Fucoxanthin–Chlorophyll *a*/*c* Light-Harvesting Complexes of *Laminaria saccharina*: Partial Amino Acid Sequences and Arrangement in Thylakoid Membranes

Dominique Douady, Bernard Rousseau, and Lise Caron*

Laboratoire Biomembranes Végétales, CNRS URA 1810, Ecole Normale Supérieure, 46 rue d'Ulm, 75230 Paris Cedex 05, France

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ABSTRACT: The N-terminus of the major polypeptide component of the light-harvesting complex (LHC) from the brown alga *Laminaria saccharina* is blocked. Two partial sequences, one near the N-terminus and the other near the C-terminus, have been obtained by chemical cleavage with acetic acid and *N*-chlorosuccinimide. Four peptides were separated after trypsin digestion of the thylakoid membranes. One fragment is not phosphorylated, is not blocked, and has been sequenced. Purification on a reversed-phase column showed two forms of the LHC protein: the more hydrophobic form appears to be bound to photosystem I. These results are compared with LHC from other Chromophytes and the CAB family of green plants.

The chloroplasts of brown algae differ from those of green plants by their ultrastructure and their pigment composition. Besides chlorophylls *a* and *c*, they contain carotenoids, especially fucoxanthin, in greater abundance than in green plants. More than 50% of chlorophylls *a* and *c* (Chl *a* and Chl *c*)[1] and 80% of fucoxanthin present in thylakoids of brown algae are bound to membrane light-harvesting complexes (LHC).

These LHC fractions have been isolated from different species and partially characterized [for review see Anderson and Barrett (1986)]. The first reports on the polypeptide components of a diatom were published by Friedman and Alberte (1984) and Fawley and Grossman (1986). We have studied the LHC from several species of macrophyte brown algae. We found that, upon denaturation of the purified macrocomplexes with detergents, typically one to two major polypeptides were observed in the molecular mass range of 17–21 kDa (Caron et al., 1988; Passaquet et al., 1991) compared to the 25–30-kDa range observed for LHC of green plants. Oligomeric forms have been purified with apparent molecular masses of 120 or 240 kDa (Passaquet et al., 1991), and even larger complexes (about 700 kDa) have also been obtained (Katoh et al., 1989; Katoh & Ehara, 1990), but the *in vivo* forms of LHC are not known.

For LHC of green plants (also known as CAB), amino acid sequences have been deduced from a large number of sequences of the *cab* gene family (Green et al., 1992). They predict the presence of three α-helical hydrophobic regions, which are thought to span the lipid bilayer. Authors have recognized a similar fold for all the CAB polypeptides. The primary structure of LHC shows an intramolecular similarity comprising parts of the first and third membrane-spanning domains. Recently, the intrinsic 22-kDa protein, associated with the oxygen-evolving photosystem II core complex in green plants, has been characterized as a four-helix protein (Kim et al., 1992; Wedel et al., 1992), which argues for the duplication–fusion hypothesis. It has been suggested that the CAB family derives from an ancestral two-helix protein and that the intramolecular similarity has come about by a gene duplication event followed by a fusion. This fusion could have given rise to a four-helix protein, with further deletion of the fourth helix.

Working on the diatom *Phaeodactylum tricornutum*, Grossman et al. (1990) have shown that the major LHC polypeptides are the products of a family of nuclear encoded genes (*fcp*). By comparison with the green plant LHC sequences, a part of the putative amino acid sequences localized in the N-terminal region of the proteins is homologous to a C-terminal region of several CAB polypeptides. But the other parts of the sequence are fairly different.

In the green plants, the LHC is obviously involved in other functions than light harvesting. Its N-terminal hydrophilic domain contains a phosphorylation site which is thought to be of regulatory importance for grana stacking (Bennett, 1980; Mullet, 1983) and distribution of energy between photosystems I and II (Knaff, 1991; Allen, 1992). In brown algae, we have shown that the LHC can be phosphorylated in the light (Caron et al., 1988). However, the green plant model cannot be straightforwardly applied to brown algae. Indeed, the thylakoids are not arranged in grana but in parallel bands of three (Gibbs, 1970; Berkaloff et al., 1983). The distribution of the LHC in the thylakoids is beginning to be studied: there is no preferential localization of the complexes in appressed membranes as is the case in green plants (Lichtlé et al., 1992).

Although, for diatoms, sequence analyses and studies on the biosynthesis of LHC are now under way by the use of molecular biological tools, little is known about the LHC of brown macroalgae. From our experience, the molecular biological approaches are not easy to apply to these algae because of difficulties in purifying nucleic acids and obtaining stable nuclear or cDNA banks. In this work, we investigated some aspects of the structural organization of brown algal LHC using proteolytic methods on the complexes. Because direct sequencing of the major LHC apoprotein from *Laminaria saccharina* was unsuccessful, indicating that the N-terminal amino acid was blocked, the protein was cleaved

* To whom correspondence should be addressed.

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[1] Abbreviations: Chl, chlorophyll; CAB, chlorophyll *a*/*b* binding protein; DTT, dithiothreitol; LHC, light-harvesting complex; PAGE, polyacrylamide gel electrophoresis; PSI, photosystem I; PSII, photosystem II; PVDF, poly(vinylidene difluoride); SDS, sodium dodecyl sulfate; TMH, transmembrane helix; Tris, tris(hydroxymethyl)aminomethane.

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by enzymatic or chemical treatments under limited proteolysis conditions in order to obtain partial sequences. Proteolysis of the labeled LHC, partially protected within the thylakoid membranes, gave information on the phosphorylation site in the protein and the arrangement of LHC complexes in the thylakoids.

MATERIALS AND METHODS

Thalli of the brown alga *Laminaria saccharina* (L.) were collected at the seashore near the marine biology laboratory at Roscoff (France).

Thylakoid and LHC Preparations. Procedures followed for the preparation of chloroplasts from brown algae have been published by our laboratory (Berkaloff & Duval, 1980). All the buffers used contained protease inhibitors (1 mM benzamidine and 1 mM PMSF). For LHC preparations, we followed procedures described previously (Caron et al., 1985). To increase the accessibility of membrane complexes to detergent, isolated chloroplasts were disrupted in a French pressure cell in the presence of digitonin (digitonin/Chl = 90). After incubation for 1 h at room temperature in the presence of digitonin, the membranes were loaded on a sucrose density gradient. The LHC fractions were recovered and diluted with TMS buffer (10 mM Tricine, 100 mM sorbitol, 5 mM $MgCl_2$, pH 7.8) containing protease inhibitors and then concentrated by centrifugation overnight at 200000*g* and 4 °C. The pellets were redissolved in suitable volumes of TMS buffer prior to cleavage treatments.

For phosphorylation and limited protease digestion of LHC *in situ*, the chloroplasts were not disrupted in order to preserve the thylakoid structure. They were pelleted, washed in TMS buffer, and resuspended (1 mg of Chl/mL) in the same buffer.

Phosphorylation of Thylakoid Proteins. The method used was from Kyle et al. (1982) modified as follows. Freshly prepared *L. saccharina* thylakoids (150 μg of Chl/mL) were resuspended in TMS buffer, pH 7.8, with 10 mM NaCl, 100 μM ATP, and 20 mM NaF (to inhibit phosphatases). [γ-^{35}S]ATP (10 μCi/mL) was added to the reaction mixture. A part of the sample was kept in the dark as reference. After 15 min of illumination by a white light (500 μE m^{-2} s^{-1}), radiolabeled membranes were pelleted at 20000*g* for 2 min and resuspended in TMS buffer plus additives as above. A sample was kept as reference for phosphorylation in the light. One sample was incubated in the presence of trypsin. Finally, all samples were pelleted and either directly solubilized for SDS–PAGE or loaded on a sucrose gradient in the presence of digitonin in an attempt to purify the LHC before PAGE. Radiolabeled proteins were located by autoradiography of the stained and dried gel or western blot membranes using Hyperfilm (Amersham).

Limited Proteolysis of Thylakoids. Trypsin (National Biochemicals Corp., Cleveland, OH) digestions of exposed parts of LHC proteins were conducted *in situ*. Thylakoids in TMS buffer were incubated for 1 h at room temperature in the dark following addition of freshly prepared protease solution to a final weight ratio of Chl/protease = 1. Digested thylakoids were centrifuged for 10 min at 20000*g*, and peptides were analyzed by denaturing Tris–Tricine PAGE at 4 °C.

Electrophoresis. Peptides were denatured by addition of solubilization buffer to obtain final concentrations of 1% SDS, 1 M β-mercaptoethanol, and 0.2 M Tris-HCl, pH 8.8, for 1 h at room temperature. Samples were loaded on a linear gradient 9–18% polyacrylamide gel. When the resolution of low molecular weight proteins was necessary, samples were applied to a Tris–Tricine gel prepared as described by Schägger and Jaggow (1987). Proteins were visualized by staining with Coomassie Brillant Blue or silver. Alternatively, proteins were electroblotted onto nitrocellulose or else peptides of interest were excised from gels.

Acid Cleavage at Asp-Pro Sites. Isolated and concentrated LHC fractions were delipidated in 80% acetone and cleaved by partial hydrolysis in 50 mM Tris-HCl, pH 8.4, 100 mM DTT, 6 M guanidinium chloride, and 10% acetic acid (in such a volume to contain 250 μg of Chl/mL) at 40 °C for 92 h. After treatment, the guanidinium salt was removed by chromatography of the sample on a filtration gel (Superose 12, FPLC), and samples were analyzed on Tris–Tricine SDS/PAGE.

N-Chlorosuccinimide Cleavage at Trp-X Sites. Isolated and concentrated LHC were delipidated as above. The pellets were resuspended in 30% w/v *N*-chlorosuccinimide in acetic acid solution and kept at room temperature for 2 h according to Shiozawa et al. (1989). Samples were loaded on 9–18% polyacrylamide gradient electrophoresis to determine their peptide composition.

Extraction of Peptides from Acrylamide Gels. After staining with Coomassie Brillant Blue, peptides of interest were removed from polyacrylamide gels by grinding in a mortar and vigorously stirring at 4 °C with FAPH (50% formic acid, 25% acetonitrile, 15% 2-propanol, 10% H_2O) for 24 h as described by Shiozawa et al. (1989).

Separation of Peptides by Chromatography. Peptides were separated on a Superose 12 column according to their molecular weight or on a reversed-phase column (Pro-RPC) according to their hydrophobic properties, using a Pharmacia FPLC system. The Superose 12 column was eluted with FAPH. The ProRPC column was equilibrated with eluent A [0.1% trifluoroacetic acid (TFA) in water, pH 2]. Peptides were eluted from the column with a gradient from eluent A to eluent B (100% acetonitrile, 0.1% TFA, pH 2, adjusted with triethanolamine). Peptides were also separated by C18 reversed-phase HPLC (eluent B: 80% acetonitrile, 0.1% TFA).

Sequencing of Peptides. The amino acid sequences were determined on an Applied Biosystems 470A protein sequencer. Direct protein microsequencing from PVDF transfer was also performed.

Electroblotting. Electroblotting was performed essentially as described in Passaquet et al. (1991). The blots were reacted with anti-*Fucus serratus* LHC antibodies prepared in our laboratory.

Alternatively, protein samples were electroblotted onto PVDF membrane. The transfer buffer was Tris–borate (50 mM Tris, 50 mM boric acid, 10% methanol).

RESULTS

Sequencing of the Major Apoprotein of the LHC Complexes: Cleavage with Proteolytic Agents. In order to sequence the major 21-kDa polypeptide of the light-harvesting complexes from *L. saccharina*, the LHC complexes were first purified by ultracentrifugation on a sucrose density gradient after solubilization of chloroplast membranes by digitonin, and then by denaturing SDS/PAGE. Finally, the protein was blotted on a PVDF membrane. Despite the precautions taken to avoid blocking proteins during purification, it was not possible to remove the N-terminal amino acid. Such a blocking is frequently due to glycosylation or acetylation of the molecule. Indeed, the *L. saccharina* LHC 21-kDa component reacted positively with a sugar-specific reagent, concanavalin A. Hence, we tried to cleave the molecule to obtain internal sequences.

(1) Cleavage after Lysine or Arginine by Trypsin. In green plants, proteolysis of thylakoid membranes by trypsin removes

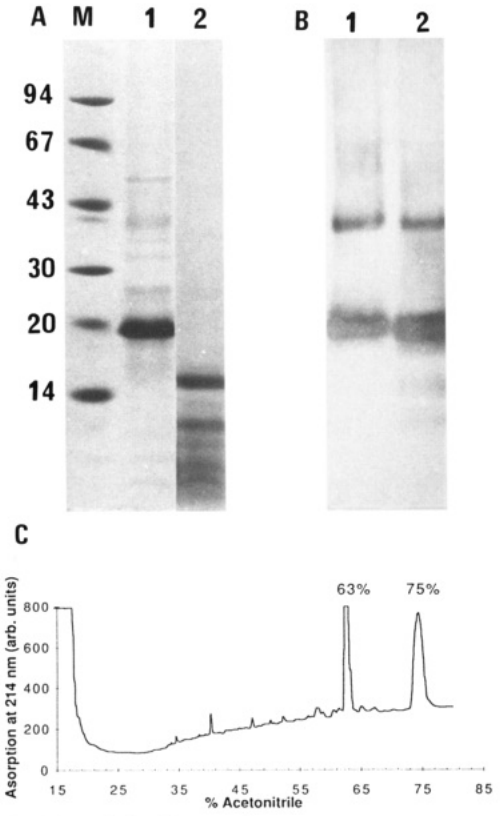


FIGURE 1: Proteolysis of *L. saccharina* chloroplast membranes by trypsin. (A) SDS/PAGE of LHC purified from chloroplast membranes, stained by Coomassie Blue. (B) Immunoblotting using polyclonal *F. serratus* anti-LHC antibodies. Lanes 1, LHC from untreated membranes; lanes 2, LHC from trypsinized membranes; lane M, molecular mass markers as indicated on the left in kDa. (C) HPLC elution profile of the excised 16-kDa component on a C18 column monitored by absorption at 214 nm. Conditions: flow rate, 0.2 mL/min; linear gradient from 20% to 100% eluent B; column temperature, ambient.

the stroma-exposed amino-terminal part of LHCII polypeptides and induces the unstacking of the membranes (Bennett, 1979; Mullet, 1983). We expected, using trypsin on *L. saccharina* chloroplast membranes, to remove the blocked N-terminal amino acid. Electrophoresis of treated membranes as well as of LHC isolated from treated membranes showed that the amount of the 21-kDa LHC polypeptide dramatically decreased whereas fragments at 16, 11, 5.5, and 5 kDa appeared (Figure 1A). As for the 21-kDa LHC polypeptide, the 16- and 11-kDa components were also recognized by polyclonal antibodies against the major LHC polypeptide of *F. serratus*, another brown alga (Figure 1B).

The peptide at 16 kDa was the most abundant component. It was excised from the gels and purified by FPLC on a Superose 12 column and then on an HPLC reversed-phase column. Two peptide fractions were eluted with 63% and 75% acetonitrile, respectively (Figure 1C). They very weakly reacted with anti-LHC antibodies. The N-termini of both peptides were blocked. By contrast, the 11-kDa fragment could be directly sequenced from PVDF transfer. Two identical amino acid sequences were obtained, one being shifted forward by two amino acids in comparison to the other one. This indicates the presence of two trypsin cleavage sites nearby. The sequence obtained was RLRYVEVKHGRIAMLAIAGHLTQQNTRLPGMLSNSA (named Lam 2). These results suggest that a part of the 21-kDa peptide is cleaved by trypsin near the C-terminus, resulting in the 16-kDa peptide, while the 11-kDa peptide originates from the 21-kDa by cleavage at both termini.

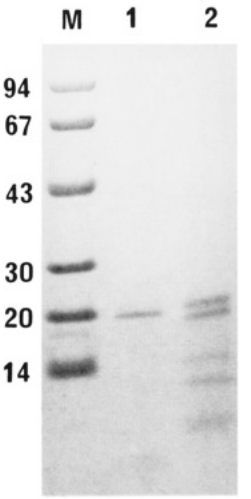


FIGURE 2: Proteolysis of purified *L. saccharina* LHC by *N*-chlorosuccinimide. Peptides were separated on SDS/PAGE and then stained by Coomassie Blue. Lane 1, untreated LHC; lane 2, LHC treated with *N*-chlorosuccinimide; lane M, see Figure 1.

(2) Cleavage after Tryptophan by N-Chlorosuccinimide. After cleavage of purified LHC with *N*-chlorosuccinimide, several peptides were released (Figure 2). Besides the uncleaved 21-kDa component, three peptides with masses of 12–19 kDa reacted with anti-*F. serratus* LHC antibodies and also concanavalin A. A peptide of about 7-kDa mass did not reacted with concanavalin A and antibodies (data not shown). This latter peptide fragment was purified on FPLC using a Superose 12 column and then submitted to sequencing. A 25 amino acid sequence was obtained: WDAMSERTEASKAIELNNGRAAQM (named Lam 3). Sequence homology with diatom LHC confirmed that this 7-kDa peptide is derived from the LHC apoprotein (see Figure 6). The lack of positive identification by the anti-*F. serratus* LHC antibodies may indicate that our antibodies recognize only certain epitopes which are not contained in this fragment.

(3) Cleavage between Aspartic Acid and Proline by Dilute Acetic Acid. The "native" purified LHC polypeptide was rather resistant to proteolysis by dilute acetic acid which did not cleave the protein under conditions of mild denaturation. In the presence of 6 M guanidinium chloride and DTT, the LHC polypeptide incurred limited proteolysis. The resulting peptides obtained were applied, first, to a Superose 12 column to remove salts, and then to a reversed-phase column. Two fractions were eluted, the first one with 55–59% acetonitrile and the second one with 63–65% acetonitrile (Figure 3A). Both fractions reacted with anti-LHC antibodies, and they both contained peptides of 21 kDa (uncleaved peptide) and 18 kDa (cleaved peptide). Small amounts of peptides with lower molecular mass coeluted with the fraction 2 (Figure 3B). A sequence of 15 amino acids from fraction 1 was obtained: DPLGLLADADQEXFF (named Lam 1).

Two Forms of LHC with a Major Apoprotein at 21 kDa. The finding of fragments with the same molecular mass but with different hydrophobic properties after acetic acid or trypsin treatment suggested that two polypeptide forms comigrate at 21 kDa. Indeed, when "native" LHC was loaded on a reversed-phase column, two peaks appeared in the elution profile and both peaks reacted with antibodies. We have previously described an LHCI fraction tightly bound to PSI (Berkaloff et al., 1990) and more recently another one, LHCII, bound to PSII (Douady et al., 1993). Both LHCs were separately loaded on a reversed-phase column, after delipidation in 80% acetone and solubilization in FAPH. The LHCI elution profile showed a predominant form eluted with 65–

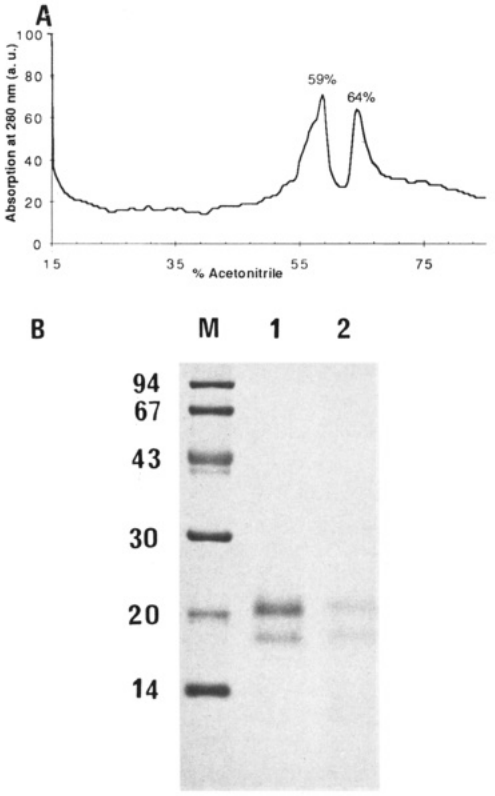


FIGURE 3: Proteolysis of purified *L. saccharina* LHC by dilute acetic acid. Peptides were separated on reversed-phase column (FPLC). (A) Elution profile of the peptidic components monitored by absorption at 280 nm. Conditions: flow rate, 0.3 mL/min; linear gradient from 10% to 100% eluent B; column temperature, ambient. (B) SDS/PAGE of fractions eluted with 59% and 64% acetonitrile (stained by Coomassie Blue): lane 1, fraction 1 eluted with 59% acetonitrile; lane 2, fraction 2 eluted with 64% acetonitrile; lane M, see Figure 1.

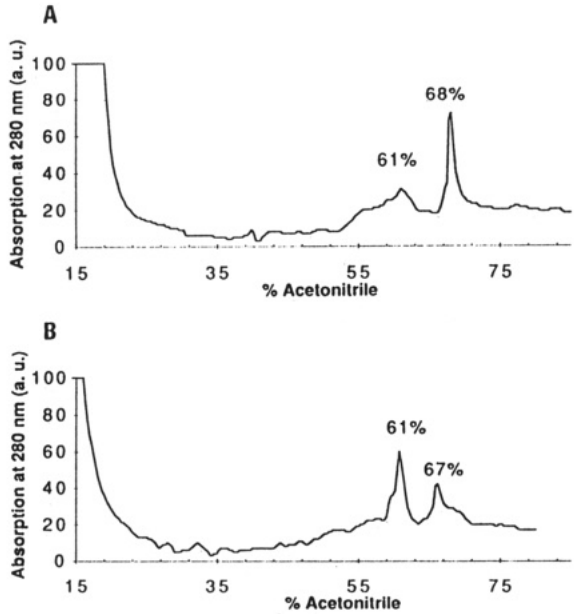


FIGURE 4: Separation of two forms of LHC from *L. saccharina* chloroplasts on a reversed-phase column (FPLC). Elution profiles monitored by absorption at 280 nm: (A) LHCI fraction; (B) LHCII fraction. Conditions as in Figure 3.

67% acetonitrile as fraction 2 described above. The LHCII elution profile showed a predominant form eluted with 58–61% acetonitrile as fraction 1 (Figure 4). We tried to distinguish the fractions by their proteolytic patterns after incubation in the presence of proteinase Lys-C and Arg-C. No differences appeared on Tris–Tricine PAGE.

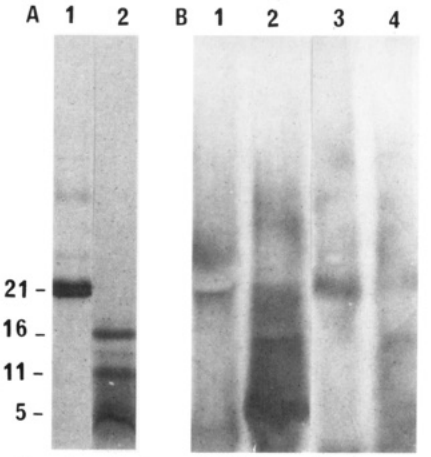


FIGURE 5: Phosphorylation of LHC peptide components from untreated chloroplast membranes or membranes treated with trypsin. Proteins were separated on SDS/PAGE. (A) Proteins stained by Coomassie Blue; (B) autoradiography of gels. Lanes 1, purified LHC from untreated membranes; lanes 2, purified LHC from trypsinized membranes; lane 3, untreated chloroplast membranes; lane 4, trypsinized membranes.

Phosphorylation of LHC Proteins. Membrane protein phosphorylation was performed *in vitro* on isolated *L. saccharina* chloroplasts using [γ-^{35}S]ATP. Aliquots of three membrane samples (dark incubated, or light exposed with or without trypsin) were directly solubilized for Tris–Tricine denaturing electrophoresis. The samples were gently solubilized with digitonin and subjected to ultracentrifugation on sucrose density gradient in order to purify the LHC complexes. Purified complexes were analyzed by PAGE and subsequent autoradiography (Figure 5). No noticeable difference between light and dark samples was observed. In both the thylakoids and the purified complexes, the most conspicuous radiolabeling was in the range of 21 kDa, where reaction with *F. serratus* LHC antibodies also was observed.

After treatment by trypsin, electrophoretic patterns showed that the 21-kDa polypeptides were cleaved, giving rise to 16- and 11-kDa fragments which still reacted with antibodies (see Figure 1) and other smaller ones at 5.5 and 5 kDa. Compared with untreated samples, labeling at 21 kDa decreased, while labeled components at 16 and 5 kDa appeared on autoradiograms (Figure 5).

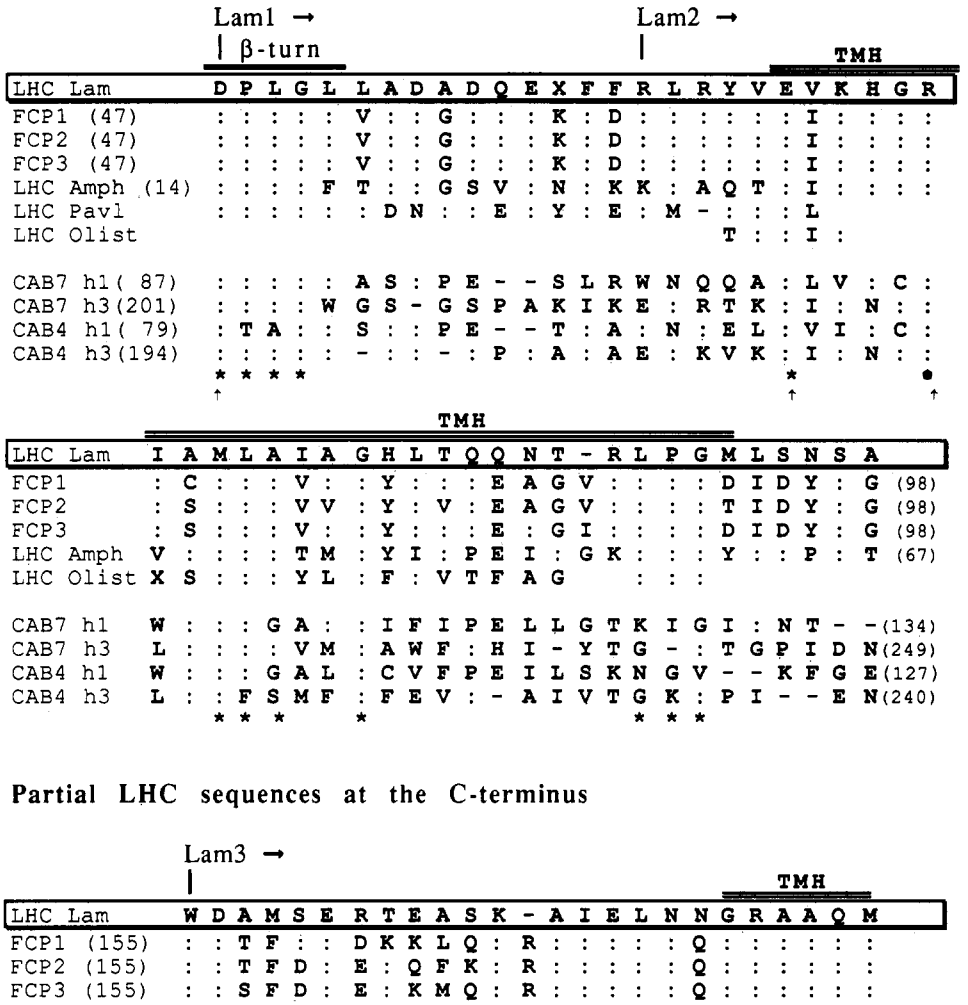
The amount of radiolabeled LHC was low compared to the total LHC loaded on electrophoresis gels, and LHC proteins were not uniformly labeled. In the diffuse labeled band, a sharp band at 21 kDa appeared on the autoradiography, indicating a strong labeling of a discrete component. Similar observations have been described by Islam (1987) working on spinach LHCII, where only 20–30% of the LHCII is fully phosphorylated, resulting in mixtures of phosphorylated and nonphosphorylated proteins. Among the LHC components two of them at 25 and 27 kDa have been shown to become phosphorylated under light [for review, see Allen (1992)].

DISCUSSION

The results obtained in limited proteolytic experiments, together with phosphorylation data of the complexes *in situ*, gave good information on the topology and the phosphorylation sites of the LHC proteins.

According to the protein sequences of diatom FCP (Grossman et al., 1990), numerous trypsin-cleavage sites are present all along the proteins, even in the loops thought to be external to the membranes. Nevertheless and surprisingly, LHC

Partial LHC sequences at the N-terminus



Partial LHC sequences at the C-terminus

FIGURE 6: Amino acid sequence comparisons. LHC Lam: *L. saccharina;* FCP1, FCP2, and FCP3: sequences deduced from three genes coding for *P. tricornutum* LHC (Grossman et al., 1990); LHC Amph: *A. carterae* LHC (Hiller et al., 1993); LHC Pavl: *Pavlova sp.* LHC (Green et al., 1992); LHC Olist: *O. luteus* (Green et al., 1992); CAB 7 and CAB 4: sequences deduced from genes *cab* 7 and *cab* 4, coding for type II LHCI and type II LHCII of *Lycoperdon esculentum*, respectively (Green et al., 1992); h1 and h3: sequences containing the first and the third TMH, respectively. The amino acid residue numbering of the beginning and the end of the shown sequences is given in parentheses to the left and right. (*) Conserved amino acids in all Chromophyte LHC. (↑) Conserved polar amino acids in all LHC. Lam 1, 2, and 3: partial sequences from *L. saccharina*.

proteins of *L. saccharina* were rather resistant to proteolysis by trypsin. Only two cleavage sites were observed under conditions in which the thylakoids remained intact although not so regularly associated in groups of three as they are in intact chloroplasts of brown algae (ultrastructural observations, not shown).

Trypsin cleavage at the C-terminus was very efficient and gave an abundant 16-kDa fragment still blocked at the N-terminus and a small peptide at 5.5 kDa. The cleavage site would be located on a stroma-exposed loop accessible to protease. The small peptide, corresponding to a transmembrane helix, protected from trypsin treatment "*in vivo*", stayed in the membrane and could be observed in the electrophoretic patterns. This hypothesis is in agreement with the predictions of Grossman et al. (1990) for diatoms.

After trypsin treatment, two other fragments at 11 and 5 kDa were found. The 11-kDa fragment was unblocked. The simplest interpretation is that a second cleavage site was localized near the N-terminus of the 16-kDa peptide which resulted in the release of these two peptides. Moreover, according to phosphorylation experiments, the labeled fragment of 5 kDa could contain the phosphorylation site near the N-terminus, as is the case in CAB proteins.

The light-harvesting complexes of Chromophyte algae exhibit weak immunological cross-reaction with antibodies to CAB proteins and a better cross-reactivity between each other (Fawley et al., 1987; Manodori & Grossman 1990; Passaquet et al., 1991; Plumley et al., 1993). These results suggest that CAB proteins and Chromophyte LHC proteins share some sequence homology.

In Figure 6, partial amino acid sequences of LHC polypeptides from *L. saccharina* and several other Chromophyte algae were arranged to give an optimal alignment. Near the N-termini, Lam 1 and Lam 2 are highly similar to amino acid sequences deduced from three nucleotide sequences of *fcp* genes from the diatom *P. tricornutum* (Grossman et al., 1990). Homologies are also present with the Prymnesiophycean alga *Pavlova species* (Green et al., 1992) and, but to a lesser extent, with a Dinophycean alga *Amphidinium carterae* (Hiller et al., 1993) and with the Raphidophycean alga *Olisthodiscus luteus* (Green et al., 1992). By reference to the other sequences, Lam 1 and Lam 2 can probably be joined end to end, Lam 2 behind Lam 1. Some motifs are remarkably conserved in all Chromophytes. They are marked with asterisks (*). Lam 2 is rich in hydrophobic residues, and according to Grossman's analyses, it includes a helical domain.

Most of the known Chromophyte sequences are located near the N-terminal portion of proteins. According to Hiller's work (Hiller et al., 1993), the *A. carterae* sequence is strongly related to sequences of *P. tricornutum* at the N-terminus, but much less at the C-terminus. The sequence of the fragment Lam 3 of *L. saccharina* showed considerable identity with the C-terminal part of the sequences from *P. tricornutum*. Two motifs are identical: AIELN and GRAAQM. The second motif could be the beginning of a transmembrane helix according to Grossman et al. (1990).

It should be interesting to know the amino acid sequence of *Pavlova sp.* LHC in the C-terminus because this alga contains fucoxanthin and Chl *c* as do the Bacillariophyceae and Phaeophyceae. Possibly, the C-terminal part of LHC proteins is specific for fucoxanthin-containing algae. However, it is unlikely that the C-termini of the LHCs might bind molecules of fucoxanthin, as has been suggested by Grossman et al. (1990), since after trypsin treatment, LHC remained in an oligomeric state with the bound pigments while the 21-kDa peptide components were cleaved at the C-termini.

By comparisons with the Chl *a/b* LHC complexes of higher plants, we were able to align sequences Lam 1 and Lam 2 with the conserved zones shared by all the CAB proteins.

In the literature, two alignments are proposed. Green et al. (1992) propose a sequence alignment of the N-terminal region of the Chromophyte LHC with the first conserved region up to the end of the first transmembrane helix of green plants. Grossman et al. (1990) indicate an alignment with the second conserved region up to the end of the third transmembrane helix of green plants. In the latter proposal, the N-terminal part of Chromophyte LHC presents homology with the C-terminal part of green plant LHC.

We tested both hypotheses for the LHC of *L. saccharina*. The sequences of the first and the third TMHs of tomato CAB 7 (LHCI II) and CAB 4 (LHCII II) proteins are shown in Figure 6. The comparison with CAB 4 gave higher degrees of homology when sequences were matched with the third transmembrane helix as proposed by Grossman. Using CAB 7, there was not noticeable difference between the two alignments. Several amino acids (marked with arrows) are conserved in all light-harvesting proteins as well as in Chromophytes and in green plants, such as the motif DPLG in the presumed stroma-exposed part and E/R residues in transmembrane helices.

These observations argue for the hypothesis that Chl *a/c* and Chl *a/b* light-harvesting proteins are derived from a common ancestral protein. However, it must be emphasized that the diatom LHC contains three putative transmembrane regions, two of which display no obvious homologies with the primary structure of Chl *a/b* proteins, althought the degree of functional conservation is probably higher than it appears (Green et al., 1992). This work extends this result to brown macroalgae since the Lam 3 sequence is very homologous to a part of the diatom sequences but cannot be aligned with CAB sequences. Moreover, the first and the third transmembrane regions in diatom LHC do not exhibit strong homology between each other as in CAB proteins. Thus, it is difficult to imagine in this case that a gene encoding a two-helix protein could have undergone a duplication and then a fusion process. These results currently support the idea that the first transmembrane helix from brown alga LHC and helices I and III of CAB are derived from the same ancestral protein.

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