Grana stacking is normal in two insect-induced cecidomyiid galls deficient in light-harvesting complex II (LHCII)

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Abstract. It has been postulated that grana stacking is mediated by the change of surface charge density of thylakoid membrane, regulated by LHCII phosphorylation and dephosphorylation. The insect-induced cecidomyiid galls lack pigment-protein complex CPI of PSI, and are totally deficient in pigment-protein complexes A1, AB1 and AB2 of PSII. Although the two galls, transformed from Machilus thunbergii Sieb & Zucc. (Lauraceae) leaf by insect, are deficient in LHCII complex, they still develop normal grana stacking. The data suggest that LHCII may be not the only factor regulating the grana stacking of thylakoid membranes.

Keywords: Grana stacking; Gall; LHCII

1. Introduction

Chloroplast thylakoids are morphologically and structurally differentiated into the appressed regions of grana, the nonappressed regions of grana end membranes and margins, and the nonappressed stroma lamellae [1]. The lateral segregation and grana stacking of thylakoid membranes in higher plants are thought to be an adaptive strategy by which green plants maximize their photosynthetic efficiency and light-harvesting capacity. Evolution has resulted in about 85% of photosystem II (PSII) being located in grana, and 90% of photosystem I (PSI) in grana end membranes and margins, and stroma lamellae. The pigments in either PSI or PSII complexes are distributed in core complex (CC) and light harvesting complex (LHC). Most of chlorophyll b (Chl b) molecules are distributed in PSII, especially in LHCII [2].

Surface charge density of the thylakoid membrane and regulation of phosphorylation-dephosphorylation cycle of LHCII apoproteins, were postulated to depict the mechanism of grana stacking [3-5]. If this is the case, the chloroplasts that are Chl b-deficient and contain little or no LHCII complex should exhibit poorly
developed grana. However, as yet the hypothesis did not explain three cases. A Chl b-lacking \textit{ch5} mutant of sweetclover exhibit normal grana to an extent similar to its wild type [6]. The lamellar system in the plastids of a barley Chl b-lacking mutant still forms macrogana [7]. A Chl-deficient LT8 mutant of rice develops normal stacked grana [8]. The three data suggest that LHCII may be not the only factor regulating the grana stacking of thylakoid membrane.

Galls are recognized as a variety of plant structure and growth form. More than 70\% of galls occur on plant leaves [9,10]. Little work has been done on the gall chloroplasts [11,12]. In this report, we show that two insect-induced cecidomyiid galls, even deficient in LHCII complexes, develop normal grana stacking in the thylakoid membranes.

2. Results and discussion

2.1. Pigment-protein Complexes

The Thornber electrophoretic system show that the infected leaf of \textit{M. thunbergii} contains both the pigment-protein complexes CPI and CPII, commonly found in all higher plant leaves, whereas the two insect-induced galls contain only CPII (Fig. 2A). The MARS system show that the two galls are totally deficient in pigment-protein complexes A1, AB1, and AB2 (Fig. 2A). The pigment-protein complex pattern was further confirmed by western blotting of antibody against LHCII apoprotein (Fig 2B). CPII and its oligomeric form, AB1, AB2, and AB3 possess LHCII apoprotein. The insect-induced gall contains only CPII and AB3, resulting in less amount of LHCIIb apoprotein. The two cecidomyiid galls are deficient in LHCII of PSII and lose pigment-protein complexes CPI and A1 of PSI, suggesting that the insect must manipulate the gene expression of photosynthetic pigment-protein complexes via an unknown mechanism.

The pigment-protein complexes pattern of the insect-induced gall is the same as that of the mungbean testa, and neither of them is found in the normal chloroplast [13]. This deficiency of pigment-protein complexes in the insect-induced gall derived from the infected \textit{M. thunbergii} leaf and in the non-leaf green tissue of mungbean testa is an interesting coincidence.

Many Chl-deficient mutants found in higher plants also possess abnormal grana and thylakoid morphology, except for three cases [8]. Insect-induced galls are transformed from insect infected leaves and they contain abnormal pigment-protein complex compositions of PSI and PSII. The pigment-protein complexes of galls are not remnant components during gall formation because of their absence during the galls’ lifetime (data not shown).

It is widely accepted that the LHCII complex regulates the formation of grana in the thylakoid membrane; that is, no normal grana are formed if no or little LHCII complex is assembled in the chloroplast [4,5]. Therefore, the reduction of LHCII apoproteins in the insect-induced cecidomyiid galls should result in a poor development of ultrastructural thylakoid morphology. The following electron microscopy, however, show that this is not the case.

2.2. Transmission Electron Microscopy
Ultrastructural studies show that the chloroplasts of the two insect-induced cecidomyiid galls possess stacked grana as normal as their host leaves (Fig. 3). The number of grana per chloroplast and the number of paired thylakoid membranes per granum are similar between the galls and their host leaf. However, the two cecidomyiis galls appear to contain fewer starch in each chloroplast than the host leaf. This is similar to the report by Rey [11] that the chloroplasts in the gall of *Pontania proxima* Lep-infected willow leaf never contain starch, but a bundle of tubules appear in their stroma, which is very often isolated in a stretched lobe. The data agrees with the results reported by Nakatani and Baliga [6] on the Chl b-lacking *ch5* mutant of sweetclover, by Ouijja et al [7] in their study of a Chl b-lacking mutant of barley, and by Yang and Chen [8] on the Chl b-deficient LT8 mutant of rice.

All chlorophyll-deficient mutants reveal reduction in chlorophyll content, higher ratio of chlorophyll a/b, immature ultrastructure of thylakoid membrane, marked changes in pigment-protein complexes, and general sensitivity to temperature, light intensity, and photoperiod [14]. Insect-induced galls in this study or mungbean testa [13] may be recognized as a kind of chlorophyll-deficient mutant of leaf or a non-leaf green tissue with abnormal morphology. The incomplete organization of PSI and PSII may affect the gall photosynthetic function of light-harvesting, energy transfer and photochemical energy conversion performed in pigment-protein complexes. However, while the chlorophyll a/b ratios of the insect-induced gall and mungbean testa are below the average, between 2.5 and 3.0, of leaf, those of chlorophyll-deficient mutants are higher than 4.0 [14].

The deficiency of LHCII pigment-protein complexes such as AB1 and AB2 does not affect the grana stacking in the chloroplasts of two insect-induced cecidomyiid galls. Therefore, factors other than LHCII may be involved in the grana stacking.

3. Conclusion

Taken together, the present study and the literature [6-8] strongly suggest that LHCII complex is not the only factor involved in grana stacking in the chloroplasts of higher plants. The role of LHCII in the regulation of grana stacking in higher plants should be further evaluated carefully. However, it is still unknown that (1) how popular the deficiency phenomena of pigment-protein complexes happen in other insect galls; (2) how the galling insects induce the lacking of some pigment-protein complexes; and (3) the physiological roles from this deficiency.

4. Methods

4.1. Plant and gall

The mature green obovate and red oval-pointed cecidomyiid galls, residing on the lower epidermis of *Machilus thunbergii* Sieb & Zucc. (Lauraceae) leaf, were collected from the Chung-Cheng Moutain of the Yang-Ming Shan National Park, in northern Taiwan. They show much different color and morphology (Figure 1). Galls were detached from the infected leaves. Surrounding healthy leaf tissues were trimmed to avoid contamination. Only the data of red oval-pointed cecidomyiid gall are presented here, because all data of both galls are the same.

4.2. Pigment-protein complexes
Thylakoid membranes isolated from both leaf and detached oval-pointed galls were analyzed for constituent pigment-protein complexes by solubilization with SDS and electrophoresis on Thornber and MARS fractionation systems [15]. The fractionated pigment-protein complexes in the Thornber and MARS gels were directly transblotted onto nitrocellulose paper. The immunoblot was incubated with antibody against LHCIIb, and visualized by means of a goat anti-mouse IgG conjugated with alkaline phosphatase and the NBT-BCIP chromogenic detection system as described by Leary et al [16].

4.3. Electron microscopy

The inner part of gall and central part of leaf were collected and cut into small cubes 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 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 [17], and then polymerized at 70°C for 8 h. Gold sections were collected and stained with ethanol uranyl acetate and lead citrate. The thylakoid morphology was examined with a Philips CM 100 transmission electron microscope at 75kV.

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References

Figure legends

Figure 1. Morphology and color of two insect-induced cecidomyiid galls residing on the lower epidermis of *M. thunbergii* leaf. One is green obovate gall and the other red oval-pointed gall.
Figure 2. Pigment-protein complexes of thylakoid membrane isolated from the infected leaf of *M. thunbergii* and the oval-pointed cecidomyiid gall. (A) Pigment-protein complexes are fractionated by Thornber and MARS electrophoretic systems. An oligomeric form of CPII complex is visible migrating between CPI and CPII. (B) Immunoblotting of antibody against LHCIIb apoprotein in the pigment-protein complexes fractionated by Thornber and MARS electrophoretic systems.
Figure 3. Ultrastructural morphology of thylakoid membranes in the chloroplasts from the infected leaf of *M. thunbergii* and the oval-pointed cecidomyiid gall. A, leaf; B and C, oval-pointed gall.