Biochimica et Biophysica Acta, 724 (1983) 370–380
Elsevier

BBA 41345

CHLOROPHYLL-PROTEIN COMPLEXES OF A *CODIUM* SPECIES, INCLUDING A LIGHT-HARVESTING SIPHONAXANTHIN-CHLOROPHYLL *a*/*b*-PROTEIN COMPLEX, AN EVOLUTIONARY RELIC OF SOME CHLOROPHYTA

JAN M. ANDERSON

CSIRO, Division of Plant Industry, P.O. Box 1600, Canberra, A.C.T. 2601 (Australia)

(Received March 23rd, 1983)

Key words: Chlorophyll-protein complex; Siphonaxanthin; Light-harvesting complex; Siphonein; (Codium sp., Chlorophyta)

Eight chlorophyll-protein complexes were isolated from thylakoid membranes of a *Codium* species, a marine green alga, by mild SDS-polyacrylamide gel electrophoresis. CP 1a^1, CP 1a^2, CP 1a^3 and CP 1a^4 were partially dissociated Photosystem (PS) I complexes, which in addition to the core reaction centre complex, CP 1, possessed PS I light-harvesting complexes containing chlorophyll (Chl) *a*, Chl *b* and siphonaxanthin. LHCP^1 and LHCP^3 are orange-brown green chlorophyll *a*/*b*-proteins (Chl *a*/Chl *b* ratios of 0.66) that contain siphonaxanthin and its esterified form, siphonein. CP a and CP 1, the core reaction centre complexes of PS II and PS I, respectively, had similar spectral properties to those isolated from other algae or higher plants. These P-680- or P-700-Chl *a*-proteins are universally distributed among algae and terrestrial plants; they appear to be highly conserved and have undergone little evolutionary adaptation. Siphonaxanthin and siphonein which are present in the *Codium* light-harvesting complexes of PS II and PS I are responsible for enhanced absorption in the green region (518 and 538 nm). Efficient energy transfer from both xanthophylls and Chl *b* to only Chl *a* in *Codium* light-harvesting complexes, which have identical fluorescence emission spectra at 77 K to those of the lutein-Chl *a*/*b*-proteins (Chl *a*/Chl *b* ratios of 1.2) of most green algae and all higher plants, proved that the molecular arrangement of these light-harvesting pigments was maintained in the isolated *Codium* complexes. The siphonaxanthin-Chl *a*/*b*-proteins allow enhanced absorption of blue-green and green light, the predominant light available in deep ocean waters or shaded subtidal marine habitats. Since there is a variable distribution of lutein, siphonaxanthin and siphonein in marine green algae and siphonaxanthin is found in very ancient algae, these novel siphonein-siphonaxanthin-Chl *a*/*b*-proteins may be ancient light-harvesting complexes which were evolved in deep water algae.

Introduction

The photosystems of photosynthetic organisms living in different environments are adapted for the optimal utilization of the available light and may be modulated in response to changing light environments. Compared to terrestrial plants, algae have peculiar problems in harvesting light energy [1–3]. Firstly, the light available in oceans or lakes rapidly decreases with increasing depth of water, due to selective absorption and scattering. Secondly, the spectral quality of the light is dramatically changed with increasing water depth, with the attenuation of far red, then red, yellow and blue light, so that at a depth of 20 metres or more the only available light is green. In coastal

Abbreviations: Chl, chlorophyll; PS, photosystem; Tricine, *N*-tris(hydroxymethyl)methylglycine; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine.

waters there is little red light even at 2 metres and none at 5 metres depth [3]. Hence, most seaweeds of the littoral zone flourish in predominantly blue-green light. Clearly, the ability of various algae to survive at different oceanic depths, depends on their successful adaptation to their particular light environment.

Siphonous marine green algae, which include the Codiales, Derbesiales and Caulerpales, are unusual orders in the Chlorophyta. In contrast to most green algae, the Siphonoaceae have uncommon storage polysaccharides, similar to the 1,3-β-glucans of brown algae, and their cell walls are 1,3-β-linked polyxylans rather than cellulose [4,5]. Some contain the novel xanthophylls siphonaxanthin and its esterified form, siphonein, instead of or as well as lutein, the predominant xanthophyll of most green algae and higher plants. Siphonaxanthin and siphonein, when complexed to protein, effectively absorb light in the blue-green region of the visible spectrum [6–9]. In a given species, the presence of siphonaxanthin or siphonein or both, correlates strongly with deeper water habitats and enhanced green light absorption in vivo [6–9]. Marine green benthic algae also have a greater content of Chl *b* than freshwater algae and higher plants [10–12] which assists them to absorb more of the available green light, since the Soret absorption peak of Chl *b* occurs at a longer wavelength than that of Chl *a*.

Two main chlorophyll-proteins of some marine green algae were first resolved by the Shibata gel electrophoretic method [13] and characterized by Nakamura et al. [13]. Later, with mild SDS electrophoresis [14], six Chl-protein bands were resolved and characterized from a marine green alga, *Caulerpa cactoides* [14]. The Chl *a*/*b*-proteins of the main light-harvesting complex from *C. cactoides* had high amounts of Chl *b* (Chl *a*/Chl *b* ratios of 0.66) [14], contrasting with those of higher plants and most Chlorophyta which have more Chl *a* than Chl *b* (Chl *a*/Chl *b* ratios of 1.2) [14,15]. Recently, we demonstrated that the Chl *a*/*b*-proteins of *C. cactoides* also had siphonaxanthin [15].

In this study, eight chlorophyll-protein complexes of a *Codium* species were isolated by mild electrophoresis and characterized by chromatography and spectrophotometry. The partially dissociated PS I complexes are interesting because they contain Chl *b* and siphonaxanthin as well as Chl *a*. The Chl *a*/*b*-proteins of the main light-harvesting complex are siphonaxanthin-siphonein-Chl *a*/*b*-proteins with Chl *a*/Chl *b* ratios of 0.66, similar to those of *C. cactoides* [14]. These light-harvesting complexes which are a new class of Chl *a*/*b*-proteins are responsible for the enhanced absorption in the blue-green and green region observed for some siphonous marine green algae.

Methods

A *Codium* species was collected at low tide from about 0–1 m below the ocean surface, at Guerilla Bay, New South Wales, and transported to Canberra, in chilled, aerated seawater which was changed at intervals.

Isolation of codium thylakoids. Younger fronds of *Codium* thalli were washed in seawater, blotted dry with paper, and 50-g lots (net weight) were homogenized in a Waring blender at top speed for 60 s in 250 ml of isolation medium containing 50 mM phosphate buffer (pH 7.2), 1 M sorbitol and 75% bovine serum albumin. The homogenate was filtered rapidly through two layers of Miracloth and gauze and centrifuged at $3000 \times g$ for 5 min. The pellet was washed twice more in the isolation medium, followed by single washes with 50 mM phosphate buffer (pH 7.2) containing 0.1 M KCl, with glass-distilled water; with 1 mM EDTA (pH 8.0) and twice with 50 mM Tricine buffer (pH 8.0). The thylakoids were then resuspended in 50 mM Tricine buffer (pH 8.0) at 0.5–5.0 mg Chl/ml for either immediate use or storage in liquid N_2.

Chl concentrations and Chl *a*/Chl *b* ratios were determined in 80% acetone [16] and protein was determined by the method of Lowry et al. [17].

Separation of the Chl-proteins of Codium thylakoids. Codium thylakoids were solubilized at 4°C prior to gel electrophoresis in 0.19 M Tris (pH 8.0) containing 20% glycerol and 0.375% SDS with an SDS/Chl weight ratio of 7.5 : 1. The extract was stirred vigorously on a vortex mixer at 25°C, centrifuged at $5000 \times g$ for 10 min and the supernatant immediately applied to pre-cooled gels according to Ref. 18. Slab gels ($140 \times 75 \times 2.4$ mm) were cast in a Pharmacia gel-casting apparatus. The separating gel (130 mm) contained an

8–12% acrylamide gradient and 0.22 M Tris-HCl (pH 9.35), 0.1% SDS, 0.01% ammonium persulphate and 0.07% (v/v) TEMED. The stacking gel (10 mm) contained 4% acrylamide, 56 mM Tris-H_2SO_4 (pH 6.14), 0.1% SDS, 0.1% ammonium persulphate and 0.05% TEMED. The acrylamide/*N,N'*-methylenebisacrylamide weight ratio was 30 : 0.8. The upper reservoir buffer (pH 8.64) was 41 mM Tris-borate buffer, 0.1% SDS and the lower reservoir buffer was 0.43 M Tris-HCl (pH 9.35). Electrophoresis was carried out at 4°C with 4–5 mA per gel using a Gradipore electrophoretic apparatus (Sydney, Australia) in which the gels are surrounded by buffer for efficient heat dissipation. Gels were photographed, and the relative distribution of chlorophyll in the chlorophyll-protein bands was determined by scanning the gels at 675 and 650 nm as previously described [19].

Absorption and fluorescence spectrometry. For absorption spectroscopy at 25°C gel slices containing the chlorophyll-proteins were placed directly on the side of the cuvette, or for spectra at 77 K, slices were incubated in glycerol/50 mM Tricine (pH 8.0) (2 : 1, v/v). Absorption spectra were recorded on a Hitachi-Perkin Elmer 557 spectrophotometer linked to a computer. Fluorescence spectra at 77 K were recorded with a Perkin Elmer MPF-44B fluorimeter.

For curve deconvolution, the absorption spectra of *Codium* chloroplasts and the isolated light-harvesting complex were obtained in digital form with a Hitachi-Perkin Elmer spectrophotometer coupled to a Digital Equipment PDP 11-03 minicomputer. A modified RESOL program (originally designed by Dr. D.D. Tunnicliff of the Shell Development Co., and obtained through the courtesy of Mr. Glenn Ford and Dr. Jeanette S. Brown of the Carnegie Institution of Washington, Stanford) was used to find component bands of the spectra. This program uses the least-squares technique to fit a sum of Gaussian distributions to each spectrum. The program was run on a Digital Equipment DPD 11-34 minicomputer.

Identification of carotenoids. The pigments of *Codium* chloroplasts and isolated chlorophyll-proteins were identified by thin-layer chromatography [20] and by their absorption spectra. The Chl-protein complexes from homogenized gel slices were extracted into 50 mM Tricine buffer (pH 8.0). Chloroplasts and isolated chlorophyll-proteins were extracted with 80% acetone and the pigments were transferred to diethyl ether at 4°C using cold 5% NaCl to create two phases. The ethereal phase was washed and dried by anhydrous Na_2SO_4 at −13°C, concentrated by evaporation with nitrogen and then applied to kieselgel plates (Merck, 250 mm). The plates were developed with benzene/acetone (3 : 1, v/v) or toluene/ethanol/acetone (3 : 0.5 : 0.5, v/v). The observed λ_{max} values were as follows: for carotenes in diethyl ether, 477, 449 and 428 nm; and for the xanthophylls in ethanol, siphonein, 445 nm; violaxanthin, 470, 440 and 419 nm; neoxanthin, 446, 439 and 417 nm; siphonaxanthin, 449 nm.

Results

Resolution of chlorophyll-proteins of Codium thylakoids by SDS-polyacrylamide gel electrophoresis

Codium chloroplasts (Chl *a*/Chl *b* ratio of 1.2–1.4) were solubilized with minimal amounts of SDS (an SDS/Chl weight ratio of 7.5 : 1), the same concentration required for the solubilization of higher plant thylakoid membranes [18] and not the higher amounts of detergent (SDS/Chl ratio of 40 : 1) needed for *C. cactoides* [14]. The Chl-protein complexes of many higher plant thylakoid membranes have been resolved by mild SDS electrophoresis which allows most of the chlorophyll and carotenoids to remain complexed with protein [18,19]. Eight major Chl-protein bands were resolved from *Codium* thylakoids (Fig. 1a) and the migration of these *Codium* chlorophyll-protein bands was compared with those resolved from spinach chloroplasts (Fig. 1b). The spinach Chl-proteins have previously been characterized by their absorption and fluorescence properties, P-700 and pigment content and polypeptide composition [18,19]. Two of the spinach chlorophyll-proteins are associated with PS I: these are CP 1 (the β-carotene-P-700-Chl *a*-protein) and CP 1a which is a partially dissociated PS I complex that contains CP 1, additional polypeptides associated with electron transport as well as minor, specific apoproteins binding Chl *a* and Chl *b* of the antenna of PS I [21–23]. The other four spinach Chl-proteins are CP a (the presumed reaction-

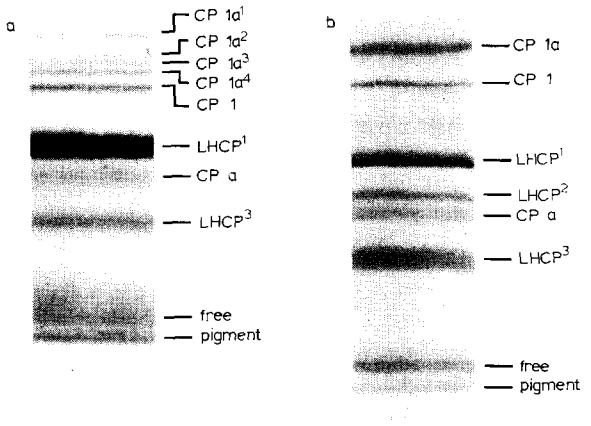

Fig. 1. Comparison of the chlorophyll-protein complexes resolved by mild SDS-polyacrylamide gel electrophoresis from a *Codium* species thylakoids (a) and spinach thylakoids (b).

centre complex of PS II) and the Chl *a*/*b*-proteins (LHCP^1, LHCP^2 and LHCP^3) of the main light-harvesting complex usually associated with the PS II reaction centre complex [18,19,24,25].

The chlorophyll-protein complexes of *Codium* (Fig. 1a) migrated to about the same positions as the spinach bands (Fig. 1b); however, there were more bands of low mobility. The *Codium* complexes listed in order of increasing mobility are designated CP 1a^1, CP 1a^2, CP 1a^3, CP 1a^4, CP 1, LHCP^1, CPa, LHCP^3 (Fig. 1a). Note that LHCP^2 usually resolved from higher plant thylakoids (Fig. 1b) was always absent in the *Codium* gels. The *Codium* chlorophyll-protein bands were characterized by spectral and pigment analyses (described below) which indicate that they are comparable to the chlorophyll-proteins characterized previously in *C. cactoides* [14] and in several higher plant thylakoids [18,19]. There was one important difference, however: the LHCP^1 and LHCP^3 bands from *Codium* were an orange-brown shade of green, which was strikingly different from the slightly yellowish shade of green of the LHCP bands of higher plants. These *Codium* bands resembled the orange-brown light-harvesting complexes isolated from brown seaweeds [20]. Indeed, the upper bands, CP 1a^2, CP 1a^2, CP 1a^3 and CP 1a^4, although present in lesser amounts also had an oragne-brown tinge. The free pigment bands at the gel front were chlorophyll-SDS and carotenoid-SDS micelles (upper band) and carotenoid-SDS micelles (lower band).

The relative distribution of chlorophyll in the chlorophyll-protein bands of *Codium* (Fig. 1a) (Table I) shows that 22% of the total chlorophyll is associated with CP 1a$^{1-4}$ and CP 1, 7% with the core reaction centre complex of PS II, and 60% with the dominant LHCP^1 and LHCP^3 bands. The amount of free Chl was low; however, the amount of free carotenoid was quite high. This distribution of chlorophyll amongst the complexes is roughly comparable to that obtained with *C. cactoides* [14]. The total amount of chlorophyll associated with PS II (CP a, LHCP^1 and LHCP^3) is 67% compared to 22% associated with PS I (CP 1a$^{1-4}$ and CP 1), giving a PS II/PS I chlorophyll content ratio of 3 (Table I). More of the total Chl is associated with PS II and less with PS I in *Codium* than is normally found in a range of sun plants which have Chl *a*/Chl *b* ratios of 2.8–3.4, and PS II/PS I chlorophyll content ratios of 2.0–2.5 [26]. Extreme shade plants (Chl *a*/Chl *b* ratios of 2.0–2.5) [27] have even higher PS II/PS I chlorophyll content ratios of 5.0–5.6 [26].

TABLE I

RELATIVE DISTRIBUTION OF CHLOROPHYLL AND THE Chl *a*/*b* RATIOS OF THE CHLOROPHYLL-PROTEINS OF *CODIUM* THYLAKOIDS

Chlorophyll-protein protein	Thylakoids	CP 1a$^{1-4}$	CP 1	LHCP^1	CP a	LHCP^3	Free pigment	PS II Chl * / PS I Chl *
Chlorophyll (%)	100	14	8	44	7	16	11	3.0
Chl *a*/Chl *b* ratio	1.2	5.0–7.0	20	0.61	6.1	0.73	–	

* PS II Chl, CP a, LHCP^1 and LHCP^3; PS I Chl, CP 1a$^{1-4}$ and CP 1.

Pigment composition of chlorophyll-proteins of Codium thylakoids

Chl *b* is present in very high amounts in marine green algae which may have Chl *a*/Chl *b* ratios as low as 1.0–1.8 [10–12], which are even lower than those found in extreme shade plants [27]. *Codium* LHCP[1] and LHCP[3] have 1.5-times as much Chl *b* as Chl *a* (Table I), as was found previously with the LHCP's isolated from *C. cactoides* [14] or *Acetabularia mediterranea* [28]. In contrast, the LHCP's isolated from most Chlorophyta and all higher plants have a fixed chlorophyll composition with slightly more Chl *a* than Chl *b*, with overall Chl *a*/Chl *b* ratios of 1.2 [15]. Significantly, substantial amounts of Chl *b* were present in the partially dissociated PS I complexes, CP 1a[1]–CP 1a[4] (Chl *a*/Chl *b* ratios of 5–7) and even CP 1 and CP *a* had traces of Chl *b* (Table I). The high Chl *b* content in *Codium* chloroplasts made it easy to detect Chl *b* in these PS I complexes. Recent evidence [21–23] suggests strongly that Chl *b* is a minor but integral component of PS I. Undissociated PS I complexes consist of the core reaction centre complex and additional light-harvesting Chl *a*/*b*-proteins, the apoproteins of which are not those of the main light-harvesting complex, LHCP[1–3] [21–23]. The *Codium* PS I complexes (Chl *a*/Chl *b* ratios of 5–7) have more Chl *b* than found in spinach CP 1a (Chl *a*/Chl *b* ratio of 11) [23].

The carotenoids present in *Codium* chloroplasts were analysed by thin-layer chromatography [20]. *Codium* thylakoids contained the major carotenoids present in most Chlorophyta and higher plant thylakoids, namely, carotenes and the xanthophylls lutein (traces only), violaxanthin and neoxanthin. The carotenoids were identified by their chromatographic mobility and absorption spectra in ethanol. Lutein was not the dominant xanthophyll; instead there were two bright orange-red pigments present in about equal proportions. The absorption miximal of these two pigments in ethanol were identical to those reported for siphonaxanthin and its esterified form, siphonein (Fig. 2). The relative chromatographic mobilities of these xanthophylls were comparable to those reported in other solvent systems with siphonein having an R_f between that of Chl *a* and Chl *b*, and siphonaxanthin having a very low R_f [29,30]. When the extracted pigments

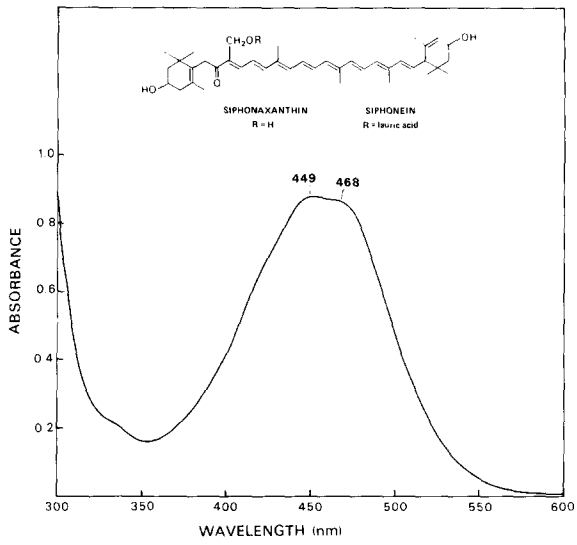

Fig. 2. Absorption spectrum in ethanol of siphonaxanthin extracted from *Codium* chloroplasts.

were saponified [20] and the carotenoids then separated on thin-layer chromatographs, only one orange-red spot was resolved; as expected it corresponded to siphonaxanthin.

The chlorophyll-protein bands resolved on preparative mild SDS-polyacrylamide gels were extracted into 50 mM Tricine buffer, pH 8.0, and the pigments extracted into 80% acetone and transferred to diethyl ether. Thin-layer chromatography of the concentrated extracts showed that the PS I complexes, CP 1a[1–4], contained siphonaxanthin and traces of siphonein. LHCP[1] and LHCP[3] had significant amounts of both siphonaxanthin and siphonein as well as minor xanthophylls, violaxanthin and neoxanthin. The molar composition of the siphonaxanthin-siphonein Chl *a*/*b*-proteins is not given, since large losses of siphonaxanthin and siphonein, ranging from 20 to 50% of the total xanthophylls, occurred during the solubilization and electrophoresis as shown by the amount of carotenoid released during the mild SDS electrophoresis. Despite these losses, siphonaxanthin and siphonein were clearly present in the Chl *a*/*b*-proteins. Previously, we did not detect these brown-green light-harvesting Chl *a*/*b*-proteins in *C. cactoides* [14] but later attempts showed limited amounts of siphonaxanthin-Chl *a*/*b*-proteins [15]. The much

lower SDS concentration required for solubilization of *Codium* compared to *C. cactoides* thylakoids premitted the isolation of Chl *a*/*b*-proteins with higher xanthophyll content. As the significance of the esterification of siphonaxanthin is unknown, *Codium* LHCP[1] and LHCP[3] will for simplicity be termed only siphonaxanthin-Chl *a*/*b*-proteins throughout.

Absorption spectra of Codium thylakoids and isolated chlorophyll-proteins

The absorption spectrum of chloroplasts isolated from *Codium* shows significant absorption in the green region (500–540 nm) (Fig. 3). These bands are similar to those observed in the intact thalli of many marine benthic green algae [6–9]. Yokohama and colleagues [6–9] showed that the xanthophylls which were responsible for the light-harvesting in vivo in the green region were siphonaxanthin, siphonein or both pigments. The enhanced absorption in the blue-green region by *Codium* chloroplasts relative to spinach chloroplasts (Fig. 3) is due to the presence of siphonaxanthin and siphonein as the main xanthophylls rather than lutein, and to their greater content of Chl *b*. The absorption by chloroplasts isolated from a brown alga, *Acrocarpia paniculata* is also enhanced in the green region, due to fucoxanthin

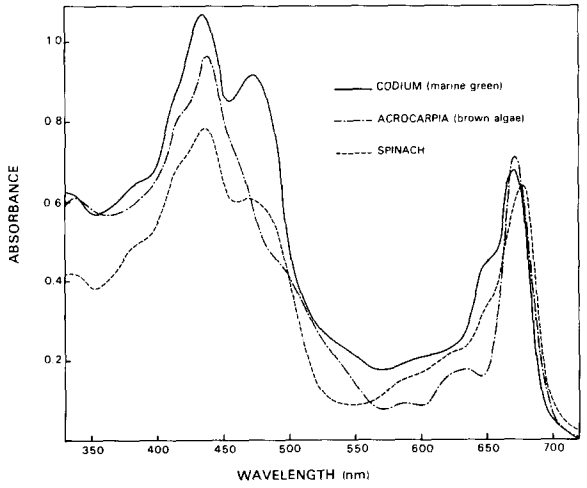

Fig. 3. Absorption spectra of chloroplasts in 50 mM Tricine buffer (pH 8.0) isolated from a *Codium* species (———), *Acrocarpia paniculata*, a brown alga (–·–·–) and spinach (- - - - -).

and in the blue-green region due to Chl c_1 and Chl c_2 [20].

CP 1, the β-carotene-P-700-Chl *a*-protein complex of *Codium* thylakoids has a similar absorption spectrum to that found for the *C. cactoides* CP 1 [14] (Fig. 4a). The absorption spectrum of *Codium* CP 1 is very similar to that of CP 1's isolated from chloroplasts of the Chlorophyta, Chromophyta or higher plants, except that the absorption maximum in the red occurs at 675 nm instead of 678 nm. The four partially dissociated PS I complexes of *Codium*, CP 1a[1–4], have relatively enhanced absorption in the blue-green and green region of the spectrum compared to CP 1 (Fig. 4a). The absorption at 475 nm is due to Chl *b* and that in the 500–540 nm region is due to siphonaxanthin and siphonein. Recent evidence suggests that the CP 1a's resolved from higher plants also contain some Chl *b* and lutein, due to the presence of minor Chl *a*/*b*-proteins specifically associated with PS I [21–23]. This may also be the case with *Codium* chloroplasts, although the actual polypeptides carrying Chl *b* and siphonaxanthin were not identified.

The absorption spectra of LHCP[1] and LHCP[3] (Fig. 4b) clearly indicate that high amounts of Chl *b*, siphonaxanthin and siphonein are present in the *Codium* light-harvesting complexes. The enhanced absorption at 475 and 652 nm (Fig. 4b) due to Chl *b* was noted also in *C. cactoides* LHCP[1–3] [14]. The bands in the 500–540 nm region seen in Fig. 4b are also characteristic of spectra of marine green algal thalli [6–9], and of *Codium* isolated chloroplasts (Fig. 3). The LHCP[1] and LHCP[3] spectra (Fig. 4b) clearly show that the bulk of the xanthophylls responsible for the enhanced green absorption in vivo are complexed with the main Chl *a*/*b*-proteins of *Codium* light-harvesting complex.

Curve deconvolution of the absorption spectra of Codium chloroplasts and isolated light-harvesting complex

One way to test whether the isolated light-harvesting complex has retained its native configuration is to analyse its in vivo absorption spectrum. The low-temperature spectra of chloroplasts or isolated chlorophyll-protein complexes can be deconvoluted by computer into a number of Gaus-

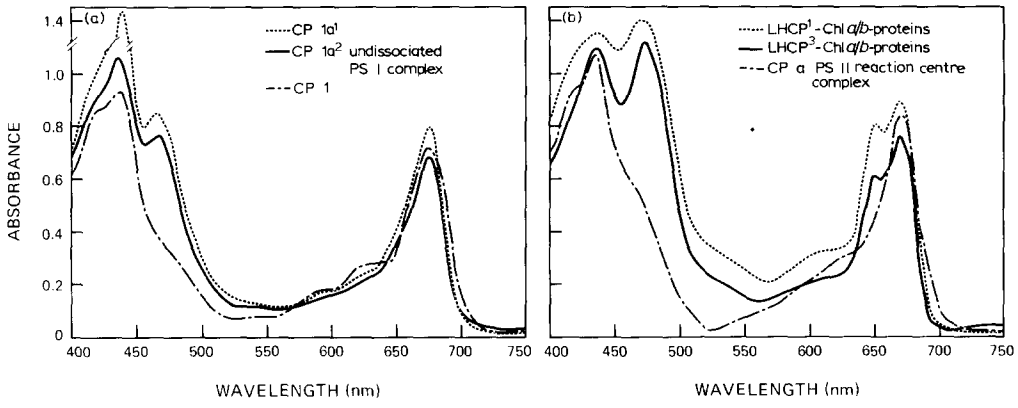

Fig. 4. Absorption spectra of purified chlorophyll-protein complexes from *Codium* on gel slices at 25°C. (a) CP $1a^2$ and CP 1. (b) $LHCP^1$ and $LHCP^3$.

sian components which represent the states or groups of chlorophyll molecules each with their own electronic transition and absorption maximum, due to their different organization within the individual chlorophyll-proteins of thylakoid membranes (Refs. 31 and 32; cf. Ref. 15). Different plants and algae have the same groups of components, four major Chl *a* groups and two to four minor components, and their absorption spectra vary due to the different proportions of these components [31]. The spectra at 77 K of *Codium* chloroplasts and isolated light-harvesting complex $LHCP^1$ (Fig. 5) have been analysed from 700 to 620 nm by computer using the modified RESOL program (see Methods). The data from the resolved curves (Fig. 5) are summarized in Table II. The peak maximum of each of the bands is given to the nearest 1 nm, and the area under each band is listed as the percentage of the total area. *Codium* chloroplasts and $LHCP^1$ have the same components, but the relative proportions of the components differ (Table II). The wavelength maxima of the major Chl *a* forms at 660, 669, 676 and 684 nm are similar to the usual four major forms of Chl *a* found in the many plant and algal chloroplasts examined at the Carnegie Institution of Washington [31,32]. $LHCP^1$ has less of the 684, 676 and 669 nm forms and more of the 660 and 650 forms relative to chloroplasts (Table II). As 63% of the total chlorophyll is associated with the light-harvesting complex in *Codium* chloroplasts, one would not expect to see marked changes in the relative percentages of the various chlorophyll components.

Fluorescence spectra of Codium chloroplasts and chlorophyll-proteins

The fluorescence emission spectrum of isolated *Codium* chloroplasts has maxima at 685, 696 and 712 nm (Fig. 6b). This spectrum is rather similar to those reported for the intact thalli of two siphonous species [8]. In higher plant chloroplasts, there are three characteristic bands: the 685 and

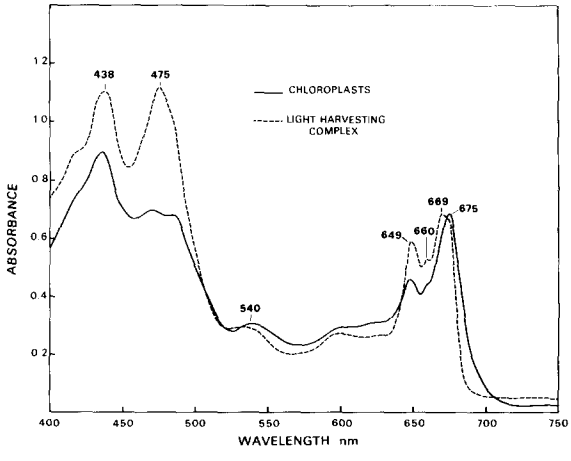

Fig. 5. Absorption spectra of *Codium* chloroplasts (———) and purified $LHCP^1$ (- - - - -) in 50 mM Tricine buffer (pH 8.0)/glycerol (1 : 2, v/v) at 77 K.

TABLE II

CURVE DECONVOLUTION ANALYSIS OF *CODIUM* CHLOROPLASTS AND ISOLATED LIGHT-HARVESTING COMPLEX, LHCP[1]

Fraction	Chlorophyll components (% of total Chl *a*) Peak wavelengths of component bands (nm):						
	625	640	650	660	669	676	684
Chloroplasts	18.2	8.4	15.5	7.2	18.3	23.1	9.3
LHCP[1]	17.6	8.0	22.2	15.3	8.3	15.8	2.8

695 nm bands are associated with PS II and the broad band in the far red at 735 nm is mainly associated with PS I [14,32]. In many green algae and species of the Chl *c*-containing algae [33,34] the far-red emission is found at lower wavelengths (710–718 nm) as in *Codium* chloroplasts. The excitation spectrum for chlorophyll *a* fluorescence emission at 712 nm of isolated *Codium* chloroplasts (Fig. 6a) shows excitation maxima at 435 and 470 nm due to Chl *a* and Chl *b*, respectively, while the bands at 512 and 538 nm are due to absorption by siphonaxanthin and siphonein. This demonstrates that all of these pigments are effective in harvesting and transferring light excitation energy to Chl *a* in the isolated chloroplasts.

The fluorescence emission spectra of CP 1 and CP 1a^2 (Fig. 7b) are unusual, particularly that of CP 1a^2 which has a single emission maximum at 698 nm. The fluorescence excitation spectrum of CP 1a^2 (Fig. 7a) with peaks at 436 nm (Chl *a*), 472 nm (Chl *b*) and 538 nm (siphonaxanthin) shows the highly organized arrangement of both Chl *b* and siphonaxanthin in CP 1a^2. The emission spectrum of CP 1 is also somewhat unusual and the band at the lower wavelength of 677 nm may indicate that there is some uncoupling or conformational change in the chlorophylls of this complex.

The fluorescence emission spectrum of LHCP[1] has a maximum at 681 nm (Fig. 6b) and that of LHCP[3] was identical (not shown). These spectra had the usual narrow half-bandwidth associated with isolated lutein-Chl *a*/*b*-proteins. Despite the enhanced Chl *b* content of this *Codium* complex, the emission spectrum is identical to those of the LHCP's of higher plants [14,18]. There is a dramatic difference, however, in the excitation spectrum of *Codium* LHCP[1] (Fig. 6a) which shows the additional absorption in the 500–540 nm region due to the siphonaxanthin and siphonein. Indeed, the similarity of the fluorescence excitation spectrum (Fig. 6a) to the absorption spectrum (Fig. 4b) in the blue and green regions indicates that the pigments of the siphonaxanthin-Chl *a*/*b*-proteins are in a highly ordered molecular arrangement permitting efficient transfer of light

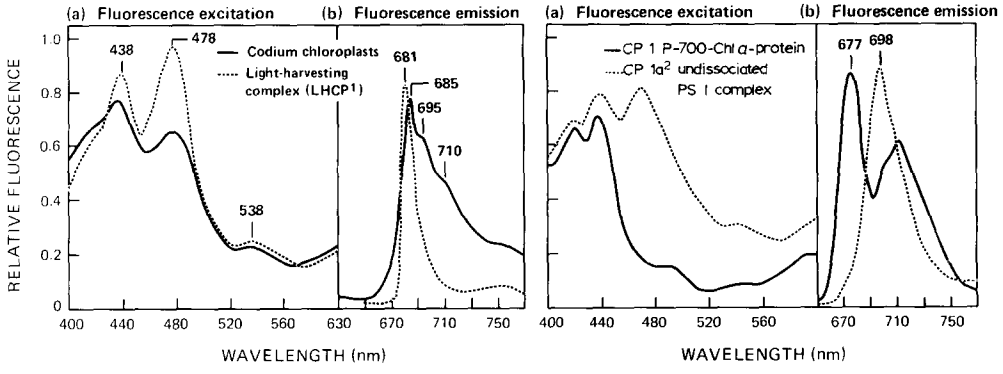

Fig. 6. Fluorescence excitation (a) and emission spectra (b) of *Codium* chloroplasts and isolated LHCP[1] at 77 K. The emission wavelengths for fluorescence excitation were 710 nm for chloroplasts and 681 nm for LHCP[1]. The excitation wavelength for fluorescence emission was 436 nm.

Fig. 7. Fluorescence emission (b) and excitation (a) spectra of purified chlorophyll-protein complexes, CP 1 and CP 1a^2 on gel slices at 77 K. The emission wavelengths for fluorescence excitation were 715 nm for CP 1 and 698 nm for CP 1a^2. The excitation wavelength for fluorescence emission was 436 nm.

excitation energy. Further, similar emission spectra to that of Fig. 6b with a maximum at 681 nm were obtained with excitation at 436 nm (Chl *a*), 474 nm (Chl *b*) or 512 and 538 nm (siphonaxanthin). Since all the fluorescence emission is at 681 and none is at 650 nm, all the Chl *b* is in its native configuration. These points, taken together, suggest strongly that both Chl *a* and *b* and siphonaxanthin and siphonein in *Codium* LHCP[1] and LHCP[3] effectively transfer their light excitation energy to Chl *a*. Hence, both siphonaxanthin and siphonein are integral components of the *Codium* Chl *a*/*b*-proteins, LHCP[1] and LHCP[3].

Discussion

The significance of relative proportions of Chl a and Chl b in light-harvesting

Although the *Codium* species investigated is not a deepwater alga, it retains a light-harvesting apparatus with enhanced ability to absorb blue-green and green light due to high amounts of Chl *b*, siphonaxanthin and siphonein. In contrast to the highly conserved P-700- and P-680-Chl *a*-proteins of the core reaction centre complexes of PS I and PS II, there is great variation between photosynthetic organisms in the chemical nature and location of the light-harvesting antenna complexes which include phycobiliproteins, peridinin-Chl *a*-proteins, fucoxanthin-Chl *a*/*c*-proteins or Chl *a*/*b*-proteins [15]. One strategy used by plants and algae to adapt to particular light environments is their ability to modulate the relative amounts of core reaction centre and light-harvesting complexes. Variations in the pigment composition of the photosynthetic apparatus may be reflected in the varying proportions of the total chlorophyll associated with reaction centre complexes on the one hand, and with antenna complexes on the other [15]. In Chl *b*-containing plants and algae, as the Chl *a*/Chl *b* ratio increases there is a concomitant decrease in the total amount of the main light-harvesting complexes and vice versa [18,26]. There is a limit to this modulation, however, since to date no more than 70% of the total Chl has been found associated with LHCP[1-3]. Hence, it appears that at least 30% of the total Chl has to be associated with the Chl *a* core reaction centre complexes. This is true for *Codium* also, since 60% of the total Chl was located in LHCP[1-3] (Table I). *Codium* chloroplasts like many marine green algae [10–12] have high amounts of Chl *b*. In such cases, another strategy for modulation of the photosynthetic apparatus has been used and the antennae of both PS I and PS II in *Codium* have greater amounts of Chl *b* than found in higher plants. The higher content of Chl *b* of *Codium* is reflected in the LHCP's which have 1.5 times as much Chl *b* as Chl *a* in contrast to the fixed overall stoicheiometry of higher plant LHCP's (Chl *a*/Chl *b* ratios of about 1.2) and in the partially dissociated PS I complexes, CP 1a[1-4], which have Chl *a*/Chl *b* ratios of 5–7 (Table I) instead of 11 found for spinach [23].

The significance of siphonaxanthin and siphonein in light-harvesting

Yet another strategy used by some green algae is the incorporation of different xanthophylls into the light-harvesting antennae, which have absorption properties specially suited to the harvesting of light in a particular niche. Thus, the orange-brown Chl *a*/*b*-proteins isolated from *Codium* contain siphonaxanthin and siphonein as the dominant xanthophylls instead of lutein found in most green algae and higher plants. They represent a new class of light-harvesting complexes in Chl *b*-containing plants. The similarity of the absorption and fluorescence excitation spectra of *Codium* LHCP[1] and LHCP[3] (Figs. 4b and 6a), together with their identical emission spectra when excited at 436 nm (Chl *a*), 474 nm (Chl *b*) or 512 or 538 nm (siphonaxanthin), indicate a highly ordered molecular arrangement of these pigments in *Codium* LHCP[1] and LHCP[3]. Moreover, the fact that the fluorescence emission spectra of *Codium* LHCP[1] and LHCP[3] are indistinguishable from those of the lutein-Chl *a*/*b*-proteins of higher plants suggests that siphonaxanthin, siphonein and Chl *b* are all transferring light excitation energy to Chl *a*, probably with 100% efficiency. The significance of siphonaxanthin and siphonein as the main xanthophylls in some siphonous algae, including *Codium*, is their ability to absorb in the green region of the spectrum (500–540 nm), the dominant light in deep oceanic waters or turbid coastal waters [1–3].

Two other xanthophylls, fucoxanthin and peri-

dinin, when complexed with proteins, are also capable of absorbing light in the green region of the spectrum [35]. Like siphonaxanthin and siphonein, fucoxanthin and peridinin have absorption maxima in organic solvents which lie between 450 and 470 nm, as indeed do all other xanthophylls. When these four xanthophylls are complexed with protein, however, their absorption maxima in vivo show a dramatic shift of 50–90 nm to longer wavelengths thereby extending their light-harvesting capacity into the green region of the spectrum. The cause of this marked red shift is unknown, but it may be significant that siphonaxanthin, fucoxanthin and peridinin all have a carbonyl group adjacent to one of the end β-ionone rings [36].

While the light-harvesting role of the siphonaxanthin is easily seen in *Codium* LHCP[1] and LHCP[3], that of lutein which absorbs in the same blue-green region as Chl *a* and Chl *b* is less evident; indeed, lutein was often ignored as a significant component of higher plant LHCP's. Siefermann-Harms and Ninnemann [37] have demonstrated that although the plant xanthophylls constituted only 25% of the total pigment of lettuce LHCP, these were responsible for 43% of the total absorption in the blue-green region, and significantly there was 100% efficiency of energy transfer from the xanthophylls and Chl *b* to Chl *a* [37]. The arrangement of Chl *a*, Chl *b* and the xanthophylls in the apoproteins of the light-harvesting complexes is now known. However, Siefermann-Harms and Ninnemann [37] elegantly showed that the chlorophylls and xanthophylls of lettuce LHCP are remarkably resistant to strong acid attack. This resistance of the complexed pigments to protons, as well as the demonstrated energy transfer of 100% from both xanthophylls and Chl *b* to Chl *a* in lettuce LHCP, suggest a highly ordered packing of the pigments within hydrophobic crevices deeply buried in the interior of the apoproteins [37]. This may be the case also for the siphonaxanthin-Chl *a*/*b*-proteins.

The distribution of siphonaxanthin and siphonein is extremely variable in the Chlorophyta. In the Siphonales, siphonaxanthin and siphonein are present in all species of Codiales, Derbesiales and Caulerpales, regardless of their growth in deep or shallow waters [6,9]. Siphonaxanthin only is present in those species of Ulvales, Chladophorales and Siphonocladales inhabiting deeper marine waters or shaded sites [6,9]. Siphonein alone is present in some Dichotomosiphonales, and neither pigment is found in the Dasycladales [6]. Since both siphonaxanthin and siphonein were found in some scaly green monads [30,38] which are considered to be the nearest extant relatives of the ancestral green algae [39] they may be of ancient evolutionary significance. Lutein, the main xanthophyll of most Chlorophyta and higher plants, may or may not occur together with siphonaxanthin and siphonein. O'Kelly [40] found that 10 out of 16 species of marine Chaetophoraceae and Chaetosiphonaceae had siphonaxanthin: four that lacked lutein inhabited the subtidal zone, those with both lutein and siphonaxanthin occurred in intermediate zones, while those with lutein only were found in upper to mid-tidal habitats. The presence of both siphonaxanthin and siphonein in ancient orders of the Chlorophyta, and their restriction in some cases to species surviving only in deeper waters or shaded sites, suggest that these pigments originated in ancient green algae restricted to deep-water habitats. They may have evolved before the ozone layer that shields algae from harmful ultraviolet radiation had fully developed. The presence of siphonaxanthin and siphonein today in some shallow water species which also contain lutein suggests that these siphonaxanthin-siphonein-chlorophyll *a*/*b*-proteins are evolutionary relics which have been retained to confer advantage on deep-water species.

Acknowledgements

I am indebted to Jack Barrett for his suggestion to study and help in collecting marine green algae, and to Dr. Jacek T. Duniec for his assistance with the curve deconvolution. The skilled assistance of Miss D. Maguire is also gratefully acknowledged.

References

1 Levring, T. (1966) in Light as an Ecological Factor (Bainbridge, R. et al., eds.), pp. 305–318, Blackwell Scientific Publications, Oxford
2 Jerlov, N.G. (1976) Marine Optics, Elsevier, Amsterdam

3 Kirk, J.T.O. (1976) Aust. J. Mar. Freshwater Res. 27, 61–71
4 Mackie, W. and Preston, R.D. (1974) in Algal Physiology and Biochemistry (Stewart, W.D.P., ed.), pp. 40–85, Blackwell Scientific Publications, Oxford
5 Craigie, J.S. (1974) in Algal Physiology and Biochemistry (Stewart, W.D.P., ed.), pp. 206–235, Blackwell Scientific Publications, Oxford
6 Kleinig, H. (1969) J. Phycol. 5, 281–284
7 Yokohama, Y., Kageyama, A., Ikawa, T. and Shimura, S. (1977) Bot. Mar. 20, 433–436
8 Kageyama, A., Yokohama, Y., Shimura, S. and Ikawa, T. (1977) Plant Cell Physiol. 18, 477–480
9 Yokohama, Y. (1981) Bot. Mar. 24, 637–640
10 Jeffrey, S.W. (1965) Biol. Bull. 135, 141–148
11 Ogawa, T., Nakamura, K. and Shibata, K. (1975) Algol. Stud. 14, 37–48
12 Yokohama, Y. and Misonou, T. (1980) Jap. J. Phycol. 28, 219–233
13 Nakamura, K., Ogawa, T. and Shibata, K. (1976) Biochim. Biophys. Acta 423, 227–236
14 Anderson, J.M., Waldron, J.C. and Thorne, S.W. (1980) Plant Sci. Lett. 17, 149–157
15 Anderson, J.M., Barrett, J. and Thorne, S.W. (1981) in Photosynthesis III (Akoyunoglou, G., ed.), Vol. 3, pp. 301–315, Balaban International Science Services, Philadelphia
16 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
17 Lowry, O.H., Rosebrough, N.J.B., Farr, A.L. and Randall, A.J. (1951) J. Biol. Chem. 193, 265–275
18 Anderson, J.M. (1980) Biochim. Biophys. Acta 591, 113–126
19 Anderson, J.M., Waldron, J.C. and Thorne, S.W. (1978) FEBS Lett. 92, 227–233
20 Barrett, J. and Anderson, J.M. (1980) Biochim. Biophys. Acta 590, 309–323
21 Wollman, F.A. and Bennoun, P. (1982) Biochim. Biophys. Acta 680, 352–360
22 Ish-Shalom, D. and Ohad, I. (1983) Biochim. Biophys. Acta 722, 498–507
23 Anderson, J.M., Brown, J.S., Lam, E. and Malkin, R. (1983) Photochem. Photobiol., in the press
24 Thornber, J.P., Markwell, J.P. and Reinman, S. (1979) Photochem. Photobiol. 29, 1205–1216
25 Burke, J.J., Ditto, C.L. and Arntzen, C.J. (1978) Arch. Biochem. Biophys. 187, 252–263
26 Anderson, J.M. (1980) FEBS Lett. 117, 327–331
27 Anderson, J.M., Goodchild, D.J. and Boardman, N.K. (1973) Biochim. Biophys. Acta 325, 573–585
28 Apel, K., Bogorad, L. and Woodcock, C.L.F. (1975) Biochim. Biophys. Acta 387, 568–579
29 Kleinig, H. and Egger, K. (1967) Phytochemistry 6, 1681–1686
30 Ricketts, T.R. (1971) Phytochemistry 10, 161–164
31 French, C.S., Brown, J.S. and Lawrence, M.C. (1972) Plant Physiol. 49, 421–429
32 Brown, J.S., Alberte, R.S. and Thornber, J.P. (1974) in Proceedings of the 3rd International Congress on Photosynthesis (Avron, M., ed.), Vol. 3, pp. 1951–1962, Elsevier, Amsterdam
33 Lavorel, J. and Etienne, L.A. (1977) in Primary Processes of Photosynthesis (Barber, J., ed.), pp. 203–268, Elsevier, Amsterdam
34 Goedheer, J.C. (1972) Annu. Rev. Plant Physiol. 23, 87–112
35 Jeffrey, S.W. (1980) in Primary Productivity in the Sea (Falkowski, P.G., ed.), pp. 33–58, Plenum Publishing Corp., New York
36 Larkum, A.W.D. and Barrett, J. (1983) Adv. Bot. Res. 70, 1–273
37 Siefermann-Harms, D. and Ninnemann, H. (1982) Photochem. Photobiol. 35, 719–731
38 Ricketts, T.R. (1970) Phytochem. 9, 1835–1842
39 Stewart, K.D. and Mattox, K.R. (1978) Biosystems 10, 145–152
40 O'Kelly, C.J. (1982) Bot. Mar. 25, 133–137