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Characterization of the thylakoid membrane in a chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana*

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Abstract. An ethylmethanesulfonate-induced *chlorina* mutant (*ch5*) of *Arabidopsis thaliana* was characterized by its chlorophyll, carotenoid, lipid, protein, and pigment-protein complexes, the surface tension of thylakoid membrane, and the influence of digitonin on the surface tension of thylakoid membrane. The pattern of the first derivative absorption spectra of chloroplast and pigment-protein complexes fractionated by Thornber and MARS gel systems revealed that the composition and organization of thylakoid membranes isolated from wild type and from *ch5* mutant were very different. The ratios of monogalactosyl diglyceride/digalactosyl diglyceride (MGDG/DGDG) in wild type and *ch5* mutant were 1.98 and 1.03, respectively. At a chlorophyll concentration lower than 70 μg ml^{-1}, the surface tension of the thylakoid membrane of wild type was higher (3-6 dyne cm^{-1}) than that of *ch5* mutant. With less than 3% digitonin, chlorophyll was more easily solubilized from thylakoid membranes of wild type than from those of *ch5* mutant, but with more than 3% digitonin the opposite result was observed. The data indicate that the thylakoid membrane of *ch5* mutant is more loosely arranged than that of wild type.

Keywords: *Arabidopsis thaliana*; Chlorophyll-deficient mutant; Lumen; Partition; Surface tension; Thylakoid.

Introduction

Many chlorophyll deficient mutants have been reported in barley, pea, maize, wheat, sweetclover, *Chlamydomonas reihardii* (for references, see Markwell et al., 1986), rice (Terao et al., 1985), soybean (Droppa et al., 1988), sugar beet (Abadia et al., 1985), *Arabidopsis thaliana* (Hirono and Redei, 1963), and other plants. The chlorophyll-deficient mutants can be placed into two groups: one with no detectable chlorophyll b, one with a reduced amount of this pigment. Many chlorophyll-deficient mutants have been used to study the biosynthetic pathway of this pigment and the biogenesis of the photosynthetic apparatus (Somerville, 1986). It has been proposed that only a minority of the chlorophyll-deficient mutants are impaired in the chlorophyll biosynthetic pathway (Nelson, 1967). This proposal has been substantiated by systematic examination of a family of chlorophyll-deficient sweetclover mutants (Yang et al., 1990). In the chlorophyll-deficient *ch4* (U394) mutant of sweetclover, one of the enzymes involved in the conversion of coproporphyrinogen III to protoporphyrin IX likely has decreased activity or altered regulatory properties (Bevins et al., 1992). In a family of chlorophyll-deficient wheat and barley mutants, the Mg-chelatase activity was found to be blocked (Falbel and Staehelin, 1994). Temperature-sensitivity has been proposed as a general phenomenon in chlorophyll-deficient mutants of sweetclover and *Arabidopsis thaliana* (Yang et al., 1990; Markwell and Osterman, 1992; Yang et al., 1993). It was suggested that a block in Mg-chelatase activity, the first enzyme committed to chlorophyll biosynthesis, could account for the other traits of their pleiotropic phenotype (Falbel and Staehelin, 1994).

The chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana* is a yellow-green mutant originally induced by ethylmethanesulfonate. Information on the biochemical and structural development of chloroplasts of this mutant is still meager (Koornneef et al., 1983). The aim of our research is the investigation of variation in thylakoid membranes of the *ch5* mutant. We examine the composition of pigments, lipids, and proteins, surface tension, the effect of digitonin on the surface tension, and digitonin solubilization of thylakoid membrane of this mutant.

Materials and Methods

Plants

Seeds of Columbia wild type (*wt2*) and *chlorina* mutant-5 (*ch5*) of *Arabidopsis thaliana* were generous gifts of Dr. H. M. Pan, Department of Botany, National Taiwan University and the Nottingham *Arabidopsis* stock center (UK), respectively. Seeds were germinated and grown for 4 weeks in a soil-vermiculite mixture in a growth chamber with a 12D/12L photoperiod, a photon flux of 250–300 μmole m^{-2} s^{-1}, a 60% relative humidity, and a temperature of 23°C.

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Determination of Pigment and Protein

Following extraction of liquid-nitrogen frozen leaf with 80% acetone, the concentrations of chlorophyll and carotenoid were determined according to the methods of Arnon (1949) and Jaspers (1965), respectively. Protein concentration was determined using the bicinchoninic acid assay of Bradford (1976). Absorbance was measured with a Hitachi U3200 UV-visible spectrophotometer.

High Performance Liquid Chromatograghy (HPLC) of Carotenoid

Pigments were extracted from leaves according to the method of Braumann and Grimme (1981). After drying in nitrogen, total pigments were dissolved in 1 ml of ether. The extract was stored in the dark at -80°C until analyzed. HPLC was performed on a Vercopak inertsil 10 ODS 25 × 4.6 mm reverse phase column (10 μm particle size). Mobile phases were pumped by a Beckman model 126AA high pressure pump at 2.5 ml min^{-1}. Peaks were detected at 450 nm by a Beckman model 166 detector. The sample (10-20 μl) was injected into the column, and two mobile phases were applied as shown in Table 1. Prior to injecting each sample, the column was equilibrated by flushing with acetonitrile:methanol (3:1, v/v, mobile phase B) for 10 min. Peaks were identified by standard methods as previously described (Val et al., 1986).

Table 1. Time, flow rate, mobile phase, and duration of HPLC.

Time (min)	Flow rate (ml min^{-1})	A phase (%)	B phase (%)	Duration (min)
0	1.5	25	75	0
22	2.5	0	100	22
70	2.5	0	100	48

Thin Layer Chromatography (TLC) of Lipid

Crude lipid was obtained by dissolving the isolated thylakoid membranes in 10 ml of chloroform:methanol (2:1, v/v) and centrifuging at 10,000 *g*, 4°C for 10 min to eliminate protein (Bligh and Dyer, 1959). The supernatant liquid was nitrogen dried and then processed twice again by the same procedure, except that the solvent was chloroform only. TLC was performed with Merck kieselgel 60 (20×20 cm), which was dried at 120°C for 15 min and developed in a solution of chloroform:methanol:acetic acid:H_2O (85:15:10:3, v/v/v/v). The lipid was made visible by staining with iodine vapor. The concentration of galactolipid, sterol glycoside, and sulfolipid were determined according to the method of Roughan and Batt (1968), and that of phospholipid was determined by the method of Chalvardjian and Rudnicki (1970).

Electrophoresis of Pigment-Protein Complexes

Leaves were harvested and thylakoid membranes isolated as previously described (Markwell, 1986). Thylakoid membranes were analyzed for constituent pigment-protein complexes by solubilization with SDS and electrophoresis with Thornber and MARS fractionation systems (Markwell, 1986). The first derivative absorption spectra of chloroplast and pigment-protein complexes were measured with a Hitachi U3200 UV-visible spectrophotometer.

Surface Tension

To study the interaction of surfactants with thylakoid membranes, 1 ml of fresh digitonin solution in 50 mM Tris-HCl (pH 8.0) was added to an equal volume of fresh thylakoid membranes containing 15 μg ml^{-1} of chlorophyll, both being twice the desired final concentration (Bartzatt et al., 1983). The mixture was incubated at 25°C for 10 min prior to the measurement of surface tension with a Kyowa face automatic surface tensiometer (CBVP-A3). All surfactant solutions were prepared on a g/ml basis. After measurement of surface tension, the mixture was centrifuged at 4000 rpm, room temperature for 5 min. The chlorophyll concentration in the supernatant liquid was determined as described above.

Results and Discussion

Chloroplast

Most of the information on the physiology of leaves is relevant to the chloroplast structure and function. Chlorophyll and its associated polypeptides are located in the thylakoid membranes of chloroplasts. Since differentiation of an absorption spectrum with respect to wavelength can reveal detail that is not seen in a straightforward absorption spectrum, we determined the first derivative absorption spectra of chloroplasts isolated from wild type and *ch5* mutant (Figure 1). No shift occurs in the trough at 676 nm, but the intensity of the wild type's is stronger than that of the *ch5* mutant. A red peak at 652 nm found in wild type chloroplasts was red-shifted to 656 nm in the *ch5* mutant, and the troughs at 425 and 440 nm in the blue regions of wild type were shifted to 430 and 450 nm, respectively. The trough ratio of 440/483 nm in wild type differs from that of the *ch5* mutant (450/483 nm). The data strongly suggest that the thylakoid membrane components of *ch5* mutant are different from those of its corresponding wild plant. The following analysis of pigment, lipid, and proteins of thylakoid membrane confirm this.

Pigment and Protein Content

It is now clear that all chlorophyll molecules are noncovalently associated with polypeptides to form several pigment-protein complexes located in the thylakoid membranes (Markwell, 1986), and that chlorophyll is not stable in the absence of its apoprotein (Terao et al., 1985). Decreased quantities of pigments may or may not result from a damaged chlorophyll biosynthetic pathway.

We determined the chlorophyll and carotenoid contents of wild type and *ch5* mutant of *Arabidopsis thaliana*. The

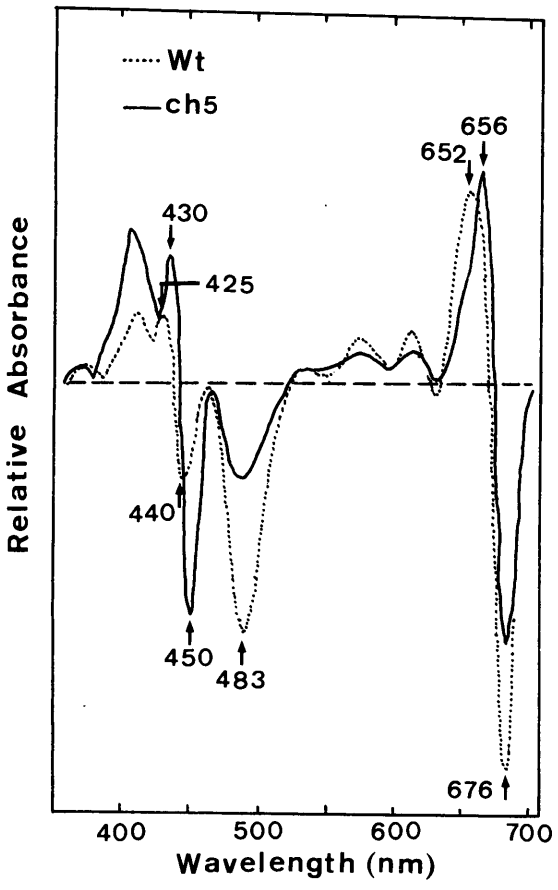

Figure 1. The first derivative absorption spectra of chloroplasts isolated from wild type and *ch5* mutant of *Arabidopsis thaliana*.

chlorophyll content (μg g^{-1} leaf) of the *ch5* mutant is 90% of that of the wild plant, whereas the carotenoid content of the *ch5* mutant is approximately 4.3 fold more than that of wild type (Table 2). The total protein content of the *ch5* mutant chloroplast is slightly higher than that of wild type (Table 2), but the wild type contains a much greater quantity of thylakoid polypeptides than does the *ch5* mutant (data not shown). The pigments were further analyzed by HPLC (Table 3). Except for antheraxanthin, pigments are more abundant in the wild type than in the *ch5* mutant. The ratios of the xanthophyll (VAZ) cycle components violaxanthin, antheraxanthin, and zeaxanthin for wild type and *ch5* mutant were 2.82, 0.81 and 2.17, respectively (Table 3). These data are in contrast to the findings of Mayfield and Taylor (1984) that a deficiency in carotenoids can cause a decreased chlorophyll content. It is likely that the changes in pigment composition of xanthophyll cycle components are an adaptation to the *ch5* mutant to dissipate excess energy.

Table 2. The chlorophyll, carotenoid, protein, and lipid contents of thylakoid membranes isolated from wild type and chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana*. The data are the mean of triplicate determinations.

Strain	Chlorophyll (μg g^{-1} leaf)	Carotenoid (μg g^{-1} leaf)	Protein (mg g^{-1} leaf)	Lipid (ng mg^{-1} Chl)	Carotenoid/ Chl ratio (%)
Wt	453±21	15.5±1.1	16.3±1.2	2667±85	3.42±0.18
Ch5	407±18	67.3±3.1	18.3±1.4	7333±286	16.53±0.28
Ch5/Wt	0.90±0.09	4.34±0.22	1.12±0.08	2.75±0.12	4.83±0.29

Table 3. HPLC anaylyses of chlorophyll and carotenoid content of thylakoid membrane isolated from wild type and *ch5* mutant of *Arabidopsis thaliana* grown under an illumination of 250–300 μmol m^{-2} s^{-1}. The data are the mean of four determinations.

Pigment	Retention time (min)	Pigment content (μg ml^{-1})	
		Wt	*h5*
Neoxanthin	16.64±0.08	0.054±0.004	0.049±0.006
Violaxanthin	17.51±0.07	0.079±0.004	0.028±0.005
Taraxanthin	20.19±0.07	0.646±0.015	0.127±0.008
Antheraxanthin	22.85±0.07	0.034±0.004	0.042±0.011
Lutein	24.73±0.03	1.309±0.013	0.403±0.013
Zeaxanthin	25.97±0.04	0.113±0.002	0.052±0.004
Pheophytin *b*	30.59±0.14	1.081±0.009	0.269±0.011
Chl *b*	32.70±0.04	8.694±0.008	2.157±0.005
Pheophytin *a*	35.76±0.17	0.740±0.005	0.258±0.008
Chl *a*	40.77±0.23	11.060±0.003	3.746±0.011
β-carotene	60.86±0.24	0.573±0.004	0.270±0.001

Lipid Content

Lipids are an integral part of the thylakoid membrane matrix in which the pigment-protein complexes are embedded. Thylakoid membrane lipids were analyzed by TLC and HPLC. Total lipids from *ch5* mutant thylakoid were approximately 2.8 fold more than those from wild type thylakoid (Table 4). The great difference in lipid composition between wild type and *ch5* mutant may affect the fluidity and surface tension of thylakoid membrane. The ratios of MGDG to DGDG in wild type and *ch5* mutant were 1.98 and 1.03, respectively. MGDG and DGDG, which are uncharged lipids, comprise about 80% of the wild type and 70% of the *ch5* mutant total lipid (Table 4). Most MGDG is located in the grana, which contains most PSII, and most DGDG is located in the stroma lamellae, which contains most PSI (O'Sullivan and Dalling, 1989). The relatively low ratio of MGDG to DGDG in thylakoid

Table 4. Lipid content of thylakoid membrane in wild type and chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana*. The Chl concentration of wild type and *ch5* mutant is 1400 mg ml^{-1}. The data are the mean of three determinations.

Lipid	Rf	Lipid content (ng mg^{-1} Chl)	
		Wt	*ch5*
Lysophosphatidyl choline(LPC)	0.15	56.7±3.1	163.1±12.3
Phosphatidyl inositol(PI)	0.18	53.4±3.2	182.3±15.6
Sulfolipid(SL)	0.28	212.6±26.7	545.3±64.5
Phosphatidyl choline (PC)	0.29	48.2±3.7	158.6±10.4
Digalactosyl diglyceride(DGDG)	0.42	708.2±24.1	2515.8±267.3
Phosphatidyl glyceride(PG)	0.44	41.9±3.8	104.0±7.8
Phosphatidyl ethanolamine(PE)	0.48	42.3±3.0	180.4±14.7
Sterol glycoside(SG)	0.74	62.8±16.1	729.7±57.8
Diphosphatidyl glyceride(DPG)	0.85	41.9±5.3	157.4±11.6
Monogalactosyl diglyceride(MGDG)	0.88	1399±66	2597±255
Total lipid		2667±85	7333±286
DGDG/total lipid		26.6%	34.3%
MGDG/total lipid		52.5%	35.4%
DGDG+MGDG/total lipid		79.1%	69.7%
MGDG/DGDG		1.98	1.03

membrane of the *ch5* mutant may lead to a decrease in membrane stacking and a decrease in fluidity of the thylakoid membrane. The thylakoid membrane of *ch5* mutant, therefore, is more rigid than that of wild type.

Pigment-Protein Complexes

Chlorophyll in the thylakoid membrane is usually bound to protein to assemble the pigment-protein complexes located in the thylakoid membrane, and it is responsible for the biophysical functions of light-harvesting, energy transduction, and photochemical energy conversion. These pigment-protein complexes of wild type and *ch5* mutant can be electrophoretically fractionated by the Thornber gel system into two pigment-protein complexes, termed CPI and CPII, and free pigment, or be resolved by the MARS gel system into four pigment-protein complexes, named A1, AB1, AB2, and AB3, and free pigment (Markwell, 1986). Among these complexes, CPI and A1 are derived from PSI and CPII, AB1, AB2, and AB3 are derived from PSII. We

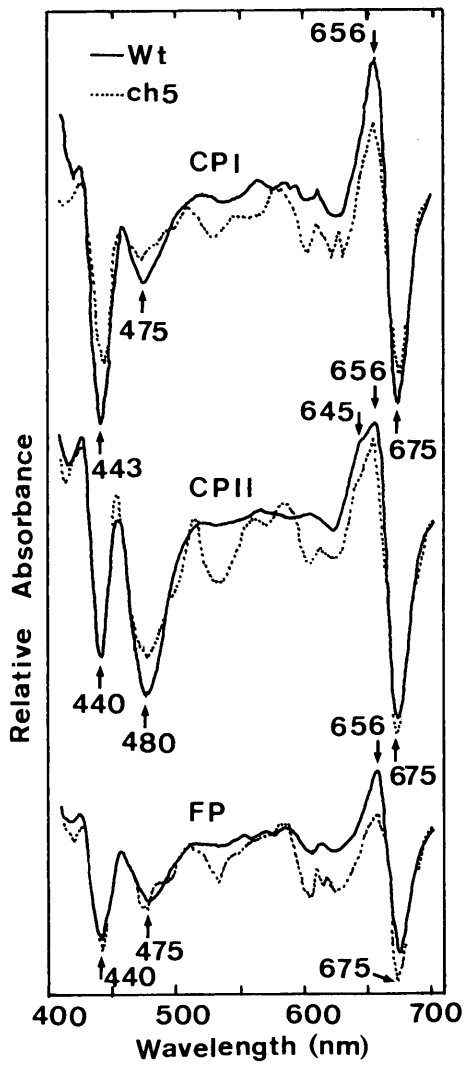

Figure 2. The first derivative absorption spectra of pigment-protein complexes resolved by the Thornber fractionation system.

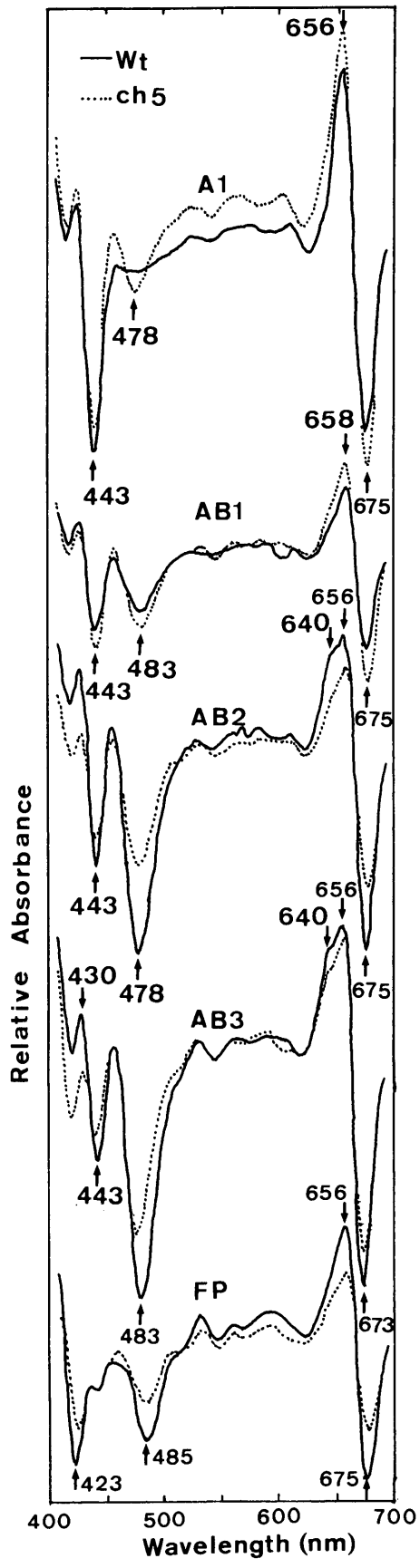

Figure 3. The first derivative absorption spectra of the pigment-protein complexes resolved by the MARS fractionation system.

determined the first derivative absorption spectra of each pigment-protein complex resolved by the two electrophoretic systems (Figures 2 and 3).

The patterns of the first derivative absorption spectra were very similar among the pigment-protein complexes fractionated by the Thornber system (Figure 2). The patterns of peak and trough in the red region were the same, the trough at 475 nm of CPI and FP was shifted to 480 nm in CPII, and the trough at 440 nm of CPII and FP was shifted to 443 nm in CPI. The intensity of each of the four major peaks and troughs (443, 475, 656, and 675 nm) of CPI in *ch5* mutant was weaker than those of wild type. The intensity of two major peaks and troughs in CPII of *ch5* mutant, i.e. 480 and 656 nm, was weaker than that of wild type, and the intensity of the trough at 675 nm was stronger than that of wild type. The intensity of the peak at 656 nm in free pigment of the *ch5* mutant was weaker than that in wild type, and the intensity of the trough at 675 nm was greater.

Excepting the patterns in the blue region, no significant difference was found between the peak and trough patterns of MARS pigment-protein complexes of wild type and *ch5* mutant (Figure 3). The peak and trough in the red region of the *ch5* mutant A1 complex were stronger than those of wild type, but it was weaker in the blue region. The four major peaks and troughs of the AB1 complex (443, 483, 658 and 675 nm) were stronger in the *ch5* mutant than in wild type, but the intensity of all four peaks and troughs of the AB2 and AB3 complexes and the free pigment of the *ch5* mutant were weaker than those of wild type. These data indicate that the stoichiometry of the four MARS pigment-protein complexes of the *ch5* mutant and the wild type may be very different.

The similarity of the peak and trough patterns of free pigments resolved by the Thornber and MARS gel systems to those of other pigment-protein complexes suggests that the free pigment may not be truly free, i.e. the free pigment zone in both systems may contain polypeptides derived from thylakoid.

Surface Tension

Surface tension is the free energy per unit surface area or the force per unit length on the surface. Substances that lower the surface tension also lower the free energy of the surface; they will preferentially migrate to the surface and concentrate at the surface. Surface tension may thus offer useful information about the composition and orientation of the molecules and structure of biological membranes (Tinoco et al., 1978). At chlorophyll concentrations less than 70 μg ml^{-1}, the surface tension of thylakoid membrane in wild type always exceeded that in *ch5* mutant by 3–6 dyne cm^{-1}, whereas at concentrations higher than 70 μg ml^{-1}, the surface tensions of thylakoid membranes were similar (Figure 4). This indicates that the quality and quantity of constituents, especially the large molecules (such as pigment-protein complexes), and the orientation of constituents that may behave much like solutes in lipid solution are greatly different in the two thylakoid membranes. This further indicates that the thylakoid membrane of the *ch5* mutant, including the stacked grana and stroma lamellae, is more loosely arranged and less compact than that of the wild type.

Influence of Digitonin on Surface Tension

Surfactants have been widely used to study the constitution and organization of biological membranes, such as the photosynthetic membranes in higher plants (Helenius and Simons, 1975). It has been reported that sub-solubilizing concentrations of surfactants have the potential to provide much useful information about the structure and organization of the thylakoid membrane (Bartzatt et al., 1983). We used digitonin at a sub-solubilizing concentration to monitor the structural differences in thylakoid membrane of wild type and *ch5* mutant of *Arabidopsis thaliana*. The influence of various concentrations of digitonin on the surface tension of thylakoid membrane is shown in Figure 5. As the amount of digitonin added to a thylakoid membrane solution (15 μg ml^{-1} of chlorophyll) of wild type or *ch5* mutant increases, there is a decrease in the surface tension of the air/liquid interface. At concentrations below approximately 5×10^{-2}%, the surface tension of thylakoid membrane isolated from the *ch5* mutant was higher than that of wild type by approximately 5 dyne cm^{-1}, whereas at concentrations higher than 5×10^{-2}%, the surface tensions were similar. It is likely that at a low concentration, the digitonin concentrates at the surface of wild type thylakoid membrane, but can intercalate into the lipid bilayer of thylakoid membrane of the *ch5* mutant, causing higher surface tension. Figures 4 and 5 show that at low concentrations, digitonin can more easily alter the interaction between the thylakoid components and lipid bilayers in the *ch5* mutant than it can that in wild type. As

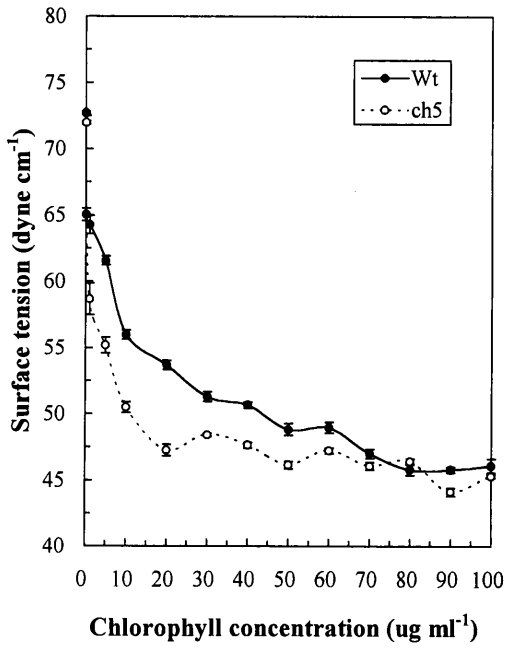

Figure 4. Surface tension at various concentrations of the thylakoid membranes isolated from wild type and *ch5* mutant of *Arabidopsis thaliana*.

the amount of digitonin increases to the critical micelle concentration, the thylakoid membranes of both wild type and *ch5* mutant are solubilized to the same degree, in spite of their original components. Since digitonin is a type B surfactant, it is probable that even at low concentrations, digitonin can intercalate into the intergrana lamellae of *ch5* mutant, but not that wild type, selectively disrupting intercomplex interaction. Further increases in digitonin produce micelles with a higher proportion of the surfactant and causes complete solubilization of the grana thylakoid of wild type and *ch5* mutant. The intramolecular interactions between pigments and proteins in grana and stromal thylakoid of wild type and the *ch5* mutant may survive incorporation into digitonin micelles, causing the surface tension of both plants to decline to a similar level.

Chlorophyll Solubilization

The perturbation of chlorophyll molecules within pigment-protein complexes is a sensitive indicator of change in the local environment of thylakoid membranes. The ability of thylakoid membrane components to remain in the supernatant fraction following centrifugation was used as the indication of intercomplex and intracomplex disruption among the pigment-protein complexes. As shown in Figures 4 and 5, digitonin can disturb the thylakoid membrane, so we investigated the influence of digitonin on chlorophyll solubilization of the *ch5* mutant and wild type (Figure 6). Chlorophyll solubilization of the *ch5* mutant begins when the digitonin concentration attains a level of 10^{-3}%, and that of wild type begins at a level of 10^{-2}%. As the concentration of digitonin increased, the release of

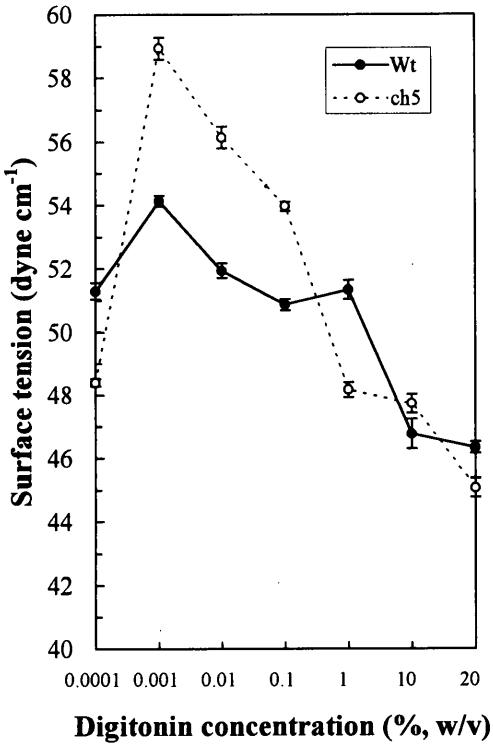

Figure 5. Influence of digitonin concentration on the surface tension of thylakoid membranes isolated from wild type and *ch5* mutant of *Arabidopsis thaliana*.

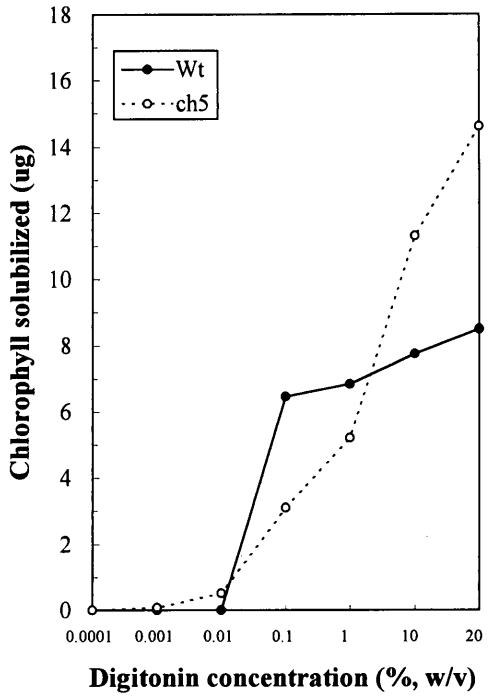

Figure 6. Patterns of the influence of digitonin concentration on solubilization of thylakoid membranes isolated from wild type and *ch5* mutant of *Arabidopsis thaliana*. Three experiments were performed, and similar patterns were observed. Amount of total chlorophyll remaining in the supernatant fraction following centrifugation is shown versus the digitonin concentration. Thylakoid membrane concentration was equivalent to 15 μg Chl ml^{-1}.

chlorophyll from thylakoid membranes increased. When the digitonin concentration was between 0.01% and approximately 3%, more chlorophyll was solubilized in the wild type than in the *ch5* mutant. In contrast, as the digitonin concentration was increased above 3%, chlorophyll was more easily solubilized in the *ch5* mutant than in the wild type. It is reasonable to presume that at digitonin concentrations below 10^{-3}%, the gross structure of thylakoid membrane is little affected. It is likely that a concentrations of digitonin less than and greater than 3%, the intercalation of the surfactant into the thylakoid membrane of wild type disturbs only the intercomplex interactions, but not the intracomplex interactions, whereas digitonin disrupts the intercomplex interactions below 3% and disrupts the intracomplex interactions above 3%. This in turn results in more chlorophyll being solubilized in the *ch5* mutant than in the wild type. The solubilization data also suggests that the intracomplex interactions in the lipid bilayer of *ch5* mutant thylakoid membrane is weaker than that in wild type, and that macromolecules, such as pigment-protein complexes, are more loosely arranged in the *ch5* mutant than in wild type.

Conclusion

Chlorophyll-deficient mutants are likely the result of decreased biochemical activity in an enzyme or component involved in biosynthesis, processing, or assembly of

the photosynthetic apparatus. In normal plants, these components are probably not rate limiting, but become so in the chlorophyll-deficient mutants because of decreased production or an altered structure (Yang et al., 1990). The mutants are more sensitive to the alteration of environmental factors such as temperature and light (Yang et al., 1993). The hypersensitivity of chlorophyll-deficient mutants to the environment may be the result of not only the deficiency in chlorophyll but also the abnormality of other components or of the thylakoid membrane structure.

In summary, we have examined the components of pigment, lipid, and protein in thylakoid membranes of *ch5* mutant of *Arabidopsis thaliana*. The change in the composition of thylakoid membranes causes the loose arrangement of thylakoid membranes in the *ch5* mutant. A forthcoming paper will discuss this aspect in more detail.

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阿拉伯芥缺葉綠素 *ch5* 突變種類囊膜的鑑定

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阿拉伯芥 *ch5* 突變種是以 EMS 誘導出的黃綠葉植物。本研究鑑定其葉綠素、類胡蘿蔔素、脂肪、蛋白質、色素蛋白複合體、類囊膜的表面張力和表面劑 digitonin 對類囊膜表面張力的影響。野生型和 *ch5* 突變種葉綠體的第一微分吸收光譜和以 Thornber 及 MARS 方法分離的色素蛋白複合體的第一微分吸收光譜顯示野生型和 *ch5* 突變種類囊膜的成份和組成極爲不同。野生型和 *ch5* 突變種的 MGDG/DGDG 比例分別是 1.98 和 1.03。當葉綠素濃度低於 70 μg ml^{-1} 時，野生型類囊膜的表面張力比 *ch5* 突變種高約 3-6 dyne cm^{-1}。當 digitonin 濃度低於 3% 時，野生型類囊膜的葉綠素比 *ch5* 突變種較易溶出；而當 digitonin濃度高於 3% 時則相反。資料顯示，*ch5* 突變種的類囊膜較野生型的更鬆垮和不緊密。

關鍵詞：阿拉伯芥；缺葉綠素突變種；空腔；間隙；表面張力；類囊膜。