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# Chlorophyll proteins of the prymnesiophyte *Pavlova lutherii* (Droop) comb. nov.: identification of the major light-harvesting complex

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**A chlorophyll *ac*-fucoxanthin light-harvesting protein has been separated by SDS-polyacrylamide gel electrophoresis and by digitonin-sucrose density centrifugation from thylakoids of *Pavlova lutherii*. It contains a single major polypeptide of 21 kDa, comprises 69% of the total chlorophyll *a* and is enriched in chlorophyll *c* compared to the thylakoids. Energy transfer from chlorophyll *c* and fucoxanthin to chlorophyll *a* was demonstrated within the protein complex. Antibodies to the 21 kDa apoprotein showed cross-reactivity with the 26–28 kDa apoproteins of higher plant light-harvesting chlorophyll *a/b* protein and with the 19 kDa apoprotein of the light-harvesting complex of diatoms, but much reduced or no cross-reactivity with the major thylakoid polypeptides of dinoflagellates and cryptophytes.**

## Introduction

Oxygenic photosynthesis requires the operation of two light reactions connected in series. In all organisms studied so far the main Chl proteins associated with the core of these two photosystems seem to be conserved. In contrast, a wide variety of light-harvesting pigment proteins has been evolved, of which the best characterised are the phycobiliproteins of Cyanophyta, Rhodophyta and Cryptophyta and the light-harvesting Chl *a/b* protein (LHC) of Chlorophyta and higher plants. For these proteins, sequences are known, as are some of the details of molecular aggregation on and within the thylakoid [1,2]. The Chromophyta, a diverse group of algae all containing Chl $c_2$, have light-harvesting Chl *ac* complexes, but little is known of their molecular structure. The best characterised are the water-soluble peridinin Chl *a* protein of dinoflagellates [3,4] and the fucoxanthin-Chl *c*-Chl *a* proteins of diatoms [5–10]. Partial purifications of light-harvesting Chl *ac* proteins have also been reported from brown algae [11], dinoflagellates [12] and cryptophytes [13], and indicates considerable heterogeneity in pigment ratios, size of complex and polypeptide composition. Our lack of knowledge stems from the paucity of workers in the field, the intractable nature of many of the more abundant examples of chromophyte algae, and the somewhat unpredictable solubilisation characteristics of their thylakoids with commonly used detergents.

We selected for study the alga *Pavlova lutherii*

Abbreviations: Chl, chlorophyll; PS I, Photosystem I; PS II, Photosystem II; LHC, light-harvesting chlorophyll *a/b* protein; PBS, phosphate-buffered saline.

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(Droop) comb. nov. which can be readily cultured and for which basic ultrastructural information is available [14]. The Prymnesiophyta were separated from the Chrysophyta largely on structural grounds [15], but their pigment composition suggests close affinities with the Phaeophyta and Bacillariophyta. Thus it was thought that a study of the light-harvesting complexes of *P. lutheri* would be of inherent interest and might show affinities with the Chl *ac*-fucoxanthin light-harvesting complexes of Phaeophyta. A preliminary account of some of this work has already been given [16].

## Materials and Methods

*Palova lutherii* (CSIRO Fisheries Collection, Hobart, No. CS-23) was grown axenically in Provasoli's enriched seawater [17] at 18° C at a light intensity of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ using fluorescent lights (Mazda 'universal white'). Cells were harvested in late log phase by centrifugation at $2000 \times g$ for 10 min. The cells were washed in 0.6 M sorbitol, 50 mM tricine, 20 mM KCl (pH 7.5), resuspended in the same medium and broken by a single passage at 4° C through a French pressure cell at 82750 MPa. Cell debris and unbroken cells were removed by centrifugation at $2000 \times g$ for 10 min. Thylakoids and thylakoid fragments were obtained by centrifugation successively at $10000 \times g$ and $30000 \times g$ for 10 min each.

*SDS polyacrylamide separation of Chl protein complexes.* Thylakoid fragments were washed twice in 0.1 M Tris-acetate (pH 9.2) and resuspended at a final concentration of 250 $\mu$g Chl *a* per ml. SDS was added to a final SDS/Chl *a* ratio of either 30:1 or 40:1 (w/v). After centrifigation at $35000 \times g$ for 15 min the supernatant was brought to 5% (w/v) sucrose and applied to cylindrical polyacrylamide gels (0.5 cm diameter; 6% polyacrylamide) at a loading of 2.5–6.0 $\mu$g Chl *a* per gel. Gels were electrophoresed at 2 mA/gel for 5 min, until all the Chl had entered the gel, and then at 6 mA/gel for 15 min. All operations were carried out at 4° C. The proportion of Chl *a* in each of the complexes was estimated from densitometer scans at 570 nm [18].

*Digitonin separation of Chl-protein complexes.* Thylakoid fragments were washed twice in 50 mM Tricine, 20 mM KCl (pH 7.5) and resuspended at a final concentration of 150 $\mu$g/ml. Digitonin was added to give 1% (w/v) final concentration and the sample was incubated with constant stirring for 3 h at 4° C. After centrifugation for 30 min at $35000 \times g$ the supernatant was applied to a linear 10–14% sucrose gradient, made up in 50 mM Tricine, 20 mM KCl (pH 7.5), containing 0.1% (w/v) digitonin. The gradients were centrifuged for 16 h at $144000 \times g$ in a Beckman SW41 rotor. Samples were removed from the gradient for further analysis as described previously [13].

*Separation of polypeptides by SDS polyacrylamide gel electrophoresis.* The method of Laemmli [19] was used with a 12% acrylamide separating gel. Molecular masses were determined by means of the following protein markers: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and $\alpha$-lactalbumin (14.4 kDa).

*Pigment and spectroscopic determinations.* Chls *a* and *c* were determined in 90% acetone using the equations of Jeffrey and Humphrey [20]. Absorbance spectra were recorded on a Philips SP 8-200 spectrophotometer. Fluorescence spectra were recorded with a Perkin Elmer MPF 44B fluorescence spectrophotometer equipped with a low-temperature attachment. Spectra were recorded in the ratio mode. Excitation spectra were corrected by factors derived from reference to Chl *a* purified by HPLC: emission spectra were not corrected. P-700 was determined from the light-induced absorbance change at 700 nm (730 nm reference wavelength) measured on a Aminco Chance dual-wavelength spectrophotometer. Samples contained 1 mM sodium ascorbate and 1 mM DCMU. An $E_{700\,nm}$ of 64 $mmol^{-1} \cdot cm^{-1}$ was used [21,22].

*Preparation of polyclonal antibodies.* The light-harvesting complex obtained by sucrose gradient centrifugation was re-isolated after concentration on an identical gradient. It was transferred to electrophoresis buffer by passage down a PD10 column of Sephadex G25. After concentration by centrifugation in an Amicon CF25 Centriflow cone, it was electrophoresed as described above. The band at 21 kDa was excised and the gel ground in a mortar. Protein was

extracted by three portions of 0.15 M NaCl, 10 mM sodium phosphate (pH 7.0) containing 0.1% SDS. Pooled extracts from many gels were combined, concentrated, emulsified with Freund's complete adjuvant and injected intramuscularly into a New Zealand white rabbit. 250 μg of antigen was injected initially followed by a second injection of 150 μg (incomplete adjuvant) after 6 weeks.

*Western blotting of polypeptides.* Polypeptides separated by electrophoresis on 12% acrylamide gels were transferred electrophoretically to nitrocellulose paper (Schleicher and Schull) using the method described by Khyse-Andersen [23]. After transfer, the membrane was washed with phosphate-buffered saline (PBS), then free protein-binding sites were blocked by treatment with 2% (w/v) dried milk powder in PBS followed by a second wash, and then incubated for 2 h with agitation with antiserum to the 21 kDa band prepared as above or preimmune serum, diluted 1:200 in the blocking buffer. The blots were washed, then incubated with 1 : 1000 dilution of goat anti-rabbit IgG (H + L) horseradish peroxidase conjugate (Bio-Rad) in blocking buffer for 1 h and then washed again. Reacted bands were stained by the method of Hawkes et al. [24] using 4-chloro-1-naphthol (Sigma) and hydrogen peroxide as substrates.

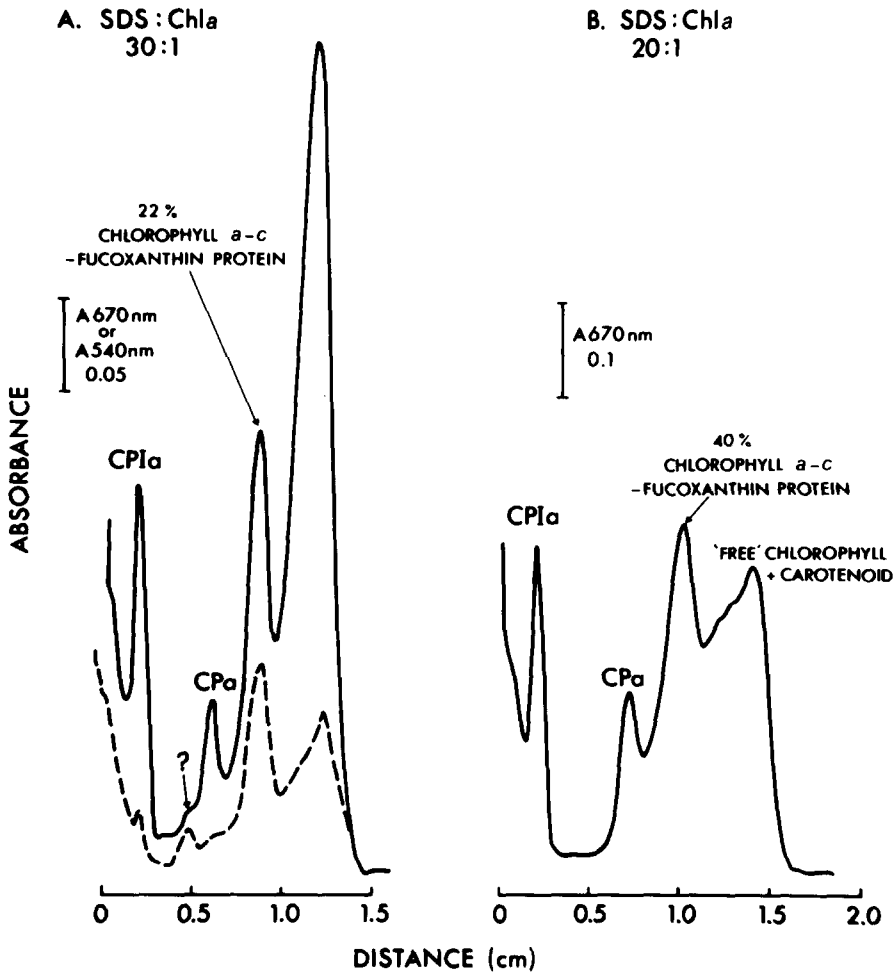


Fig. 1. Densitometer tracings of the Chl-protein complexes of *P. lutherii* separated by SDS-polyacrylamide gel electrophoresis. (A) SDS/Chl *a* of 30:1 (w/w); (B) SDS/Chl *a* of 20:1 (w/w). ———, 670 nm; ------, 540 nm.

## Results

To achieve solubilisation of *P. lutherii* thylakoids it proved necessary to use approx. 3-fold higher ratios of SDS to Chl than are effective on higher plant thylakoids. When SDS extracts (SDS/Chl *a* ratio, (30:1) were analysed by SDS polyacrylamide electrophoresis four distinct bands containing Chl were observed together with a minor shoulder (Fig. 1a). Bands denoted CP1a and CPa were bright green and were assigned to Photosystems I and II, respectively, by their absorbance and fluorescence spectra and mobility relative to the bands obtained on electrophoresis of higher-plant extracts. The band denoted as Chl *ac*-fucoxanthin protein was green at its upper edge and brown at this lower. A rescan of the gel at 540 nm, the long-wavelength absorption band of fucoxanthin in vivo, demonstrated that the minor shoulder in front of CPa may also be a Chl-fucoxanthin protein. Reduction of the ratio of SDS/Chl *a* to 20:1 during solubilisation of the thylakoids resulted in an increase in the proportion of total Chl *a* in the Chl *ac*-fucoxanthin complex from 22% to 40% although the resolution was poor (Fig. 1b). Absorbance spectra for the Chl *ac*-fucoxanthin protein and the thylakoids from which it was obtained are shown in Fig. 2. Compared to the thylakoids the Chl *ac*-fucoxanthin protein is enriched in pigments absorbing at 460 nm (Chl *c* and carotenoid), 490 nm (carotenoid) and 636 nm (CHl *c*). The ratio of Chl *a* to Chl *c* was variable (approx. 4), but this was a 2-fold enrichment over that of the thylakoids (approx. 8), suggesting that the complex could represent as much as 50% of the total chlorophyll *a*.

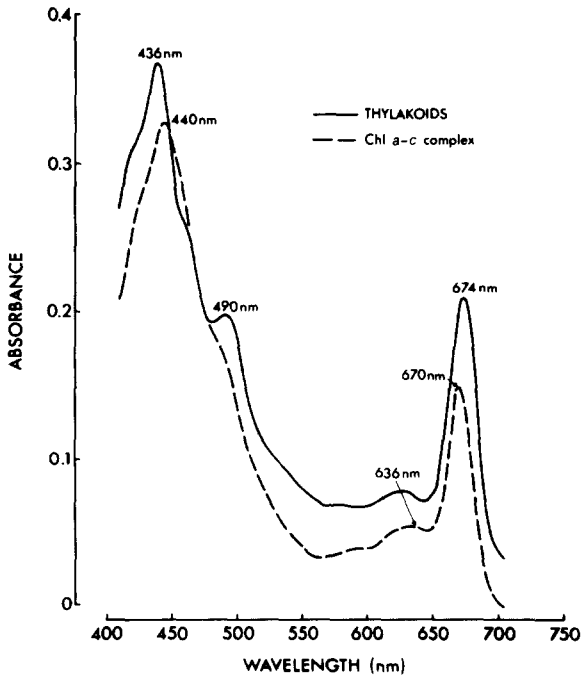


Fig. 2. Absorbance spectra of thylakoids and the Chl *ac*-fucoxanthin complex of *P. lutheri*. ———, thylakoids; ------, Chl *ac*-fucoxanthin complex.

Fluorescence emission at 77 K of *P. lutherii* cells (results not shown) and thylakoids was at 691 nm whether the excitation wavelength was 435 nm or 460 nm, whereas that of the Chl *ac*-fucoxanthin was at 676 nm, when excited at 435 nm (Fig. 3a and b). When the Chl *ac* fucoxanthin complex was excited at 460 nm there was only a minor emission from Chl *c* at 640 nm; however, when the complex in the gel was heated to 60 °C prior to freezing in liquid nitrogen, excitation at 460 nm produced the major emission from Chl *c* at 640 nm and there was only a minor emission from Chl *a* (Fig. 3b), due to destruction of energy transfer. The fluorescence excitation spectra of both thylakoids (Fig. 3c) and the Chl *ac*-fucoxanthin complex (Fig. 3d) demonstrate the involvement of both Chl *c* and carotenoids in transferring energy to Chl *a*. Absorption bands at 512 nm and 540 nm are ascribed to fucoxanthine, since these bands are red-shifted by about 40 nm compared to the

TABLE I

PROPERTIES OF FRACTIONS OF *PAVLOVA LUTHERII* THYLAKOIDS SOLUBILISED WITH DIGITONIN AND SEPARATED BY SUCROSE DENSITY GRADIENT CENTRIFUGATION

A, B and C refer to light, medium and heavy fractions as shown in Fig. 4. n.d.; not detectable. Results are presented as mean ± standard deviation ($n = 5$).

| Fraction | % of total Chl *a* | Chl *a* / Chl *c* | Chl *a* / P-700 | $A_{max}$ (nm) |
|---|---|---|---|---|
| Thylakoids | 100 | 7.47 ± 0.41 | 726 ± 38 | 674 |
| A | 67.9 ± 6.0 | 4.66 ± 0.25 | n.d. | 670 |
| B | 18.6 ± 5.2 | 9.13 ± 0.51 | 1093 ± 189 | 673 |
| C | 13.5 ± 2.1 | >12 | 171 ± 18 | 677 |

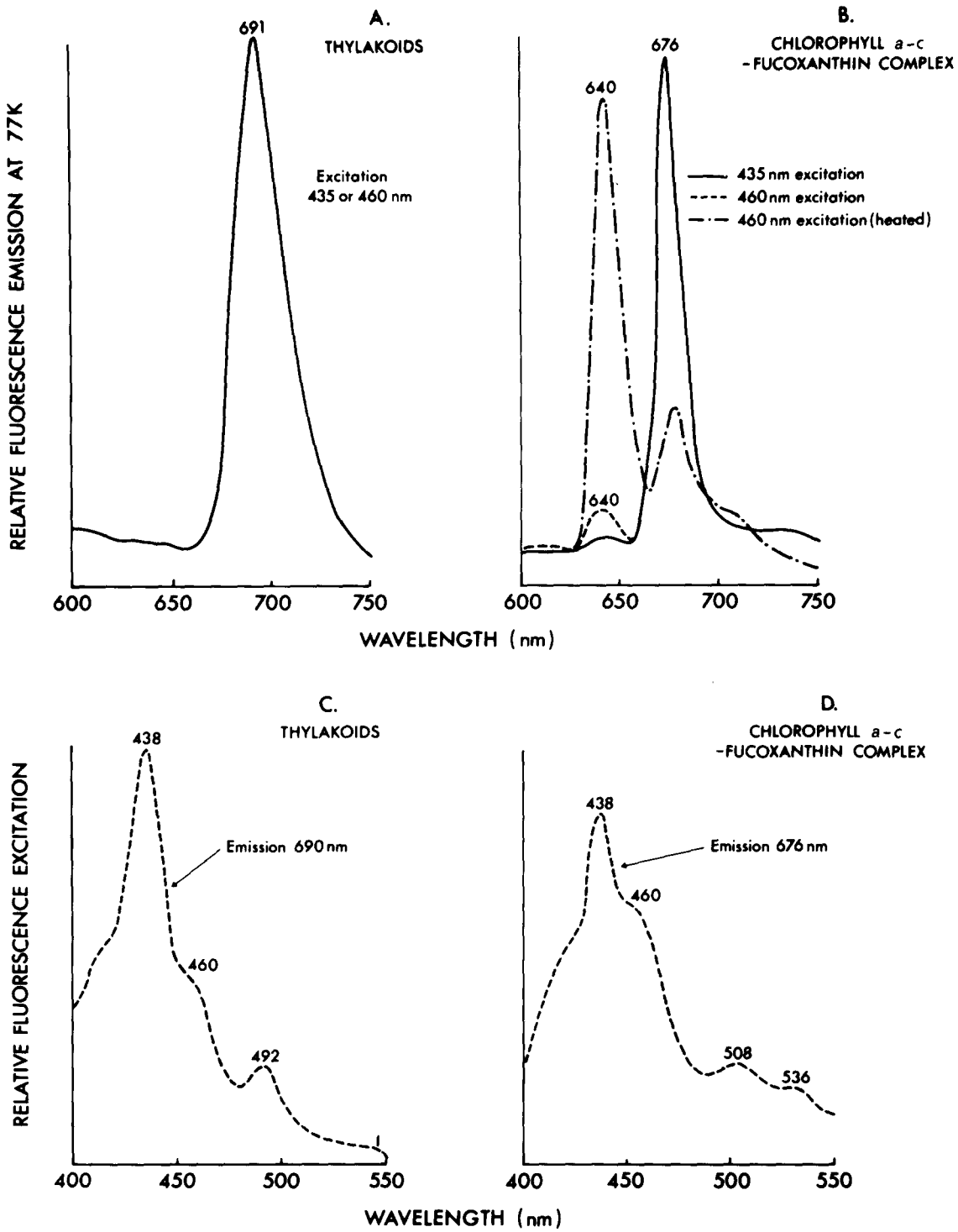


Fig. 3. Fluorescence emission and excitation spectra at 77 K of thylakoids and the Chl *ac*-fucoxanthin complex of *P. lutherii*. (A) Emission spectrum for the thylakoids; (B) emission spectrum for Chl *ac*-fucoxanthin complex; (C) excitation spectrum for thylakoids (the emission spectrum for 460 nm excitation is identical to that for 430 nm at wavelengths (longer than 660 nm); (D) excitation spectrum for Chl *ac*-fucoxanthin complex.

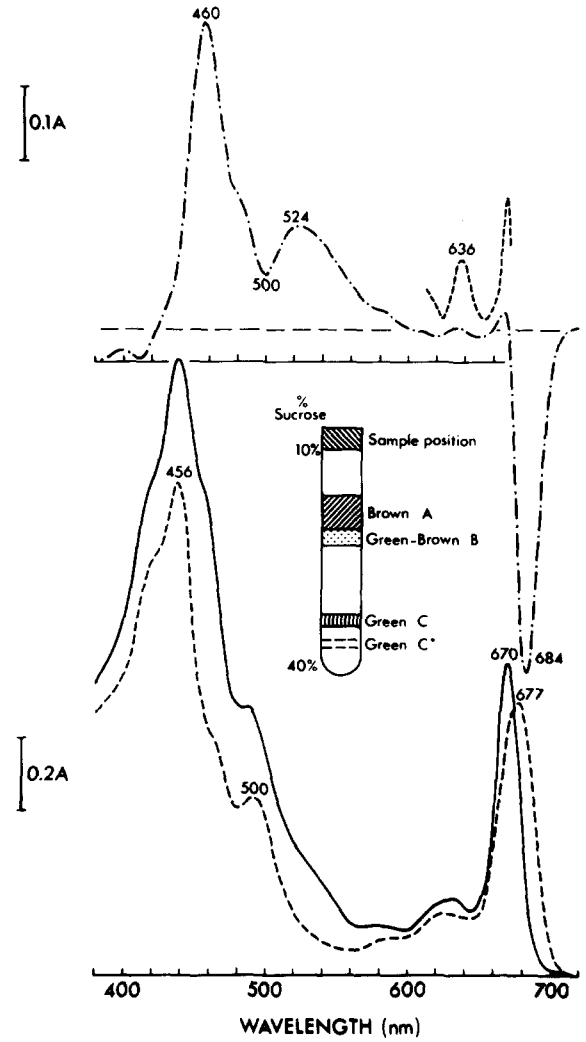


Fig. 4. Diagram of separation of pigment-protein complexes of *P. lutherii* by sucrose gradient centrifugation together with absorbance spectra of zones A and C and the difference between them. ———, zone A; ------, zone C; – · – · –, zone A minus zone C normalised at 670 nm (red portion of spectrum enlarged ×5).

free pigment when it is conjugated with protein [24,25].

Isolation of Chl-protein complexes by means of SDS-polyacrylamide gels often results in their partial destruction as well as isolating forms at or near their minimum relative molecular weight. For these reasons we solubilised thylakoids in digitonin in an attempt to obtain a larger yield of the light-harvesting complex in an aggregated form. Samples of thylakoids solubilised in digitonin were separated into different components by centrifugation through a linear sucrose gradient. This separated three principle coloured zones designated A, B and C as shown in Fig. 4. A minor component C* was often present. The properties of the bands are listed in Table I. Zone A, which was brown in colour, had about 68% of the total Chl *a*, lacked P-700, was enriched in Chl *c* compared to the starting material, and contains the major light-harvesting pigment protein. Zone B was brownish-green and was probably a mixture of the light-harvesting Chl *ac* complex and PS II complex with smaller amounts of PS I complex. Zone C was green, contained little Chl *c*, some carotenoid absorbing at 500 nm, and was enriched in P-700 and in long-wavelength forms of Chl *a*, indicating the presence of PS I protein complex (Table I and Fig. 4). Zone C* has not been studied in detail, but its properties seemed to be very similar to those of zone C.

Room-temperature fluorescence spectra of zone

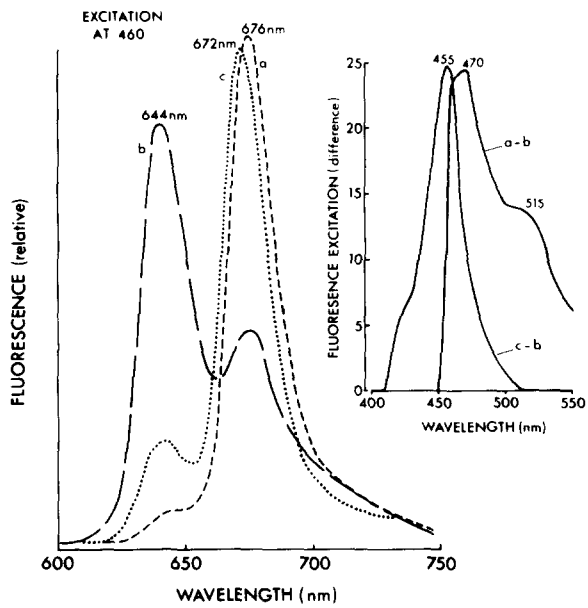


Fig. 5. Fluorescence emission spectra at room temperature of the light-harvesting Chl ac-fucoxanthin complex (zone A of Fig. 4) and the effect of Triton X-100 and its subsequent removal. (a) ------, zone A; (b) – – – –, zone A plus 0.05% Triton X-100; (c) · · · · · ·, as for (b) plus 20 min exposure to Biobeads. Inset: fluorescence excitation difference spectra measured at 675 nm; a−c, original zone A (a, above) minuis Biobead treatment (c, above); c−b, Biobead treatment (c, above) minus Triton X-100 treatment (b, above).

A showed that there was energy transfer from carotenoids and Chl *c* to Chl *a*. Excitation with 460 nm light resulted in a principal emission at 676 nm with a minor peak at 644 nm. On addition of 0.05% (w/v) Triton X-100 the relative magnitudes were reversed, the 644 nm emission due to Chl *c* predominating. Removal of Triton X-100, by incubating the sample with Biobeads (Bio-Rad) for 20 min restored energy transfer from Chl *c* to Chl *a* (Fig. 5). Analysis of the excitation spectra for the effect of Triton X-100 on the energy transfer within the light-harvesting complex indicated that although Triton destroys the energy transfer from both carotenoids and Chl *a* to Chl *a* removal of Triton only restores energy transfer from Chl *c* (Fig. 5, inset). At 77 K, zone C had two main fluorescence emission peaks at 684 nm and 708 nm, although the overall fluorescence was extremely low. The excitation spectrum showed a strong contribution from a carotenoid absorbing at 500 nm. After heating with SDS, much increased emission at 640 nm due to Chl *c* was observed.

*Polypeptide analysis*

SDS polyacrylamide gel electrophoresis of the digitonin-solubilised thylakoid preparations gave rise to many bands after staining with Coomassie blue, with one 21 kDa being particularly prominent (Fig. 6). This band was particularly prominent in zone A from the sucrose gradient and much reduced in zone C which also contained a peptide of 67 kDa, probably that of the main intrinsic polypeptide of PS I (Fig. 6). Zone B (results not shown) contained small amounts of both 21 and 67 kDa polypeptides together with several prominent bands in the range 43–52 kDa which were tentatively attributed to PS II. When the Chl *ac*-fucoxanthin protein, isolated on non-denaturing polyacrylamide gels, was extracted and rerun under denaturing conditions it also gave a principal polypeptide of 21 kDa.

*Western blotting of polypeptides*

Western blotting was used to check antiserum raised to the 21 kDa polypeptide of the Chl *ac*-fucoxanthin complex for cross-reactivity to thylakoid proteins from a range of algae including *Phaeodactylum* and one from higher plants

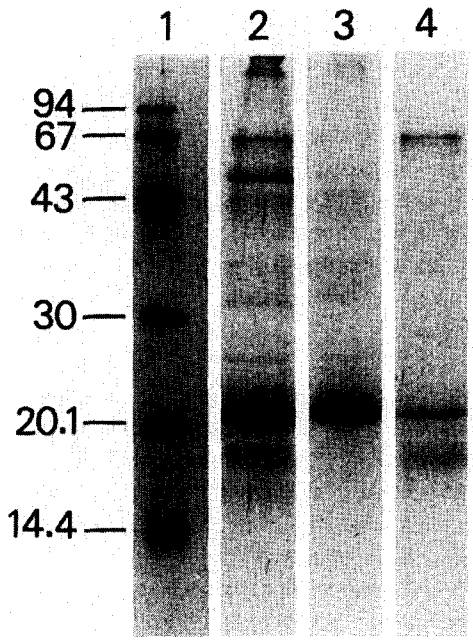


Fig. 6. Polypeptides of *P. lutherii* thylakoid preparations separated by SDS polyacrylamide gel electrophoresis under fully denaturing conditions and stained with Coomassie Blue. Lane 1, protein standards; lane 2, thylakoids solubilised in digitonin; lanes 3 and 4, zones A and C of sucrose gradient centrifugation.

(spinach) (Fig. 7). None of the other algae nor the spinach thylakoids had a 21 kDa polypeptide which reacted with the antibody to the 21 kDa

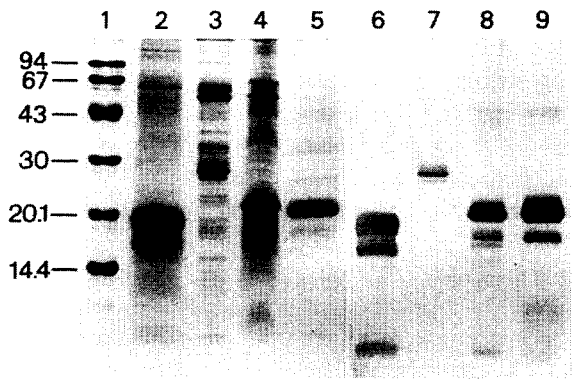


Fig. 7. Cross-reactivity of antibody raised to the 21 kDa polypeptide of the light-harvesting Chl *ac*-fucoxanthin protein of *P. lutherii*. Lanes 1–5 stained with Coomassie Blue; lanes 6–9 Western blotted. Lane 1, standard proteins, lanes 2 and 6, *Phaeodactylym* thylakoids; lanes 3 and 7, spinach thylakoids; lanes 4 and 8, *Pavlova lutherii* thylakoids; lanes 5 and 9, Chl *ac*-fucoxanthin complex.

polypeptide of *P. Lutherii*. However, *Phaeodactylum* had polypeptides in the 18–19 kDa region which cross-reacted. The polypeptides at 26–28 kDa of the light-harvesting Chl *a/b* binding protein of spinach cross-reacted significantly (Fig. 7). The light-harvesting polypeptides from *Amphidinium carterae* (Dinophyta) reacted very weakly and there was no reaction with those of *Chroomonas* sp (Cryptophyta) (results not shown).

## Discussion

Although previous workers have emphasised the difficulties in solubilising chromophyte algal thylakoids using those detergents which have proved successful with higher plants [25,26], in *P. lutherii* this was not a problem. Both SDS and digitonin were effective; the former at 2–3-fold the levels required for spinach thylakoids. The SDS-solubilised thylakoids contained a Chl *ac*-fucoxanthin protein complex which had an electrophoretic mobility greater than that of $LHCP_3$ from higher plants on non-denaturing SDS polyacrylamide gels and a single polypeptide of 21 kDa on denaturing gels. We conclude from this evidence, and from the absorbance and fluorescence spectra, that the monomer form of the complex can bind Chl *a*, Chl *c* and fucoxanthin and retain energy transfer between all three chromophores. The form of this pigment-protein separated by means of digitonin and sucrose gradient centrifugation is clearly an aggregate of many such monomers and this probably more nearly represents its condition in the thylakoids. We estimate the complex to contain about 70% of the total Chl *a* and most of the Chl *c* and fucoxanthin of the thylakoids. It is thus very comparable to the light-harvesting complex of diatoms [6]. Although only 33% of the Chl *a* was recovered from sucrose gradients in the latter work, a considerable proportion of Chl *a* was unaccounted for and the enrichment of Chl *c* indicated that 63% of total Chl *a* is in the light-harvesting complex. Gugliemelli et al. [5] independently isolated the light-harvesting complex of *Phaeodactylum* and found it to contain 76% of the total Chl *a*.

The relative molecular weights of all the apoproteins of Chl *ac*-carotenoid complexes as determined by mobility on denaturing SDS polyacrylamide gel lie in the region of 20 kDa [6–9]; however, the differences between them seen to be real, since the polypeptide of light-harvesting complexes of *Pavlova lutherii* (Prymnesiophyta), *Chroomonas* sp. (Cryptophyta), *Amphidinium carterae* (Pyrrhophyta) and *Phaeodactylum tricornutum* (Bacillariophyta) migrated at different rates under identical conditions in our hands (results not shown). Furthermore, cross-reactivity of antibodies to the 21 kDa polypeptide of *P. lutherii* was reduced or absent with the major polypeptidesof the Chl *ac*-carotenoproteins of these organisms, with the exception of *P. tricornutum*, despite the fact that cross-reactivity occurred with the 26–28 kDa polypeptides of LHCP from a higher plant.

In Cyanophyta and Rhodophyta as well as in higher plants [27], the major light-harvesting systems transfer energy preferentially to PS II. There are indications that this may not be so in *P. lutherii*. We always found some of the 21 kDa Chl *ac*-fucoxanthin apoprotein to be associated with PS I in zones B and C from the sucrose gradient separation, and heating this complex resulted in a considerable increase in fluorescence from Chl *c*. In addition, the excitation spectra at 77 K for the thylakoids shows little evidence of the presence of the Chl *ac*-fucoxanthin complex believed to make up 70% of the total Chl. This could be explained if half the complex were connected directly to PS I and its fluorescence quenched as indicated by the lack of a separate long-wavelength PS I fluorescence.

Neither this study nor that of Friedman and Alberta [6] attempted to distinguish between Chl $c_1$ and Chl $c_2$. Barrett and Anderson [28] solubilised brown algal thylakoids in Triton X-100 and separated three pigment-protein complexes by sucrose density centrifugation and hydroxyapatite chromatography. These results suggested that the Chl $c_2$ may be preferentially associated with fucoxanthin whereas both Chl $c_1$ and Chl $c_2$ were associated with violaxanthin. In a subsequent study [29] in which the light-harvesting complexes were further treated with detergents, Barrett suggested that Chl *a*, Chl $c_2$ and fucoxanthin may be bound to separate polypeptides of differing molecular weights.

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## References

1 Glazer, A.N. (1984) Biochim. Biophys. Acta 768, 29–51.
2 Thornber, J.P. (1986) Encycl. Plant Physiol. (N.S.) 19, 98–142.
3 Siegelman, H.W., Kycia, J.H. and Haxo, F.T. (1976) Brookhaven Symp. Biol. 28, 162–169.
4 Song, P.-S., Koka, P., Prezelin, B.B. and Haxo, H.T. (1976) Biochemistry 15, 4422–4427.
5 Gugliemelli, L.A., Dutton, H.J., Jursinic, P.A. and Siegelman, H.W. (1981) Photochem. Photobiol. 33, 903–907.
6 Friedman, A.L. and Alberte, R.S. (1984) Plant Physiol. 76, 483–489.
8 Gugliemelli, L.A. (1984) Biochim. Biophys. Acta 766, 45–50.
8 Fawley, M.W. and Grossman, A.R. (1986) Plant Physiol. 81, 149–155.
9 Friedman, A.L. and Alberte, R.S. (1986) Plant Physiol. 80, 43–51.
10 Owens, T.G. and Wold, E.R. (1986) Plant Physiol. 80, 732–738.
11 Barrett, J. and Anderson, J.M. (1980) Biochim. Biophys. Acta 540, 309–323.
12 Baczar, B.A., Prezelin, B.B., Markwell, J.P. and Thornber, J.P. (1980) FEBS Lett. 120, 243–247.
13 Ingram, K. and Hiller, R.G. (1983) Biochim. Biophys. Acta 772, 310–319.
14 Green, J.C. (1975) J. Mar. Biol. Ass. U.K. 55, 785–793.
15 Hibberd, D.J. (1976) Bot. J. Linn. Soc. 72, 55–80.
16 Hiller, R.G. and Larkum, A.W.D. (1983) Abstract of the Workshop on Molecular Structure and Function of Light-Harvesting Pigment Protein Complexes and Photosynthetic Reaction Centres (Zürich, July 23–28, 1987).
17 Provasoli, L. (1963) Proceedings of the Fourth International Seaweed Symposium (De Virville, D. and Feldmann, J., eds.), pp. 9–17, Pergamon Press, Oxford.
18 Genge, S., Pilger, D. and Hiller, R.G. (1974) Biochim. Biophys. Acta 346, 22–30.
19 Laemmli, U.K. (1970) Nature 227, 680–685.
20 Jeffrey, S.W. and Humphrey, G.D. (1975) Biochim. Physiol. Pflanzen 167, 191–194.
21 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171.
22 Alberte, R.S., McClure, P.R. and Thornber, J.P. (1976) Plant Physiol. 58, 341–344.
23 Kyhse-Andersen, J. (1984) Biochem. Biophys. Methods 10, 203–209.
24 Hawkes, R., Niday, E. and Gordon, J. (1982) Anal. Biochem. 119, 142–47.
25 Goedheer, J.C. (1970) Photosynthetica 4, 97–106.
26 Kirk, J.T.O. (1977) Plant Sci. Lett. 9, 373–380.
27 Larkum, A.W.D. and Barrett, J. (1983) Adv. Bot. Res. 10, 1–219.
28 Barrett, J. and Anderson, J.M. (1977) Plant Sci. Lett. 9, 275–283.
29 Barrett, J. and Thorne, S.W. (1981) Photosynthesis (Proceedings of the Fifth International Photosynthesis Congress) (Akoyonoglou, G., ed.), Vol. 3, pp. 347–356, Balaban Science Services, Philadelphia, PA.