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Grana stacking is normal in a chlorophyll-deficient LT8 mutant of rice

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Abstract. Thylakoid proteins and chloroplasts isolated from chlorophyll-deficient rice mutant LT8 and its Norin 8 parent type (*Oryza sativa*) were compared using SDS-PAGE and electron microscopy. The LT8 mutant contained approximately 37% of the amount of chlorophyll found in the Norin 8; the ratio of chlorophyll a/b for LT8 and Norin 8 were 6.4 and 3.2, respectively. SDS-PAGE profiles showed that LT8 mutant contained approximately 10% of the amount of LHCII polypeptide found in the Norin 8, although both biotypes produced similar levels of LHCI and other thylakoid proteins. Electron microscopy showed that the grana stacks were similar in the LT8 mutant and Norin 8, suggesting that LHCII is not the only factor regulating the grana stacking of thylakoid membrane.

Keywords: Chlorophyll-deficient mutant; Grana stacking; LHCII; *Oryza sativa*.

Abbreviations: Chl, chlorophyll; **CF**, coupling factor; **Cyf**, cytochrome f; **PSI**, photosystem I; **PSII**, photosystem II; **LHCI** and **LHCII**, light-harvesting complexes associated with PSI and PSII, respectively; **SDS-PAGE**, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Introduction

Grana stacks are predominant ultrastructural characteristics of thylakoid membranes in higher plants and green algae. It has been postulated that grana stacking is mediated by the surface charge density of thylakoid membrane (Barber, 1982), which is regulated by the phosphorylation and dephosphorylation of LHCII apoproteins in the thylakoid membrane (Staehelin and Arntzen, 1983; Murphy, 1986; Bennett, 1991; Allen, 1992ab). If this is the case, the chloroplast mutants that are Chl b-deficient and contain little or no LHCII complex should exhibit poorly-developed or no grana stacks in the thylakoid—as in etiolated chloroplasts. A Chl b-lacking *ch5* mutant (U374) of sweetclover, however, exhibits normal grana to an extent similar to its wild type, indicating that components other than LHCII are involved in grana stacking (Nakatani and Baliga, 1985). It has been suggested that the intraplastidial lamellar system in the plastids of a Chl b-lacking mutant in barley reorganized to form macrograna and optimize energy distribution between PSI and PSII (Ouijja et al., 1988).

In this report, we show that LT8 mutant, although containing little LHCII apoprotein, developed a normal grana stacking in the thylakoid membranes.

Materials and Methods

Seeds of rice mutant LT8 and its Norin 8 parental type were germinated and grown in a greenhouse for 5 weeks in a soil-vermiculite mixture under natural light. Thylakoid membranes were obtained as previously described (Markwell, 1986). The concentrations of Chl and protein were determined using the method of Porra et al. (1989) and the Bio-Rad protein assay (Bradford, 1976), respectively. SDS-PAGE fractionation was performed with 12% acrylamide, using the method of Laemmli (1970).

For transmission electron microscopy, the central part of all leaves of both biotypes was collected and cut into small cubes (approx. 1 mm) in fixation buffer containing 2.5% glutaraldehyde. After incubation at 4°C for 2 h in 0.1 M cacodylate buffer (pH 7.0) containing 2.5% glutaraldehyde, the leaf samples were washed three times in plain buffer, postfixed in 1% osmium tetraoxide for 2 h, dehydrated through an ethanol series, infiltrated and embedded in Spurr's resin (Spurr, 1969), and then polymerized at 70°C for 8 h. Gold sections were collected by ultramicrotomy and stained with ethanol uranyl acetate and lead citrate. The thylakoid morphology was examined with a Hitachi H-600 transmission electron microscope at 75 kV.

Results and Discussion

SDS-PAGE Profiles

In normal green plants and green algae, about 50% of all Chl molecules are distributed in LHCII, which has a lower Chl a/b ratio than does LHCI (Murphy, 1986). Five-week-old LT8 mutant contained approximately 37% of the amount of Chl found in its parent type, Norin 8. Chl a/b ratios for LT8 and Norin 8 were 6.4 and 3.2, respectively

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Table 1. The chlorophyll content and a/b ratio in LT8 mutant and its Norin 8 parent type.

Strain	Chl (μg g^{-1} fresh leaf)	Chl a/b ratio
Norin 8	2730 ± 118	3.2 ± 0.2
LT8	1011 ± 69	6.4 ± 0.3

(Table 1). Given the low Chl content and the high Chl a/b ratio, if Chl levels were decreased equally in each Chl-protein complex, LT8 mutant would contain less LHCI and LHCII polypeptides. SDS-PAGE analysis of thylakoid polypeptides isolated from leaves indicated that the polypeptide composition was similar in both biotypes, but the quantity of LHCII polypeptides in LT8 mutant was about 10% of that found in Norin 8 (Figure 1). LHCI and other proteins were stoichiometrically similar in the two biotypes. If LHCII regulates the grana stacking of thylakoid membrane in higher plants (Staehelin and Arntzen, 1983; Murphy, 1986; Allen, 1992a and 1992b), the reduction of apoproteins associated with LHCII complex in LT8 mutant rice should result in a poor development of thylakoid morphology. Electron microscopy, however, showed that this was not the case.

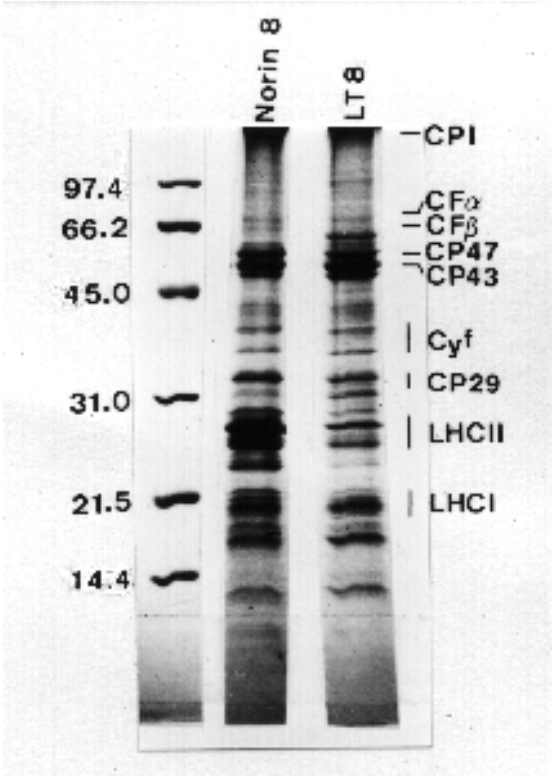

Figure 1. SDS-PAGE profile of thylakoid polypeptides isolated from LT8 mutant and Norin 8 parental type. All leaves in same plant were sampled. Equal amount of chlorophyll was applied into each well.

Transmission Electron Microscopy

Ultrastructural studies showed that the chloroplasts of both biotypes possessed normal stacked grana (Figure 2). Though the number of grana per chloroplast in both biotypes was varied, the number of paired thylakoid membranes per granum of LT8 mutant was less than that per granum of Norin 8 (i.e. the grana of LT8 mutant was thin-

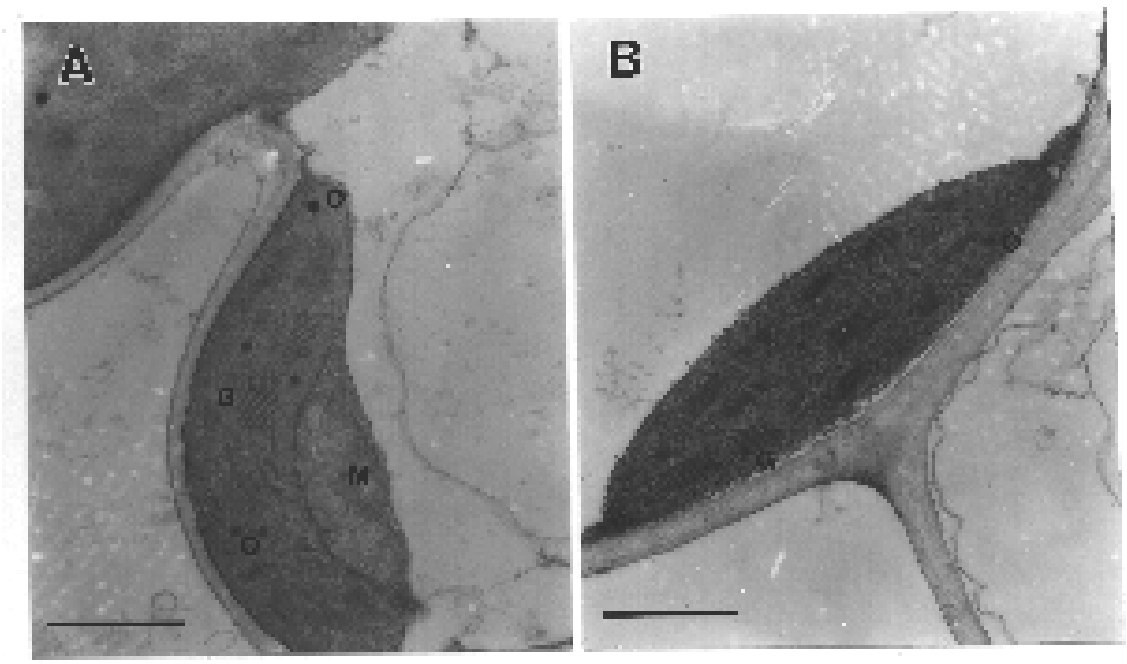

Figure 2. Ultrastructural morphology of chloroplasts from the the mesophyll cells of Norin 8 parental type (A) and LT8 mutant (B). Notes: G, grana; M, mitochondria; O, oil droplet. All bars are in μm.

ner than that of Norin 8). The LT8 mutant appeared to contain many fewer oil droplets in each chloroplast than did the Norin 8 biotype. Although this observation differs from the results reported by Goodchild et al. (1966), it agrees with the results reported by Nakatani and Baliga (1985) on the Chl b-lacking *ch5* mutant of sweetclover and by Ouijja et al. (1988) in their study of a Chl b-lacking mutant of barley. The sweetclover mutant contained more oil droplets than did the wild type (Nakatani and Baliga, 1985), and its membrane pairs showed slightly greater separation.

Taken together, the present study and the literature (Nakatani and Baliga, 1985; Ouijja et al., 1988) strongly suggest that LHCII is not the only factor involved in grana stacking in higher-plant chloroplasts. The role of LHCII in the regulation of grana stacking in higher plants should be reevaluated carefully.

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水稻缺葉綠素突變種 LT8 類囊膜是正常的摺疊

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本文以 SDS-PAGE 和電子顯微鏡比較水稻缺葉綠素 LT8 突變種及其正常品系農林 8 號的類囊膜蛋白及葉綠體之異同。 LT8 突變種的葉綠素含量只有農林 8 號的 37% ；兩者之葉綠素 a/b 比分別是 6.4 和 3.2 。 SDS-PAGE 顯示， LT8 突變種的 LHC II 含量只有農林 8 號的 10% ，但其它類囊膜蛋白及 LHC I 的含量則相似。電子顯微鏡顯示 LT8 突變種及其正常種農林 8 號的葉綠餅摺疊非常相似，顯示 LHC II 可能不是類囊膜上葉綠餅摺疊機制的唯一因子。

關鍵詞：缺葉綠素突變種；葉綠餅摺疊； LHC II ；水稻。