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LIGHT-HARVESTING SYSTEMS OF BROWN ALGAE AND DIATOMS

ISOLATION AND CHARACTERIZATION OF CHLOROPHYLL *a*/*c* AND CHLOROPHYLL *a*/FUCOXANTHIN PIGMENT-PROTEIN COMPLEXES

RANDALL S. ALBERTE [a,*], ALAN L. FRIEDMAN [a], DAREL L. GUSTAFSON [b], MARLA S. RUDNICK [c] and HARVARD LYMAN [d]

Experimental Marine Botany Program, Marine Biological Laboratory, Woods Hole, MA 02543 (U.S.A.)

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Summary

The present study examined the protein associations and energy transfer characteristics of chlorophyll *c* and fucoxanthin which are the major light-harvesting pigments in the brown and diatomaceous algae. It was demonstrated that sodium dodecyl sulfate (SDS)-solubilized photosynthetic membranes of these species when subjected to SDS polyacrylamide gel electrophoresis yielded three spectrally distinct pigment-protein complexes. The slowest migrating zone was identical to complex I, the SDS-altered form of the P-700 chlorophyll *a*-protein. The zone of intermediate mobility contained chlorophyll *c* and chlorophyll *a* in a molar ratio of 2 : 1, possessed no fucoxanthin, and showed efficient energy transfer from chlorophyll *c* to chlorophyll *a*. The fastest migrating pigment-protein zone contained fucoxanthin and chlorophyll *a*, possessed no chlorophyll *c*, and showed efficient energy transfer from fucoxanthin to chlorophyll *a*. It is demonstrated that the chlorophyll *a*/*c*-protein and the chlorophyll *a*/fucoxanthin-protein complexes are common to the brown algae

* To whom reprint requests should be addressed.
[a—d] Permanent addresses: [a] Barnes Laboratory, The University of Chicago, 5630 S. Ingleside Ave, Chicago, IL 60637, U.S.A. [b] Department of Biology, University of Bridgeport, Bridgeport, CT 06602; [c] Department of Pharmacology, Yale School of Medicine, New Haven, CT 06510; [d] Department of Biology, S.U.N.Y., Stony Brook, NY 11794, U.S.A.

Abbreviations: LDAO, lauryldimethylamine oxide; Chl, chlorophyll; SDS, sodium dodecyl sulfate.

and diatoms examined, and likely share similar roles in the photosynthetic units of these species.

Introduction

The photosynthetic pigments in oxygen-evolving plants can be divided functionally into two categories. The major group (99%) serves to harvest and transfer light energy to the photochemical reaction centers. This group of chlorophylls (Chls *a*, *b*, and *c*) and other accessory pigments such as peridinin, fucoxanthin, and the phycobilipigments, are associated with distinct protein moieties and are aggregated into large multimacromolecular arrays in the photosynthetic unit. The second group represents only a very small portion of the total pigment (less than 1%), and serves in the reaction centers of Photosystems I and II. These Chl *a* molecules are associated with intrinsic membrane proteins, and are able, upon excitation, to donate electrons to an acceptor molecule and accomplish charge separation, and hence the primary photochemistry of photosynthesis.

Recent studies have shown that the Photosystem I reaction center pigment-protein, the P-700-Chl *a*-protein, is ubiquitous in the photosynthetic unit of Chl *a*-containing organisms [1—6]. Light-harvesting pigment-proteins, in contrast, are highly diverse in different plant groups [7,8]. Three light-harvesting pigment systems have been characterized to date. The light-harvesting Chl *a*/*b*-protein (also termed LHCP) is the major light-harvesting system in green algae and higher plants. It is a hydrophobic, intrinsic membrane protein which accounts for up to 65% of the total lamellar pigment and protein, and all of the Chl *b* [2,7]. In the cyanobacteria, red and cryptomonad algae, the water-soluble phycobilipigments (allophycocyanin, phycocyanin, and phycoerythrin) constitute the major light-harvesting system [9]. The dinoflagellate algae also contain a water-soluble pigment-protein termed peridinin-Chl *a*-protein [10, 11]. This caroteno-chlorophyll protein is present in these species with uncharacterized water-insoluble Chl *a*/*c* complexes [3—5]. Recently, Anderson and Barrett [5] have described components from a brown alga which are enriched in Chl *c*, and Prézelin and Alberte [6] reported the presence of a Chl *c*-rich band on SDS gel electrophoresis of dinoflagellate lamellae. The diatoms and brown algae share the presence and functional role of Chl *c* with the dinoflagellates as shown from action spectra for oxygen evolution [12,13]. In these latter groups the carotenoid fucoxanthin has also been shown [13,14] to function in harvesting light energy for photosynthesis, though its specific protein associations are unclear (see Refs 5 and 14).

As a part of an interest in the nature, composition, and comparative biochemistry of light-harvesting systems, we sought to characterize the specific protein associations and the role of Chl *c* and fucoxanthin in light capture and excitation energy transfer within the photosynthetic unit of brown algae and diatoms. The present investigation demonstrates that in these algal species Chl *a* and Chl *c* are associated together in a pigment-protein complex lacking fucoxanthin which shows efficient energy transfer from Chl *c* to Chl *a*. Another pigment-protein containing fucoxanthin and Chl *a* is identified which lacked

Chl *c* and shows energy transfer from fucoxanthin to Chl *a*. In addition, it is shown that these two pigment-protein complexes are common to diatoms and brown algae and likely share the same functional role in the organization of the photosynthetic unit.

Materials and Methods

Plant material. *Laminaria saccharinia*, *Leathesia difformis* and *Chorda filum* were collected at 10 m off the north shore jetty of the Cape Cod Canal, Sandwich, MA, and *Pylellia littoralis* was collected from surface waters in Nahant Bay, MA. If plant material was not used immediately, it was maintained for short periods aerated, on an illuminated sea table with running seawater. *Skeletonema costatum* (culture provided by R.R.L. Guillard) was grown in axenic culture in f/2-enriched seawater medium [13] under day/night (16 h/8 h) cycles at 15°C. Cells in log phase were harvested by centrifugation and stored at −20°C.

Pigment analysis. Chl *a* and Chl *c* were estimated spectrophotometrically by extracting in 90% acetone, with $MgCO_3$, and using the equations of Jeffrey and Humphrey [16]. No attempt was made to separate or quantitate Chl c_1 and Chl c_2. Pigment content of gel slices was obtained, after pulverization, in 90% acetone. In most cases, gel slices were extracted four times or until a 98% recovery in pigment was obtained. Pigments were separated by thin-layer chromatography (TLC) following the procedures of Jeffrey [17,18].

Preparation of photosynthetic membranes. Tissue of *Laminaria*, *Chorda*, *Leathesia* or filaments of *Pylellia* were cut into small pieces using a razor blade and frozen rapidly in liquid nitrogen. The frozen tissue was pulverized in a mortar and pestle, and 100 mM Tris-borate (pH 8.0), 10 mM $MgCl_2$, was added and the material further ground until a slurry was obtained. The slurry was homogenized (4°C) using a Polytron tissue disintegrator with 5-s pulses at 40 000 rev./min for 2—3 min. This homogenization step was repeated using an ultrafine Polytron generator. The homogenate was passed through a French pressure cell twice at 12 000—15 000 lb/inch^2. The resultant brei was filtered twice through three thicknesses of Miracloth removing most of the cellular debris and the bulk of the mucopolysaccharides.

The green-brown filtrate was centrifuged at 1000 × *g* for 1 min and the pellet was discarded. The supernatant was centrifuged at 40 000 × *g* for 10 min to pellet membrane fragments. The pigmented pellets were resuspended in 100 mM Tris/borate buffer (pH 8.0) and centrifuged first at 1500 × *g* for 5 min and the supernatant collected and recentrifuged at 40 000 × *g* for 10 min. These steps were repeated twice or three times. The final washed pellets were either frozen (−20°C) in the Tris-borate buffer or used immediately for gel electrophoresis.

Cells of *Skeletonema* were suspended in 100 mM Tris-borate buffer, pH 8.0, and broken by two passages through a French pressure cell at 15 000 lb/inch^2. The cellular brei was filtered through Miracloth and the filtrate centrifuged at 1000 × *g* to remove unbroken cells and debris. The supernatant was centrifuged at 40 000 × *g* for 15 min. The pelleted membranes were washed twice in the Tris-borate buffer and collected by centrifugation at 40 000 × *g* for 10 min.

The pelleted membranes were either stored frozen (−20°C) or used immediately for electrophoresis.

Polyacrylamide gel electrophoresis. All membrane preparations were pelleted at 40 000 × *g* for 10 min and then solubilized in 0.5% SDS in 50 mM Tris-HCl (pH 8.0) using a Kontes tissue homogenizer. Sufficient volumes of 0.5% SDS were added to the membranes to yield a final detergent/chlorophyll ratio of 20 : 1 (w/w). The resultant green-brown solution was centrifuged at 40 000 × *g* for 20 min. The pigmented supernatant (less than 10% of the pigment remained in the pellet) was used immediately for electrophoresis. The use of 0.1, 0.5 or 1% lauryldimethylamine oxide (LDAO) or Deriphat (General Foods) did not yield as complete solubilization as equivalent amounts of SDS.

Preparative tube gel electrophoresis was used to separate the pigment-proteins. Gel tubes were 10 cm × 2 cm and contained 25% acrylamide, 0.5% bisacrylamide, 0.05% SDS and 4 mM $MgCl_2$ in a 50 mM Tris-HCl buffer (pH 8.0). The running and gel buffer systems were continuous and samples were run at 25—35 mA per tube for 2—3 h at 14—18°C. Approx. 100—150 μl of sample in glycerol was applied to the gels.

Electrophoresis was terminated after the pigmented zones were clearly separated (2—3 h) and the respective colored zones were sliced out. Electrophoresis of LDAO- or Deriphat-solubilized membranes in the described SDS gel systems did not yield distinct pigmented zones. Gel slices were examined for their absorbance and fluorescence (emission and excitation) characteristics and then extracted into 90% acetone for pigment analysis. Identical gels were stained for protein in 0.2% Coomassie Blue R for 1 h and destained in methanol/water/acetic acid (5 : 5 : 1, v/v).

Spectral characterizations. Extracted pigments were quantified on a Pye Unicam 1800 spectrophotometer. Absorption spectra of gel pigmented slices were not well resolved by absorption spectrophotometry so room temperature fluorescence excitation spectrophotometry was used with high sensitivity and resolution. An Aminco SPF-500 Corrected Spectrum fluorometer was used to obtain fluorescence emission and excitation spectra. Emission spectra were scanned with 1 nm slits with excitation slits between 2 and 10 nm while excitation spectra were scanned with 1 nm slits with emission slits at 2—5 nm.

Light-induced oxidation and dark reduction of P-700 was measured in 1% Triton-solubilized membranes of the macrophytes and 0.1% Triton-solubilized membranes of *Skeletonema* according to the procedures outlines by Shiozawa et al. [19]. Using the same sample, the molar ratios of chlorophyll to P-700 were measured as an estimate of photosynthetic unit size [20].

Results

High yields of chloroplast lamellae were obtained from several species of macrophytic brown algae (e.g., *P. littoralis*, *C. filum*, *L. saccharina*, and *L. difformis*) using procedures modified from those previously described [20] for the isolation of photosynthetic membranes of gymnosperms. The use of both Tris-borate buffer and filtration through Miracloth aided in the removal of the abundant mucopolysaccharides present in these species. The chloroplast lamellae obtained were readily solubilized in 0.5% SDS, though a 20 : 1 (w/w) deter-

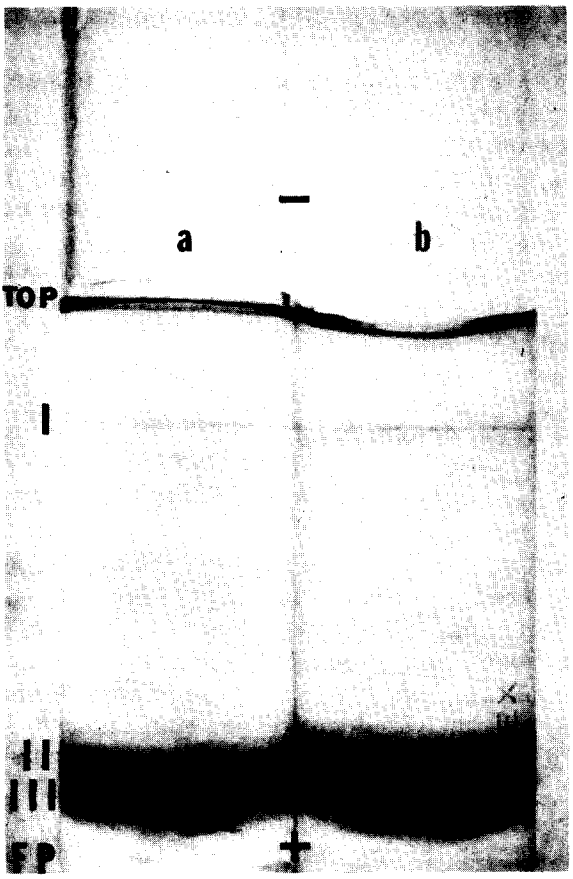

Fig. 1. SDS-polyacrylamide gel electrophoretic pattern of *L. saccharina* (a) and *S. costatum* (b) lamellae solubilized in 0.5% SDS and run as described in the text. The gel is not stained for protein, the bands visualized are from the associated pigments. Zone I was blue-green, zone II was olive-green, zone III was deep-orange, and the free pigment zone (FP) was pale yellow-green in appearance. The gels were overloaded for zones II and III to obtain photographable complex I (zone I) in native unstained gels.

gent/chlorophyll ratio was needed to obtain complete solubilization in contrast to previous work (see Refs. 1 and 20).

Examination of solubilization efficiency of LDAO and Deriphat revealed that neither detergent was as effective as SDS at the same low concentrations.

TABLE I

DISTRIBUTION OF PIGMENT IN POLYACRYLAMIDE GEL ELECTROPHORESIS ZONES OF *L. SACCHARINA* LAMELLAE

Gel zone	% total chlorophyll	Chl *a*/Chl *c*	% total fucoxanthin
I	7	—	0
II	22	0.5	0
III	40	—	98 *
Free pigment	31	—	2
Starting material	100	5.9	100

* In zone III a Chl *a*/fucoxanthin ratio of 1 : 4—5 was estimated.

SDS-polyacrylamide gel electrophoresis run with LDAO or Deriphat membrane extracts in these detergents or with SDS in the gel did not yield satisfactory results. It was found that both LDAO and Deriphat solubilization and/or electrophoresis produced high levels of non-protein-associated pigment and streaking in the gel.

Polyacrylamide gel electrophoresis of SDS detergent extracts in 25% acrylamide preparative gels yielded four distinct pigmented bands after 2—3 h of electrophoresis (Fig. 1). The gel pattern was identical in all the brown algae examined and identical to that obtained for the diatom species. Recently, the same gel pattern has been obtained from photosynthetic membranes of *Chaetoceros* and *Asterionella* species (Perry, M.J. and Alberte, R.S., unpublished results). The slowest migrating zone (zone I) contained only Chl *a* and accounted for between 6 and 9% of the total chlorophyll (Table I). This gel zone stained for protein and possessed the same absorption and electrophoretic properties as complex I, the SDS-altered form of the P-700-Chl *a*-protein [1,7]. The second pigmented zone (zone II) on the gel column contained Chls *a* and *c* in a molar ratio of 1 : 2 (Table I); no fucoxanthin could be detected in hydrocarbon extracts. This component has characteristics similar to sucrose gradient or hydroxyapatite fractions of Triton-solubilized *Ecklonia radiata* lamellae, however, the Triton fractions contained fucoxanthin [3]. This zone stained for protein and accounted for between 21 and 24% of the total chlorophyll. The apparent molecular weight of the native complex was 35—40 kdaltons. Pigmented zone III accounted for between 36 and 42% of the total chlorophyll, stained for protein, contained no Chl *c*, and was the major location of fucoxanthin on the gel. The native complex had an apparent molecular weight of 22—27 kdaltons. Barrett and Anderson [3] found that the fucoxanthin-enriched component of *Ecklonia* accounted for approx. 40% of the total chlorophyll. Fucoxanthin was not quantified precisely because a reliable extinction coefficient for this carotenoid in the presence of other pigments is not available. From extracts we estimated the fucoxanthin/Chl *a* ratio to be approx. 4—6. Kirk [14] has obtained a fucoxanthin-enriched fraction from the brown alga *Hormosira* sp. though Chl *c* was also present in his preparation. The fastest migrating pigmented zone did not stain for protein, contained Chl *a* and traces of fucoxanthin, contained no detectable Chl *c*, and accounted for between 29 and 34% of the total chlorophyll. This zone was termed free pig-

TABLE II

DISTRIBUTION OF CHLOROPHYLL IN THE PHOTOSYNTHETIC UNIT OF *L. SACCHARINA*

Data are expressed as the number of molecules. Total chlorophyll/P-700 = 610 (an independent measurement of total Chlorophyll/P-700).

Component	% total	Chl *a*	Chl *c*	Chl (*a* + *c*)	Chl *a*/Chl *c*
Complex I	7	43	0	43	
Chl *a*/*c* complex	22	45	91	136	0.5
Chl *a*/fucoxanthin complex	40	244	0	244	
Free pigment	31	189	0	189	
Total	100	519	91	610	5.7

ment; pigment arising from detergent-induced dissociation from its native proteins [2,8].

The pigment distribution and composition of the pigment gel zones from *L. saccharina* are summarized in Table I. The relative proportion of pigment in gel zones for all the macrophytic brown algae were qualitatively identical though quantitative differences were seen since all tissue was field collected. Identical values were obtained from *Skeletonema* cells possessing a Chl *a*/Chl *c* ratio of 5.9. The recovery of Chl *c* in zone II (15% of the total chlorophyll) accounts for essentially all of the Chl *c* present in the starting material. A Chl *a*/Chl *c* ratio of 5.9 indicates that 17% of the total chlorophyll should be Chl *c*. These data confirm the sole location of Chl *c* in this gel zone. The total chlorophyll to P-700 ratio of membranes from this species was 610 (±10). Considering a photosynthetic unit size of 610, the percentages of total chlorophyll present in the respective gel zones can be converted to numbers of chlorophyll molecules and the distribution of chlorophyll in the photosynthetic unit can be examined as done previously for green plants [7] and dinoflagellates [6]. Gel zone I accounted for 7% of the chlorophyll which is equal to 43 mol Chl *a* (e.g., 7% of 610 = 43). This amount of chlorophyll is precisely what has been found to be associated with complex I in a wide variety of plants [7]. In zone II, 15% of the chlorophyll is Chl *c* and 7% is Chl *a* (Table II); therefore, this Chl *a*/*c* component accounts for 22% of the chlorophyll or 134 mol chlorophyll (43 mol

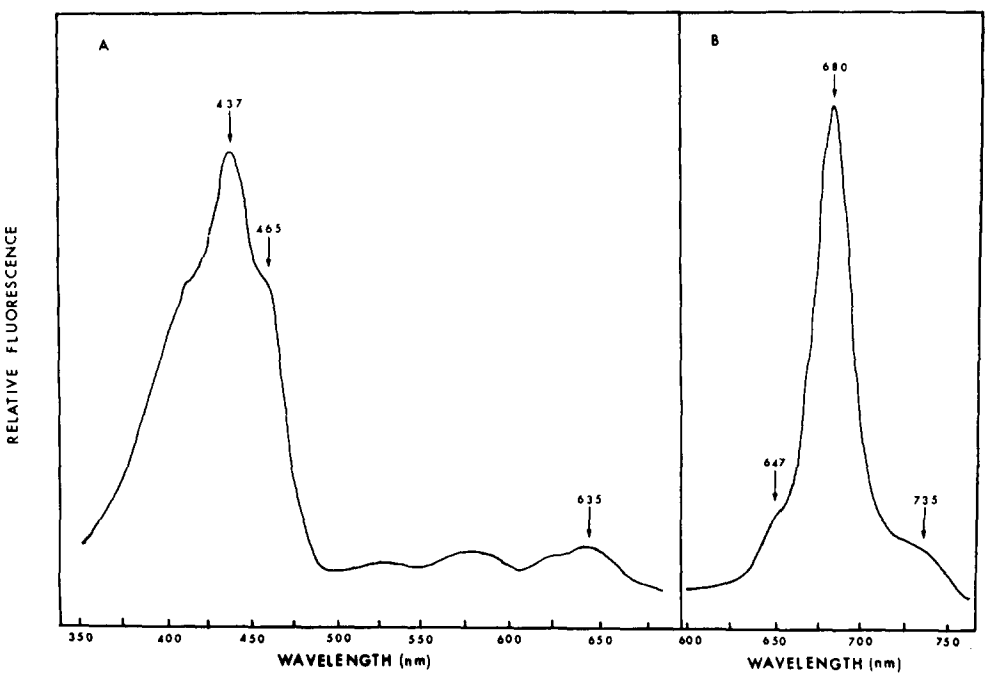

Fig. 2. Fluorescence excitation and emission spectra (273 K) of gel zone II of *L. saccharina*. The absorbance of the sample at the red peak was 0.22. (A) Fluorescence excitation spectrum for the 680 nm emission showing Soret bands for Chl *a* (437 nm) and Chl *c* (465 nm) and the red absorption band of Chl *c* (635 nm). (B) Fluorescence emission spectrum when exciting with 430—440 or 460—470 nm light showing a dominant Chl *a* emission at 680 nm, a shoulder at 647 nm attributable to Chl *c* and long-wavelength fluorescence at 735 nm.

Chl *a* and 91 mol Chl *c*). Zone III accounts for 40% of the chlorophyll, equalling 244 mol Chl *a*. The free pigment zone accounted for 31% of the total chlorophyll, equalling approx. 189 mol chlorophyll. Therefore, there are 519 mol Chl *a* and 91 mol Chl *c* in the photosynthetic unit. The calculated Chl *a*/Chl *c* ratio from the pigment distribution in the gel zones would be 5.7; the starting material had a Chl *a*/Chl *c* ratio of 5.9. These values agree quite well considering the errors in obtaining accurate Chl *a*/Chl *c* ratios. The amount of chlorophyll found as free pigment (31%, or 189 mol) is similar to the amounts measured in other studies (e.g., 180—210 mol Chl *a*/P-700). This chlorophyll has been designated as the Chl *a* which surrounds the reaction centers of Photosystems I and II and appears to be common to all Chl *a*-containing organisms (see Refs. 6 and 7).

Assuming that there are 4—5 fucoxanthins per Chl *a* in zone III, then the functional photosynthetic unit in *Laminaria* would contain 519 mol Chl *a*, 91 mol Chl *c*, and approx. 1200 mol fucoxanthin, yielding a size of about 1800 mol. Photosynthetic unit sizes in this range have been found in both red algae and gymnosperms (Refs. 1 and 20; Kursar, T., unpublished results).

The fluorescence analyses of the pigmented gel zones revealed that in zone II Chl *c* efficiently transferred light energy to Chl *a* (Fig. 2). When the Soret band of Chl *c* was excited (475 nm), an emission band at 680 nm, due to Chl *a*, was observed. A small shoulder at 647 nm was also observed in the emission indi-

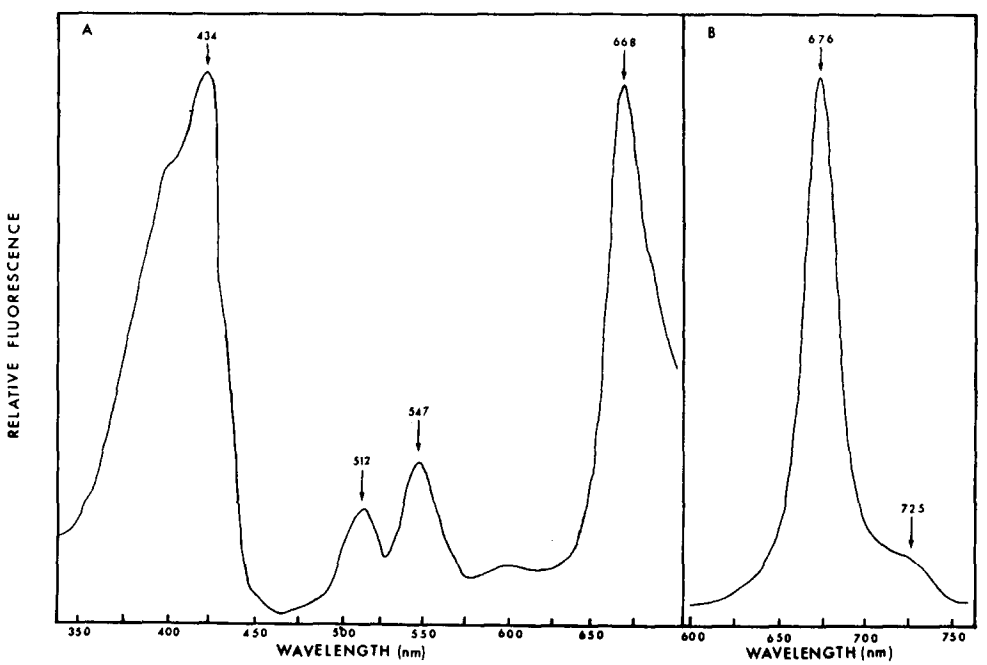

Fig. 3. Fluorescence excitation and emission spectra (273 K) of gel zone III of *L. saccharina*. The absorbance of sample at the red absorption peak was 0.31. (A) Fluorescence excitation spectrum for the 676 nm emission, showing a Chl *a* Soret band at 434 nm and bands at 512 and 547 nm attributable to fucoxanthin. The shoulder at approx. 445 nm is likely also attributable to fucoxanthin. (B) Fluorescence emission spectrum when exciting with 430—445 or 510—525 nm light showing Chl *a* emission at 676 nm and long-wavelength fluorescence at 725 nm.

cating that the transfer efficiency was less than 100%, giving rise to Chl *c* emission. The characteristic long-wavelength fluorescence emission band at 735 nm was also seen. Excitation across the Chl *c* Soret band (460—475 nm) yielded the same results. The excitation spectrum for the 680 nm emission (Chl *a*) (Fig. 2A) shows the Chl *a* Soret (437 nm), the Chl *c* Soret (465 nm), but no contribution from fucoxanthin, which would have absorption bands at 515 and 545 nm. These fluorescence properties are similar to those obtained in another brown alga [3]. Though not shown, the excitation spectrum for the 643—647 nm emission revealed primarily the Soret for Chl *c* (465 nm). These observations confirm the absence of fucoxanthin in this pigment-protein and demonstrate that in the isolated complex energy transfer occurs from Chl *c* to Chl *a*.

Zone III which contained fucoxanthin and Chl *a* showed a single emission peak at 676 nm, due to Chl *a*, when excited by light of 510 or 545 nm (those absorbed by fucoxanthin) (Fig. 3). No shoulder at 647 nm (Chl *c* emission) was observed though the long-wavelength fluorescence (725 nm) was still seen (Fig. 2B). The excitation spectrum for the 676 nm emission revealed the Soret for Chl *a*, no 465 nm Chl *c* Soret and bands at 512 and 547 nm attributable to fucoxanthin. These observations demonstrate that chlorophyll *c* is absent from this complex and that light energy absorbed by fucoxanthin is transferred to Chl *a* in the isolated complex. The fluorescence emission and excitation spectra for zones II and III were identical for the brown algae *Laminaria*, *Pylellia*, *Leathesia* and *Chorda* and for the diatom *Skeletonema*.

Discussion

Considering the position of Chl *c*- and fucoxanthin-containing species in marine primary productivity, it is surprising that so little is known about their pigment systems. Wassink and Kersten [21] and Tanada [22] demonstrated that fucoxanthin was as effective as Chl *a* in driving oxygen evolution and producing Chl *a* fluorescence. In fact, Tanada [22] demonstrated that the quantum yield of fucoxanthin was equal to that of Chl *a*. Goedheer [13] confirmed the roles of Chl *c* and fucoxanthin in contributing to Chl *a* fluorescence in brown algae and diatoms. More recently, Barrett and Anderson [4,5] and Kirk [14] have described Chl *c*- and fucoxanthin-enriched components from membranes of brown algae. In Triton X-100-solubilized membranes of a brown alga three main fractions were obtained by sucrose density centrifugation [4]. These included a P-700-Chl *a* complex, an orange-brown complex which had a Chl *a*/Chl c_2/fucoxanthin molar ratio of 2 : 1 : 2, and a green complex which had a Chl *a*/Chl c_1/Chl c_2/violaxanthin molar ratio of 8 : 1 : 1 : 1. The two latter complexes differed from those described here by their methods of preparation and isolation, and in their pigment composition and proportions. It is possible that our preparations represent smaller entities of those described by Barrett and Anderson [4] in as much as SDS is a far more vigorous detergent than Triton. In the present study we demonstrate in a distinct pigment-protein complex, separated by SDS-polyacrylamide gel electrophoresis, containing Chls *c* and *a* (2 : 1) and no fucoxanthin (gel zone II), that light energy absorbed by Chl *c* is transferred to Chl *a*, giving rise to Chl *a* fluorescence. The complex has an apparent molecular weight of 35 000—40 000, accounts for approx. 22% of

the total chlorophyll, and contains all of the chlorophyll *c* present in the photosynthetic lamellae. Jeffrey [23] has reported for brown algae and diatoms that Chl *c* ($c_1 + c_2$) usually accounts for approx. 20% of the total pigment. Holdsworth and Arshad [24] have described a metalo-pigment-protein complex containing Chl *c* isolated from *Pheaodactylum tricornutum*, a diatom, which they describe as analogous to complex II of green plants [2,8]. We assume that the analogy is only to the apparent molecular weight and/or electrophoretic migration behavior since complex II contains, in addition to Chl *a*, Chl *b* (see Ref. 2) which is not present in diatoms or brown algae. The complex from *Pheaodactylum* possessed Chl *a* and Chl *c* and fucoxanthin in a 2 : 1 : 1 molar ratio, and contained both copper and manganese. The authors suggest this complex is a component of Photosystem II; the relationship between this complex and those described here is unclear.

Analysis of the fluorescence properties of this Chl *a*/*c* complex indicates that energy transfer from Chl *c* to Chl *a* in the complex is tightly coupled as little emission from Chl *c* can be observed. The quantum yield for this transfer has yet to be measured. Because of the spectroscopic properties of this pigment-protein, we term the complex a light-harvesting Chl *a*/*c*-protein. Its occurrence in several species of brown algae and one diatom species examined here and in at least two others examined more recently (Perry, M.J. and Alberte, R.S., unpublished results) suggests that this complex may prove to be as ubiquitous among the diatoms and brown algae as is the light-harvesting Chl *a*/*b*-protein among green plants [2,7,8].

A brief report by Boczar et al. [25] has indicated the presence of a Chl *a*/*c* pigment-protein complex in dinoflagellates which contains 5—6 Chl *c* molecules per Chl *a*, in contrast to the 2 : 1 ratio observed here in diatoms and brown algae. These differences may arise due to generic characteristics or may be due to the fact that the dinoflagellates possess only Chl c_2 in contrast to the presence of both Chl c_1 and c_2 in the brown and diatomeous algae [23,26]. In species examined which contain both Chl *c* forms, both are present in approximately equal molar amounts [23]. It is possible that Chl c_1 and Chl c_2 are associated with Chl *a* on different polypeptide chains. Further, there may exist generic differences in the Chl c_2 polypeptide to account for the observed difference in Chl *a*/Chl *c* ratios of pigment-proteins from dinoflagellates, and brown algae and diatoms. In the present study we have not attempted to distinguish between Chl c_1 and Chl c_2 in our isolated complex as they are not readily distinguished by absorption spectrophotometry.

The pigment-protein containing Chl *a* and fucoxanthin (zone III) showed energy transfer from fucoxanthin to Chl *a*, though the efficiency of energy transferred cannot be assessed. From hydrocarbon pigment extracts of this gel zone we have estimated the fucoxanthin content to be 4—5 fucoxanthin molecules per Chl *a*. An accurate determination of the fucoxanthin content will require the calculation of reliable differential extinction coefficients of fucoxanthin in the presence of chlorophyll. This complex accounts for approx. 40% of the total chlorophyll in the diatoms and brown algae species examined. Preliminary results [27] have indicated that this complex is specifically lost in a diatom species in response to increased light intensity during growth and nitrogen limitation. Similar pigment changes have been observed in other diatom

and haptophyte species [27]. It is likely that this complex functions in a manner similar to that described for the peridinin-Chl *a*-protein of the dinoflagellates [10] or the Chl *a*/*b*-protein of green plants [7,8], which have been shown to vary in response to growth light intensity [11,28].

The apparent molecular weight of this pigment-protein is 22 000—27 000. It is most likely that Chl *a* and fucoxanthin are associated with the same polypeptide, as is the case for the peridinin-Chl *a*-protein. Such a situation would ensure and enhance the efficiency of energy transfer between fucoxanthin and Chl *a*. Based on the properties of this complex we have named this carotenochlorophyll-protein a light-harvesting Chl *a*/fucoxanthin-protein, and have found it to be indistinguishable in diatoms and brown algae.

Complex I (zone I) observed here was determined to be identical to complex I, the SDS-altered form of the P-700-chlorophyll *a*-protein, described in green plants, cyanobacteria, and other major algal groups [1—3,8]. This complex accounted for approximately the same amount of chlorophyll as that reported in other groups of plants [1,6,7]. The amount of pigment migrating in the non-protein zone, termed free pigment (see Refs. 1 and 5), was approximately the same as that described for other algae and higher plants, although recently, gel electrophoresis systems using Deriphat have succeeded in essentially eliminating non-protein-bound pigment [29]. Since this zone contained only Chl *a*, we attribute it to chlorophyll lost from light-harvesting Chl *a*-protein complex(es) closely associated with the reaction centers of Photosystems I and II, and Photosystem II reaction centers not resolved in our gel system (see Refs. 6—8).

The isolation of fucoxanthin and Chl *c* in separate and distinct Chl *a*-containing protein complexes indicates that in vivo it is unlikely that the majority of light energy absorbed by fucoxanthin is transferred to Chl *c*, even though these pigments have closer absorption bands than fucoxanthin and Chl *a*. We assume that light energy absorbed by fucoxanthin and Chl *c* is transferred first to the Chl *a* molecules associated with the respective complexes, and that energy transfer between the Chl *a*/fucoxanthin system and the Chl *a*/Chl *c* system occurs via Chl *a*. Ultimately, the bulk of this energy is transferred to the Chl *a* in the reaction center of Photosystem II to drive photosynthesis [13]. Based on these characteristics and the distribution of pigments in the different pigment-protein complexes, we propose that the Chl *a*/fucoxanthin complex is the most peripheral component of the photosynthetic unit occupying the same position proposed for the peridinin-Chl *a*-proteins of the dinoflagellates [6]. This notion is supported by the observations that (i) the Chl *a* emission band of the Chl *a*/fucoxanthin complex is at a shorter wavelength than Chl *a* in the Chl *a*/*c* complex and should be located furthest from the long-wavelength traps [30]; (ii) this complex is most readily removed or added under environmental stress analogous to peridinin-Chl *a*-protein [6,27], and (iii) it shares several features with peridinin-Chl *a*-protein in that it is a caroteno-chlorophyll-protein and it accounts for approximately the same amount of Chl *a* and carotenoid as peridinin-Chl *a*-protein. We envisage that in vivo this complex transfers the bulk of the light energy it absorbs to Chl *a* in the Chl *a*/*c*-protein. The Chl *a*/*c* component we place in close association with the Chl *a* surrounding the reaction centers of Photosystems I and II as proposed by Prézelin and Alberte [6]. In

this complex, light energy transferred from the Chl *a*/fucoxanthin complex or that absorbed directly by Chl *c* or Chl *a* in the complex would then be transferred primarily to the reaction center of Photosystem II.

Measurements of the ratio of total Chl (*a* + *c*) to P-700 in the *Laminaria* data presented are similar to those found in dinoflagellates [6] and to a number of diatom and haptophyte species [31]. Our preliminary observations of photosynthetic unit size determined by oxygen yield under short flashes revealed that *Laminaria* has approx. 600 chlorophylls per Photosystem II (assuming four electron transfers), a value identical to the chlorophyll/P-700 ratio obtained here. If we use the estimate of 4—5 fucoxanthin per Chl *a* in the Chl *a*/fucoxanthin-protein and the observed proportion of this complex present in photosynthetic lamellae (40%), we would estimate there are 1200 mol fucoxanthin per photosynthetic unit in addition to the 519 mol Chl *a* and 91 mol Chl *c* (see Table II). A similar calculation from the data of Prézelin and Alberte [6] for an estimate of the number of peridinin molecules associated with the photosynthetic unit in dinoflagellates would give approximately the same number. These calculations suggest that perhaps as many as 1800 chromophores function in the photosynthetic unit of these species. Such large pigment arrays may prove essential to the success of these marine species which are subjected to either large fluctuations in light environment due to vertical mixing in the water column [31] or to light-limited growth conditions due to their benthic distribution. A more complete understanding of the functional organization of light-harvesting pigment-proteins in such species may provide insight and molecular explanations for the evolutionary success of major photoplankton taxa and benthic algae in the oceans.

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References

1 Brown, J.S., Alberte, R.S. and Thornber, J.P. (1975) Proc. 3rd Int. Congr. Photosynth. Res. (1974) pp. 1951—1962
2 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127—158
3 Barrett, J. and Anderson, J.M. (1977) Plant Sci. Lett. 9, 275—283
4 Barrett, J. and Anderson, J.M. (1980) Biochim. Biophys. Acta 590, 309—323
5 Anderson, J.M. and Barrett, J. (1979) Ciba Found. Symp. 61, 81—104
6 Prézelin, B.B. and Alberte, R.S. (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 1837—1841
7 Thornber, J.P., Alberte, R.S., Hunter, F.A., Shiozawa, J.A. and Kan, K.-S. (1977) Brookhaven Symp. Biol. 28, 132—148

8 Thornber, J.P., Markwell, J.P. and Reinman, S. (1979) Photochem. Photobiol. 29, 1205—1216
9 Gantt, E. (1975) Bioscience 25, 781—785
10 Haxo, F.T., Kycia, J.H., Somers, G.F., Bennett, A. and Siegleman, H.W. (1976) Plant Physiol. 57, 297—303
11 Prézelin, B.B. and Haxo, F.T. (1976) Planta 128, 133—141
12 Duysens, L.N.M. (1952) Doctoral Thesis, University of Utrecht, The Netherlands
13 Goedheer, J.C. (1970) Photosynthetica 4, 97—106
14 Kirk, J.T.O. (1977) Plant Sci. Lett. 9, 373—380
15 Guillard, R.R.L. (1973) In Handbook of Phycological Methods (Stein, J.R., ed.), pp. 289—312, Cambridge University Press
16 Jeffrey, S.W. and Humprey, G.F. (1975) Biochem. Physiol. Pflanz 167, 191—194
17 Jeffrey, S.W. (1968) Biochim. Biophys. Acta 177, 456—467
18 Jeffrey, S.W. (1972) Biochim. Biophys. Acta 279, 15—33
19 Shiozawa, J.A., Alberte, R.S. and Thornber, J.P. (1974) Arch. Biochem. Biophys. 165, 388—397
20 Alberte, R.S., McClure, P.R. and Thornber, J.P. (1976) Plant Physiol. 58, 341—344
21 Wassink, E.C. and Kersten, J.A.H. (1946) Enzymologia 12, 3—32
22 Tanada, T. (1951) Am. J. Bot. 38, 267—283
23 Jeffrey, S.W. (1976) J. Phycol. 12, 349—354
24 Holdsworth, E.S. and Arshad, J.H. (1977) Arch. Biochem. Biophys. 183, 361—373
25 Boczar, B., Prézelin, B.B., Markwell, J.P. and Thornber, J.P. (1979) Photochem. Photobiol. 29, 80 (Abstr.)
26 Strain, H.H., Cope, B.T., McDonald, G.N., Svec, W.A. and Katz, J.J. (1971) Phytochemistry 10, 1109—1114
27 Gustafson, D.L., Friedman, A.L., Rudnick, M.S., Lyman, H. and Alberte, R.S. (1979) Biol. Bull. 157, 371 (Abstr.)
28 Prézelin, B.B. and Sweeney, B.S. (1978) Mar. Biol. 48, 27—35
29 Markwell, J.P., Thornber, J.P. and Boggs, R.T. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 1233—1235
30 Seely, G. (1973) J. Theor. Biol. 40, 189—199
31 Perry, M.J., Talbot, M.C. and Alberte, R.S. (1981) Mar. Biol., in the press