/P-700 ratio of 86) at 715 nm (Fig. 5). Once again the excitation spectra (not shown) resembled the absorption spectra and showed decreased amounts of chlorophyll *b* and siphonaxanthin compared to the original PS I complex. These fluorescence emission spectra of the partly dissociated PS I complexes are somewhat similar to thos of *Codium* CP1a[1] and CP1a[2] resolved by mild SDS polyacrylamide gel electrophoresis [8].

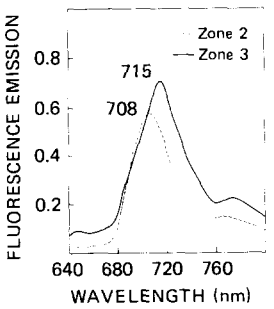

Fig. 5. The fluorescence emission spectra at 77 K of the *Codium* PS I complexes partly depleted in LHC I (zones 2 and 3). The excitation wavelength was 428 nm.

Comparison of absorption and fluorescence spectra of Codium LHC I with spinach LHC I

For comparative purposes, a purified spinach Triton X-100 PS I complex was isolated [11] and fractionated with zwittergent-316 : dodecyl-β-D-maltoside and sucrose density gradients to yield spinach LHC I: this had similar properties to pea LHC I [6]. A comparison of the absorption spectra of *Codium* LHC I and spinach LHC I which have been normalized to give equal absorbance at their red maximum (Fig. 6a) clearly shows the enhanced content of chlorophyll *b* (650 and 470 nm) in *Codium* LHC I. The enhanced absorption in the green region of the spectrum of *Codium* LHC I compared to spinach LHC I is also obvious in the difference spectrum (Fig. 6b). This is due to the predominant xanthophyll of *Codium* LHC I being siphonaxanthin while that of spinach LHC I is lutein. Spinach LHC I has a fluorescence emission spectrum at 77 K with a maximum at 735 nm, as observed previously for pea LHC I {6]. The emission maximum of *Codium* LHC I has never been observed at such high wavelengths (Fig. 4) and instead occurs at 686 nm.

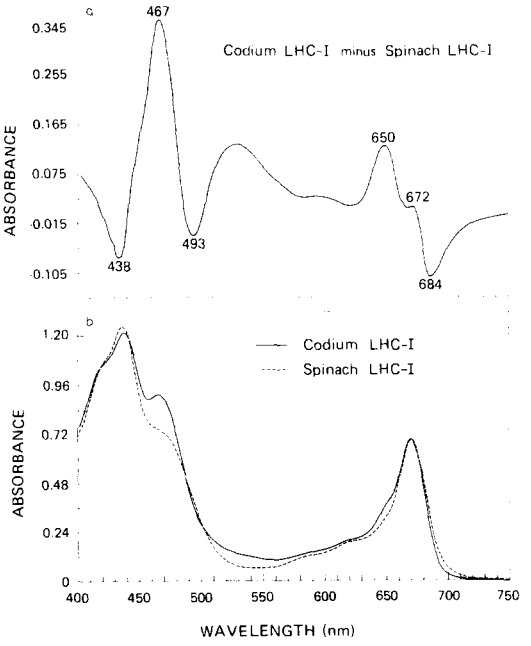

Fig. 6. Comparison of (a) the absorption spectrum of *Codium* LHC I and spinach LHC I at 25°C, and (b) a difference spectrum of *Codium* LHC I minus spinach LHC I.

Discussion

This is the first time that a specific Chl *a/b*-protein complex which serves as the peripheral light-harvesting assembly of PS I has been isolated from a siphonaceous alga. Our results clearly show that siphonaceous algae, like other orders of the Chlorophyta and higher plants, have chlorophyll *b* associated with PS I. Triton X-100 fragmentation of well-washed *Codium* thylakoids followed by sucrose density-gradient centrifugation in the absence of cations allows the separation of an undissociated PS I complex, a reaction centre PS II complex with small amounts of Chl *a/b*-protein of PS II, and LHC II [10]. Using the method introduced by Haworth et al. [6] to dissociate partly LHC I from a PS I complex isolated from pea thylakoids, we have separated and characterized a discrete Chl *a/b*-protein complex, LHC I from *Codium* PS I complex. This method is successful only if, in the initial Triton X-100 solubilization of *Codium* thylakoids, PS I complex has no traces of LHC II. The success of these two detergent solubilization procedures with *Codium* thylakoids indicates that the arrangement and distribution of surface charges of the supramolecular intrinsic thylakoid complexes at the outer thylakoid membrane surface must be rather similar in siphonaceous algae to that of other *Chlorophyta* and higher plant thylakoids.

Our studies demonstrate that *Codium* LHC I is distinctly different from the previously characterized *Codium* LHC II [9,10]. *Codium* LHC I has a Chl *a*/Chl *b* ratio of 1.7 and no P-700. It contains five polypeptides of molecular mass ranging from 25 to 19 kDa; this group of polypeptides is depleted in the partly dissociated PS I complexes isolated in the sucrose density gradients. In contrast, the *Codium* LHC II has polypeptides of 35.5–27 kDa [10].

The siphonaceous algae have marked absorption in the blue-green and green region of the visible spectrum due to enhanced amounts of chlorophyll *b*, siphonaxanthin and siphonein. These two xanthophylls when complexed to proteins in vivo have their absorption shifted some 100 nm to the red compared to their absorption in organic solvents [9]. *Codium* LHC I has less chlorophyll *b* and siphonaxanthin relative to chlorophyll *a* than *Codium* LHC II (Fig. 3). This difference is further increased in *Codium* thylakoids, since about 60% of the total chlorophyll is distributed in *Codium* LHC II, while only 10–15% of the total chlorophyll is estimated to occur in *Codium* LHC I. Nevertheless, it is clear that the PS I light-harvesting assembly of *Codium* is more capable of absorption of blue-green and green light than the corresponding lutein-Chl *a/b*-protein complex of PS I of higher plant and *Chlamydomonas* thylakoids (see Fig. 7 of Ref. 6).

The fluorescence emission spectrum of isolated *Codium* LHC I (686 nm) (Fig. 4) is very different from that of pea [6] or spinach LHC I (approx. 730–735 nm). It is not known whether this is due to either an alteration of the pigment orientations in *Codium* LHC I compared to its in vivo state, or alternatively, *Codium* thylakoids have a different arrangement of their Chl-proteins in PS I, since they do not have the long-wavelength fluorescence at 735 nm characteristic of higher plants and some green algae [16].

As suggested previously for higher plants [5], we propose that the role of *Codium* LHC I will be to enhance the light-harvesting capacity of PS I under light-limiting conditions and to extend the spectral range of light available for PS I to regions other than those where chlorophyll *a* has maximal absorbance. This is of great importance for marine algae where the light available is often greatly diminished and has altered spectral qualities. Although the *Codium* sp. studied here is an intertidal and not a deep water alga, some members of the Siphonales live in deeper oceanic waters or shaded coastal habitats [17,18]. Since the dominant light penetrating to deeper oceanic waters or turbid coastal habitats is in the blue-green or green region [see Ref. 16], the Siphonales are well-adapted to live in these environments. It is probably essential that the light-harvesting assembly of PS I, as well as that of PS II [8,9] of siphonaceous algae has siphonaxanthin and increased chlorophyll *b*, and hence enhanced ability to absorb blue-green light.

Finally, despite the differences in the pigment composition of *Codium* LHC I compared to spinach LHC I the apparent molecular masses of their polypeptides are rather similar. It will be interesting to see the extent of homology of the primary structures of these light-harvesting pro-

teins of PS I, as the siphonaceous algae are more ancient than most of the other Chlorophyta and terrestrial plants (cf. Ref. 9). A comparison of the deduced primary amino acid sequences and secondary structural homologies of the proteins of *Codium* LHC I with those of spinach LHC I will also be interesting to see if the arrangements of the different xanthophylls and extra chlorophyll *b* molecules in *Codium* LHC I can be deduced.

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