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Light-harvesting complexes of brown algae

Biochemical characterization and immunological relationships

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The pigment composition of the light-harvesting complexes isolated from several brown algae belonging to different orders has been analysed by reverse-phase HPLC. Relative to whole chloroplasts, they were markedly enriched in Chl *c*, fucoxanthin and violaxanthin and conversely depleted in Chl *a*. The relative molar proportions of the 4 main pigments (Chl *a*/Chl *c*/fucoxanthin/violaxanthin) ranged from 100:18:76:6 to 100:30:107:17. The protein moiety of LH complexes of all the species studied were composed of one or two main polypeptide components in the range of 19–22 kDa. These polypeptide subunits were arranged in polymeric particles about 240 kDa in *Laminaria saccharina*. A polyclonal antibody raised against the LH polypeptide of *Fucus serratus* has been tested on LH apoproteins of other Chromophytes and Chlorophytes. Phylogenic implications of these results are discussed.

Brown algae; Fucoxanthin; Light-harvesting complex; Polyclonal antibody

1. INTRODUCTION

In thylakoid membranes, pigments, electron carriers and proteins are conponents of several well-defined complexes. By contrast with the highly conserved reaction centers, the outer antennae light-harvesting complexes exhibit a great diversity of pigments and associated proteins. Amongst the photosynthetic eucaryotes, 3 main different types are recognized: (i) the extrinsic antenna of Rhodophytes, the phycobilisome, a highly ordered heterogeneous structure mainly comprising phycobilin proteins, (ii) the LH complexes (frequently termed LHCP) of Chlorophytes (green plants and green algae) which are intrinsic protein complexes associated with Chl *a* and *b* and xanthophylls, mainly lutein, and (iii) the LH complexes of Chromophytes, which are also intrinsic, but contain very abundant special xanthophylls (fucoxanthin, peridinin, diadinoxanthin...) along with Chl *a* and Chl *c*. The first two, the phycobilisome and the LHCP, have been extensively studied (for review see [1,2]). In contrast, the knowledge on LH complexes of Chromophytes lags far behind. Furthermore, almost all the published papers deal with unicellular phytoplanktonic species of different groups (for review see [3]) and few are concerned with the macrophytic brown seaweeds, i.e. *Phaeophyceae* [4–11] although these kelps play a major role in intertidal primary production. In fact, the isolation of well-preserved pigment-protein complexes from brown algae has proven to be difficult, due to their polysaccharide walls and the great lability of the pigment-protein link in their chloroplasts [12].

In two previous papers [7,9] we described the purification, by density gradient centrifugation after deriphat or digitonin treatment, of well-defined LH complexes of *Cystoseira mediterranea* and *Fucus serratus* and their characterization by spectroscopy. More recently, we have analysed the polypeptide composition of LH complexes from several species belonging to different orders of *Phaeophyceae* [10]. These LH fractions exhibited one or two major polypeptide components. Electrophoresis of the different samples clearly showed that the M_w of these polypeptides varied from 17 to 21 kDa according to the species (versus the 25–30 kDa range observed in green plants). These results have led us to ask two further questions: first, what are the relationships among the LH proteins of different orders of brown algae, and between those of brown algae and other Chromophytes? And secondly, although they differ noticeably in their molecular mass, is there a molecular analogy between the LH of brown algae and LHCP of green plants? In order to determine whether there is a molecular analogy in the polypeptidic composition of LH complexes from Chlorophytes and

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Abbreviations: LH, light-harvesting complex; LHCP, light-harvesting complex of higher plants; M_w, molecular mass; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; LiDS, lithium dodecyl sulphate

brown algae, an immunological study was initiated using a maiza LHCP polyclonal antibody. Although LH apoproteins of brown algae have noticeably lower M_w than higher plants, the maize LHCP antibody clearly recognized the different Chromophyte LH. This indicated that at least some peptidic sequences are common to Chromophyte and Chlorophyte LH proteins [10].

In the present work, we have studied the pigment and polypeptide composition of LH fractions from several species of brown algae, and have raised an antibody against the LH fraction of one of them, *Fucus serratus* (L.). Herein we report its immunological relationships with the LH complexes from other Chromophytes and from Chlorophytes.

2. MATERIALS AND METHODS

2.1. *Materials*

Thalli of 4 species of brown algae (*Fucus serratus* (L.), *Dictyota dichotoma* (Hudson) Lamouroux, *Laminaria saccharina* (L.) Lamouroux and *Pelvetia canaliculata* (L. Decsne and Thur) were collected at the sea shore near the Biological Station at Roscoff (France). An axenic strain of *Pylaiella littoralis* (L.) Kjellm was kindly provided by S. De Goër. Chloroplasts were prepared according to [13]. Diatom species (*Phaedactylum tricornutum* Bohlin, *Nitzschia acicularis* (Kützing) W. Smith from the algal culture collection of Laboratoire Arago (Banyuls, France) were cultivated according to [14]. *Giraudyopsis stellifer* Dangeard, from the culture collection of Caen University (France) was cultivated in Erdschreiber enriched sea-water.

2.2. *Isolation of LH fractions*

Chloroplasts of macrophytic brown algae, or cells of phytoplanktonic algae, were resuspended in a 10 mM Hepes-Na buffer (pH 7.4), with 2 mM $MgCl_2$, 2 mM $MnCl_2$, 10 mM KCl, 1 M sorbitol and protease inhibitors (1 mM benzamidine and 1 mM phenylmethanesulfonylfluoride). They were then disrupted in a French pressure cell (136 MPa) in the presence of digitonin, at a detergent/Chl ratio of 100, and incubated for one hour in the same medium. The homogenate was loaded on the top of a sucrose gradient (15–55%) and centrifuged at 140 000 × *g* during 15 h at 4°C.

2.3. *Gel electrophoresis*

The LH complexes obtained in the 15–20% fraction of the gradient were diluted by 0.5 M Tris-HCl buffer (pH 8.0) with the same protease inhibitors as above, then concentrated by centrifugation overnight at 200 000 × *g* and 4°C. The pellets were denatured for one hour at room temperature in 0.06 M Tris-HCl buffer, 5% LiDS and 1 M mercaptoethanol,. Samples were loaded on a 10–22% polyacrylamide gel gradient with 0.1% SDS pH 8.8 with the buffer system of Laemmli [15] and 0.1% SDS was added to the upper reservoir. Polypeptides were stained with Coomassie brilliant blue G250.

2.4. *Antibody preparation*

The *Fucus* LH fractions from the digitonin gradient were pelleted, resuspended in Tris-HCl buffer (pH 7.4) containing the zwitterionic detergent Deriphat 160, and then dialysed overnight against the same buffer before being loaded on a DEAE-cellulose (DE-52) column equilibrated with the same buffer. Proteins were eluted by increasing concentrations of NaCl. One main fraction, obtained with 0.75 M NaCl, was shown by LiDS-PAGE to contain only the 21-kDa polypeptide. After ultracentrifugation, it was resuspended in water, then mixed with Freund adjuvant (1 vol/1 vol) and injected into female New-Zealand rabbits at two week intervals (150 μg protein/injection). The final serum was collected 10 weeks after the first injection.

2.5. *Western blotting of polypeptides*

Polypeptide fractions were separated by LiDS-PAGE in a 9–18% acrylamide gradient. After electrophoresis, the proteins were electrophoretically transferred to nitrocellulose in an X-blot apparatus (Ceralabo). The blots were reacted with the anti-21-kDa serum, followed by incubation with goat anti-rabbit IgG conjugated with horse-radish peroxidase (Biorad) and were subsequently developed by paraphenylene diamine/pyrocatechol-H_2O_2.

2.6. *HPLC analysis of pigments*

The LH fractions were extracted with cold acetone at a final concentration of 90%. The extract was evaporated to dryness under nitrogen, resuspended in methanol and injected into a Zorbax ODS (Du Pont Instruments) HPLC column. The apparatus, the elution procedure (linear gradient from 90% acetonitrile/10% water/0.5% triethylamine to 100% ethylacetate) and the extinction coefficients were the same as in [16].

2.7. *Amino acid analysis*

Samples were hydrolysed in 6 N HCl for 17 h at 112°C under vacuum and were then analysed on a 130A Applied Biosystem automated amino acid analyser.

2.8. *Amino acid analysis*

Emission and excitation fluorescence spectra were recorded using an Hitachi F 3010 spectrofluorimeter with 5 nm slit width for both emission and excitation. Excitation spectra were corrected from 400 to 800 nm using hexamethylindotricarbocyanine as probe [17].

3. RESULTS AND DISCUSSION

After centrifugation, the upper part of the gradient appeared colorless, indicating that no pigment was released during the solubilization by digitonin. Thus, the pigment composition of the discrete bands observed on the gradient can be considered to be representative of the native state of the complexes. The general distribution of these bands on the gradient has been presented in [9] and the PSI-enriched, heavy fractions in [16]. The present work is focused on the LH fractions, harvested in the 15–20% sucrose layer.

3.1. *Pigment composition*

The LH fractions contained the 5 main pigments present in the whole chloroplast, but were enriched in chorophyll *c* and fucoxanthin, relative to chlorophyll *a* and β-carotene. They fluoresced intensively at rather short wavelength (maximum near 680 nm). The characteristic in vivo absorption bands of Chl *a*, Chl *c* and fucoxanthin were observed in the fluorescence excitation spectrum (Fig. 1) demonstrating the light-harvesting efficacy of these pigments. More than 60% of the Chl *c* and fucoxanthin of the chloroplast extract loaded on the gradient was associated with the LH fraction.

The composition of LH from different species belonging to several orders of *Phaeophyceae* are presented in Table I. The LH fractions, in contrast to their very simple protein moiety (only one or two main polypeptides, see [10]) retained molecules of all 5 main pigments present in the chloroplast, and only β-carotene was present in a percentage slow enough to be

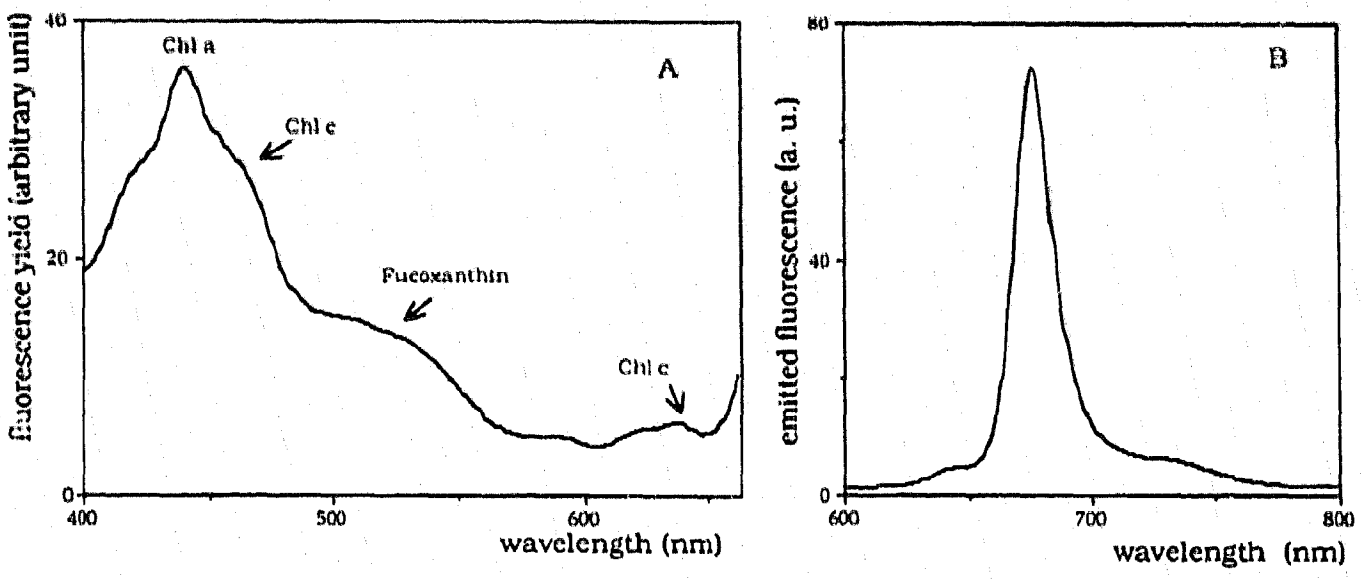

Fig. 1. Fluorescence emission and excitation spectra at room temperature of the 240-kDa LH fraction from *Fucus serratus*. A: excitation spectrum of the fluorescence emission band at 676 nm (excitation bandpass: 5 nm; emission bandpass: 5 nm). B: emission spectrum (excitation at 440 nm ± 5 nm; emission bandpass 3 nm).

considered as contaminant. Therefore, any model of these protein complexes must take into account the existence of linkage sites for all these pigment molecules.

By contrast with green plants, where the Chl *a*/Chl *b* ratio in LHCP is thought to be 1, the pigment stoichiometry of the LH complexes of brown algae appears rather variable. For example the Chl *c*/ Chl *a* and fucoxanthin/Chl *a* ratios are much higher in *Dictyota* than in *Fucus*. Furthermore these ratios can vary noticeably within one species, according to the environmental conditions, as is the case for *Dictyota* (Table I). This last observation rules out the possibility of this variability in pigment composition being linked to variations in amino acid composition.

3.2. *Polypeptide and amino acid composition*

Two discrete bands of about 240 kDa and 120 kDa were obtained after centrifugation of the LH fraction of *Fucus* and *Laminaria* on a linear sucrose density gradient.

Excitation spectra indicated that energy transfers from Chl *c* and xanthophylls were still operating in the 240-kDa particle (Fig. 1) but were significantly decreased in the 120-kDa one (data not shown). Thus the heavy fraction suumed to be closer to the native state of the complex. A molecular mass of 240 kDa is similar to the value obtained by Guglielmelli for the LH of a diatom [18]. In a more closely related species, *Dictyota dichotoma*, Katoh et al. [11] found twice this M_w for particles extracted with mild detergent (dodecylsucrose) and 75 kDa after Triton treatment. As previously described in [10], and as is shown in Fig. 3, the LH fractions from *Phaeophyceae* have a very simple protein moiety with one or two main polypeptides with M_w near 20 kDa. These results support the hypothesis of an in vivo arrangement of polypeptides in dodecameric structures resulting from an aggregation of trimeric subunits, in a similar way to the LHCP of green plants.

The amino acid compositions of the dominant polypeptide from *Fucus* and *Laminaria* have been

Table I

Pigment composition of LH complexes from several brown algae, obtained by HPLC

The results are expressed in molar percentages relative to Chl *a*

	*Fucus** *serratus*	*Pelvetia*** *canaliculata*	*Laminaria** *sacharina*	*Dictyota** *dichotoma*	*Pylaiella*** *littoralis*
Chlorophyll *a*	100	100	100	100	100
Chlorophyll *c*	18 ± 1	8	30 ± 4	30 ± 10	30
β-Carotene	4 ± 1	3	2 ± 1	4 ± 1	2
Fucoxanthin	77 ± 3	61	76 ± 7	107 ± 12	85
Violaxanthin	17 ± 2	30	10 ± 1	10 ± 2	6

* Mean of several experiments (more than 5) ± standard variation

** Data from one experiment

Table II

Amino-acid composition of the *Fucus serratus* and *Laminaria saccharina* LH polypeptides (molar %)

	Fucus	*Laminaria*
Asparagine + Aspartic acid	10.1	11.9
Glutamine + Glutamic acid	12.4	12.0
Serine	6.8	4.8
Glycine	12.4	12.3
Histidine	1.4	1.4
Threonine	4.4	3.3
Alanine	10.4	12.7
Proline	5.8	5.8
Tyrosine	1.2	1.1
Valine	5.9	2.2
Methionine	1.7	1.9
Leucine	10.2	9.8
Isoleucine	4.6	4.4
Phenylalanine	4.5	4.6
Lysine	4.7	4.5
Arginine	3.0	3.4
Cysteine	n.d.	0.3
Tryptophan	0.5	n.d.

determined (Table II). They are rather similar to one another and to those of *Dictyota* [11], *Phaeodactylum* [19] and also the LHCP of green plants [20]. Each has the same 5 most abundant components (corresponding to near 10% or more of the total amino acid content, expressed as molar %). Conversely, the amino acid composition of these species is quite different from that of *Gleodinium (Dinophyceae)* [21]. Histidine molecules, which are now considered to be Chl *a* binding sites, are present at a similar molar % as in spinach LHCP [20].

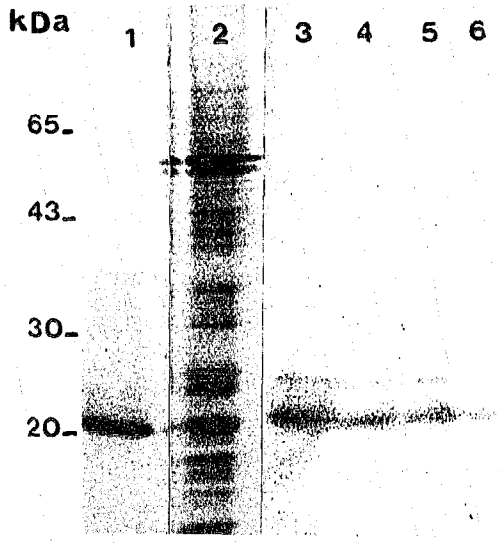

Fig. 2. Specificity of the antibody. Test on *Fucus serratus* fractions. Lane 1, Coomassie blue-stained gel of LH; lane 2, Coomassie blue-stained gel of thylakoids; lanes 3–6, immunoblots of thylakoids reacted with antibody to LH *Fucus*. Decreasing amounts of proteins, estimated by Chl *a*, were loaded on the gel: 10, 5, 2.5 and 1.25 μg Chl *a* in lanes 3, 4, 5 and 6, respectively.

3.3. *Immunological study*

First, the specifity of the antibody raised against the LH complex of *Fucus serratus* was assessed by Western blotting of total *Fucus* thylakoids (Fig. 2). The antibody reacted strongly with the LH fraction.

In the thylakoid fraction, it was bound most strongly to only one band, at the same M_w as the LH fraction, and to a lesser entent to a band of somewhat higher M_w, the nature of which remains to be determined.

The antibody reacted with the LH of a number of *Phaeophyceae*, including representatives of Fucales, Laminariales, Dictyotales and even Ectocarpales (Fig. 3). It was also used to determine whether antigenically related apoproteins were present in other Chl *c*-containing algae (Fig. 3): (i) the antibody clearly cross-reacted with the LH polypeptide of *Giraudyopsis* (Chrysophyta), (ii) among the *Bacillariophyceae*, it showed reactivity with the LH polypeptides of *Nitzschia*. With the LH of other diatoms (*Phaeodactylum, Asterionella, Chaetoceros*), a very faint or in some cases negative reaction was obtained, (iii) the antibody

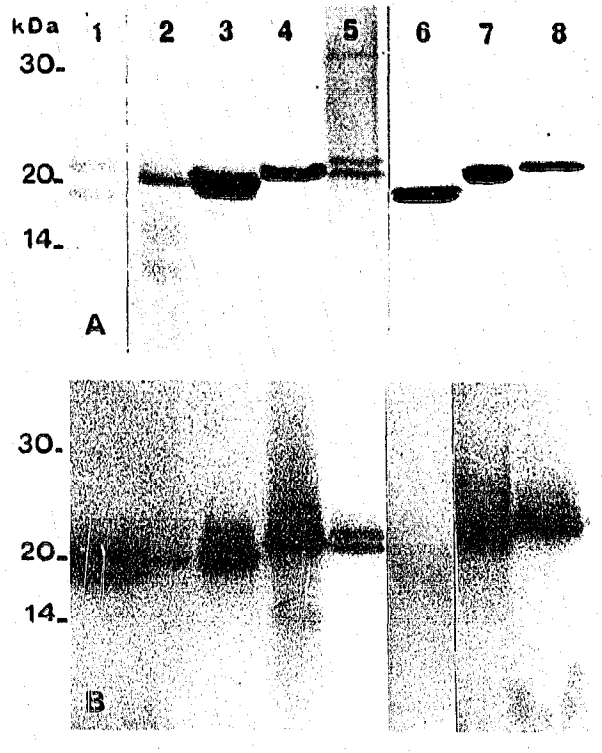

Fig. 3. Cross-reactivity of *Fucus* LH antibody with LH of other Chromophytes. A: Coomassie blue-stained gel, except for lane 1 which was stained with Ponceau red. B: Immunoblots reacted with antibody against *Fucus* LH. Lane 1, *Nitzschia acicularis* LH; lane 2, *Giraudyopsis stellifer* LH; lane 3, *Dictyota dichotoma* LH; lane 4, *Pylaiella littoralis* LH; lane 5, *Pelvetia canaliculata* LH; lane 6, *Phaeodactylum tricornutum* LH; lane 7, *Laminaria saccharina* LH; lane 8, *Fucus serratus* LH.

Table III

Immunological relationships between the LH complexes of fucoxanthin-containing algae and of other photosynthetic organisms

	PHAEOPHYCEAE	BACILLARIOPHYCEAE	PRYMNESIOPHYCEAE	CHRYSOPHYCEAE	EUSTIGMATOPHYCEAE	DINOPHYCEAE	CHLOROPHYTA	CRYPTOPHYTA
Anti LH *Fucus serratus* *(Phaeophyceae)* This work	++	+ according species		+	+		ε	−
Anti LH *Phaeodactylum* *(Bacillariophyceae)* Friedman § Alberte, 1984,87		++		−		−	−	−
Anti LH *Phaeodactylum* *(Bacillariophyceae)* Fawley et al., 1987	+	++	± according species	± according species				
Anti LH *Phaeodactylum* *(Bacillariophyceae)* Manadori & Grossman, 1990						+	+	
Anti LH *Pavlova* *(Prymnesiophyceae)* Hiller et al., 1987	+	++	++		−	+	+	−
Anti LH *Zea mays* *(Chlorophyta)* Caron et al., 1988	+	+			−		++	
Anti LH *Spinach (Chlorophyta)* Chrystall & Larkum, 1987					−			
Anti LH *Chroomonas (Cryptophyta)*					−			

raised against *Fucus* did not recognize the LH of *Cryptomonas (Cryptophyceae)* nor that of *Mantoniella (Prasinophyceae)*, (iv) interestingly, a well-defined reaction was also observed in our laboratory (W. Arsalane, personal communication) with the LH complex of an *Eustigmatophycea, Monodus subterraneus*, which confirms the relationship of these algae with Chromophytes even though they do not possess Chl *c* and (v) Western blotting was also used to test the relatedness of the *Fucus* LG with the LHCP of green plants (maize, spinach, pea) and of a green macrophyte alga, *Ulva sp.* In the majority of the experiments, no cross-reactivity was obtained. However, in some cases, when using a rather high concentration of antibody, or using the biotin reagent, a weak positive reaction was observed. As we have previously shown a reactivity of an antibody raised against maize LHCP with the LH polypeptide of 3 different *Phaeophyceae* [10], the question of the relatedness of these two types of light-harvesting complexes remains speculative.

3.4. *Immunological overview*

More generally, considering our data with those presented by other authors [22-26], we can summarize (Table III) the present state of knowledge regarding the immunological relationships between the light-harvesting complexes of fucoxanthin-containing algae and those of other photosynthetic organisms. The data summarized in this table clearly demonstrate the relationships between the LH of various Chl *c*-fucoxanthin-containing groups of algae, although it should be noted that some of these results are conflicting, especially those from the *Bacillariophyceae*.

Concerning the other groups of algae (containing no

Chl *c* or no fucoxanthin) it is worth noting that cross-reactivity has been observed by Hiller et al. [24] between *Prymnesiophyceae* and fucoxanthin-containing algae, and that there is also a very clear reactivity of anti-*Fucus*-LH antibody with the LH of *Monodus*, an alga containing neither fucoxanthin nor Chl *c*.

In fact, the paucity of data at this time does not afford a general insight into the relationships among the LH complexes of Chromophytes. More precise studies of the composition of LH fractions are still lacking, and no sequence data are yet available. Further work is in progress to improve this situation.

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