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Low Temperature Development of Winter Rye Leaves Alters the Detergent Solubilization of Thylakoid Membranes[1]

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ABSTRACT

Thylakoids isolated from leaves of winter rye (*Secale cereale* L. cv Puma) grown at either 20 or 5°C were extracted with the nonionic detergents Triton X-100 and octyl glucoside. Less total chlorophyll was extracted from 5°C thylakoids by these detergents under all conditions, including pretreatment with cations. Thylakoids from either 20 or 5°C leaves were solubilized in 0.7% Triton X-100 and centrifuged on sucrose gradients to purify the light harvesting complex (LHCII). Greater yields of LHCII were obtained by cation precipitation of particles derived from 20°C thylakoids than from 5°C thylakoids. When 20 and 5°C thylakoids were phosphorylated and completely solubilized in sodium dodecyl sulfate, no differences were observed in the ^{32}Pi-labeling characteristics of the membrane polypeptides. However, when phosphorylated thylakoids were extracted with octyl glucoside, extraction of LHCII associated with the 5°C thylakoids was markedly reduced in comparison with the extraction of LHCII from 20°C membranes. Since 20 and 5°C thylakoids exhibited significant differences in the Chl content and Chl *a/b* ratios of membrane fractions produced after solubilization with either Triton X-100 or octyl glucoside, and since few differences between the proteins of the two membranes could be observed following complete denaturation in sodium dodecyl sulfate, we conclude that the integral structure of the thylakoid membrane is affected during rye leaf development at low temperature.

The ability of winter rye plants to photosynthesize efficiently at low temperatures is dependent on plant growth and development at low temperature (20). When Puma rye is grown at temperatures near freezing (2–5°C), leaves develop which are morphologically, anatomically and functionally distinct from leaves produced by plants grown at warmer temperatures (20°C). These low temperature leaves have shorter blades, fewer stomates, thickened epidermal cell walls, increased epicuticular wax deposits and larger, multivacuolate mesophyll cells (9, 12, 17, 18).

When examined functionally, leaves developed at 5°C exhibit CO_2 exchange rates comparable to those seen in 20°C leaves whether measured at 10°C or at 20°C (21). Further studies of electron transport and energy distribution within these leaves revealed marked differences in leaves acclimated to low temperature. For example, thylakoids isolated from 5°C leaves exhibit light-saturated rates of whole chain electron transport ($H_2O \rightarrow$ MV) that are 40% higher than similar measurements of 20°C thylakoids (19). In addition, studies of Chl fluorescence emission spectra measured at 77°K reveal a decrease in the fluorescence emission associated with PSI relative to that associated with PSII in 5°C thylakoids compared with 20°C thylakoids. Emission spectra at 77°K also revealed emission bands at 680 nm and at 695 nm in 5°C thylakoids not present in 20°C thylakoids. These results are thought to reflect changes in energy distribution between LHCII[2] and PSII reaction centers, and between the reaction centers of PSI and PSII (13).

The compositions of photosynthetic membranes isolated from leaves developed at either 5 or 20°C were examined and found to be similar in thylakoid polypeptide (8, 10) and lipid (NPA Huner, JP Williams, personal communication) compositions, with the exception of the accumulation of Chl, carotenoids and plastoquinone in membranes developed at 5°C (11, 18). No changes have been observed in Chl *a/b*, protein/Chl, Chl/P_{700} or PSII photosynthetic unit size (8, 10, 11, 18) between the two membranes.

In the absence of dramatic changes in membrane composition, it was hypothesized that structural modifications of thylakoid membranes might account for the increased electron transport capacity which arises during the development of low temperature leaves. Ultrastructural analyses of chloroplasts isolated from low temperature leaves show that they have the same number of granal stacks, although the grana tend to be smaller in size, than those of chloroplasts developed at warmer temperatures (18). Both sets of plastids exhibit similar particle densities on the exoplasmic and protoplasmic freeze-fracture faces of the thylakoids; however, the particle size distributions are significantly different on both fracture faces (18). Other modifications of rye membranes were revealed by SDS-PAGE of Chl-protein complexes extracted from thylakoids (8). For example, when 5°C thylakoids are solubilized by the detergent SDS (SDS:Chl = 1) and the Chl-protein complexes are separated electrophoretically, only 37% of the LHCII is present in the oligomeric form, whereas 50% of the LHCII solubilized from 20°C thylakoids can be electrophoresed as the oligomer (8). In addition, both LHCII and PSII reaction center complexes solubilized from 5°C thylakoids are thermally less stable *in vitro*, as shown by the loss of Chl when the complexes are electrophoresed at room temperature (8).

The changes which occur in thylakoid membranes associated in leaves produced at 5°C are not gross compositional changes,

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[2] Abbreviations: CPI, chlorophyll *a*-protein complex associated with the reaction center of PSI; LHCII, light harvesting Chl *a/b*-protein complex associated with PSII; RNH, nonhardened rye; RH, cold-hardened rye.

rather they represent more subtle alterations in the structure of the membranes. We hypothesized that changes in the structures of integral membrane proteins or in the interactions between membrane protein complexes could account for the observed changes in thylakoid structure and function during leaf development at 5°C (8). In this paper, we explore this hypothesis by using anionic and nonionic detergents to determine the extractability of the pigment-protein complexes from thylakoids assembled at 5°C and at 20°C.

MATERIALS AND METHODS

Plant Materials. Winter rye (*Secale cereale* L. cv Puma) was grown in vermiculite watered with modified Hoagland nutrient solution (16). Seeds were germinated in growth chambers programmed for a 16 h photoperiod with a light intensity of 200 μE m^{-2} s^{-1} and a temperature regime of 20°C/16°C (day/night) for 7 d. Rye plants grown under these conditions for an additional 3 weeks are termed unhardened rye. Plants shifted instead to a temperature regime of 5°C/5°C for an additional 7 to 8 weeks with other conditions kept constant were considered to be cold-hardened rye. Krol and coworkers (22) have shown that RNH and RH plants were of similar physiological age when grown under these conditions.

Triton X-100 Extraction. Thylakoids were isolated from RNH and RH leaves and extracted with Triton X-100 essentially as described by Mullet and coworkers (24). Puma rye leaves were homogenized in 0.4 M sorbitol, 50 mM Tricine-NaOH (pH 7.8), with two 5 s bursts of a Waring Blendor, filtered through two layers of Miracloth and centrifuged at 3,000*g* for 5 min. The pellet was washed in 0.05 M sorbitol and 5 mM EDTA (pH 7.8) and centrifuged at 10,000*g* for 5 min. After resuspending the pellet to 0.8 mg Chl/ml in distilled H_2O, the thylakoids were solubilized by adding Triton X-100 to a final concentration of 0, 0.2, 0.4, 0.7, 1.0, and 1.4% (w/v) and stirring for 30 min at room temperature. The detergent-treated thylakoids were centrifuged at 32,000*g* for 40 min. Two ml of the resulting supernatant were layered on an 8 ml linear sucrose gradient (0.1–1.0 M sucrose with a 2 M sucrose cushion) containing 0.02% Triton X-100, and centrifuged for 24 h at 100,000*g* in a swinging bucket rotor (SW41). Following centrifugation, each sucrose gradient was illuminated and sketched to illustrate differences in densities of the Chl-containing bands at varying Triton X-100 concentrations. Subsequently, the density, total Chl recovered and the Chl *a/b* of each fraction were determined. Highly fluorescent Chl-containing bands were removed from each gradient and mixed with $MgCl_2$ and KCl at final concentrations of 10 and 100 mM respectively (4). After 30 min at 4°C, the preparations were centrifuged for 10 min at 10,000*g* to estimate the amount of LHCII precipitated by cations. PSI was collected from the non-fluorescent band obtained after sucrose density centrifugation of 0.7% Triton X-100 extracts. The sample was diluted in 10 mM Tricine (pH 7.8) and then centrifuged at 45,000*g* for 15 min. The fractions containing LHCII and PSI were then prepared for electrophoresis of Chl-protein complexes as described below. Chl concentrations and Chl *a/b* ratios of thylakoids and detergent extracts were calculated according to Arnon (3).

Electrophoresis of Chl-Protein Complexes. Chloroplasts were isolated at 4°C from RH and RNH leaves in 50 mM Tricine (pH 7.8), containing 0.4 M sorbitol and 10 mM NaCl. After filtration through two layers of Miracloth, chloroplasts were collected by centrifugation at 3000*g* for 5 min, and washed once in double distilled H_2O, once in 1 mM EDTA (pH 8.0), and twice in 50 mM Tricine (pH 8.0). Finally, thylakoids were resuspended in 0.3 M Tris (pH 8.8), containing 13% (w/v) glycerol and 1% SDS (w/v), followed by the addition of 2% (w/v) deoxycholate (DOC) in 0.3 M Tris (pH 8.8) to give a final DOC:SDS:Chl of 20:10:1. The solubilized membranes were then subjected to SDS-PAGE in the dark at 4°C using a 7.5% (w/v) SDS-polyacrylamide gel according to Waldron and Anderson (28). Room temperature absorbance scans of the separated complexes were obtained immediately after electrophoresis using a Shimadzu UV-250 spectrophotometer.

Thylakoid Isolation and Cation Treatment. Rye leaves were homogenized in 0.1 M Tricine-NaOH (pH 7.8) and 0.4 M sorbitol, filtered through four layers of Miracloth, and pelleted at 6,000*g* for 2 min. The pellets were washed twice with 10 mM $Na_4P_2O_7$ (pH 7.4) and twice with 0.4 M sucrose and 2 mM Tricine-NaOH (pH 7.8). The washed thylakoids were resuspended in 10% (w/v) glycerol, 65 mM Tris-HCl (pH 6.8), and 5 mM DTT, at a concentration of 1 mg Chl/ml, and stored at −70°C (6). For cation treatments, thylakoids were thawed, washed in low salt medium (0.1 M sorbitol, 10 mM Tricine-NaOH [pH 7.8], 10 mM NaCl), and resuspended in a minimal volume of low salt medium (25). An appropriate volume of 1.0 M NaCl was added to the thylakoids (0.2 mg Chl) in low salt medium to provide a final volume of 2.0 ml and a concentration range of 10 to 200 mM NaCl (6). The thylakoids were allowed to stand at room temperature for 20 min, and the degree of stacking was estimated by measuring the absorbance of the thylakoid suspension at 550 nm (23).

Octyl Glucoside Extraction. Cation treated thylakoids (0.2 mg Chl aliquots) were pelleted at 10,000*g* for 10 min and solubilized in 0.45 ml of 30 mM octyl-β-D-glucopyranoside (detergent:Chl was 20:1) in 2 mM Tris-maleate (pH 7.0) at room temperature.

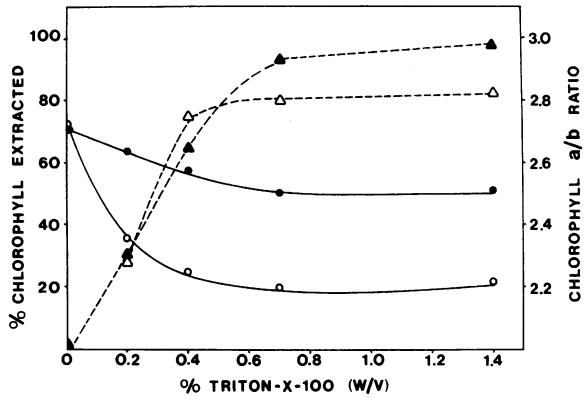

FIG. 1. Triton X-100 extraction of RNH and RH thylakoids. Points represent the means of three separate experiments. (▲---▲) Percent total Chl extracted from RNH thylakoids using the given concentration of Triton X-100; (●——●) Chl *a/b* ratio of the RNH extracts; (△---△) Percent total Chl extracted from RH thylakoids with Triton X-100; (○——○) Chl *a/b* ratio of the RH extracts.

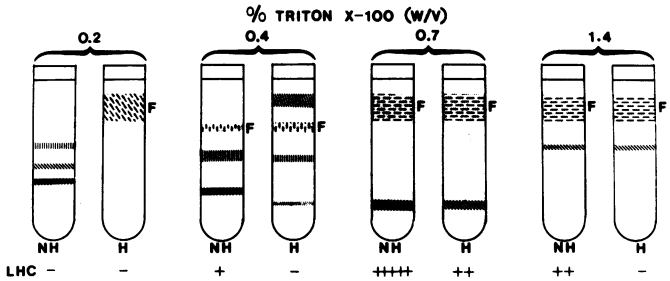

FIG. 2. Purification of light harvesting complex from Triton X-100 extracts of RNH and RH thylakoid membranes centrifuged on sucrose gradients. Line drawings illustrate separations of detergent extracted RNH and RH thylakoid membrane particles on linear sucrose gradients (0.1–1.0 M). The relative proportion of LHCII which could be cation-precipitated from fluorescent bands (F) is shown along the bottom of the diagram.

Table I. *Summary of Results of Sucrose Density Centrifugation of Triton X-100 Extracts of RNH and RH Thylakoids*

Final Triton X-100 Concn	Treatment	Density of Thylakoid Fractions	Chl Recovered	Chl *a/b*	Fluorescence[a]
% w/v		*g ml^{-1}*	*%*	*ratio*	
0.2	RNH	1.0507	2.8	2.60	NF
		1.0702	20.5	2.76	NF
		1.0897	7.4	2.71	NF
		1.1053	22.4	3.17	NF
	RH	1.0338	52.1	2.34	F
0.4	RNH	1.0540	25.3	2.17	F
		1.0780	36.1	3.30	NF
		1.1310	7.0	3.63	NF
	RH	1.0260	29.7	2.71	NF
		1.0540	10.1	2.21	F
		1.0890	24.2	3.46	NF
		1.1352	2.1	4.70	NF
0.7	RNH	1.0338	50.2	1.93	F
		1.1352	8.2	6.13	NF
	RH	1.0338	55.6	1.89	F
		1.1352	5.1	6.83	NF
1.4	RNH	1.0338	55.8	2.20	F
		1.0741	2.3	4.80	NF
	RH	1.0338	52.3	2.00	F
		1.0741	1.8	4.08	NF

[a] NF, nonfluorescent; F, fluorescent.

Solubilized thylakoids were centrifuged at 100,000*g* for 30 min, and the resulting supernatant (octyl glucoside extract) was removed by pipette from the octyl glucoside pellet (6). Chl-protein complexes in the octyl glucoside extracts were separated by SDS-PAGE on 10% (w/v) acrylamide slab gels as described by Camm and Green (5).

Phosphorylation. Thylakoids were isolated in 0.4 M sorbitol, 10 mM NaCl, and 50 mM Tricine-NaOH (pH 7.8); washed with 10 mM NaCl, 5 mM $MgCl_2$, and 15 mM Tricine-NaOH (pH 7.8); and resuspended to 0.2 mg Chl/ml in 0.1 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 50 mM Tricine-NaOH (pH 7.8), 0.1 mM ATP, 0.2 mCi [γ-^{32}P]ATP/μmol ATP, and 10 mM NaF (26). Control thylakoids (nonphosphorylated) were placed in foil-wrapped vials in a 20°C water bath. Phosphorylation of thylakoid polypeptides was activated by light at an intensity of 200 μE m^{-2} s^{-1} focused on the same 20°C water bath. After 15 min, aliquots of phosphorylated and nonphosphorylated thylakoids were pelleted in a microfuge, washed twice in resuspension buffer, and solubilized in 0.06 M Tris-HCl, pH 6.8, containing 5% (w/v) glycerol, 5% (v/v) β-mercaptoethanol, and 2% (w/v) SDS. Polypeptides were separated on slab gels containing a 5% (w/v) stacking gel and a 12 to 18% (w/v) acrylamide gradient separating gel at a constant current of 18 mamp. Gels were stained with 1% (w/v) Coomassie brilliant blue R-250 and destained in methanol:acetic acid:water (3:1:10). Molecular mass markers included BSA (69 kD), alcohol dehydrogenase (37.5 kD), cowpea chlorotic motte virus (CCMV) coat protein (19.4 kD), and Cyt *c* (12.5 kD). For octyl glucoside extraction, phosphorylated and control thylakoids were washed four times in 10 mM $Na_4P_2O_7$ (pH 7.4) to unstack the membranes prior to solubilization in the detergent and electrophoresis of the Chl-protein complexes.

RESULTS

Effects of Triton X-100 Concentration on Chl Extraction. At Triton X-100 concentrations between 0.7 and 1.4% (w/v), extracts of RNH thylakoids consistently contained a greater percentage of the total Chl than extracts of RH thylakoids (Fig. 1). Approximately 100% of the total Chl could be extracted from RNH thylakoids, while only 80 to 85% of the total Chl could be extracted from RH thylakoids, with Triton X-100. Although both RNH and RH thylakoids reached maximal detergent extraction at a Triton X-100 concentration of 0.7%, RH thylakoid extracts consistently contained a greater proportion of Chl *b* than RNH extracts (Fig. 1). Centrifugation of these extracts on linear sucrose gradients revealed differences in the Chl-containing components released from the thylakoid membrane by detergent solubilization. For example, when RNH and RH thylakoids were extracted by 0.2% Triton X-100, approximately 50% of the total Chl was released from the membranes. However, RNH extracts exhibited four nonfluorescent Chl bands of varying density (Fig. 2 and Table I) with Chl *a/b* between 2.6 and 3.2. In contrast, RH extracts exhibited only one fluorescent band (Fig. 2 and Table I) with a Chl *a/b* of 2.3. Bands differing in density, in the proportion of Chl recovered and in Chl *a/b* were also observed in RH and RNH thylakoids extracted by 0.4% Triton X-100.

At a detergent concentration of 0.7%, however, RNH and RH extracts exhibited Chl bands of similar densities with one large, fluorescent band with a Chl *a/b* of about 1.9, and a smaller, nonfluorescent, Chl band with a Chl *a/b* of 6 to 7 (Fig. 2 and Table I). The fractions containing the fluorescent bands were removed, and K^+ and Mg^{2+} were added to these fractions at final concentrations of 100 and 10 mM, respectively. The cation precipitates from both RNH and RH samples had a Chl *a/b* between 1.1 and 1.2, and consisted of three major Chl-protein complexes (Fig. 3, LHC scan), each exhibiting characteristic absorption maxima at 438 and 480 nm with a red absorption maximum at 672 nm coupled with a prominent shoulder at 652 nm (data not presented). These three complexes corresponded to the oligomeric, dimeric, and monomeric forms of the light harvesting Chl *a/b*-protein complex associated with PSII. How-

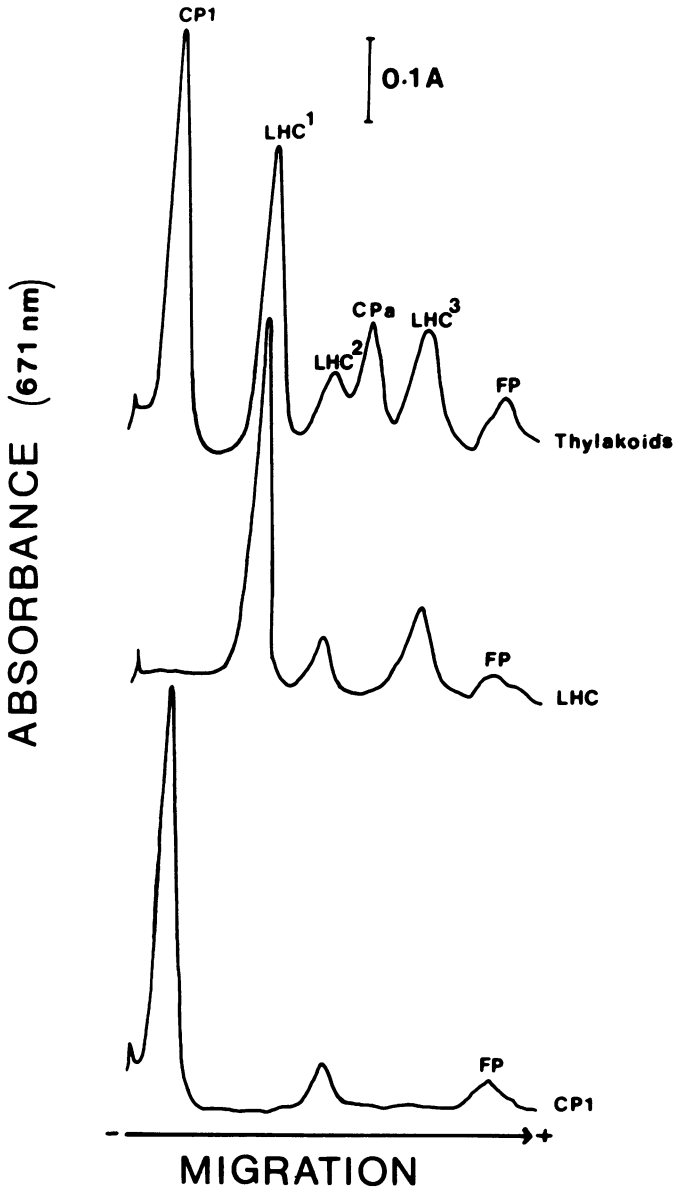

FIG. 3. Electrophoretic separations of Chl-protein complexes from RNH samples, and scanned spectrophotometrically at 670 nm. LHCII and CPI were obtained after sucrose density centrifugation of RNH thylakoids solubilized in 0.7% Triton X-100. Whole thylakoids, LHCII, and CPI were prepared and electrophoresed according to Waldron and Anderson (28). CPI, P_{700} Chl *a*-protein complex; CPa, PSII reaction center Chl *a*-protein complex; LHC^1, LHC^2, LHC^3, oligomeric, dimeric, and monomeric forms of the Chl *a/b* light harvesting complex associated with PSII; FP, free pigment.

ever, addition of cations consistently yielded less precipitate in RH samples than in RNH samples. Typically, 25 to 30% of the Chl in the fluorescent band could be precipitated as LHCII from RNH samples, whereas only 10 to 15% of the Chl from the fluorescent band was precipitated from RH samples. Increasing the cation concentration, incubation time, or incubation temperature did not significantly alter these results. In addition, RNH thylakoid extracts consistently exhibited a greater amount of the nonfluorescent Chl than RH extracts when a concentration

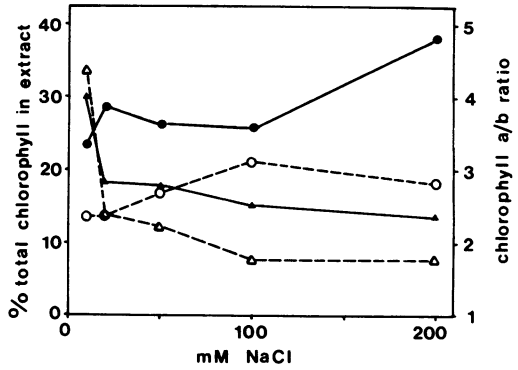

FIG. 4. Effect of cation pretreatments of RNH and RH thylakoids on subsequent octyl glucoside extraction. The points represent the means of five separate experiments for RNH thylakoids, and four experiments for RH thylakoids. (▲——▲) Percent total Chl solubilized from RNH thylakoids by octyl glucoside; (●——●) Chl *a/b* ratio of RNH extract; (△---△) Percent total Chl solubilized from RH thylakoids by octyl glucoside; (○—○) Chl *a/b* ratio of RH extract.

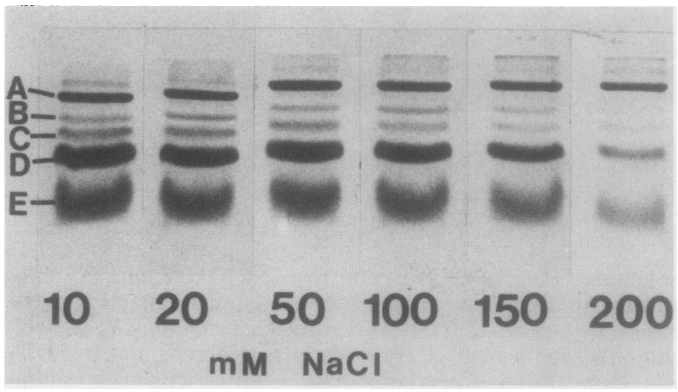

FIG. 5. Effect of cation pretreatments of RNH thylakoids on octyl glucoside extraction of Chl-protein complexes. The Chl-protein complexes were separated by SDS-PAGE. The gel is unstained. The Chl-proteins complexes were identified as: A, reaction center of PSI (CPI); B, oligomer of light harvesting complex; C, reaction center of PSII (CPIV); D, monomer of light harvesting complex (LHCII); and E, free pigment.

of 0.7% Triton X-100 was employed (Fig. 2 and Table I). This band exhibited two major pigment-protein complexes upon electrophoresis (Fig. 3, CPI scan). One migrated as CPI and exhibited a characteristic absorption maximum at 436 nm and a red absorption maximum at 675 nm; the other migrated as the dimeric form of LHC and exhibited absorption maxima at 438 and 480 nm and a red absorption maximum at 671 nm with a shoulder at 652 nm. This latter complex is consistent with the light harvesting Chl *a/b* protein associated with PSI (1, 14). Detergent extractions of rye thylakoids yielded variable results when averaged over all experiments, however, all trends reported here were consistent when RNH and RH thylakoid membranes were extracted by detergents in paired experiments.

Effects of Cation Concentration on Chl Extraction with Octyl Glucoside. When RNH and RH thylakoids were washed with "low salt medium" containing 10 mM NaCl, approximately 30% of the total Chl could be extracted by octyl glucoside (Fig. 4). When these same membranes were treated with 20 to 200 mM NaCl prior to solubilization in octyl glucoside, the amount of Chl extracted by the detergent decreased dramatically. The Chl in cation-treated RH thylakoids was less extractable with octyl glucoside than similarly treated RNH thylakoids. Generally, octyl glucoside extracts of RH thylakoids averaged 6% less Chl

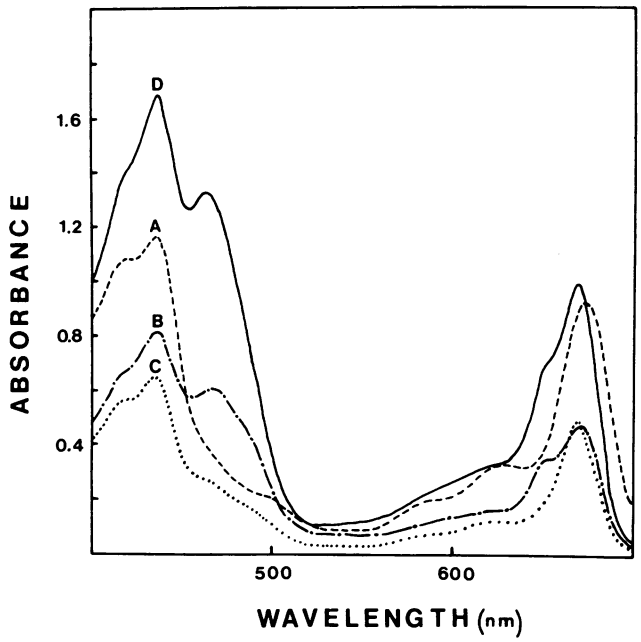

FIG. 6. Absorption spectra of Chl-protein complexes. A, P_{700} Chl *a*-protein complex; B, oligomeric form of the light harvesting Chl *a/b*-protein complex; C, the PSII reaction center Chl *a*-protein complex (CPa or CPIV); D, monomeric form of the light harvesting Chl *a/b*-protein complex (LHCII).

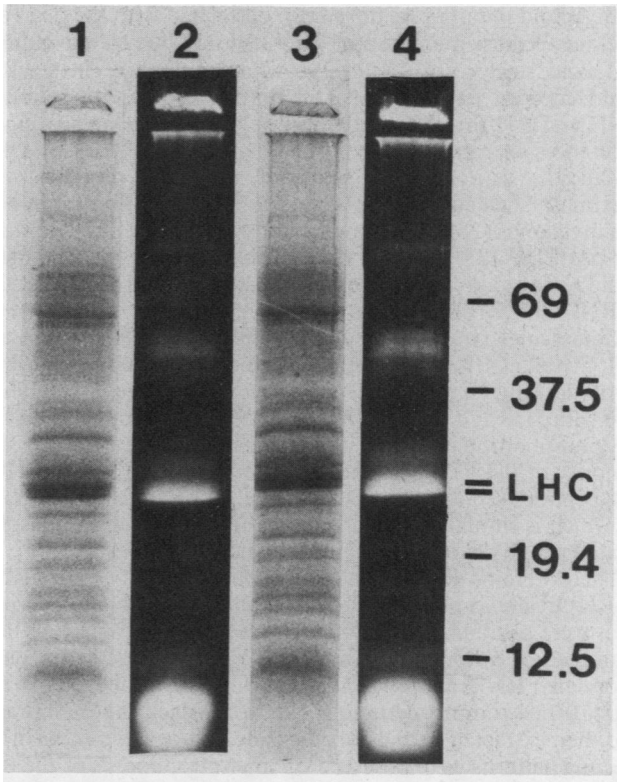

FIG. 7. Phosphorylation of RNH and RH thylakoid polypeptides. Thylakoid polypeptides were phosphorylated, solubilized in SDS, separated by SDS-PAGE, and subjected to autoradiography. The apparent molecular masses of four standard proteins separated on the same gel are shown on the right-hand side of the figure. The major phosphorylated polypeptides are associated with LHCII. Lanes 1 and 3 are stained SDS-polyacrylamide gels of RNH and RH-thylakoid polypeptides, respectively. Lanes 2 and 4 are autoradiographs of the same gels showing the phosphorylated polypeptides.

than RNH thylakoid extracts over the entire concentration range of 20 to 200 mM NaCl (Fig. 4).

The Chl *a/b* ratios of the octyl glucoside extracts were also affected by the cation treatment. Prior to detergent extraction, the Chl *a/b* ratio of RNH thylakoids was 3.10 ± 0.16, and the ratio for RH thylakoids was 2.78 ± 0.19. Regardless of the cation concentration employed prior to octyl glucoside extraction, the Chl *a/b* always tended to be lower in octyl glucoside extracts of RH thylakoids than RNH thylakoids (Fig. 4). Over the cation concentration range of 10 to 200 mM, the Chl *a/b* increased from 3.3 to 4.8 for octyl glucoside extracts of RNH thylakoids, whereas the Chl *a/b* increased only slightly from 2.4 to 2.8 for octyl glucoside extracts of RH thylakoids.

The increase in Chl *a/b* observed for octyl glucoside extracts of RNH thylakoids was presumably due to the fact that the Chl *a/b*-protein complexes of RNH thylakoids became less extractable with this detergent at higher cation concentrations. This was tested by SDS-PAGE of the octyl glucoside extracts (Fig. 5). Each pigment band was identified according to its characteristic absorption spectrum (Fig. 6) and its relative electrophoretic migration. Band A corresponded to CPI with a red absorption maximum at 675 nm; bands B and D corresponded to the oligomeric and monomeric forms of the light harvesting Chl *a/b* protein with red absorption maxima at 652 and 672 nm; band C corresponded to CPa or CPIV with a red absorption maximum at 671 nm. There were few changes in the relative proportions of Chl-protein complexes over the range of 20 to 100 mM NaCl in RNH extracts with LHCII monomer, the major Chl *a/b*-protein complex, being the most prominent band. At higher concentrations of NaCl, however, the CPI band, the major Chl *a*-protein complex, was the most prominent pigment-protein complex observed. In contrast, RH thylakoids pretreated with 200 mM NaCl exhibited a loss of all four Chl-protein complexes (data not shown). Thus the electrophoretic results are consistent with the differential effect of cations on the extractability of Chl from RH and RNH thylakoids with octyl glucoside.

Effects of Cations on Stacking. Changes in the extractability

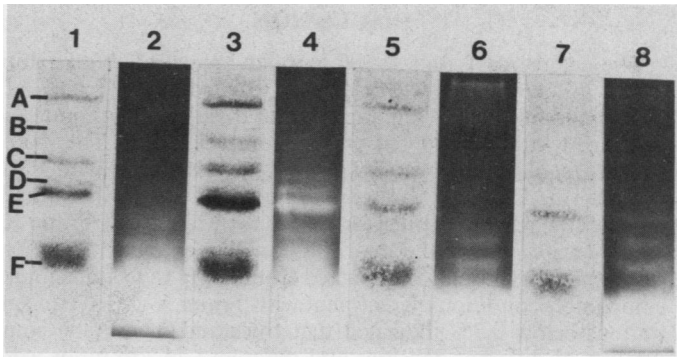

FIG. 8. Effect of phosphorylation on octyl glucoside extraction of Chl-protein complexes from thylakoid membranes of RNH and RH. Lanes 1, 3, 5, and 7 are unstained SDS-polyacrylamide gels of the Chl-protein complexes separated from octyl glucoside extracts of nonhardened (lanes 1 and 3) and cold-hardened (lanes 5 and 7) rye thylakoid membranes. Lanes 2, 4, 6, and 8 are autoradiographs of each gel. The thylakoid membranes in lanes 1 and 5 were phosphorylated in the dark, and the thylakoids in lanes 3 and 7 were phosphorylated in the light. A, CPI; B, LHCII oligomer; C, CPIV; D, CP29; E, LHCII monomer, and F, free pigment.

of thylakoid membranes have been correlated with the degree of stacking of those membranes (7, 25). We studied cation-induced thylakoid stacking to determine whether changes in stacking could account for differences in octyl glucoside extraction of RNH and RH thylakoid membranes. There were no significant differences in cation-induced stacking between RNH and RH thylakoids (data not shown). Both sets of membranes were maximally stacked at 150 mM NaCl as has been observed in both pea thylakoids (25) and in phosphatidyl choline vehicles containing pea LHC (23). Thus it appears that stacking characteristics could not account for differences in octyl glucoside extractability of RNH and RH thylakoids.

Detergent Extractability of Phosphorylated LHC. RNH and RH thylakoids were phosphorylated under conditions which favor stacking (5 mM $MgCl_2$). Following membrane solubilization with SDS and separation of the polypeptides by SDS-PAGE, the phosphoproteins were visualized by autoradiography. The majority of the ^{32}Pi label was incorporated into the two LHCII polypeptides (26 and 25 kD) of RNH and RH thylakoids (Fig. 7), which is similar to the labeling pattern observed in phosphorylated pea thylakoids by Steinback and coworkers (26).

There were no differences in phosphorylation of either RNH or RH thylakoid polypeptides when all the membrane polypeptides were solubilized in SDS, separated by electrophoresis, and examined by autoradiography (Fig. 7). In contrast, when phosphorylated RNH and RH membranes were washed extensively with 10 mM sodium pyrophosphate to unstack the membranes and then extracted with octyl glucoside, major differences in the labeling patterns were observed upon electrophoresis of the Chl-protein complexes. Phosphorylation of the LHCII polypeptides of RNH thylakoids appeared to be light dependent (Fig. 8, lanes 2 and 4). In contrast, the octyl glucoside extracted LHCII of RH membranes exhibited minimal light dependent ^{32}Pi incorporation (Fig. 8, lanes 6 and 8), even though SDS solubilization of RH and RNH thylakoids indicated no significant difference in the total ^{32}Pi incorporation into membrane polypeptides (Fig. 7). A greater proportion of the total label in RH thylakoids was found to be associated with the pellet rather than the supernatant after octyl glucoside extraction. Electrophoresis of the Chl-protein complexes associated with the pellet indicated that the majority of the label was associated with a nonpigmented polypeptide complex with a molecular mass greater than CPI (data not presented).

DISCUSSION

The detergents Triton X-100 and octyl glucoside both exhibited a differential ability to extract Chl from RNH and RH membranes. The Chl contained within thylakoid membranes is associated with specific Chl protein complexes (27). The major Chl *a/b* containing complex is the LHC associated with PSII, whereas the major Chl a containing complex is CPI which includes the reaction center for PSI. In the presence of Triton X-100, 5 to 20% less of the total Chl could be extracted from RH thylakoids than could be extracted from RNH thylakoids under comparable conditions. Treatment with Triton X-100 at concentrations below 0.7% indicated that thylakoid membrane components of different densities and different Chl *a/b* ratios were solubilized from RH thylakoids and RNH membranes subjected to identical solubilization conditions. At 0.7% Triton X-100, both RNH and RH thylakoids exhibited the presence of membrane components of similar densities and Chl *a/b* ratios. The fluorescent band (1.0 g ml^{-1}) was shown to contain the Chl-protein complexes associated with LHCII whereas the nonfluorescent band (1.2 g ml^{-1}) contained the Chl-protein complexes associated with PSI. However, cation precipitation of LHCII from the fluorescent band consistently resulted in a 50% greater yield from the 0.7% Triton X-100 extract of RNH thylakoids compared with RH thylakoids. Furthermore, solubilization of RNH thylakoids in 0.7% Triton X-100 consistently yielded 50 to 60% more PSI than RH thylakoids based on the total Chl recovered from the nonfluorescent band (Table I).

Cation pretreatment of RNH and RH thylakoids decreased the ability of octyl glucoside to extract Chl from both RNH and RH thylakoids. However, octyl glucoside preferentially solubilized Chl *a* from RNH thylakoids pretreated with increasing concentrations of cations. This was indicated by the fact that the Chl *a/b* of RNH thylakoid extracts was higher than that for RH extracts at all cation concentrations tested. This did not appear to be due to a differential effect of cations on thylakoid stacking. Thus, we conclude that rye thylakoid membranes developed at low temperature can be distinguished from those developed at warm temperature on the basis of the differential detergent solubilization of thylakoid membranes.

This was further supported by examining the detergent solubilization of phosphorylated LHCII from RNH and RH membranes. When RNH and RH thylakoids were phosphorylated and completely solubilized in the ionic detergent SDS, no apparent differences were observed in the labeling characteristics of the LHCII polypeptides from the two sets of membranes (Fig. 7). However, when phosphorylated thylakoids were extracted with the nonionic detergent octyl glucoside, LHCII solubilized from RNH thylakoids was heavily labeled, whereas minimal label was associated with LHCII solubilized from RH thylakoids (Fig. 8). These results indicate that light-dependent protein phosphorylation differentially affected the ability of octyl glucoside to solubilize LHCII from RH thylakoids. Solubilization with SDS clearly showed that LHCII polypeptides were labeled to the same extent in RH and RNH thylakoids, and thus we conclude that protein phosphorylation of LHCII alters protein-protein interactions of this complex within RH thylakoids such that the labeled LHCII complex is no longer solubilized by the nonionic detergent octyl glucoside. Protein phosphorylation has been shown to affect protein-protein interactions associated with LHCII of thylakoid membranes (2, 25, 26).

Nonionic detergents such as Triton X-100 and octyl glucoside appear to disrupt primarily hydrophobic interactions between membrane lipids and polypeptides. In contrast to denaturing detergents such as SDS, nonionic detergents are typically inefficient in affecting protein-protein interactions (15). In earlier experiments (8), and again in our phosphorylation experiments described above, we have found that RNH and RH thylakoid membranes solubilized in SDS exhibit few differences in the profiles of denatured polypeptides. Yet RNH and RH thylakoids exhibited significant differences in the Chl content and Chl *a/b* ratios of membrane fractions produced after solubilization with Triton X-100, with octyl glucoside, and with very low concentrations of SDS. Therefore, we conclude that development of rye leaves at low temperature must affect thylakoid membrane protein complexes at the level of protein-protein interactions.

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