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# The Novel Light-Harvesting Pigment-Protein Complex of *Mantoniella squamata* (Chlorophyta): Phylogenetic Implications

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**Summary.** A light-harvesting pigment–protein complex has been isolated from *Mantoniella squamata* (Micromonadophyceae, Chlorophyta) by non-denaturing polyacrylamide-gel electrophoresis. The complex runs as two bands of molecular weights 54,000 and 55,000. There are two constituent polypeptides of molecular weights 20,500 and 22,000. Antibodies were raised to the 20,500-dalton polypeptide from this complex and to the 24,500-dalton polypeptide from the analogous complex of *Pedinomonas minor* (Micromonadophyceae). The antibodies to the *M. squamata* polypeptide are specific for both polypeptides of the *M. squamata* light-harvesting complex, as well as for a 27,000-dalton polypeptide of undetermined function. The antibodies to the *P. minor* polypeptide are specific for polypeptide components of the light-harvesting complex of that alga. The antibodies specific for the *M. squamata* light-harvesting complex polypeptides do not cross react with any polypeptides of *P. minor* thylakoid membranes, as demonstrated by crossed immunoelectrophoresis. Similarly, no polypeptides of *M. squamata* thylakoids cross react with the antibodies specific for *P. minor* light-harvesting complex polypeptides. These results indicate that the light-harvesting complex of *M. squamata* is structurally very different from that of *P. minor*. In a survey of several land plants and green algae, including representatives of all classes of green algae, a light-harvesting complex homologous to that of *M. squamata* was found only in *Micromonas pusilla*. All other organisms tested possessed a light-harvesting complex homologous to that of *P. minor*. The evolutionary and taxonomic implications of the novel *M. squamata* light-harvesting complex are discussed.

**Key words:** Chlorophyta — Micromonadophyceae — *Mantoniella* — *Micromonas* — *Pedinomonas* — Pigment–protein complexes — Phylogeny — Immunochemistry

## Introduction

Photosynthetic pigments occur in cells as macromolecular complexes with proteins. There are several functional types of such complexes, including those that make up the reaction centers P700 and P680 and those involved in light harvesting (Glazer 1983). The major light-harvesting chlorophyll–protein complex of green algae and land plants (LHC, LHCP, CP-2, or chlorophyll a/b–protein complex) displays only minor variation in molecular weight, polypeptide composition, and spectral characteristics (Glazer 1983). Antisera to polypeptides of the light-harvesting complex from the green algae *Chlamydomonas reinhardtii* (Chua and Blomberg 1979; Schmidt et al. 1981; Tobin 1981) and *Acetabularia mediterranea* (Apel and Kloppstech 1978) cross react with light-harvesting complex polypeptides and precursor polypeptides of higher plants at the level of double-diffusion assays, crossed immunoelectrophoresis, and immunoprecipitation. As

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both *C. reinhardtii* (Chlorophyceae) and *A. mediterranea* (Ulvophyceae) are far removed from the evolutionary line of green algae (Charophyceae) that led to land plants (Mattox and Stewart 1984), it is evident that there is a high degree of conservation of both the immunological determinants of the constituent polypeptides and the functional aspects of the complex.

It is therefore of interest that the light-harvesting chlorophyll–protein complex of the green flagellate *Mantoniella squamata* has been shown to be of much higher molecular weight than "normal" light-harvesting complexes and to possess unusual spectral features (Brown 1983; J.S. Brown, personal communication). *M. squamata* is currently placed in the Micromonadophyceae (Mattox and Stewart 1984), a group of primitive green algae of uncertain affinities and generally considered to be the ancestral stock for all other classes of Chlorophyta. We have now determined the importance of the observed differences by biochemical and immunochemical comparison of the light-harvesting complex from *M. squamata* with those from another member of the Micromonadophyceae, *Pedinomonas minor,* and other green algae and higher plants.

## Materials and Methods

*Cultures and Growth Conditions.* Microalgae were obtained from the Culture Collection of Algae at the University of Texas at Austin (UTEX) and the Culture Collection of Marine Phytoplankton at Bigelow Laboratory for Ocean Sciences (CCMP). Species and strains investigated are given in Table 1. Marine algae were grown in the artificial-sea-water medium ESAW (Harrison et al. 1980) buffered with 10 mM Tris-HCl, pH 7.8, and enriched with 10-times-normal phosphate ($KH_2PO_4$) and nitrate ($NaNO_3$) plus 1% soil extract. Freshwater algae were cultured in a mixture of 9 parts Woods Hole medium (Nichols 1973) and 1 part soil extract. Cultures were grown at 19°C with a 14 h : 10 h light/dark period and continuous aeration. Barley (*Hordeum vulgare*) was grown in vermiculite at 25°C. *Chara* species and *Cladophora* species were collected from local populations.

*Isolation and Characterization of Light-Harvesting Complexes.* Microalgae were harvested by centrifugation; resuspended in isolation medium consisting of 300 mM sucrose, 125 mM potassium phosphate (pH 7.2), 10 mM KCl; and broken using a chilled French pressure cell at the minimum force required to break the majority of the cells. The lysed-cell suspension was diluted fourfold with 50 mM Tricine-NaOH (pH 7.5) and brought to 100 mM NaCl. Membranes were pelleted by centrifugation at 40,000 × g for 15 min. The supernatant was discarded, and the pellet was resuspended in 2 mM ethylenediaminetetraacetate (EDTA), 50 mM Tricine-NaOH (pH 7.5), 10 mM KCl, and centrifuged at 40,000 × g for 15 min to obtain an EDTA-washed membrane-enriched fraction. Macrophytes (including *Cladophora* species) were washed with water to remove contaminating organisms and blended in isolation medium (100 ml medium/15 g tissue) for 15 s in a Waring blender. *Cladophora* species cells were broken with a glass homogenizer after blending. The homogenate was filtered through two layers of Miracloth (Calbiochem), and EDTA-washed membranes were prepared as for microalgae. Thylakoid membranes were purified by flotation in sucrose gradients using the method of Chua and Bennoun (1975), except that all buffers contained 20 mM Tricine-NaOH (pH 7.5) instead of 20 mM Hepes. This method did not work with *Cladophora* species, and EDTA-washed membranes of that organism were used for subsequent experiments. All buffers contained the protease inhibitors benzamidine-HCl (5 mM) and 6-amino-n-hexanoic acid (1 mM), and all steps were carried out at 0–4°C. Pigment–protein complexes from *M. squamata* and *P. minor* were separated by lithium dodecyl sulfate polyacrylamide gradient (7.5–15%) gel electrophoresis at 4°C (Delepelaire and Chua 1981). Pigmented bands were excised, electroeluted, or diffused from the gel, and light-harvesting complexes identified by spectral characteristics. The method of Delepelaire and Chua (1981) was used to compare the native molecular weights of the light-harvesting complexes from the various organisms, as well as to determine the polypeptide compositions of isolated complexes. Molecular-weight markers (Sigma) were bovine serum albumin (66,000), egg albumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), trypsin inhibitor (20,100), and α-lactalbumin (14,200).

Absorption spectra of the isolated complexes were determined at room temperature using a Cary 210 absorption spectrophotometer. Chlorophyll was determined by the method of Arnon (1949).

*Production and Characterization of Antibodies to Polypeptides of the Light-Harvesting Complexes.* The major polypeptides identified as constituents of the light-harvesting complexes of *P. minor* (molecular weight 24,500) and *M. squamata* (molecular weight 20,500) were isolated by denaturing preparative sodium dodecyl sulfate polyacrylamide gradient (7.5–15%) gel electrophoresis of purified thylakoids at 4°C. Thylakoid membranes equivalent to 250 μg chlorophyll from *P. minor* and 125 μg chlorophyll from *M. squamata* were electrophoresed on 130 × 210 × 1 mm gels. Gels were stained with Coomassie Brilliant Blue R250, destained, and dried. Polypeptides to be used as antigens were excised from the gels and electroeluted. Polypeptides electroeluted from 14 gels were homogenized with 0.6 ml 0.1 M NaCl and 0.6 ml Freund's complete adjuvant and injected into female New Zealand White rabbits. Booster injections were given using incomplete adjuvant at 4- to 5-week intervals with bleeding 7–8 days following each booster. Immunoglobulin G (IgG) was purified from serum using Bio Rad DEAE Affigel Blue according to the manufacturer's instructions, followed by concentration by precipitation with 50% (of saturation) ammonium sulfate. IgG fractions were dialyzed extensively against Tris–acetate–EDTA buffer (pH 8.6) (Chua and Blomberg 1979) and characterized by double immunodiffusion (Ouchterlony and Nilsson 1973) and crossed immunoelectrophoresis (Chua and Blomberg 1979).

## Results

Several pigmented bands were detected when purified thylakoid membranes of *M. squamata* and *P. minor* were separated using lithium dodecyl sulfate polyacrylamide-gel electrophoresis under nondenaturing conditions. For *M. squamata,* the two major pigmented complexes, which were of molecular weights 54,500 and 54,000 (Fig. 1, lane 1), corresponded to the light-harvesting complex described by Brown (1983). These bands were isolated by excision from the gels followed by either electroelution

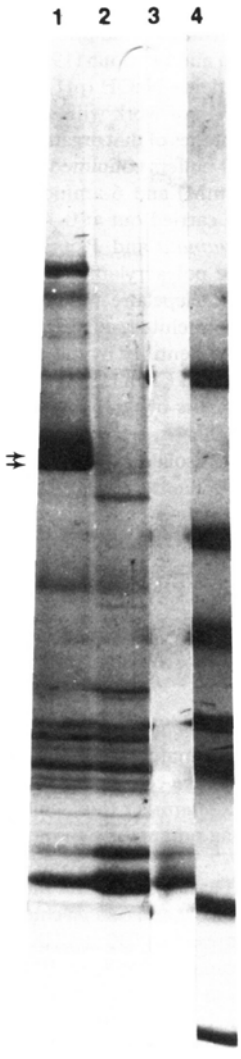


**Fig. 1.** Isolation of the light-harvesting pigment–protein complex of *Mantoniella squamata* by lithium dodecyl sulfate polyacrylamide gradient (7.5–15%) gel electrophoresis at 4°C. Lane 1: Thylakoid-membrane polypeptides separated under nondenaturing conditions. Arrows mark the light-harvesting pigment–protein complexes. Lane 2: Thylakoid-membrane polypeptides denatured by heating at 100°C for 1 min. Lane 3: Polypeptide components of the light-harvesting complex isolated by preparative polyacrylamide gel electrophoresis. The 54,000-dalton complex was excised from gels, electroeluted, and applied to the gel after denaturation by heating at 100°C for 1 min. Lane 4: Molecular weight markers, as described in Materials and Methods; cf. also Fig. 2

or diffusion from the gel slices, and the polypeptide constituents were determined by reelectrophoresis after denaturation of the complexes by heating at 100°C for 1 min. The molecular weights of the polypeptide components of the isolated complex were 22,000 and 20,500 (Fig. 1, lane 3). The major polypeptides that appeared on denaturation of the thylakoid membranes also had molecular weights of 22,000 and 20,500 (Fig. 1, lane 2).

Three pigmented bands were detected from *P. minor* thylakoid membranes in the molecular-weight range 25,000 to 30,000 (Fig. 2, lane 5), corresponding to the light-harvesting complexes of *Chlamydomonas reinhardtii* and other green algae and land plants (Delepelaire and Chua 1981; Glazer 1983). The light-harvesting complexes of molecular weights 25,000 and 26,000 from *P. minor* were isolated, and three major constituent polypeptides, of molecular weights 26,500, 25,000, and 24,500, were detected (Fig. 2, lanes 2 and 3); these also corresponded to major polypeptides of the denatured thylakoid membranes (Fig. 2, lane 4). Although the two light-harvesting complexes contained the same polypeptides, the stoichiometries of these polypeptides were different. *P. minor* light-harvesting complex appears to be very similar to the light-harvesting complexes of other green algae and land plants, which generally produce three to five pigmented bands on nondenaturing gels and contain three to six constituent polypeptides (Delepelaire and Chua 1981).

The molecular weights of both the native *M. squamata* light-harvesting complex and the constituent polypeptides, however, are very different from those of the complexes of any other green algae or land plants that have been examined, including those ulvophycean algae that have light-harvesting complexes with large quantities of carotenoids (Anderson et al. 1980; Anderson 1983; Benson and Cobb 1983).

The room-temperature absorption spectra of the light-harvesting complexes of *M. squamata* and *P. minor* are given in Fig. 3. As has been noted by Brown (1983), the spectrum of the light-harvesting complex of *M. squamata* differs in several ways from the absorption spectra of other green algae and of land plants. The chlorophyll-b maximum of *M. squamata* is shifted 5 nm toward shorter wavelengths from the *P. minor* chlorophyll-b maximum (643 nm vs 648 nm). The absorption maximum near 630 nm may be due to a third chlorophyll species, magnesium 2,4-divinylphaeoporphyrin $a_5$ monomethyl ester, which is present in *M. squamata* (Ricketts 1970). The carotenoid prasinoxanthin, also present in *M. squamata* (Foss et al. 1984), probably contributes to absorption in the region 500–550 nm. Although carotenoids are not generally such an important part of the light-harvesting complexes of green algae and higher plants, they do play an important role in light harvesting in some ulvophycean algae (Anderson et al. 1980; Anderson 1983; Benson and Cobb 1983). The absorption spectrum of the light-harvesting complex of *M. squamata* can be considered different from those of complexes from other green algae in both the shift of the chlorophyll-b absorption and the presence of the chlorophyll-c-like pigment. The absorption spectrum of the light-

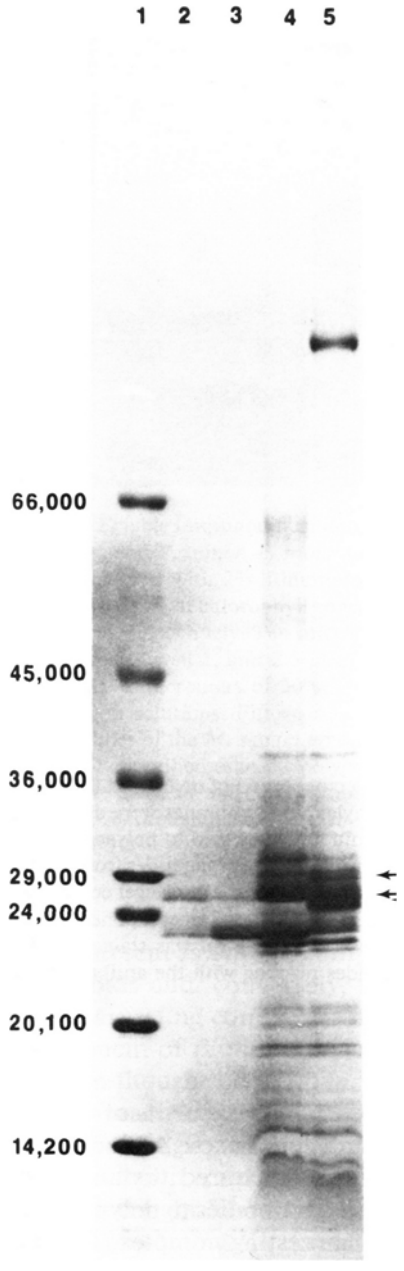


**Fig. 2.** Isolation of the light-harvesting pigment–protein complex of *Pedinomonas minor* by lithium dodecyl sulfate polyacrylamide gradient (7.5–15%) gel electrophoresis at 4°C. Lane 1: Molecular-weight markers. Lane 2: Polypeptide components of the 25,000-dalton complex. Lane 3: Polypeptide components of the 24,500-dalton complex. Lane 4: Thylakoid-membrane polypeptides denatured by heating at 100°C for 1 min. Lane 5: Thylakoid-membrane polypeptides separated under nondenaturing conditions. Light-harvesting pigment–protein complexes are indicated by arrows. Samples prepared as in Fig. 1

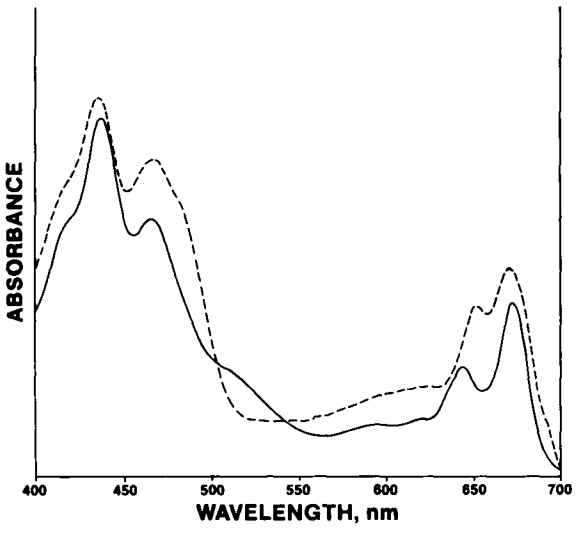


**Fig. 3.** Absorption spectra of light-harvesting pigment–protein complexes. Solid line: *M. squamata* light-harvesting complex. Dashed line: *P. minor* light-harvesting complex. Complexes were isolated by preparative lithium dodecyl sulfate polyacrylamide gel electrophoresis and diffused from gel pieces into 50 mM Tricine-NaOH, pH 7.5. Absorption spectra were taken at room temperature using a Cary 210 spectrophotometer

harvesting complex from *P. minor* is essentially identical to those of many other green algae and higher plants (Delepelaire and Chua 1981; Brown 1983).

However, differences in the spectra and molecular weights of the native complexes and the constituent polypeptides do not necessarily indicate that the complexes or polypeptides are fundamentally different. The lower-molecular-weight polypeptides present in the *M. squamata* complex could be a result of modification of a portion of the normal polypeptide and subsequent evolutionary development of a new arrangement resulting in a higher-molecular-weight complex. Also, the *M. squamata* type might represent a primitive condition from which the light-harvesting complexes of other plants were derived. A third possibility is that the light-harvesting complex found in *M. squamata* is present as a minor component of other green-algal thylakoids, or, conversely, that the normal light-harvesting complex is present as a minor component of *M. squamata* thylakoids.

Antibodies to both the 20,500-dalton polypeptide from the light-harvesting complex of *M. squamata* and the 24,500-dalton polypeptide from *P. minor* were raised in rabbits. Since these antibodies were produced using sodium dodecyl sulfate-denatured polypeptides as antigens, the determinants should be specific for the primary structure of the polypeptides, rather than the conformation (Chua and Blomberg 1979). The antibodies to the *M. squamata* polypeptide were specific for the intact light-harvesting complex and for both the 20,500- and 22,000-dalton polypeptides of the *M. squamata* light-harvesting complex, as demonstrated by crossed immunoelectrophoresis using purified thylakoid membranes, either nondenatured (data not shown) or denatured (Fig. 4), in the first dimension. One other polypeptide, of molecular weight 27,500, also reacted with the antibodies (Fig. 4). The nature of this polypeptide is unknown. At least three poly-

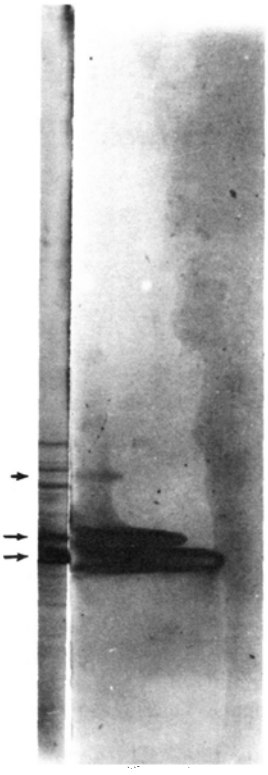

**Fig. 4.** Crossed immunoelectrophoresis of denatured thylakoid membranes of *M. squamata*. Thylakoid membranes were denatured by heating at 100°C for 1 min and subjected to polyacrylamide gradient (7.5–15%) gel electrophoresis. Polypeptides from a strip of the gel were then electrophoresed into agarose gel containing antibodies prepared against the 20,500-dalton component of the *M. squamata* light-harvesting complex. The gel was washed extensively with 0.15 M NaCl followed by several changes of distilled water. Precipitin lines were then visualized by staining with Coomassie Brilliant Blue R250. Major polypeptides reacting with the antibodies are marked with large arrows and the minor reacting polypeptide with a smaller arrow

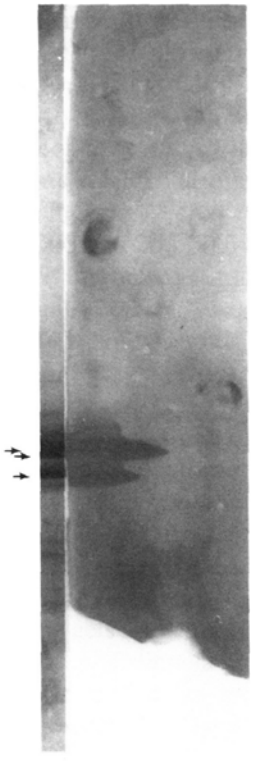

**Fig. 5.** Crossed immunoelectophoresis of denatured thylakoid membranes of *P. minor*. Thylakoid membranes were denatured by heating at 100°C for 1 min and subjected to polyacrylamide gradient (7.5–15%) gel electrophoresis. Polypeptides from a strip of the gel were then electrophoresed into agarose gel containing antibodies prepared against the 24,500-dalton component of the *P. minor* light-harvesting complex. The gel was stained as described in Fig. 5. Polypeptides reacting with the antibodies are marked with arrows

peptides from *P. minor* thylakoid membranes, including the polypeptides of molecular weights 24,500 and 26,500 identified as constituents of the light-harvesting complex, reacted with antibodies to the *P. minor* 24,500-dalton polypeptide (Fig. 5). The 27,000-dalton polypeptide, which also reacts with these antibodies, may also be a component of a light-harvesting complex. The techniques used may not have resolved the 24,500- and 25,000-dalton constituents of the light-harvesting complex well enough for us to determine whether both or only the former was reacting with the antibodies. The intact *P. minor* light-harvesting complex also reacted with the antibodies (data not shown). These data indicate that the antibodies produced to polypeptide constituents of the light-harvesting complexes of *M. squamata* and *P. minor* are polyspecific for up to four thylakoid-membrane polypeptides, including those known to be components of light-harvesting complexes.

These antibodies were then used to test the relatedness of the polypeptide constituents of the two types of light-harvesting complexes. As judged by double immunodiffusion, denatured thylakoids of *P. minor* reacted with the antibodies to polypeptides of the *P. minor* light-harvesting complex, but not with the antibodies specific for polypeptides of the light-harvesting complex of *M. squamata* (Fig. 6). Conversely, denatured thylakoids of *M. squamata* reacted with the *M. squamata* antibodies, but not with the *P. minor* antibodies (Fig. 6).

Results were similar with the more sensitive method of crossed immunoelectrophoresis. When thylakoid membranes from *M. squamata* were tested against antibodies to the *P. minor* polypeptide using crossed immunoelectrophoresis, no precipitin lines were observed (data not shown). Using denatured *P. minor* thylakoid membranes against antibodies to *M. squamata* polypeptides also produced no precipitin lines (data not shown).

These results indicate that the *M. squamata* light-harvesting complex polypeptides are structurally very different from those of *P. minor*. They also show that the light-harvesting complex of *P. minor*

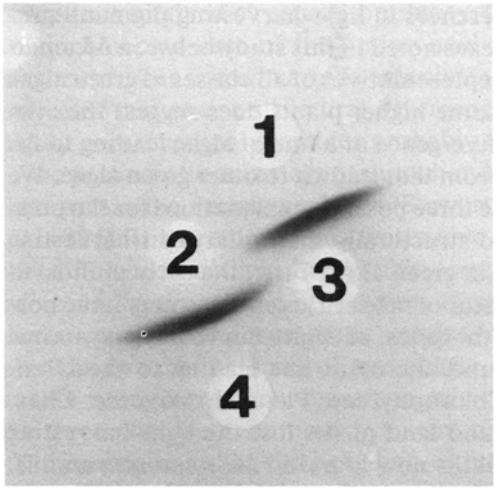


**Fig. 6.** Double immunodiffusion of denatured thylakoids of *M. squamata* and *P. minor*. *P. minor* and *M. squamata* thylakoids were denatured with 2% lithium dodecyl sulfate and heated at 100°C for 1 min at chlorophyll concentrations of 500 μg/ml and 250 μg/ml, respectively. Ten microliters of *P. minor* thylakoids was placed in well 1, and 2.5 μl of *M. squamata* thylakoids in well 4. Five microliters of 20% Triton X-100 was mixed with the antigen solutions. Fifteen microliters of antibodies to the polypeptides of the *M. squamata* complex was placed in well 2, and 15 μl of antibodies to the polypeptides of the *P. minor* complex was placed in well 3. Diffusion was carried out for about 24 h at room temperature in an agarose gel containing 1% Triton X-100

is not present as a minor component of *M. squamata* thylakoids and, conversely, that the *M. squamata* light-harvesting complex is not present as a minor component of *P. minor* thylakoids.

Even though the *P. minor* light-harvesting complex appears to be similar in every way to those of other green algae and of higher plants, the possibility remains that it is structurally different. We therefore compared light-harvesting complexes from organisms representing the five classes of Chlorophyta and from higher plants with those of *M. squamata* and *P. minor*. When subjected to nondenaturing polyacrylamide gel electrophoresis, all the organisms studied except the minute flagellate *Micromonas pusilla* (Micromonadophyceae) produced multiple pigmented bands in the 25,000–35,000 molecular-weight range (Table 1).

The molecular weight of the light-harvesting complex of *M. pusilla* is similar to that of the *M. squamata* complex, and in addition the denatured-thylakoid-membrane profile for *M. pusilla* contains two major polypeptides of molecular weights similar to those of the *M. squamata* light-harvesting complex (data not shown). The absorption spectra (Brown 1983) and pigment compositions (Ricketts 1970, Foss et al. 1984) of *M. squamata* and *M. pusilla* are known to be comparable.

Of the other organisms tested, only the *Micro-*

**Table 1.** Comparison of light-harvesting pigment–protein complexes of green plants

| | Reaction with anti-*Mantoniella* | Reaction with anti-*Pedinomonas* | Complex size |
|---|---|---|---|
| Micromonadophyceae | | | |
| *Asteromonas gracilis* Artari [CCMP STGRA] | − | + | 25–35,000 |
| *Mantoniella squamata* (Manton & Parke) Desikachary [UTEX 990] | + | − | ca. 55,000 |
| *Micromonas pusilla* Manton & Parke [UTEX 991] | + | − | ca. 54,000 |
| *Nephroselmis rotunda* (Carter) Manton [UTEX 996] | − | + | 25–35,000 |
| *Pedinomonas minor* Korsh. [UTEX 1350] | − | + | 25–35,000 |
| Chlorophyceae | | | |
| *Chlamydomonas reinhardtii* Dang. [UTEX 89] | − | + | 25–35,000 |
| Charophyceae | | | |
| *Chara* sp. [local collection] | − | + | 25–35,000 |
| *Klebsormidium flaccidum* (Kutz.) Silva, Mattox & Blackwell [Mattox] | − | + | 25–35,000 |
| Pleurastrophyceae | | | |
| *Microthamnion* sp. [UTEX 237] | − | + | 25–35,000 |
| *Tetraselmis suecica* (Kylin) Butcher [UTEX 2286] | − | + | 25–35,000 |
| Ulvophyceae | | | |
| *Cladophora* sp. [local collection] | − | + | 25–35,000 |
| Others | | | |
| Barley (*Hordeum vulgare* L.) | − | + | 25–35,000 |

Results of double-diffusion assays using purified thylakoid membranes of each organism against the antibodies raised to the 20,500-molecular-weight polypeptide of the light-harvesting complex of *Mantoniella squamata* are given in the first column. Results of double-diffusion assays using the antibodies raised to the 24,500-molecular-weight polypeptide of the light-harvesting complex of *Pedinomonas minor* are given in the second column. The formation of a precipitin line is indicated by a +; no reaction, by a −. The approximate molecular-weight range of the light-harvesting complex as determined by nondenaturing lithium dodecyl sulfate polyacrylamide gradient (7.5–15%) gel electrophoresis is given in the third column. Sources of organisms are given in brackets (for abbreviations, see Materials and Methods)

*thamnion* species was unusual, in that the light-harvesting complex was yellow–green, perhaps due to the presence of the carotenoids siphonaxanthin and siphonein, which are known to be present in *Microthamnion kützingianum* (Goodwin 1980).

Using double immunodiffusion, we tested purified thylakoid membranes from several organisms for cross reactions with antibodies to the light-harvesting complex polypeptides of *P. minor* and *M. squamata*. The results are presented in Table 1. Only *M. pusilla* and *M. squamata* produced precipitin lines against the *M. squamata* antibodies, whereas all other organisms produced precipitin lines against the *P. minor* antibodies. Generally, either no spurs or very small spurs were produced in these assays, and no basis presently exists for their interpretation. These results are convincing evidence that the light-harvesting complex of *P. minor* is homologous with the light-harvesting complexes found in all other classes of green algae and higher plants, whereas that of *M. squamata* is very different, with a homologous complex known so far only in *M. pusilla*.

## Discussion

The phylogeny of the Chlorophyta and land plants as interpreted by Mattox and Stewart (1984) is based largely on characteristics of the flagellar apparatus and cell division, with little reference to chloroplast structure. Indeed, the only chloroplast characters that have been found useful for green-algal classification pertain to pyrenoid structure and location of stored starch. This is largely because so little variation is seen in the basic characteristics of the chloroplast throughout the Chlorophyta and land plants. The chloroplasts of all green algae and land plants have some development of grana stacks, possess chlorophyll b as an accessory pigment, and are surrounded by a two-membrane envelope. Starch is generally present as a storage polysaccharide, but its location in the chloroplast varies. Pyrenoids are present in most green algae and some primitive land plants, but not in higher plants. In all of these ways, the chloroplast of *M. squamata* resembles those of other green algae. In fact, *M. squamata* possesses all those characteristics that are considered to define the Chlorophyta, namely, the "star" and "H" in the flagellar transition region, chlorophyll b, and starch storage (Manton and Parke 1960; Salisbury and Floyd 1978; Barlow and Cattolico 1980, 1981). There are, however, some differences in scales, pyrenoid structure, flagellar apparatus, and cell division between *M. squamata* and other green algae (Manton and Parke 1960; Barlow and Cattolico 1980, 1981), but these are certainly not enough to suggest removal of *M. squamata* from the Chlorophyta.

The differences in light-harvesting pigment–protein complexes noted in this study between *M. squamata* and representatives of all classes of green algae as well as some higher plants does suggest the evolutionary divergence of a line of algae leading to *M. squamata* from that leading to other green algae. We can propose three possible explanations for the presence of two structurally dissimilar light-harvesting complexes in green algae. First, the early photosynthetic ancestor of modern green algae may have possessed both types of light-harvesting systems. Through time, the main line leading to the Ulvophyceae, Chlorophyceae, Pleurastrophyceae, Charophyceae, and land plants lost the light-harvesting complex that is now found in *M. squamata* and *M. pusilla*, while the evolutionary line leading to *M. squamata* lost the complex found in *P. minor* and other green algae. Second, the complex found in *M. squamata* may have evolved directly from the form found in *P. minor*, or vice versa. Third, the chloroplast of *M. squamata* may have had an independent origin from a photosynthetic prokaryote that was structurally similar to the prokaryotic precursor of other green-algal chloroplasts, but possessed a different light-harvesting complex.

If the first explanation, that the ancestor of all green algae had two structurally distinct forms of light-harvesting complexes, is true, such an organism might be found among extant green algae. We are continuing to examine members of the Micromonadophyceae to test this possibility. The current best candidate for the prokaryotic precursor of green-algal chloroplasts is *Prochloron* or a similar organism (Lewin 1977). *Prochloron* has been shown to possess a light-harvesting complex very similar to that of *P. minor*, with no apparent complex corresponding to the light-harvesting complex of *M. squamata* (Withers et al. 1978; Shuster et al. 1984). Although it is possible that *Prochloron* has lost the complex now found in *M. squamata*, and that the actual prokaryote that became green-algal chloroplasts had both types of complex, the presence of only a single light-harvesting system in *Prochloron* is indirect evidence against such a mode of evolution.

It seems to us unlikely that the light-harvesting complex of *M. squamata* evolved by modification of the amino acid sequences of the polypeptide constituents of the light-harvesting complex of other green algae, or vice versa. Our results show that the light-harvesting complex, including the structure of the constituent polypeptides, is highly conserved in green algae and land plants. It seems unlikely that the light-harvesting complex of one line of evolution would have changed so much that structural similarity would not be detected by the sensitive method of crossed immunoelectrophoresis, while all other

complexes retained structural similarity. In the better-documented case of the amino acid sequences of cytochromes c from various organisms, evidence indicates that major structural divergence of these highly conserved polypeptides probably took place in the prokaryotic ancestors of mitochondria, rather than within the eukaryotic hosts (Stewart and Mattox 1984). It therefore seems reasonable to us that the differences in light-harvesting complexes between *M. squamata* and other green algae could indicate separate endosymbiotic origins of chloroplasts in two distinct lines of green algae. However, at this time, there is no reason to rule out any of the three possible evolutionary origins presented.

It is apparent that the light-harvesting complex of *M. squamata* and *M. pusilla* represents a unique character not found in other green algae and that the light-harvesting complex is a highly conserved character in green algae and land plants. *M. squamata* is also different from established classes of green algae in scale structure, pyrenoid structure, flagellar apparatus, chloroplast pigments, and some aspects of cell division (Manton and Parke 1960; Barlow and Cattolico 1980, 1981). *M. pusilla* shares some of these characteristics with *M. squamata* (Manton 1959; Manton and Parke 1960; Ricketts 1970). Recently, Moestrup (1984) has proposed the order Mamiellales, including the flagellates *Mamiella, Mantoniella,* and *Dolichomastix* (Manton 1977), based on the unusual body and flagellar scales found on these algae. *Micromonas* could not be included in this order, because it lacks scales. *Mamiella gilva* resembles *M. squamata* also in details of the flagellar apparatus, pyrenoid (Moestrup 1984), and chloroplast pigments (Ricketts 1970). Other algae have been shown to possess pigmentation similar to that of *Mantoniella, Micromonas,* and *Mamiella* (Ricketts 1970), including some coccoid forms that are important in the oceanic nanoplankton (Foss et al. 1984), or to have scales similar to those of *Mantoniella* and *Mamiella* (Johnson and Sieburth 1982). If these algae were shown to possess the *M. squamata* type of light-harvesting pigment–protein complex, they could be placed into a line of evolution separate from that of other green algae and characterized by reduction from *Mamiella,* with two equal flagella (Moestrup 1984), to minute, nonflagellate, coccoid organisms (Johnson and Sieburth 1982; Foss et al. 1984). This group would probably deserve class-level recognition. We are currently working to determine the types of light-harvesting complexes present in these organisms.

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