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Polypeptide composition of light-harvesting complexes from some brown algae and diatoms

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The polypeptide composition of some Chromophyte light-harvesting complexes was investigated by SDS-PAGE and compared to LHCP of higher plants. According to the species, one or two major polypeptides were found in the range 17–25 kDa, which is noticeably lower than for higher plant LHCP polypeptides. Evidence is provided for three species LH polypeptides possessing different molecular masses that they all possess some immunological analogy with a maize LHCP. In addition, *Fucus serratus* LH was proved to be able to phosphorylate.

Immunodetection; Light-harvesting complex; Phosphorylation; Polypeptide; (Brown alga; Diatom)

1. INTRODUCTION

The Chromophytes (or chlorophyll *c*-containing algae) play a major role in ocean productivity. Their photosynthetic apparatus differs from that of green plants, in both ultrastructure and pigment composition. Besides chlorophyll *a* and *c*, their chloroplasts contain carotenoids in greater abundance than in green plants. The light-harvesting (LH) function of these carotenoids has been proved for at least two of them, peridinin in Dinophyceae [1] and fucoxanthin in brown algae [2,3] and diatoms [4]. The major LH pigment protein complex of Dinophyceae, extrinsic to thylakoid membranes, has been rather extensively studied [1,5]. In contrast, although LH fractions containing fucoxanthin, Chl *a* and Chl *c* have been isolated from different materials by means of density gradient or electrophoresis (reviews [6–8]) some confusion remains about their number and pigment composition, probably owing to their high sensitivity to detergents. Their polypeptide composition has been studied in two species, the diatom *Phaeodactylum tricornutum* and the Prymnesiophyte *Pavlova gyrans* [9]. For *P. tricornutum*, several reports exist [9–12] that do not describe the same number or exactly the same molecular mass of the constituent polypeptides. In addition, polyclonal antibodies against this last LH complex have been raised [11,12] and tested against total membrane polypeptides of a large variety of diatoms [11].

In green plants, the LHCP complex has been proven to be implicated in the regulation of the energy distribution between the two photosystems. The overreduction of the plastoquinone pool triggers the phosphorylation of LHCP, which modifies the charge of LHCP molecules and leads to disconnection of a part of them from the PS II centers (review [13]). This kind of state transition has not been demonstrated in brown algae or diatoms and, to date, it appears rather that LH of these algae normally transfers energy to both photosystems [4,14]. Furthermore, their chloroplasts do not possess the granal structure needed

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Abbreviations: LH, light-harvesting complex; LHCP, light-harvesting complex of higher plants; PAGE, polyacrylamide gel electrophoresis; PS, photosystem

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for this regulation process. However, the variability of light intensity and quality in the natural habitat of these organisms suggests that some kind of equilibration of energy distribution must operate in their photosynthetic apparatus. In Cyanobacteria, in which the thylakoid arrangement is also agranal another kind of regulation occurs, which seems to implicate protein phosphorylation processes [13].

Here, we show that the molecular mass of major polypeptides of LH from different brown algae and diatoms may vary between 17 and 22 kDa, that they exhibit immunological cross-reactions with antibodies of green plant LHCP and moreover that brown algae LH are capable of light-driven phosphorylation.

2. MATERIALS AND METHODS

Thalli of different species of brown algae were collected at the seashore near the marine laboratories of Roscoff (for *Fucus serratus* L. *Dictyota dichotoma* (Hudson) Lamouroux, *Laminaria saccharina* (L.), Lamouroux, or Banyuls (for *Cystoseira mediterranea* (Sauvageau) (France). Chloroplasts were prepared according to [15]. Diatom species (*P. tricornutum* Bohlin, *Skeletonema costatum* (Greville) Cleve) were cultivated as in [16] and harvested by centrifugation. Chloroplasts or diatom cells, resuspended in a 10 mM Hepes-Na buffer (pH 7.4), 2 mM $MgCl_2$, 2 mM $MnCl_2$, 10 mM KCl, 1 M sorbitol, were fragmented with a French pressure cell (136 MPa) in the presence of digitonin at a detergent/Chl ratio of 100 and incubated for 1 h in the same medium. The homogenate was loaded on the top of a sucrose gradient and centrifuged at 140000 × *g* for 15 h.

The LH complexes obtained in the upper part of the gradient were diluted with 0.5 M Tris-HCl buffer, pH 8.0, with protease inhibitors (1 mM benzamidine and phenylmethanesulfonyl fluoride), then concentrated by centrifugation overnight at 200000 × *g* and 4°C. The pellets were incubated in dissolution buffer, containing 0.06 M Tris, 5% SDS, 1 M mercaptoethanol, for 1 h at room temperature. Samples were loaded on electrophoresis slabs to determine polypeptide composition. The stacking gel was 5% acrylamide with 0.1% SDS, pH 6.8, the running gel comprising a 10–22% acrylamide gel gradient with 0.1% SDS, pH 8.8. The buffer system of Laemmli [17] was used and 0.1 SDS was added to the upper reservoir. Proteins were stained with Coomassie brilliant blue R250.

Immunological detection of electroblotted LH apoproteins was based on techniques described by Towbin et al. [18] and Burnette [19]. A polyclonal antibody raised in rabbits against maize LHCP apoproteins was used [20]. A horseradish peroxidase-conjugated IgG (Biosys) was used as antibody directed against the immunoglobulins of the first antiserum. Revelation of the peroxidase activity was performed using 0.015% H_2O_2, 0.05% 4-chloronaphthol, in 0.02 M Tris, pH 8.2, and 0.9% NaCl.

For thylakoid phosphorylation, the method in [21] was followed except that chlorophyll concentration in the incubation medium was 150 μg/ml and ATP was 100 μM. Illumination was provided by a white light (500 $\mu E \cdot m^{-2} \cdot s^{-1}$). 10 μCi/ml of [γ-^{32}P]ATP (Amersham, spec. act. 3000 Ci/mM) was added to the reaction mixture. Dark phosphorylation was performed by adding 20 mM Na dithionite. To inhibit phosphatases, 20 mM NaF was added to the reaction mixture. Following phosphorylation (15 min), the membranes were immediately pelleted at 20000 × *g* for 5 min and solubilized for SDS-PAGE [17]. Phosphoproteins were located after 15 days of autoradiography of the stained and dried gel, using X-ray film (Agfa-Gevaert Curix).

3. RESULTS AND DISCUSSION

3.1. *Isolation of the LH fractions*

After digitonin treatment, similar gradient patterns were obtained from all the screened species. As already described for *Fucus* [3], three major types of bands could be observed: (i) one or two heavy green, PS I-enriched fractions; (ii) in some cases a few abundant median fractions with PS II characteristics; (iii) in the upper part of the gradient a large zone with an intense orange-brown color: in some experiments two different colored layers could be distinguished in this zone. In this case, only the lower of the two layers was used for the present study, since excitation fluorescence spectra had already [3] demonstrated the actual light-harvesting role of this fraction. Its pigment composition had been analysed by means of HPLC. It contained all the main pigments present in the whole chloroplast but with an enrichment in chlorophyll *c* and fucoxanthin, and conversely a depletion in chlorophyll *a* and β-carotene [22].

3.2. *Polypeptide composition of LH fractions*

The polypeptide compositions observed in LH fractions from the 6 studied species are listed in table 1. The LH fractions of all the tested species exhibited one or two major polypeptide components. Electrophoresis of the different samples (fig.1) clearly showed that the molecular mass of these polypeptides varied from 17 to 21 kDa according to the species. The number of bands in this molecular mass range varied from one to three and their relative abundance differed.

Some other polypeptides were present beyond this range, but in much lower quantities; in brown algae they were only seen on overloaded gels. They were relatively more abundant and also more

Table 1

Presently reported polypeptide compositions of LH complexes from brown algae and diatoms

Species	Major LH polypeptides (kDa)	
	This work	Other reports
Brown algae		
Fucus serratus	21.5, 23	
Cystoseira mediterranea	21, 22	
Dictyota dichotoma	20, 19	
Laminaria saccharina	20.5, 21.5	
Diatoms		
Phaeodactylum tricornutum	18.5, 18	16, 16.4, 17, 17.5 [9] 16.4, 16.9 [10] 17.5, 18 [11] 19, 19.5 [12]
Skeletonema costatum	22, 19.5	

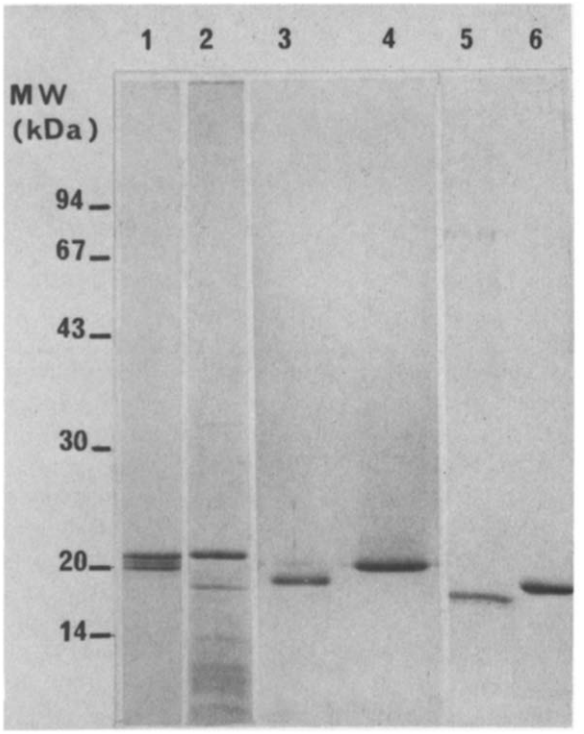

Fig.1. Coomassie blue-stained gel loaded with 2 μg chlorophyll. LH from (lanes): (1) *Cystoseira mediterranea*, (2) *Skeletonema costatum*, (3) *Laminaria saccharina*, (4) *Fucus serratus*, (5) *Phaedactylum tricornutum*, (6) *Dictyota dichotoma*.

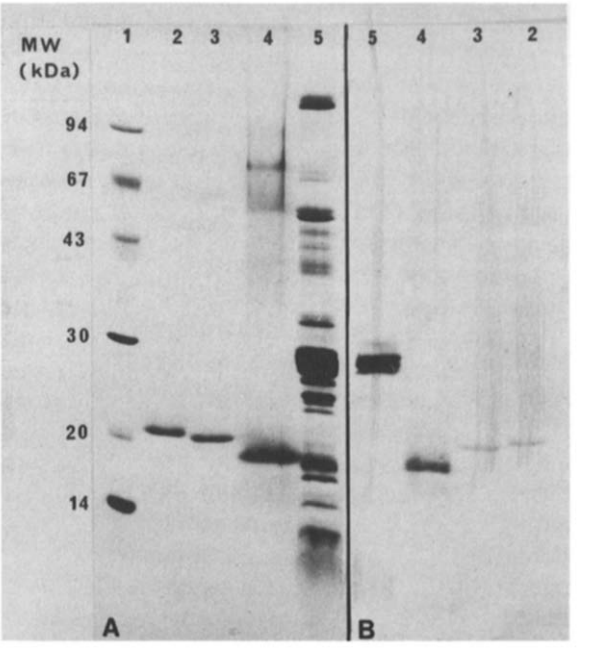

Fig.2. (A) Coomassie blue-stained gel. (B) Western blot and immunodetection using LHCP antiserum. Lanes: (1) protein markers, (2) *Fucus serratus* LH, (3) *Dictyota dichotoma* LH, (4) *Phaedactylum tricornutum* LH, (5) polypeptides from pea thylakoids. In order to obtain clear immunodetection, lane 5 in B was loaded with 5-times less protein than the corresponding lane in A.

numerous in the two diatoms' LH (figs 1,2). Probably, these minor bands were not constituents of LH itself, but originated from other membranes components still present in the gradient fraction. It must be noted that, whereas in *P. tricornutum* two successive detergent incubations seem to be needed to purify the 20 kDa range polypeptides [11,12], we obtained the same result after only digitonin action in the brown algal fractions.

Concerning *P. tricornutum*, if we compare our results with published analyses (table 1), we note that the molecular mass estimations of the major polypeptide were closer to the value given by Friedman and Alberte [11] than to those reported in [9,10]. It is noteworthy that the polypeptide composition of *S. costatum* LH was very different from that observed in *P. tricornutum*. Concerning brown algae LH, to our knowledge no molecular mass estimation has yet been published. All species tested here showed LH polypeptide with lower molecular mass than the current value observed for LHCP of green plants.

3.3. *Immunochemical analogy with higher plant LHCP*

In order to determine whether there was a molecular analogy in the polypeptide composition of LH complexes from Chlorophytes and Chromophytes, an immunological study was performed using a maize LHCP polyclonal antibody. The resulting immunoblot is presented in fig.2. As a control the cross-reaction with pea LHCP polypeptides is given in lane 5. Although LH apoproteins of brown algae (lanes 2,3) and diatoms (lane 4) have noticeably lower molecular masses than higher plants, it is clear that the maize LHCP antibody recognizes the different Chromophyte LH. This indicates that at least some peptide sequences are common to Chromophyte and Chlorophyte LH proteins. However, as a polyclonal antibody was used, it cannot be ascertained whether it was the same peptide sequence that was recognized in the different LH investigated. Recently, very interesting data were published showing that a monospecific polyclonal antibody raised against the two apoproteins (17.5 and 18 kDa) of the major light-harvesting pigment-protein from the diatom *P. tricornutum* [23] cross-reacted with neither LH polypeptides of three Chlorophyte algal divisions (Chrysophyta, Cryptophyta, Pyrrophyta) nor two classes of Chlorophyta algae. This probably indicated that the antibodies used in [23] recognize by chance a very sharp sequence. The maize LHCP antibody used in our work showed monospecific reactions with three constituent polypeptides of 29, 27 and 25 kDa of the maize LHCP [20] and with similar polypeptides of pea LHCP; therefore, larger sequences were probably recognized, which could explain why a cross-reaction was found here with *P. tricornutum* LH.

As the different polypeptides of green plant LHCP are known to be coded by a multiple family of genes [24], it might be suggested that an analogous family is present in brown algae and diatoms, and that different genes of this family are expressed in the different genera or species, explaining the variability in molecular mass from one to another.

3.4. *Phosphorylation of brown alga LH*

Owing to this newly demonstrated homology between LH of brown algae and green plants, it seemed interesting to investigate whether brown alga LH was able to be phosphorylated and perhaps to play a role in light adaptation. Fig.3 demonstrates that *Fucus* LH was able to phosphorylate under light, or in darkness in the presence of dithionite, as higher plant LHCP. If photosynthetic membrane phosphorylation has now been demonstrated in organisms other than higher plants such as Cyanobacteria [25] and some purple photosynthetic bacteria [26,27], it is to our knowledge the first time that such a possibility has been reported with regard to brown algae. At present, it is not possible to ascertain if this phosphorylation is related with modification of the light energy distribution between the two photosystems.

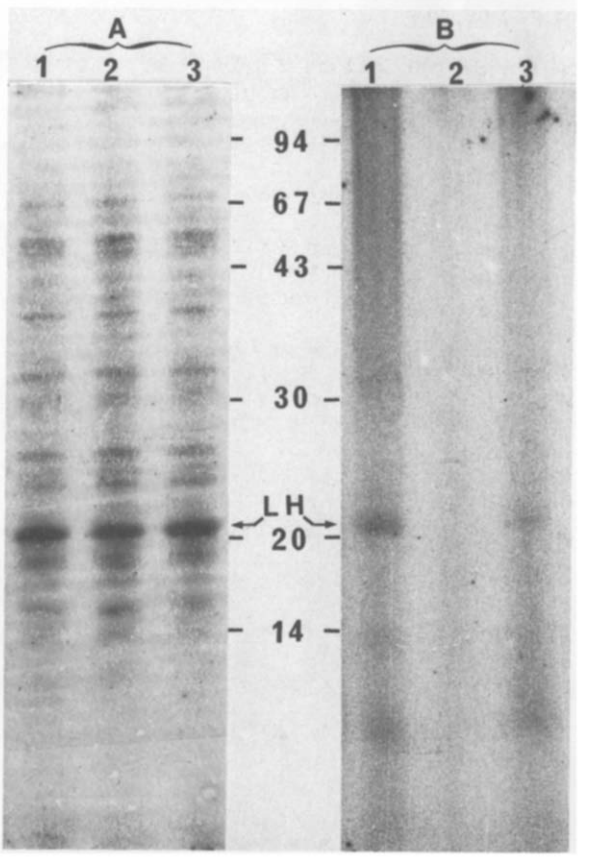

Fig.3. Evidence showing that *Fucus serratus* LH can be phosphorylated. Isolated *Fucus* membranes were phosphorylated in vitro with [γ-^{32}P]ATP. (A) Coomassie blue-stained gel loaded with 5 μg chlorophyll; lanes: 1, light-phosphorylated membranes; 2, dark control; 3, dark plus dithionite. (B) Autoradiogram of gel in A.

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