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Development at Cold-Hardening Temperatures[1]

THE STRUCTURE AND COMPOSITION OF PURIFIED RYE LIGHT HARVESTING COMPLEX II

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ABSTRACT

Light harvesting complex II (LHCII) was purified from cold-hardened (RH) and nonhardened winter rye (RNH) (*Secale cereale* L. cv Puma) employing a modified procedure of JJ Burke, CL Ditto, CJ Arntzen (Arch Biochem Biophys 187: 252–263). Triton X-100 solubilization of thylakoid membranes followed by three successive precipitations with 100 mM KCl and 10 mM $MgCl_2$ resulted in yields of up to 25% on a chlorophyll (Chl) basis and a purity of 90 to 95%, based on polypeptide analysis within 4 hours. Polypeptide and pigment analyses, 77 K fluorescence emission and room temperature absorption spectra indicate the LHCII obtained by this modified method is comparable to LHCII obtained by other published methods. Comparison of purified RH and RNH LHCII indicated no significant differences with respect to polypeptide, amino acid, Chl, and carotenoid compositions as well as no differences in lipid content. However, RH LHCII differed from RNH LHCII specifically with respect to the fatty acid composition of phosphatidyldiacylglycerol only. RH LHCII exhibited a 54% lower *trans*-Δ^3-hexadecenoic acid level associated with PG and a 60% lower oligomeric LHCII:monomeric LHCII ($LHCII_1$:$LHCII_3$) than RNH LHCII. Both RH and RNH LHCII exhibited a 5-fold enrichment in PG specifically. Complete removal of PG by enzymic hydrolysis resulted in a significant reduction in the oligomeric content of both RH and RNH LHCII such that $LHCII_1$:$LHCII_3$ of RH and RNH LHCII preparations were the same. This confirms that this specific compositional change accounts for the structural differences between RH and RNH LCHII observed *in situ* and *in vitro*.

In a previous report, Huner *et al.* (12) reported that growth and development of winter rye at low RH[3] resulted in a specific 67% decrease in the *trans*-16:1 acid level of PG although the overall thylakoid lipid content was not significantly altered. This was correlated with an oligomeric LHCII:monomeric LHCII that was 2-fold lower in RH than RNH thylakoids.

In the present report, we examine the structure and composition of purified LHCII from RH and RNH thylakoids in order to answer the following questions: (a) Is PG specifically associated with purified rye LHCII? (b) If so, does the fatty acid composition of PG represent the only compositional difference between RH and RNH LHCII? (c) Can this compositional difference account for the observed difference in the organization of RH and RNH LHCII?

MATERIALS AND METHODS

Plant Materials. Winter rye (Secale cereale L. cv Puma) was grown in vermiculite watered with Hoagland nutrient solution. Seedlings were initially grown in controlled environment growth chambers at cold-hardening (5°C) and nonhardening temperatures as described previously (12). Bean (*Phaseolus vulgaris* L.), spinach (*Spinacia oleracea* L.), and corn (*Zea mays* L.) seedlings were grown under controlled environment conditions of a 16 h photoperiod, at a light intensity of 200 mol photons m^{-2} s^{-1} (PAR) and a temperature regime of 20°C/16°C (day/night). Periwinkle (*Vinca rosa* L.) was field grown.

Isolation and Purification of LHCII Complex. Leaf material (100 gm fresh weight) from each plant species was homogenized in 50 mM Tricine-NaOH buffer (pH 7.8) containing 0.4 M sorbitol, filtered through four layers of Miracloth and centrifuged at 5,000*g* for 5 min. The pellet was washed once with 5 mM EDTA, 50 mM sorbitol (pH 7.8), and centrifuged at 10,000*g* for 10 min.

The thylakoid membrane pellet was resuspended with cold distilled H_2O to a Chl concentration of 0.8 mg/ml and then Triton X-100 was added from 5% (w/v) stock solution to final detergent concentration of 0.7% (w/v). The suspension was incubated at room temperature with continuous stirring for 30 min and centrifuged at 30,000*g* for 40 min KCl and $MgCl_2$ from 1 M stock solutions were added to Triton X-100 supernatant to final concentrations of 100 and 20 mM, respectively, and stirred gently for the precipitation of crude LHCII. The suspension was then layered on a 0.5 M sucrose solution (the volume of sucrose should exceed three times the volume of LHCII suspension) and centrifuged at 10,000*g* for 10 min. The pellet was resuspended in 50 mM Tricine-NaOH buffer (pH 7.8) containing 100 mM sorbitol to a Chl concentration about 0.8 mg/ml. Triton X-100 (5% w/v stock solution) was then added to obtain a detergent to Chl ratio of 10:1. After short stirring, LHCII was precipitated

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[3] Abbreviations: RH, cold-hardened rye; RNH, nonhardened rye: LHCII, light harvesting chlorophyll *a/b* protein complex associated with photosystem II; $LHCII_1$, oligomeric form of the light harvesting complex; $LHCII_2$, dimeric form of the light harvesting complex; $LHCII_3$, monomeric form of the light harvesting complex; GL, galactolipase; PLC, phospholipase C; PLA_2, phospholipase A_2; PG, phosphatidyldiacylglycerol; PC, phosphatidylcholine, PE, phosphatidyl-ethanolamine; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacyl-glycerol; SQDG, sulfoquinovosyldiacylglycerol; *trans*-16:1, *trans*-Δ^3-hexadecenoic acid; 16:0, palmitic acid.

with K^+ and Mg^{2+} salts as described above. The suspension was gently stirred to allow good precipitation of LHCII and then it was layered on 0.5 M sucrose solution and centrifuged at 30,000*g* for 40 min.

For the final purification of LHCII, the pellet was suspended in 50 mM Tricine-NaOH buffer (pH 7.8) containing 100 mM sorbitol and precipitated with 1 M KCl and 1 M $MgCl_2$ as described above. Triton X-100 was omitted in this step of purification. The suspension containing LHCII was layered on 0.5 M sucrose solution, centrifuged at 10,000*g* for 10 min and finally, the purified LHCII was suspended in the appropriate buffer.

Electrophoresis. SDS-PAGE of purified LHCII was performed on 10 to 20% acrylamide gradient slab gels. Prior to electrophoresis LHCII preparations were solubilized in 60 mM Tris-HCl buffer (pH 7.8) containing 1 mM EDTA, 60 mM DTT, 2% (w/v) SDS, and 12% (w/v) sucrose to a final SDS:Chl (mg:mg) ratio of 10:1. Gels were stained with 0.1% (w/v) Coomassie brilliant blue R-250 and destained in methanol:acetic acid:water (3:1:10).

To estimate the percentage purity of each LHCII preparation, the SDS-PAGE slab gels were scanned at 555 nm on Shimadzu UV-250 spectrophotometer equipped with a scanning attachment. Peaks representing LHCII and any impurities were excised from the scans and their areas were measured with a LI-COR portable area meter (model LI-3000). The purity of LHCII was expressed as percentage of the LHCII peak area *versus* total area of the scan.

Chl-protein complexes were separated as described by Huner *et al.* (12). Gels were scanned at 671 nm on Shimadzu UV-250 spectrophotometer. The relative Chl contents of $LHCII_1$, $LHCII_2$, and $LHCII_3$ were determined by relative peak areas.

Protein content was estimated using the procedure of Bradford (4) with the commercially available reagents from Bio-Rad Laboratories, Inc.

Absorption and Fluorescence Emission Spectra. Absorption spectra of LHCII were recorded at 25°C with Shimadzu UV-250 spectrophotometer. In this case, LHCII preparations were suspended in 50 mM Tris-HCl buffer (pH 8.0).

Low temperature (77 K) fluorescence emission spectra of LHCII were obtained with a Perkin-Elmer 650-40 fluorometer modified to contain the sample chamber of the CTI-Cryogenics model 21C Cryodyne cryostat.

LHCII and thylakoid preparations were suspended in 50 mM Tricine-NaOH buffer (pH 7.8), containing 10 mM $MgCl_2$ and 50% (w/v) glycerol to Chl concentration of 5 μg/ml.

Excitation was at 440 through 4 nm slits and emission spectra were collected with either 4 or 6 nm slits for LHCII and thylakoids, respectively. The data were plotted using Perkin-Elmer supplied software.

Pigment Analyses. Chl content was measured in 80% (v/v) acetone according to Arnon (2). β-Carotene and xanthophylls were separated by TLC using the method of Hager and Bertenrath (10). β-Carotene and xanothophylls were estimated spectrophotometrically according to Davies (7).

Lipid and Fatty Acid Analyses. Purified RH and RNH LHCII were extracted and analyzed for their lipid and fatty acid compositions as previously described (12).

Enzymatic Hydrolysis of Lipids from RH and RNH LHCII. To stabilize the oligomeric form of LHCII, the cation concentration should be minimized (1). The cation concentrations of RH and RNH LHCII preparations were minimized by resuspension in a solution containing 100 mM EDTA at pH 7.8 and a Chl concentration of 0.1 mg^{-1} ml. After stirring for 5 min, LHCII was collected by centrifugation at 10,000*g* for 10 min. This washing procedure was repeated three times. LHCII preparations were then incubated in 50 mM Tricine-NaOH (pH 7.5) containing lipolytic enzymes for 90 min at 25°C in the dark as described previously (14). Bean galactolipase was present at a concentration of 500 μg protein/mg Chl. Phospholipase C (Sigma, *Bacillus cereus*, EC 3.1.4.3) and Phospholipase A_2 (Sigma, *Vipera russelli*, EC 3.1.1.4) were present at concentrations of 50 units/mg Chl. Subsequently, LHCII preparations were collected by centrifugation, analyzed for acyl lipid composition and the relative proportions of $LHCII_1$, $LHCII_2$, and $LHCII_3$ determined after separation by SDS slab gel electrophoresis as described above. Acyl lipids were extracted from LHCII and separated by TLC as described by Khan and Williams (13).

Bean galactolipid lipase (lipolytic acyl hydrolase) was purified from bean leaves as described previously (14) except that a Sephacryl S-200 column (1.6 × 100 cm) was used instead of Sephadex G-100.

Amino Acid Analyses. RH and RNH LHCII were precipitated with cold acetone and centrifuged at 10,000*g* for 10 min. This procedure was repeated five times to remove pigments and acyl lipids present. The protein pellets were then dried under N_2 and hydrolyzed in 6 N HCl for 18 h at 115°C. The hydrolysates were analyzed on a Beckman model 119CL amino acid analyzer.

RESULTS

Protein and Pigment Compositions of Purified LHCII Preparations. Figure 1 illustrates that RH and RNH LHCII were indistinguishable and exhibited the presence of 26,000 and 27,000 mol wt polypeptides. Amino acid analyses indicated that RH and RNH LHCII exhibited similar amino acid compositions (Table I) with 32 ± 2% polar and 64 ± 2% nonpolar amino acids calculated according to Capaldi (6). The minor differences in the mol wt of LHCII polypeptides from various plant sources (Table II) is consistent with previous reports (5, 9, 16). We consistently observed the presence of additional minor, low mol wt polypeptides when Triton X-100 was included in the third and final precipitation (data not shown). Thus, for maximum purity which ranged from 90 to 95% based on SDS-PAGE of LHCII polypeptides (Table II), Triton X-100 was omitted from the final purification step.

The Chl*a*/*b*, Chl:protein, and purity based on polypeptide composition of RH and RNH LHCII was not significantly different and similar to those observed for LHCII isolated from other plant species (Table II). In addition, we observed no significant difference in carotenoid:protein between RH

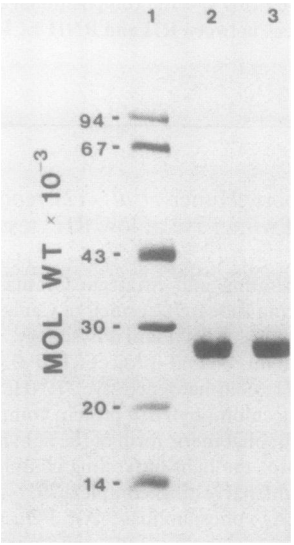

FIG. 1. SDS-PAGE of purified RH and RNH LHCII. Lane 1, mol wt standards; lane 2, RH LHCII; lane 3, RNH LHCII. Fifteen μg of protein were electrophoresed for each of RH and RNH LCHII.

Table I. *Amino Acid Composition of Purified RH and RNH LHCII*

The data represent the means of 4 different isolations ± SD.

Amino Acid	RNH	RH
	mol % of total	
Asp	9.7 ± 0.2	10.1 ± 0.1
Thr	2.8 ± 1.1	3.0 ± 0.6
Ser	3.7 ± 1.8	4.2 ± 0.9
Glu	9.4 ± 0.5	9.8 ± 0.5
Pro	4.8 ± 1.2	4.8 ± 1.0
Gly	12.2 ± 0.6	12.3 ± 0.8
Ala	12.5 ± 0.4	12.5 ± 0.3
Val	7.6 ± 0.2	7.6 ± 0.1
Met	2.0 ± 0.6	1.9 ± 0.9
Isoleu	4.1 ± 0.6	4.4 ± 0.4
Leu	11.4 ± 0.5	10.9 ± 0.4
Tyr	2.9 ± 0.2	2.8 ± 0.2
Phe	6.0 ± 0.4	5.7 ± 0.5
His	1.1 ± 0.1	1.4 ± 0.5
Lys	5.2 ± 0.8	5.2 ± 0.8
Arg	3.2 ± 0.3	3.3 ± 0.2

and RNH LCHII. The ratio of Chl a:β-carotene: lutein:violaxanthin:neoxanthin was 100:0.7:17:2:6.5 on a mol basis. In general, purified LHCII constituted from 13 to 23% of the total thylakoid membrane chlorophyll. However, RNH thylakoids consistently provided a 1.33-fold higher yield of purified LHCII on a Chl basis than RH thylakoids.

Room temperature absorption spectra of the purified LHCII preparations exhibited characteristic Soret peaks at 436 to 438 and 478 to 480 nm and red maxima of 652 to 653 and 678 to 680 nm. Figure 2 illustrates that purified spinach LHCII exhibited the expected 77 K fluorescence emission maximum at 681 nm and the absence of the prominent emission band at 734 nm (15). Thus, our modified procedure results in purified, cation-aggregated, LHCII preparations which exhibit characteristic pigment and polypeptide compositions. More important, RH and RNH LHCII are indistinguishable with respect to polypeptide, amino acid, and pigment composition.

Lipid Content and Fatty Acid Composition of Purified RH and RNH LHCII. The total lipid content of RH and RNH LHCII was not significantly different (Table III). However, the fatty acid composition of RH and RNH LHCII differed significantly with respect to PG only. RH LHCII exhibited a 54% lower *trans*-16:1 content and a concomitant doubling in palmitic acid (16:0) level compared to RNH LHCII. The 5-fold decrease in the *trans*-16:1/16:0 ratio associated with PG of RH LHCII compared to RNH LHCII is consistent with that observed for the PG composition of whole leaves and isolated thylakoids from RH and RNH seedlings (12).

The results summarized in Table III indicate that the proportion of PG associated with purified RH and RNH LHCII (31–33 mol%) had significantly increased relative to the PG content of whole thylakoids (8–11 mol%) (12). The results of Table IV illustrate that as the Chl *a/b* of RNH LHCII decreased from an initial value of 3.0 to a value of 1.1 for purified RNH LHCII, the proportion of PG relative to MGDG + DGDG associated with purified rye LHCII exhibited a 5- to 6-fold increase on a mol·mol^{-1} basis. In contrast, both SQDG/MGDG+DGDG and PC+PE/MGDG+DGDG exhibited minimal increases from 0.04 to 0.05 mol mol^{-1} for whole thylakoids to 0.11 to 0.17 mol·mol^{-1} for purified RNH LHCII. Purification of RH LHCII also resulted in a 5- to 6-fold enrichment of PG (data not shown). Thus, the lipid enrichment observed upon purification of rye LHCII appears to be specific for PG although MGDG and DGDG remained significant components of purified LHCII.

Chl-Protein Complexes Associated with Purified RH and RNH LHCII. Both RH and RNH LHCII exhibited the presence of three chlorophyll-protein complexes (Fig. 3A) with absorption spectra characteristic of the Chl *a/b* light harvesting protein complex (Fig. 3B) and exhibited electrophoretic migration typical for $LHCII_1$, $LHCII_2$, and $LHCII_3$ (12). However, RNH LHCII consistently exhibited oligomer ($LHCII_1$):monomer ($LHCII_3$) ratios (2.59 ± 0.2) which were 60% higher than those observed for RH LHCII (1.62 ± 0.15). This difference in $LHCII_1$:$LHCII_3$ was not due to differences in the Chl content associated with $LHCII_1$ and $LHCII_3$ after electrophoretic separation since the free pigment (FP) observed was negligible (5–10% of the total CHl) for both RH and RNH LHCII preparations (Fig. 3A).

Effects of Lipid Hydrolysis on the Lipid Composition and Structure of RH and RNH LHCII. The only compositional differences observed between purified RH and RNH LHCII was with respect to the *trans*-16:1/16:0 associated with PG. Similarly, the only structural difference observed between RH and RNH LHCII was the relative $LHCII_1$:$LHCII_3$. We hypothesized that if the differences in $LHCII_1$:$LHCII_3$ observed between RH and RNH LHCII were due solely to the differences in the fatty acid composition of the associated PG, subsequent removal of PG should result in similar $LHCII_1$:$LHCII_3$ for both RH and RNH LHCII. Purified preparations of RH and RNH LHCII were incubated in the presence of various combinations of acyl lipid hydrolases as described in "Materials and Methods": PLC alone; PLA_2 plus PLC; bean GL plus PLA_2 and PLC; control samples were incubated in the absence acyl lipid hydrolases. Controls indicated that the incubation conditions did not significantly affect the lipid content of RH and RNH LHCII. PLC treatment specifically hydrolyzed all of the PC only and left 98% of the other thylakoid lipids (MGDG, DGDG, SQDG, PG) associated with LHCII. Incubation with PLA_2 and PC completely removed all of the PG and PC but did not affect the content of MGDG, DGDG, or SQDG. After treatment of RH and RNH LHCII in the presence of bean GL+PLA_2+PLC, only SQDG (85–90%)

Table II. *Characteristics of LHC II Preparations Purified from Various Sources by the Method of Successive Precipitation*

Where indicated values represent the mean ± SD (n = 6–10).

Sample	Chl *a/b*	Chl *a* + *b* Protein	Carotenoid Protein	Purity	Yield	Polypeptides
		mol/mol		*% of total protein*	*% of total Chl*	*kD*
RNH	1.09 ± 0.01	10.4 ± 0.2	1.4 ± 0.2	91.5 ± 0.9	20.0 ± 2.1	27, 26
RH	1.09 ± 0.02	9.7 ± 1.4	1.5 ± 0.01	90.8 ± 1.2	15.1 ± 0.1	27, 26
Corn	1.14 ± 0.02	11.3	ND[a]	93.9 ± 0.7	20.2	27.5, 26
Spinach	1.13 ± 0.02	13.7 ± 0.4	ND	95.4 ± 0.5	22.9	27.5, 26
Phaseolus	1.14 ± 0.01	7.5	ND	92.9 ± 1.2	12.9	27, 26
Periwinkle	1.13 ± 0.01	6.1	ND	89.5 ± 2.1	23.4	28, 26

[a] Not determined.

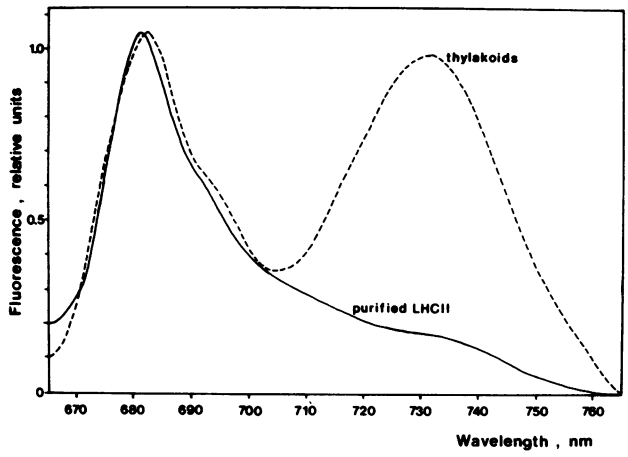

FIG. 2. Fluorescence emission spectra of spinach LHCII (——) and spinach thylakoids (· · · ·) recorded at 77°K.

and minor amounts of DGDG (15%) remained associated with LHCII (data not shown).

After various pretreatments, RH and RNH LHCII were subjected to SDS-PAGE at 4°C in the dark to determine the relative proportions of $LHCII_1$:$LHCII_3$ (Fig. 4). Washing with 100 mM EDTA (pH 7.8) caused an 8 to 14% decrease in $LHCII_1$:$LHCII_3$ (Fig. 4, B and C). Incubation at 25°C in the dark did not significantly affect the ratio for either RH or RNH LHCII (Fig. 4D). Complete hydrolysis of PC had little effect on the oligomer content of either RH or RNH LHCII (Fig. 4E). The $LHCII_1$:$LHCII_3$ was 1.84 for RNH LHCII and 1.14 for RH LHCII. Complete hydrolysis of PG and PC (Fig. 4F) from purified RNH LHCII caused a 21% decrease in RNH oligomer content and a concomitant 9 and 11% increase in dimer and monomer content, respectively. Similarly, complete hydrolysis of PG and PC from purified RH LHCII caused a 14% decrease in oligomer content and a concomitant 8 and 6% increase in dimer and monomer content, respectively. Thus, enzymic removal of PG from RH and RNH LHCII caused a quantitative conversion of oligomeric LHCII to its dimeric and monomeric forms. These changes in $LHCII_1$:$LHCII_3$ were not due to destabilization of protein-Chl associations since pretreatment of RH and RNH LHCII with PLC or PLC + PLA_2 did not result in significant changes in the free Chl observed. Furthermore, after PLC + PLA_2 treatment, the $LHCII_1$:$LHCII_3$ was 0.66 for purified RNH LHCII and 0.64 for purified RH LHCII.

Hydrolysis of PC, PG, MGDG, and a major proportion of the DGDG content resulted in no additional alterations in the oligomer content of RH and RNH LHCII (Fig. 4G) but did cause a reduction in the monomer content. This resulted in a slight increase in the $LHCII_1$:$LHCII_3$ for both RH (0.70) and RNH LHCII (0.74). The presence of bean GL in the incubation medium appeared to cause a destabilization of protein-Chl associations in the monomeric, and to lesser extent, the dimeric forms of RH and RNH LHCII which gave rise to a significant increase in the free Chl observed. However, even after treatment with a mixture of bean GL, PLC, and PLA_2 24% of the RNH LHCII and 19% of the RH LHCII was still present in the oligomeric form (Fig. 4G).

DISCUSSION

On the basis of lipid and fatty acid analyses of RH and RNH thylakoids, Huner *et al.* (12) concluded that development at cold-hardening temperatures modulates LHCII organization by specifically altering the *trans*-16:1 content of PG. This implies that PG is an important structural component of rye LHCII. The specific, 5- to 6-fold enrichment of PG during the purification of RH and RNH LHCII strongly supports this notion and is consistent with that proposed by Dubacq and Tremolieres (8). The only compositional difference detected between RH and RNH LHCII was a specific, 4.4-fold decrease in the ratio of *trans*-16:1/16:0 associated with PG or RH LHCII. Furthermore, the only significant structural difference observed was the 1.6-fold higher oligomeric LHCII/monomeric LHCII associated with RNH than RH LHCII. These structural and compositional differences are consistent with those observed between RH and RNH thylakoids.

However, can this compositional difference account for the observed difference in the organization of RH and RNH LHCII? Complete removal of PG from both RH and RNH LHCII by acyl lipid hydrolysis resulted in the reduction of oligomeric LHCII content to the extent that the $LHCII_1$:$LHCII_3$ of RH and RNH LHCII were the same. This is strong support for the concept that the difference in the supramolecular organization between RH and RNH LHCII is directly related to the difference in the *trans*-16:1 content of PG.

After complete removal of PG, about 20 to 25% of the purified RH and RNH LHCII was still present in the oligomeric form with a resultant $LHCII_1$:$LHCII_3$ of about 0.65. This experimentally determined value is consistent with the value predicted from the results for whole thylakoids (0.63) (12). This may be due to the presence of two populations of oligomeric LHCII in rye thylakoids; one which is totally dependent on PG and its fatty acid composition for its stabilization and another population of LHCII whose structural stability is completely independent of PG. Thus, one would expect the lipid composition of the former to exhibit the presence of PG but not the latter. Low temperature development in rye would modulate only the organization of that population of LHCII which is directly dependent upon PG composition by reducing the *trans*-16:1 content. Alternatively, only one population of oligomeric LHCII may be present in rye

Table III. *Lipid Content and Fatty Acid Composition of Purified RH and RNH LHCII*

The data represent the means of three different isolations ± SD.

	RNH LHCII							RH LHCII						
	Lipid	Fatty acid profile						Lipid	Fatty acid profile					
		16:0	16:1	18:0	18:1	18:2	18:3		16:0	16:1	18:0	18:1	18:2	18:3
	mol %	*mol %*						*mol %*	*mol %*					
PC	7 ± 3	31 ± 2		2 ± 2	3 ± 1	27 ± 3	36 ± 3	10 ± 3	36 ± 3		2 ± 3	3 ± 0	29 ± 3	31 ± 3
PE	2 ± 0.6	35 ± 1		tr[a]	tr	23 ± 2	43 ± 2	3 ± 1	41 ± 9		tr	tr	21 ± 3	38 ± 6
SQDG	6 ± 1	37 ± 1		tr	tr	6 ± 1	57 ± 3	5 ± 2	41 ± 7		tr	tr	2 ± 3	57 ± 4
DGDG	25 ± 3	9 ± 3	1 ± 0.3	1 ± 0	1 ± 0.3	2 ± 0	87 ± 3	23 ± 3	8 ± 2		1 ± 0.6	tr	1 ± 0	90 ± 1
PG	33 ± 1	20 ± 2	37 ± 4	1 ± 0.3	1 ± 0	2 ± 0	40 ± 6	31 ± 3	40 ± 2	17 ± 2	1 ± 0.3	1 ± 0	3 ± 1	38 ± 2
MGDG	28 ± 5	3 ± 0.6	2 ± 0	1 ± 0.3	1 ± 0	5 ± 0	90 ± 2	27 ± 4	3 ± 0		1 ± 0.3	1 ± 0	4 ± 0.6	93 ± 3

[a] tr = <0.5%.

Table IV. *Effect of Purification on PG Content of RNH LHCII*

	Chl *a/b*	PG / MGDG + DGDG
		mol/mol
Whole thylakoids	3.07	0.13
Precipitate 1	1.95	0.16
Precipitate 2	1.26	0.20
Precipitate 3	1.09	0.62

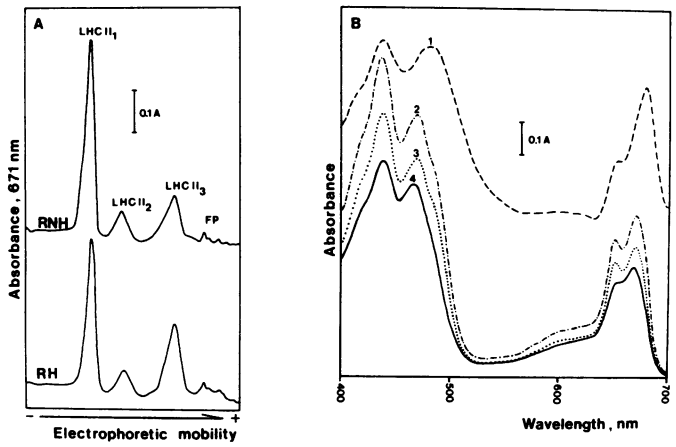

FIG. 3. A, Gel scans Chl-protein complexes associated with purified RNH and RH LHCII. $LHCII_1$, oligomer; $LHCII_2$, dimer; $LHCII_3$, monomer; FP, free pigment. In each case 20 μg of Chl was electrophoresed. B, Room temperature absorption spectra of purified RNH LHCII prior to electrophorese (curve 1). Spectra of $LHCII_1$ (curve 2), $LHCII_2$ (curve 3), and $LHCII_3$ (curve 4) were obtained after electrophoresis.

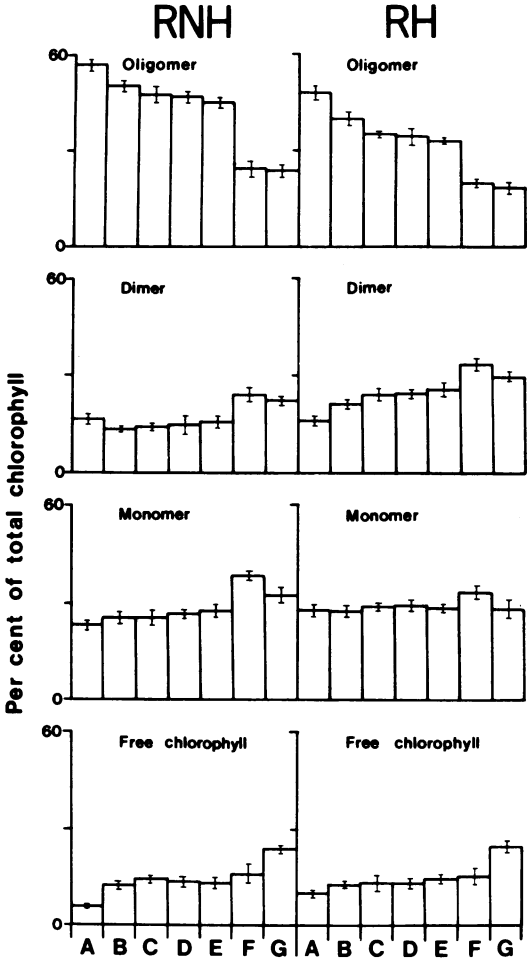

FIG. 4. The effects of EDTA washing and the hydrolysis of acyl lipids on the structure of purified RNH and RH LHCII. LHCII complexes were washed with EDTA and treated with lipolytic enzymes as described in "Materials and Methods." Where indicated, values represent mean ± SD (n = 3–6). A, control nontreated LHCII; B, LHCII washed with 5 mM EDTA (pH 7.8); C, LHCII washed with 100 mM EDTA (pH 7.8); D, LHCII washed with 100 mM EDTA (pH 7.8) then incubated in the dark at 25°C for 90 min without enzyme; E, LHCII washed with 100 mM EDTA (pH 7.8) and incubated in the presence of PLC; F, LHCII washed with 100 mM EDTA (pH 7.8) and incubated with PLC plus PLA_2; G, LHCII washed with 100 mM EDTA (pH 7.8) and incubated with bean GL plus PLC and PLA_2. In each case 20 g of Chl was electrophoresed. Relative proportion of each Chl-protein complex was calculated from the peak areas of gel scans obtained at 671 nm.

thylakoids which is dependent upon PG for stability. In this case, we would conclude that other factors such as pH and cation concentration (1) must be important in stabilizing $LHCII_1$ in addition to PG content. In this latter alternative, PG would be a constituent of all LHCII complexes and would be considered a factor which enhances the stability of oligomeric LHCII rather than an absolute requirement for oligomeric organization. Low temperature development would then decrease the stability of the entire population of oligomeric LHCII by reducing the *trans*-16:1 content of PG. Presently, we do not have sufficient data to discriminate between these alternatives. This is presently under investigation.

Although purified RH and RNH LHCII contained significant levels of PC, SQDG, MGDG, and DGDG, removal of PG had the most significant effect on the organization of LHCII. The presence or absence of PC had no observable effect on-$LHCII_1$:$LHCII_3$. Removal of the bulk of the MGDG and DGDG and all of the PG had the same effect on$LHCII_1$:$LHCII_3$ as the removal of PG alone. Thus, MGDG and DGDG do not appear to be intimately involved in the stabilization of oligomeric LHCII in rye thylakoids. However, MGDG and DGDG may be involved in the stabilization of Chl-protein associated within rye LHCII since treatment with bean GL+PLA_2+PLC caused a significant increase in the free pigment observed. It is interesting to note that the free pigment appeared to originate from the destabilization of the monomeric and dimeric forms but not oligomeric LHCII. This possible role for the major galactolipids of thylakoid membranes contradicts the recent report of Heinz and Siefermann-Harms (11) who concluded that MGDG and DGDG are not integral components of spinach LHCII. This apparent discrepancy may be due to species differences and/or differences in the procedures for the purification and separation of the Chl-protein complexes associated with LHCII. It is now widely accepted that all of the Chl within thylakoid membranes is specifically associated with Chl-protein complexes. Thus, the presence of free Chl during electrophoretic separation is an indicator of the *in vitro* instability the Chl-protein complexes. In contrast to the report of Heinz and Siefermann-Harms (11), we attempted to examine the possible role of the various thylakoid lipids in LCHII organization under conditions which minimize the presence of free Chl *in vitro*.

After treatment with bean GL+PLA_2+PLC, SQDG was the major lipid present in both RH and RnH LHCII. Since 20 to 25% of LHCII was still present as oligomer after such treatment, it may be argued that SQDG is also involved in stabilizing $LHCII_1$. However, the association of SQDG with LHCII was not specific since there was no observed enrichment of this lipid relative to the bulk bilayer lipid associated with purified rye LHCII. Further work is required to substantiate a specific role

for SQDG.

The purified preparations of RH and RNH LHCII did exhibit minimal levels of PE(2 ± 1%). Recently, Block *et al.* (3) showed that spinach chloroplast membranes contain no PE. Thus, the presence of this phospholipid in our LHCII preparations may represent minimal contamination by extrachloroplastic membrane lipids during purification. However, a similar minimal level of PE was observed in rye thylakoid membranes (12) and PE showed no enrichment during purification of rye LHCII. Therefore, we suggest that the observed minimal levels of PE do not invalidate our results and conclusions.

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