

# Influence of Fe- and Mg-deficiency on the thylakoid membranes of a chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana*

Yih-Kuang Lu<sup>1</sup>, Yung-Reui Chen<sup>1</sup>, and Chi-Ming Yang<sup>2,3</sup>

<sup>1</sup> Department of Botany, National Taiwan University, Taipei, Taiwan 106, Republic of China

<sup>2</sup> Institute of Botany, Academia Sinica, Nankang, Taipei, Taiwan 115, Republic of China

(Received January 7, 1995; Accepted May 13, 1995)

**Abstract:** We characterized the thylakoid membranes of a chlorophyll-deficient *ch5* mutant and a wild type of *Arabidopsis thaliana* grown in Murashige and Skoog medium with and without Fe or Mg by their composition and surface tension, and by the influence of surfactant on the thylakoid membranes. Deficiency in Fe and Mg decreases the amount of chlorophyll and protein in the *ch5* mutant and wild type, but dramatically increases the amount of carotenoid. The pattern of the first-derivative absorption spectra of chloroplast revealed that the composition and organization of thylakoid membranes were very different in the two biotypes. In both biotypes, the surface tension of Fe- or Mg-deficient thylakoid membranes was greater than that in plants grown in normal media, indicating that the thylakoid membranes of Fe- or Mg-deficient plants are more easily accessed by surfactants than those of normally-grown plants. In both biotypes, chlorophyll was more easily solubilized from the thylakoid membranes of Mg-deficient plants than that of Fe-deficient plants, which was more easily solubilized than normally grown plants. We concluded that Fe- or Mg-deficiency results in a looser organization of thylakoid membranes than in the controls, and that Mg-deficiency results in a more disorganized thylakoid membrane than does Fe-deficiency. Results suggest that Fe- and Mg-deficient thylakoids contain smaller amounts of the light-harvesting complexes associated with photosystem II (LHCII) and an abnormal ratio of monogalactosyl diglyceride to digalactosyl diglyceride (MGDG/DGDG).

**Keywords:** Chlorophyll-deficiency; Fe-deficiency; Light-harvesting complex II; Mg-deficiency; Surface tension; Surfactant; Thylakoid membranes.

## Introduction

Iron (Fe) is intimately involved in oxidation-reduction reactions in plants. Magnesium (Mg) is an integral component of the chlorophyll molecule and is a divalent cation cofactor for many enzymatic reactions (Evans and Sorger, 1966). Fe-deficiency causes changes in the structure and composition of thylakoid membranes (Terry and Abadia, 1986; Abadia, 1992), such as a drastic reduction in the amount of thylakoid membrane that has few grana stacks, a general decrease of chlorophyll and carotenoid contents (Stocking, 1975; Pushnik and Miller, 1982; Pushnik et al., 1984; Abadia et al., 1988; Abadia et al., 1989; Guller and Krucka, 1993), a decrease of the ratio of monogalactosyl diglyceride to digalactosyl diglyceride (MGDG/DGDG) (Nishio et al., 1985b), and a depletion of thylakoid membrane proteins—especially pigment-protein complexes (Nishio et al., 1985a). Plants suffering from Mg-deficiency suffer pronounced changes in the chloroplasts. Photosynthesis, net assimilation, and transpiration rates are decreased under Mg-deficiency (Terry and Ulrich, 1974; Cao and Tibbits, 1992).

The chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana* was originally induced by ethylmethane-

sulphonate (Koornneef et al., 1983). In a previous study, we examined the composition and biophysical characteristics of thylakoid membranes and concluded that the thylakoid membranes of *ch5* mutant were less compact than those of the wild type (Lu et al., 1995a). In a further study, we presented evidence to show that the drastic reduction of light harvesting complex I and II and of MGDG/DGDG ratio affected the grana stacking and swelling (Lu et al., unpublished). This chlorophyll-deficient mutant is able to operate the xanthophyll cycle to respond to the alteration of light intensity, but within a narrower range than does the wild type (Lu et al., 1995b). The aim of our research is to investigate the influence of Fe- and Mg-deficiency on the structure and organization of thylakoid membranes of the *ch5* mutant and wild type.

## Materials and Methods

Seeds were germinated and grown in Murashige and Skoog (1962) medium for 4 weeks in a growth chamber providing a photoperiod of 12 h L/12 h D, a photoflux of 250–300  $\mu\text{mol m}^{-2} \text{S}^{-1}$ , a relative humidity of 60%, and a temperature of 25°C. Leaves were harvested and the chloroplast and thylakoid membranes isolated as previously described (Markwell, 1986). The concentrations of chlorophyll and carotenoid were determined according to the methods of Arnon (1949) and Jaspers (1965), respectively,

<sup>3</sup> Corresponding author.

following extraction of liquid-nitrogen-frozen leaf with 80% acetone. Protein concentration was determined using the Bio-Rad protein assay of Bradford (1976). Absorbance and the first derivative spectra were obtained with a Hitachi U3200 UV-visible spectrophotometer.

To study the interaction of surfactants with the 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 membrane containing  $15 \mu\text{g ml}^{-1}$  of chlorophyll, both being twice the desired final concentration (Bartzatt et al., 1983). The mixture was incubated at  $25^\circ\text{C}$  for 10 min prior to the determination of surface tension with a Kyowa face automatic surface tensiometer model CBVP-A3. After measuring the surface tension, the mixture was centrifuged at  $5,000 g$  for 10 min at room temperature. The chlorophyll concentration released in the supernatant fraction was determined as described above.

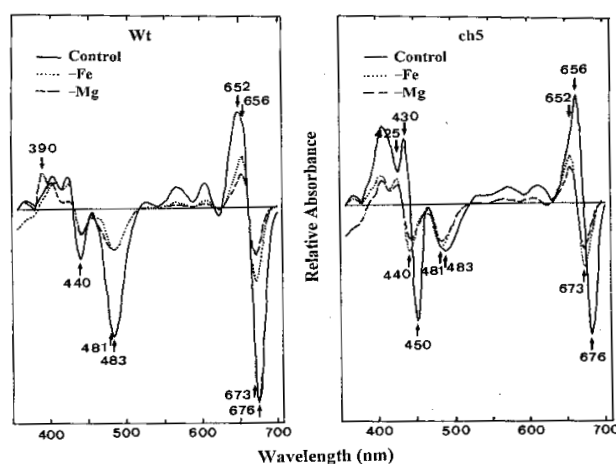
## Results and Discussion

### Chloroplasts

The first-derivative of the absorption spectrum can reveal details that are not seen in a straightforward absorption spectrum (Figure 1). In both biotypes, the intensity of all peaks and troughs in the first-derivative absorption spectrum of Fe- and Mg-deficient chloroplasts was much lower than that associated with normally-grown plants. That of Mg-deficient chloroplast was weaker than that of Fe-deficient chloroplast. The peak at 652 nm and the trough at 676 nm found in normally-grown plants were shifted to 656 and 673 nm, respectively, in Fe- and Mg-deficient wild type. In the *ch5* mutant, the peak at 656 nm and the trough at 676 nm were shifted to 652 and 673 nm, respectively. The peak ratio of 390/430 nm and trough ratio of 440/483 nm in the wild type were different than those in the *ch5* mutant, but the blue-region peak and trough patterns of Fe- and Mg-deficient plants were similar to those of normally-grown plants. These data strongly suggest that in both biotypes the thylakoid membrane components of Fe- and Mg-deficient plants are different than those in the control plants.

### Influence of Fe- or Mg-Deficiency on Thylakoid Components

We determined the concentrations of chlorophyll, carotenoid, and protein in thylakoid membranes of Fe- and Mg-deficient *ch5* mutant and wild type (Table 1). Fe- and Mg-deficiency resulted in a decrease in chlorophyll and protein and an increase in carotenoid in both biotypes. Our results conflict with reports that chlorophyll and carotenoid are simultaneously decreased by Fe- and Mg-deficiency, and that the decrease of carotenoid is less than that of chlorophyll (Terry and Abadia, 1986; Abadia, 1992). They also conflict with a report that a deficiency in carotenoids may cause a decrease in chlorophyll content (Mayfield and Taylor, 1984). The chlorophyll content of the *ch5* mutant is 85% of that of wild type, whereas the carotenoid content of the *ch5* mutant is approximately 4.3-fold greater than that of wild type. It is obvious that Fe- and Mg-deficiency cause a higher ratio of carotenoid to chlorophyll. In both biotypes, Mg-deficiency seems more



**Figure 1.** The first-derivative absorption spectra of chloroplasts isolated from wild type and *ch5* mutant of *Arabidopsis thaliana* grown in Murashige and Skoog solid medium in the absence and presence of Fe or Mg.

**Table 1.** Effect of Fe- or Mg-deficiency in Murashige and Skoog medium on the components of thylakoid membrane isolated from wild type and *ch5* mutant of *Arabidopsis thaliana*. The results are the mean of three determinations. Numbers in parenthesis present percent.

Strain	Treatment	Total Chl ( $\mu\text{g g}^{-1}$ leaf)	Carotenoids ( $\mu\text{g g}^{-1}$ leaf)	Protein ( $\text{mg g}^{-1}$ leaf)	Carotenoid/ Chl ratio (%)
Wt	Control	454 $\pm$ 21 (100)	15.5 $\pm$ 1.1 (100)	16.3 $\pm$ 1.2 (100)	100
	-Fe	441 $\pm$ 31 (97)	70.9 $\pm$ 4.9 (457)	14.9 $\pm$ 1.8 (91)	474
	-Mg	317 $\pm$ 26 (70)	63.6 $\pm$ 6.2 (410)	8.2 $\pm$ 1.9 (50)	591
<i>ch5</i>	Control	387 $\pm$ 18 (100)	67.3 $\pm$ 3.1 (100)	18.3 $\pm$ 1.4 (100)	100
	-Fe	317 $\pm$ 24 (82)	80.6 $\pm$ 5.3 (120)	11.1 $\pm$ 1.3 (61)	154
	-Mg	172 $\pm$ 13 (44)	95.3 $\pm$ 7.6 (142)	10.0 $\pm$ 1.4 (55)	336

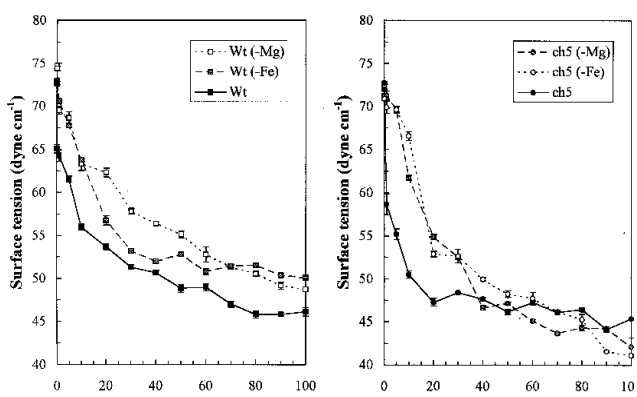
effective than Fe-deficiency in decreasing chlorophyll and protein levels, but the ratio of carotenoid to chlorophyll in Mg-deficient plant is higher than that in Fe-deficient plant. The above data suggest that in both biotypes, Mg-deficiency causes more changes in the composition and organization of thylakoid membranes than does Fe-deficiency, which indicates that the deficiency in chlorophyll is not directly accompanied by a loss of carotenoids.

### Influence of Fe- or Mg-Deficiency on Surface Tension

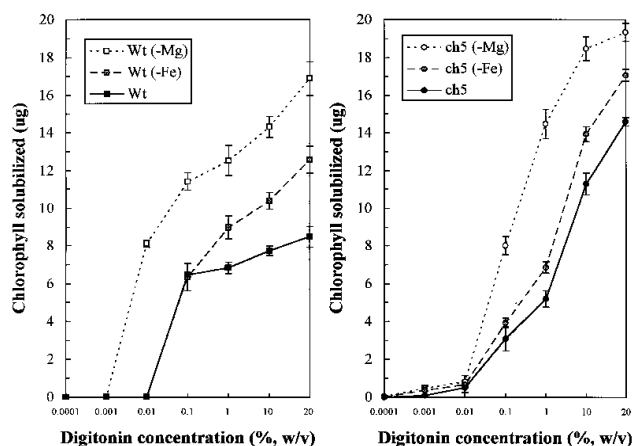
Surface tension was used to monitor the composition and orientation of the molecules and the structure of biological membranes (Tinoco et al., 1978). The surface tension of thylakoid membranes at various concentrations was measured (Figure 2). Deficiencies of Fe and Mg caused higher surface tension than in the control at all concentrations of thylakoid membranes in wild type and at concentrations less than  $50 \mu\text{g ml}^{-1}$  in the *ch5* mutant. This indicates that the quality and quantity of constituents of thylakoid membranes, especially the large molecules such as pigment-protein complexes, and the orientation of constituents differ greatly between Fe- and Mg-deficient and control plants in both biotypes. This further indicates that the thylakoid membranes of Fe- and Mg-deficient *ch5* mutant are more accessible to surfactants than are those of the wild type.

### Influence of Fe- or Mg-Deficiency on Chlorophyll Solubilization

Digitonin was used to study the constituents and organization of thylakoid membranes in the *ch5* mutant and wild type (Lu et al., 1995a). The influence of various digitonin concentrations on the solubilization of Fe- and Mg-deficient thylakoid membranes is shown in Figure 3. In the wild type, Mg-deficient thylakoids were solubilized when digitonin concentration was  $10^{-3}\%$ , whereas Fe-deficient and control thylakoids were solubilized at a digi-



**Figure 2.** Surface tension of various concentrations of the thylakoid membrane isolated from wild type and *ch5* mutant of *Arabidopsis thaliana* grown in Murashige and Skoog solid medium in the absence and presence of Fe or Mg.



**Figure 3.** Influence of digitonin concentration on the solubilization of the thylakoid membrane isolated from wild type and *ch5* mutant of *Arabidopsis thaliana*. Amount of total chlorophyll remaining in the supernatant fraction following centrifugation is shown versus digitonin concentration. Thylakoid membrane concentration was  $15 \mu\text{g ml}^{-1}$ .

tonin concentration of  $10^{-2}\%$ . Fe-deficient thylakoid is more easily solubilized than the control at digitonin concentrations greater than  $10^{-1}\%$ . In the *ch5* mutant, Fe- and Mg-deficient thylakoids were slightly solubilized at a concentration of  $10^{-4}\%$ , whereas the control thylakoids were solubilized at a concentration of  $10^{-3}\%$ . All kinds of thylakoids, however, were greatly solubilized when digitonin concentration was greater than  $10^{-2}\%$ . In both biotypes, Mg-deficient thylakoids are more easily solubilized than Fe-deficient thylakoids, which are more easily solubilized than the controls. The solubilization data suggest that Fe- and Mg-deficient thylakoids are more loosely arranged than the controls in both biotypes.

In the present investigation, we examined certain biochemical and biophysical characteristics of Fe- and Mg-deficient thylakoid membranes isolated from a chlorophyll-deficient mutant. Our results show that: a) the absorbance in the first derivative spectra of Fe- and Mg-deficient chloroplasts is lower than that of the control; b) a deficiency in chlorophyll is not directly accompanied by the loss of carotenoids; and c) Fe- and Mg-deficient thylakoids are more accessible to surfactants.

In conclusion, we found that the Fe- and Mg-deficient thylakoids are more disorganized or swollen than the controls, suggesting that mineral deficiency may result in the presence of only very small amounts of LHCII, which is assumed to regulate the grana stacking (Bennett, 1991; Allen, 1992), and that MGDG and DGDG and the ratio of MGDG to DGDG, which was proposed to affect the grana stacking and lumen swelling (Lu et al., unpublished), may greatly change.

**Acknowledgements.** This research was supported by National Science Council of the Republic of China grants NSC81-0211-B-002-04 (to Y.R.C.) and NSC82-0211-B-001-051 (to C.M.Y.).

## Literature Cited

- Abadia, A., F. Ambard-Bretteville, R. Remy, and A. Tremolieres. 1988. Iron-deficiency in pea leaves: effect of lipid composition and synthesis. *Physiol. Plant.* **72**: 713–717.
- Abadia, A., Y. Lemoine, A. Tremolieres, F. Ambard-Bretteville, and R. Remy. 1989. Iron-deficiency in pea: effects on pigment, lipid and pigment-protein complex composition of thylakoids. *Plant Physiol. Biochem.* **27**: 679–687.
- Abadia, J. 1992. Leaf responses to Fe deficiency: a review. *J. Plant Nutri.* **15**: 1699–1713.
- Allen, J. F. 1992. Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta* **1098**: 275–335.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24**: 1–15.
- Bartzatt, R.A., C. M. Yang, and J. P. Markwell. 1983. The interaction of surfactants with the chloroplast thylakoid membrane at sub-solubilizing concentrations. *Biochim. Biophys. Acta* **725**: 341–348.
- Bennett, J. 1991. Protein phosphorylation in green plant chloroplasts. *Annu. Rev. Plant. Physiol.* **42**: 281–311.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254.
- Cao, W. and T. W. Tibbits. 1992. Growth, carbon dioxide exchange and mineral accumulation in potatoes grown at different magnesium concentrations. *J. Plant Nutri.* **15**: 1359–1371.
- Evans, H. J. and G. J. Sorger. 1966. Role of mineral elements with emphasis on the univalent cations. *Annu. Rev. Plant Physiol.* **17**: 47–76.
- Guller, L. and M. Krucka. 1993. Ultrastructure of grape-vine (*Vitis vinifera*) chloroplasts under Mg- and Fe-deficiency. *Photosynthetica* **29**: 417–425.
- Jaspers, E. M. W. 1965. Pigmentation of tobacco crown-gall tissues cultured in vitro in dependence of the composition of the medium. *Physiol. Plant.* **18**: 933–940.
- Koornneef, J. van E., C. J. Hanhart, P. Stam, F. J. Braaksma, and W. J. Feenstra. 1983. Linkage map of *Arabidopsis thaliana*. *J. Hered.* **74**: 265–272.
- Lu, Y.K., C. M. Yang, and Y. R. Chen. 1995a. Characterization of the thylakoid membrane in a chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana*. *Bot. Bull. Acad. Sin.* **36**: 33–40.
- Lu, Y.K., C. M. Yang, and Y. R. Chen. 1995b. Response of the xanthophyll cycle to changes in low irradiance in a chlorophyll-deficient *ch5* mutant of *Arabidopsis thaliana*. *Photosynthetica* **31**: (in press).
- Markwell, J. P. 1986. Electrophoretic analysis of photosynthetic pigment-protein complexes. In M.F. Hipkins and N.R. Baker (eds.), *Photosynthesis Energy Transduction: a practical approach*. IRL press, Oxford, England, pp. 27–49.
- Mayfield, S. P. and W. C. Taylor. 1984. Carotenoid-deficient maize seedlings fail to accumulate light-harvesting chlorophyll a/b binding protein (LHCP) mRNA. *Eur. J. Biochem.* **144**: 79–84.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15**: 473–497.
- Nishio, J. N., S. E. Taylor, and N. Terry. 1985a. Changes in thylakoid galactolipids and proteins during iron nutrition-mediated chloroplast development. *Plant Physiol.* **77**: 705–711.
- Nishio, J. N., J. Abadia, and N. Terry. 1985b. Chlorophyll-proteins and electron transport during iron nutrition-mediated chloroplast development. *Plant Physiol.* **78**: 296–299.
- Pushnik, J. C. and G. W. Miller. 1982. The effects of iron and light treatments on chloroplast composition and ultrastructure in iron-deficient barley leaves. *J. Plant Nutri.* **1**: 311–322.
- Pushnik, J. C., G. W. Miller, and J. H. Manwaring. 1984. The role of iron in higher plant chlorophyll biosynthesis, maintenance and chloroplast biogenesis. *J. Plant Nutri.* **7**: 733–757.
- Stocking, C. R. 1975. Iron deficiency and the structure and physiology of maize chloroplasts. *Plant Physiol.* **55**: 626–631.
- Terry, N. and A. Ulrich. 1974. Effects of magnesium deficiency on the photosynthesis and respiration of leaves of sugar beet. *Plant Physiol.* **54**: 379–381.
- Terry, N. and J. Abadia. 1986. Function of iron in chloroplast. *J. Plant Nutri.* **9**: 609–646.
- Tinoco, I., K. Sauer, and J. C. Wang. 1978. *Physical Chemistry: Principles and Applications in Biological Sciences*. Prentice-Hall, Inc., Englewood Cliffs, N.J., USA.

## 缺鐵或缺鎂對阿拉伯芥缺葉綠素 *ch5* 突變種類囊膜的影響

盧義光<sup>1</sup> 陳榮銳<sup>1</sup> 楊棋明<sup>2</sup>

<sup>1</sup>國立台灣大學植物學系

<sup>2</sup>中央研究院植物研究所

本研究探討阿拉伯芥正常種和缺葉綠素 *ch5* 突變種於缺鐵或缺鎂時其類囊膜的組成和表面張力，及表面劑對類囊膜的影響。缺鐵或缺鎂導致正常種和突變種葉綠素和蛋白質量的減少，但促成類胡蘿蔔素含量的增加。葉綠體的第一微分光譜顯示正常種和突變種的類囊膜之組成和構造差異很大。不論正常種或突變種，缺鐵或缺鎂導致類囊膜的表面張力較正常生長者大，顯示缺鐵或缺鎂植物的類囊膜比正常生長者更容易被表面劑接近其表面。缺鎂植物的葉綠素比缺鐵植物更易自類囊膜上溶出，後者的葉綠素則比正常植物更易溶出。故而，缺鐵或缺鎂導致類囊膜較正常者鬆散，而且缺鎂比缺鐵更易導致類囊膜的鬆散。結果顯示缺鐵或缺鎂植物的類囊膜只含少量的捕光複合體 II 和不正常的 MGDG/DGDG 比例。

**關鍵詞：**缺葉綠素；缺鐵；缺鎂；捕光複合體 II；表面劑；表面張力；類囊膜。