The Role of Acyl Lipids in Reconstitution of Lipid-Depleted Light-Harvesting Complex II from Cold-Hardened and Nonhardened Rye

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**ABSTRACT**

The role of acyl lipids in the in vitro stabilization of the oligomeric form of light-harvesting complex II of winter rye (Secale cereale L. cv MusKateer) grown at 5 or 20°C was investigated. Purified light-harvesting complex II was enzymically delipidated to various extents by treatment with the following lipolytic enzymes: phospholipase C, phospholipase A₂, and galactolipase. Complete removal of phosphatidylcholine plus phosphatidylglycerol caused a decrease in the ratio of oligomeric:monomeric forms from 1.86 ± 0.17 to 0.85 ± 0.17 and 3.51 ± 0.82 to 0.81 ± 0.29 for purified cold-hardened and nonhardened light-harvesting complex II, respectively, with no change in free pigment content. Incubation of delipidated cold-hardened or nonhardened light-harvesting complex with purified thylakoid phosphatidylglycerol containing trans-Δ⁶-hexadecenoic acid resulted in 48% reconstitution of the oligomeric form on a total chlorophyll basis with an oligomer:monomer of about 1.90. Incubation in the presence of di-16:0 or di-18:1 phosphatidylglycerol, phosphatidylcholine, monogalactosyldiacylglyceride, or digalactosyldiacylglyceride caused no oligomerization, but rather a further destabilization of the monomeric form. These lipid-dependent structural changes were correlated with significant changes in the 77K fluorescence emission spectra for purified light-harvesting complex II. We conclude that the stabilization of the supramolecular organization of light-harvesting complex II from rye is specifically dependent upon molecular species of phosphatidylglycerol containing trans-Δ⁶-hexadecenoic acid.

The LHCII of PSII was one of the first pigment proteins to be characterized in higher plants (3, 27, 28). Generally, about 50% of the Chl and about 30% of the protein present in chloroplast thylakoids is associated specifically with this complex (27, 28). In addition, the xanthophylls lutein, violaxanthin, and neoxanthin are associated with LHCII (23). These pigments are noncovalently bound to LHCII polypeptides. The remainder of the Chl and carotenoids are associated with LHCl core antennae and reaction center complexes of PSII and PSI (21). LHCl, with an apparent molecular mass of 72 kD and a Chl a/b of 1.0 to 1.3, is considered to be the primary form of this complex. This is supported by recent electron crystallographic data (17, 18) as well as circular differential scattering of LHCII aggregates (5, 6). Upon denaturation of the holocomplex with SDS, typically three to four polypeptides are observed in the molecular mass range of 25 to 30 kD. These polypeptides are the products of a family of light-regulated, nuclear-encoded cab genes (8). LHClII functions not only as the major antenna for PSI, but also regulates energy distribution between PSII and PSI and thylakoid membrane appression (26).

The role of thylakoid lipids in the stabilization of oligomeric LHClII is controversial. Plumley and Schmidt (23) reported that the presence of thylakoid lipids such as MGDG, DGDG, PG, or PC, separately or in combination, inhibited the reconstitution of LHClII. They concluded that in vitro assembly of LHClII was specifically dependent upon the presence of Chl a, Chl b, and xanthophylls and that the fact that diacylglycerols are not integral components of LHClII. In addition, Somerville’s group (2, 20, 22), using a mutant of Arabidopsis thaliana specifically lacking t-16:1 in thylakoid PG, concluded that PG containing t-16:1 was not required for either grain stacking or stabilization of oligomeric LHClII in vitro.

In contrast to the above, data from several laboratories support a functional association between PG and the stabi-
lization of LHCII (4, 7, 11, 14–16, 24). Trémolières et al. (4, 24) were the first to present evidence that the t-16:1 content of the primary phospholipid of higher plant thylakoids, PG, is correlated with the stabilization of LHCII. Using a mutant of Chlamydomonas reinhardtii that lacks t-16:1 and does not exhibit LHCII, they reported that in vivo uptake and incorporation of exogenous PG containing t-16:1 by the mutant resulted in the restoration of LHCII (7).

Huner et al. (11, 14, 16) have shown that exposure of rye to low, cold-hardening growth temperature (5°C) (RH) results in a specific 72% lower t-16:1 content in thylakoid PG compared with the same plants grown at 20°C (RNH). No other significant changes were observed in either thylakoid lipid content or fatty acid composition. In vitro separation of the Chl-protein complexes from thylakoids indicate that LHCII, predominates, whereas LHCII, predominates when Chl-protein complexes are separated from RNH thylakoids. This has been confirmed for several cultivars of rye, wheat, triticale, and barley exposed to cold hardening under natural field conditions (N.P.A. Huner and J.P. Williams, unpublished results). Furthermore, stabilization of LHCII during chloroplast biogenesis indicated that optimal levels of LHCII occurred only after maximum, light-dependent synthesis of t-16:1 in thylakoid PG had occurred (14). This is observed with no change in poly peptide or pigment composition of the thylakoid membranes (10, 11). The in vitro data indicating a change in the supramolecular organization of LHCII in response to low growth temperature is corroborated by in situ data of isolated thylakoids by differential scanning calorimetry, 77K fluorescence emission spectroscopy, room temperature and 77K linear dichroism spectroscopy (J. Lazenby, D. Bruce, N.P.A. Huner, unpublished results), as well as freeze fracture (10, 14, 16). Furthermore, PG has been shown to be tightly and specifically associated with LHCII during purification (16). It was concluded that the difference in the stability between RH and RNH LHCII could be totally accounted for by the difference in the t-16:1 content of PG. Indeed, a recent electron spin resonance study reported by Li et al. (19) indicates that a unique feature of thylakoid membranes is the specificity of interaction of thylakoid PG for thylakoid proteins.

According to our previous results (11, 16), the stability of rye LHCII appears to be specifically dependent upon PG and its t-16:1 content. As an extension of this work, we now describe the in vitro reconstitution of the oligomeric form from lipid-depleted RH and RNH LHCII and address the following questions. First, is the in vitro oligomerization of rye LHCII dependent upon PG containing t-16:1 specifically? Second, how does the molecular species composition of PG and other thylakoid lipids affect the reconstitution of LHCII? Last, is the reconstitution of LHCII correlated with specific changes in the spectral properties of LHCII?

MATERIALS AND METHODS

Plant Material

Winter rye (Secale cereale L. cv Muskateer) was grown in vermiculite watered with Hoagland nutrient solution in controlled environment growth chambers at cold-hardening (5/5°C day/night) and nonhardening (20/16°C day/night) temperatures and a 16-h photoperiod at an irradiance of 250 μmol m⁻² s⁻¹ PAR, as described in detail elsewhere (13). Based upon comparative growth kinetics (13), cold-hardened and nonhardened plant material of comparable physiological age were used throughout.

Isolation and Purification of LHCII

LHCII complex was isolated and purified by successive cation precipitation as described in detail previously (16). To minimize the cation concentration in purified RH and RNH LHCII, preparations were resuspended and washed three times in 100 mM EDTA (pH 7.8) at a Chl concentration of 0.1 mg ml⁻¹. Purified LHCII preparations were then resuspended in 50 mM Tricine-NaOH (pH 7.8) containing 50% (v/v) glycerol and stored at −80°C until further use.

Enzymic Hydrolysis of Lipids from Purified LHCII

RH and RNH LHCII preparations were incubated in 50 mM Tricine-NaOH buffer (pH 7.5) containing lipolytic enzymes for 90 min at 25°C in the dark, as described previously (16). Bean galactolipase was present at a concentration of 500 μg protein mg⁻¹ Chl. PLC (Sigma, Bacillus cereus, EC 3.1.4.3) and PLβ2 (Sigma, Vipera russelli, EC 3.1.1.4.) were present at concentrations of 50 units mg⁻¹ Chl. Subsequently, LHCII preparations were collected by centrifugation, washed twice with incubation buffer, and analyzed for acyl lipid content and fatty acid composition. The relative proportions of LHCII₁, LHCII₁₂, and LHCII₃ determined after separation by SDS/DOC-PAGE as described below.

Bean galactolipid lipase (lipolytic acyl hydrolase) was purified from bean leaves as described previously (16).

Reconstitution of Lipid-Depleted RH and RH LHCII

The following acyl lipids used for reconstitution experiments were purchased from Sigma: dipalmitoyl-PG, dioleoyl-PG, dipalmitoyl-PC, linoleoylpalmitoyl-PC, MGDG, and DGDG. t-16:1 containing PG was isolated from total lipid extracts of RNH thylakoids and purified by preparative TLC as described by Khan and Williams (12). The lipids used for reconstitution of RH and RNH LHCII were dissolved in chloroform:methanol (98:2, v/v) and evaporated under nitrogen. Subsequently, 5 mM Tricine-NaOH buffer (pH 7.5) containing 50 mM glycerol and 0.5 mM EDTA was added to the lipid samples and the mixtures were sonicated under nitrogen for 2 h at 10°C in an ultrasonic water bath (Branson Co.). Suspensions of untreated or lipid-depleted LHCII in the above sonication buffer were added to the sonicated lipids at a final concentration of 0.5 mg ml⁻¹. This mixture was sonicated for 60 s and then slowly cooled from 20 to −40°C over 90 min in a methanol bath (Endocal, Neslab). The sample was then immediately allowed to warm gradually to 20°C over a 30-min period in the same bath and subsequently sonicated for 60 s at 10°C. After three repetitions, the reconstituted LHCII was collected by centrifugation, washed once with sonication buffer, resuspended in 50 mM Tricine-NaOH (pH 7.8) containing 50% (v/v) glycerol, and stored at −80°C.
Chl-protein complexes associated with the untreated, lipid-depleted, and reconstituted LHCII preparations were separated using the SDS/DOC detergent system described in detail earlier (11, 14). Gels were scanned at 671 nm with a Shimadzu UV-160 spectrophotometer. Relative Chl contents of the individual complexes were estimated as (individual peak area/total area of scan) × 100%.

Denaturing SDS-PAGE of purified LHCII was performed on 12% (w/v) polyacrylamide gels as described earlier (16). Sample purity was estimated by scanning the Coomassie-stained gel and calculating (peak area for the LHCII polypeptides/total area of the scan) × 100%.

77K Fluorescence Emission Spectra

Steady-state fluorescence emission spectra of control, lipid-depleted, and reconstituted RH and RNH LHCII were recorded at 77K using an LS-1 Fluorescence spectrophotometer (Photon Technology International Inc.). Excitation was at 440 nm with a 12-nm slit width, and emission was collected with a 4-nm slit width. LHCII samples were resuspended in 25 mM Tricine-NaOH (pH 7.8) containing 50% glycerol to a concentration of 1 μg Chl mL⁻¹ and frozen in liquid N₂. All spectra were corrected for the spectral sensitivity of the instrument and processed using PTI dedicated software. Unless otherwise stated, all spectra represent the average of at least three independent experiments.

Acyl Lipid Analyses

Purified RH and RNH LHCII were extracted before and after treatment with the lipolytic enzymes and analyzed for their lipid and fatty acid composition, as described by Williams et al. (29).

Chl Analyses

Chl analyses were performed according to Arnon (1).

RESULTS

Composition and Structure of Purified RH and RNH LHCII

SDS-PAGE indicated the presence of two major polypeptides of 26 and 27 kDa (data not shown), an overall purity of 95%, Chl a/b of 1.19 ± 0.06, and Chl/protein of about 10 ± 1 for both RH and RNH LHCII. Both RH and RNH LHCII exhibited 62 mol % MGDG + DGDG, 30 mol % PG, 2 mol % SQDG, and 5 mol % PC. Thus, purified RH and RNH LHCII exhibited a 3-fold enrichment specifically in PG that is typically present at a level of about 10 mol % in rye thylakoids (11, 14). Although the content of the major lipids was not significantly different, RNH LHCII exhibited a higher level of t-16:1 (40 mol %) in PG than RH LHCII (13 mol %) at the expense of the 16:0 content of PG. This resulted in a ratio of t-16:1/16:0 of 2.4 for RH LHCII and 0.3 for RH LHCII. These results for the polypeptide, pigment and lipid content, and composition of RH and RNH LHCII are similar to the more detailed results published earlier (16).

RH and RNH LHCII exhibited the presence of three Chl-protein complexes upon mildly denaturing gel electrophoresis in the presence of SDS and DOC: LHCII, apparent LHCII₂, and LHCII₃ (Fig. 1). Untreated, control RH LHCII consistently exhibited a higher ratio of LHCII₃/LHCII₁ (3.51 ± 0.82) than untreated, control RH LHCII (1.86 ± 0.63) (Table I).

Fluorescence emission spectra for purified LHCII were collected at 77K. At Chl concentrations of 1 μg mL⁻¹ or less, purified RNH LHCII exhibited a strong emission band at 679 and at 694 nm and low emission at 730 nm (data not shown). This is consistent with an earlier report by Sarvari and Nyitray (25). However, at Chl concentrations greater than 1 μg mL⁻¹, the fluorescence emission at 694 nm was significantly reduced relative to that at 679 nm (data not shown). Thus, we employed a Chl concentration of 1 μg mL⁻¹ for all comparative fluorescence emission spectra presented.

In contrast to RH LHCII, purified RH LHCII exhibited a stronger emission at 694 nm than 679 nm (Fig. 2A) with both preparations at a concentration of 1 μg mL⁻¹. These spectra have been checked and found to be consistent using three different instruments in three different laboratories. Thus, it appears that a higher ratio of LHCII₁:LHCII₃ is correlated with a higher ratio of F₆₇₉:F₆₉₄. However, it is interesting to note that the 77K emission spectra of LHCII₁, LHCII₂, and LHCII₃ are very similar after solubilization with SDS/DOC, electrophoretic separation, and extraction from polyacrylamide gels containing SDS and DOC (Fig. 2B).
Table I. The Effects of Lipid Hydrolysis and Reconstitution with PC on LHCII1:LHCII3 Ratios for Purified RH and RNH LHCII

The data are from reconstitution experiments in which a 5-fold excess of the lipid was used. Values represent the mean ± SD for three to six experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lipid Added</th>
<th>LHCII1:LHCII3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RH LHCII:RNH LHCII</td>
</tr>
<tr>
<td>Control</td>
<td>None</td>
<td>3.51 ± 0.82:1.86 ± 0.63</td>
</tr>
<tr>
<td>PLC</td>
<td>None</td>
<td>3.28 ± 0.61:1.88 ± 0.40</td>
</tr>
<tr>
<td>PLC</td>
<td>di-16:0-PC</td>
<td>3.38 ± 0.43:2.17 ± 0.55</td>
</tr>
<tr>
<td>PLC</td>
<td>18:2/16:0-PC</td>
<td>3.40 ± 0.20:1.88 ± 0.33</td>
</tr>
<tr>
<td>PLC/PLA2</td>
<td>None</td>
<td>0.81 ± 0.29:0.85 ± 0.17</td>
</tr>
<tr>
<td>PLC/PLA2</td>
<td>di-16:0-PC</td>
<td>0.34 ± 0.09:0.34 ± 0.13</td>
</tr>
<tr>
<td>PLC/PLA2</td>
<td>18:2/16:0-PC</td>
<td>0.74 ± 0.19:0.77 ± 0.29</td>
</tr>
<tr>
<td>PLC/PLA2/CL</td>
<td>None</td>
<td>0.68 ± 0.11:0.72 ± 0.19</td>
</tr>
</tbody>
</table>

Effects of Acyl Lipid Hydrolysis on the Structure of Purified LHCII

PLC treatment caused the total hydrolysis of PC with negligible effects on the other lipid constituents of both RH and RNH LHCII, as reported previously (16). This resulted in minimal changes in the ratio of LHCII1:LHCII3 (Table I) or in the 77K fluorescence emission spectra (data not shown).

Simultaneous treatment of purified RH and RNH LHCII with PLC and PLA2 removed all of the PC and PG, as reported previously (17). This was accompanied by a significant reduction in the ratio of LHCII1:LHCII3 for RH and RNH LHCII (Fig. 1). After removal of PC and PG, RH and RNH LHCII were similar based on the ratio of LHCII1:LHCII3 (Table I) and 77K fluorescence emission spectra (Fig. 3A; PLC, PLA2). Removal of PC and PG resulted in a significant change in the ratio of F679: F620. The hydrolysis of these lipids did not result in any significant change in the amount of free Chl (<10%) observed after electrophoresis in the presence of SDS and DOC (Fig. 1).

Treatment of purified RH and RNH LHCII with PLC + PLA2 + GL caused the total hydrolysis of PC, PG, and MGDG, and reduced the DGDG content to 20 to 25% of control and SQDG to 90 to 95% of control. This resulted in a further 19% reduction in the ratio of LHCII1:LHCII3 for both RH and RNH LHCII (Table I). The 77K fluorescence emission spectra indicated that the long wavelength emission band was red-shifted from 694 to 696 nm for RNH LHCII and to 698 nm for RH LHCII. In addition, there was a concomitant decrease in the ratio of the 679-nm emission peak relative to the long wavelength emission peak (Fig. 3B; PLC, PLA2, GL). However, this latter treatment also caused a significant increase in the level of free pigment to about 20% of the total Chl after electrophoresis in the presence of SDS and DOC (data not shown).

Reconstitution of Lipid-Depleted RH and RNH LHCII

To study the role of acyl lipids in the reconstitution of LHCII, from lipid-depleted RH and RNH LHCII, we tested several commercially available lipids (Sigma), either synthetic (di-16:0-PC, 18:2/16:0-PC, di-16:0-PC, di-18:1-PC) or isolated from plant sources (MGDG, DGDG). We isolated and purified PG containing t-16:1 from RNH rye thylakoids. The fatty acid compositions of all acyl lipids used in the following reconstitution were checked and the results are shown in Table II.

First, we checked the effects of all lipids at levels normally found in LHCII (1X). This represented a concentration of 30 mol % for PG, 32 mol % for MGDG, and 30 mol % for DGDG. In addition, we examined lipid concentrations of 5- and 10-fold excess (5X and 10X, respectively) on the structure of the nonlipid-depleted samples of RH and RNH LHCII using the identical reconstitution procedure described in "Materials and Methods." Even a 10-fold excess of the lipid did not cause any significant change in the ratio of LHCII1:LHCII3 for RH (3.33 ± 0.34 to 3.59 ± 0.44) and RH LHCII (1.81 ± 0.45 to 2.10 ± 0.57) for all lipids tested or the level of FP released (10%) (data not shown). Thus, RH and RNH LHCII appear to be very stable under our purification and reconstitution protocol.

Subsequently, we tested each lipid separately for its ability to regenerate LHCII after lipid depletion. Reconstitution of the PLC-treated samples in the presence of a 5- or 10-fold excess of either di-16:0-PC or 18:2/16:0-PC did not have any significant effects on the ratio of LHCII1:LHCII3 for either RH or RNH LHCII (Table I). The ratios were similar to those observed for controls (Table I).

The reconstitution of PLC/PLA2-treated samples with di-16:0-PC (Fig. 1) caused a 60% reduction, whereas reconstitution with 18:2/16:0-PC caused only a 10% reduction in the ratio of LHCII1:LHCII3 for both RH and RNH LHCII (Table I). Similarly, increasing concentrations of di-16:0-PC caused a destabilization of LHCII1, and a concomitant trend toward an increase in LHCII2 and LHCII3 such that the LHCII1:LHCII3 decreased from 0.81 ± 0.29 to 0.24 ± 0.06 with no change in FP for both RH and RNH LHCII (Fig. 4).

Figure 2. Corrected 77K fluorescence emission spectra of purified RH and RNH LHCII (A) and their component Chl-protein complexes after separation by SDS/DOC-PAGE (B). Excitation was at 440 nm. Chl concentration was 1 µg ml⁻¹.
The effect of hydrolysis of acyl lipid components of purified RH and RNH LHCII on their corrected 77K fluorescence emission spectra. Purified RH and RNH LHCII were treated with either PLC and PLA2 (A) or with PLC, PLA2, and GL (B), as indicated. Chl concentration was 1 μg mL⁻¹ with excitation at 440 nm.

Increasing concentrations of di-18:1-PG also caused a destabilization of LHCII₁ and LHCII₂ for both RH and RNH LHCHII. However, this could be accounted for by a significant increase in free pigment rather than any significant change in the level of LHCII₁ (Fig. 4). In contrast, reconstitution with PG containing t-16:1 (Fig. 1) caused a 2.3-fold increase in the ratio of LHCII₁:LHCII₂ for both RH (from 0.81 ± 0.29 to 1.92 ± 0.41) and RH LHCII (from 0.85 ± 0.17 to 1.87 ± 0.30) (Fig. 4). The 15% increase in LHCII₁ relative to control (PLC, PLA2-treated alone) occurred concomitant with a 15% decrease in LHCII₂. However, we noted that incubation in the presence of PG containing t-16:1 did increase the FP from 10 to about 15%, similar to that observed for di-18:1-PG (Fig. 4).

Reconstitution of PLC, PLA₂, GL-treated samples with either MGDG or DGDG caused a destabilization of LHCII₁ and LHCII₂, with a concomitant increase in LHCII₁ and an increase in free pigment for both RNH and RH LHCII (Fig. 5). However, we do not have sufficient data at this time to discount the possibility that galactolipids may stabilize LHCII₁ when present in combination with PG.

Figure 6 illustrates the effects of reconstitution of RNH (solid line) and RH (dashed line) with PG containing t-16:1 (Fig. 6A), di-16:0-PG (Fig. 6B), or MGDG (Fig. 6C) on the

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**Table II. Fatty Acid Composition of Acyl Lipids Used for Reconstitution of RNH and RH LHCII**

All lipids were purchased from Sigma except t-16:1-PG, which was isolated and purified from RNH plants. nd, Not detected.

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Fatty Acid</th>
<th>16:0</th>
<th>t-16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>di-16:0-PC</td>
<td>100</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>18:2/16:0-PC</td>
<td>50</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>50</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>di-16:0-PG</td>
<td>100</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>di-18:1-PG</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>100</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>t-16:1-PG</td>
<td>26</td>
<td>34</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>MGDG</td>
<td>9</td>
<td>nd</td>
<td>1</td>
<td>9</td>
<td>3</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>DGDG</td>
<td>28</td>
<td>nd</td>
<td>2</td>
<td>9</td>
<td>3</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>
they were able to show only relatively low levels (15–20%) of reconstitution of LHCII; on a total Chl basis using t-16:1 containing PG. Furthermore, their reconstitution procedure utilizing SDS-PAGE LHCII3 was accompanied by the release of large amounts (50%) of free pigments. In contrast, our method, which starts with Triton X-100-purified LHCII followed by repetitive slow freezing and slow thawing in the presence of liposomes of known composition, exhibits a reconstitution of 48% on a total Chl basis and minimal release (10–15%) of FP.

The composition of purified RNH and RH LHCII differ only in the fatty acid composition of PG associated with LHCII. The higher t-16:1 level in PG of RH LHCII is correlated with a significantly higher ratio of LHCII;:LHCII,. This difference in LHCII organization is associated with a higher ratio of F<sub>694</sub>:F<sub>679</sub> in RH LHCII than RH LHCII. However, the results of our experiments clearly show that the organization of RH and RNH LHCII exhibit similar

**DISCUSSION**

Rémy et al. (24) have reported the reconstitution of LHCII; from LHCII, isolated by preparative SDS-PAGE. However, fluorescence emission spectra. The spectra for di-16:0-PG- and MGDG-reconstituted LHCII (Fig. 6) indicate that reconstitution with these lipids caused an apparent decrease in the resolution of the 679-nm emission peak relative to that observed for the PLC,PLA<sub>2</sub>-treated and PLC,PLA<sub>2</sub>GL-treated samples in Figure 3. GL treatment also induced a red shift in the major emission peak from 694 nm to about 700 nm. In contrast, the spectra for t-16:1-PG-reconstituted LHCII (Fig. 6) illustrate a significant increase in the ratio of F<sub>694</sub>:F<sub>679</sub> relative to those observed for the PLC,PLA<sub>2</sub>-treated samples in Figure 3 and are similar to those for untreated RH LHCII (Fig. 2). Thus, the ratio of F<sub>694</sub>:F<sub>679</sub> appears to be positively correlated to the ratio of LHCII;:LHCII,.

![Figure 4](image_url)  
**Figure 4.** Chl content of the Chl-protein complexes of RH and RNH LHCII reconstituted with PG molecular species. Initially, all samples were treated with PLC and PLA<sub>2</sub> ( ). Subsequently, these delipidated samples were reconstituted with either di-16:0-PG ( ), t-16:1-PG ( ), or di-18:1-PG ( ), as indicated. The Chl content of each Chl-protein complex is expressed as a percent of the total Chl that was determined as described in "Material and Methods." Concentration (mol PG/mol Chl) of each PG molecular species used during reconstitution was based on that normally found associated with rye LHCII (1X = 30 mol % PG), 5X or 10X that normally associated with LHCII. Data represent the average ± SD for three to six experiments.

![Figure 5](image_url)  
**Figure 5.** Chl content of Chl-protein complexes of RH and RNH LHCII reconstituted with MGDG and DGDG. Initially, all samples were treated with PLC, PLA<sub>2</sub>, and GL ( ). Subsequently, these delipidated samples were reconstituted with MGDG ( ) or DGDG ( ). The Chl content of each Chl-protein complex is expressed as a percent of the total Chl, which was determined as described in "Materials and Methods." The concentration (mol MGDG or mol DGDG/mol Chl) of each lipid used was equal to that normally found associated with LHCII (1X = 32 mol % for MGDG; 1X = 30 mol % for DGDG), 5X or 10X that normally associated with rye LHCII. Data represent the average ± SD for three to six experiments.
sensitivities to lipid hydrolysis and subsequent reconstitution of LHCII, with different lipids (Table I, Figs. 2–5). Thus, we conclude that the stabilization of the supramolecular complex of LHCII is specifically dependent upon the molecular species of PG containing t-16:1.

Plumley and Schmidt (23) recently reported that the minimum components required for the reconstitution of LHCII from LDS/heat-denatured or acetone-extracted spinach LHCII were Chl a, Chl b, and xanthophyll. They report that all the lipids that they tried caused a destabilization of LHCII. Our results for reconstitution of LHCII from either PLC, PLA$_2$-treated or PLC, PLAA, GL-treated LHCII using commercially available molecular species of PC, MGDG, DGDG, and PG are also consistent with this. PG containing t-16:1 was the only lipid that successfully stabilized and reconstituted LHCII. This illustrates the extreme specificity for t-16:1-PG in the stabilization of LHCII. Our results are consistent with the recent report of Garnier et al. (7). They showed that uptake of t-16:1-PG by a mutant of Chlamydomonas reinhardtii specifically lacking t-16:1-PG restored LHCII.

We noted that even though t-16:1-PG reconstituted LHCII, incubation in the presence of this lipid did cause a 5% increase in FP. This is probably due to the fact that our preparation of PG was a mixture of PG molecular species (Table II). Our results show that the presence of molecular species of PG other than those containing t-16:1 can destabilize Chl-protein interactions.

Previously (11, 16), we noted that even after complete removal of PG from RNH or RH LHCII, about 25% of the total Chl was still associated with LHCII; and resulted in a LHCII/LHCII ratio of about 0.65. The results presented in this report are consistent with this (Table I; Figs. 1, 3, and 4) and indicate that PG may not be the only factor important in stabilizing rye LHCII. Alternatively, our results may be interpreted to indicate that there may be at least two distinct populations of LHCII in rye: one population (75% of the total on a Chl basis) that requires PG to stabilize the oligomeric form, and another subpopulation (25% of the total on a Chl basis) that does not require the presence of PG to stabilize LHCII. Hence, only that subpopulation that is dependent upon the presence of PG for the stabilization of LHCII would be sensitive to delipidation and reconstitution with t-16:1-PG. Further work is required to substantiate these hypotheses.

It appears that the differences in the organizational states of RNH and RH LHCII give rise to distinct differences in the 77K fluorescence emission spectra (Fig. 2A). Thus, it appears that a high ratio of LHCII$_{RNH}$:LHCII$_{RH}$ is correlated with a high ratio of $F_{679}$:$F_{695}$. This was an unexpected result because oligomerization implies aggregation of LHCII. If oligomerization of LHCII were a simple aggregation phenomenon, we would expect an increase in the 694-nm emission band relative to the 679-nm emission band and, thus, a low ratio of $F_{694}$:$F_{679}$, as reported by Horton et al. (9) for in vitro aggregation of octylglucoside/digitonin-solubilized LHCII. Thus, we suggest that LHCII oligomerization mediated by t-16:1-PG does not reflect a simple aggregation phenomenon, but a more subtle organizational change in LHCII. It is difficult to compare our results with those of Horton et al. (9) because our reconstitution system differed in terms of protocol and detergents employed.

Krol et al. (14) reported that biogenesis of rye thylakoids at 20°C resulted in a significantly higher $F_{679}$:$F_{695}$ than biogenesis at 5°C. However, after purified RH and RNH LHCII were solubilized in SDS/DOC and the component Chl-protein complexes separated electrophoretically, LHCII$_{RNH}$ and LHCII$_{RH}$ exhibited similar 77K emission spectra (Fig. 2B). Thus, exposure to detergents after purification may alter the structure of RH and RNH LHCII, which affects the 77K fluorescence emission spectra. This was confirmed by incubation of RH and RNH LHCII with various concentrations of Triton.
X-100 prior to collection of the 77K fluorescence emission spectra (Z. Krupa and N.P.A. Huner, unpublished data).

In summary, the results presented support the thesis that t-16:1-PG has an important role in stabilizing LHClI. The specific requirement for the t-16:1-containing molecular species of PG for this stabilization indicates a precise role for this unique thylakoid lipid in the supramolecular organization of rye LHClI.

LITERATURE CITED