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Light delays the degradation of pigment-protein complexes in imbibed mungbean testa

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Abstract. We examined spectral differences in extracts of the thylakoid membranes of mungbean leaf and testa, and the influence of light on the chlorophyll (Chl), protein, and pigment-protein complexes in thylakoid membranes of imbibed mungbean testa. Absorption spectra of mungbean testa thylakoid were blue-shifted from 679.5 and 437 nm, as usually found in leaf, to 663.5 and 432.5 nm. Thornber and MARS extracts of mungbean testa thylakoid were blue-shifted from 672.5, as usually found in leaf, to 670 nm. When the thylakoid membrane of mungbean testa was illuminated for 8 or 16 h with a light intensity of 400 μmol m^{-2} s^{-1} it had higher levels of Chl, protein, and pigment-protein complexes than when illuminated with dim light (<15 μmol m^{-2} s^{-1}) for the same period. Testa illuminated for 24 h, however, showed the opposite result. We concluded that the composition and structure of pigment-protein complexes in dry mungbean testa thylakoid are very different from those of pigment-protein complexes in the leaf, that strong illumination for less than 16 h delays the degradation of pigment-protein complexes of mungbean testa, and that strong illumination for more than 16 h accelerates the degradation.

Keywords: Chlorophyll; Mungbean; Pigment-protein complexes; Testa; *Vigna radiata*.

Introduction

While much attention has been focused on the physiology, biochemistry, anatomy, morphology, and molecular biology of developing seeds, relatively little research has been done on the anatomy, morphology, and ultrastructure of testa. Of the little information available about testa, most concerns the permeability or impermeability (Dure, 1975; Wolswinkel, 1990; Wolswinkel and Ammerlaan, 1985), the genetics of pigmentation of developing testa (Buzzell et al., 1987), or the ultrastructure of the dry seed (Webb and Arnot, 1982; Opik, 1985). These reviews all discuss the thylakoid pigment-protein complexes isolated from leaf (Markwell, 1986; Thornber, 1986). Few researchers had studied the biochemical analysis of dry testa. Using the Thornber electrophoretic system, mungbean testa has been found to contain only pigment-protein complex CPII, and using the MARS fractionation system, it has been found to contain only pigment-protein complexes A2 and AB3. Darkness or dim light (<15 μmol $m^{-2}s^{-1}$) for 24 h delays the degradation of Chl in the mungbean testa, whereas intense light (400 μmol m^{-2} s^{-1}) accelerates it (Yang et al., 1994). In this paper, we further examine the influence of intense illumination on the Chl, protein, and pigment-protein complexes in thylakoid membranes of imbibed mungbean testa.

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Materials and Methods

Seeds of mungbean (*Vigna radiata* L.) were germinated and grown in a greenhouse in a soil-vermiculite mixture for approximately 4 weeks. Leaves were harvested and the thylakoid membranes isolated as previously described (Markwell, 1986). Extracts of thylakoid membranes produced with the Thornber and MARS fractionation systems have been described previously (Markwell, 1986). Seeds of mungbean were immersed in distilled water in the dark or in dim light (<15 μmol m^{-2} s^{-1}) and in intense light (400 μmol m^{-2} s^{-1}), then the testa were detached from the embryo. Chl and protein concentrations were determined using the method of Porra et al. (1989) and the bicinchoninic acid assay of Smith et al. (1985), respectively. Room-temperature absorbance was measured with a Hitachi U2000 UV-visible spectrophotometer. Thylakoid membranes isolated from leaf and detached testa were analyzed for constituent pigment-protein complexes by solubilization with sodium dodecylsulfate (SDS) and electrophoresis with the Thornber fractionation gel system (Markwell, 1986).

Results and Discussion

The Chl content and Chl a/b ratio in dry mungbean testa are very different from those in fresh leaf. The dry testa of mungbean contains half as much Chl as does the fresh leaf (mg Chl/g dry testa or fresh leaf) and is enriched in Chl b. Mungbean testa lacks some light-harvesting pigment-protein complexes of both PSI and PSII (Yang et al., 1994), indicating the thylakoid composition and structure of testa may be very different from those of leaf. Absorption spectra of the thylakoids, and Thornber and MARS extract of thylakoids isolated from leaf and testa confirmed this.

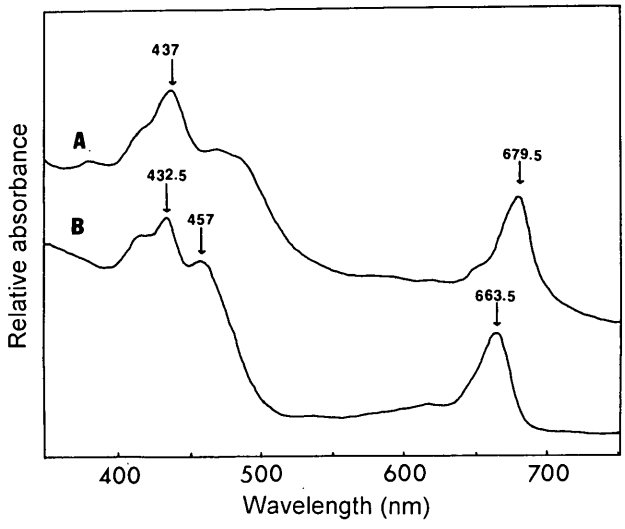

Figure 1. Room temperature absorption spectra of thylakoid isolated from mungbean leaf (**A**) and testa (**B**).

Two major peaks of testa thylakoid, when compared to those of leaf thylakoid, were blue-shifted from 679.5 to 663.5 nm and from 437 to 432.5nm; the shoulders in blue and red regions were also blue-shifted (Figure 1). While the two major peaks at 437.5 and 470 nm were always same, the absorption in the red region of testa pigment-protein complexes was blue-shifted from 672.5 nm, as usually found in leaf, to 670 nm. The shoulder in the red region was blue-shifted from 653.5 to 651 nm (Figure 2). The blue-shift phenomena of thylakoid membranes and of the Thornber or MARS extract of thylakoid membranes of testa apparently result from a deficiency or absence of the pigment-protein complexes of photosystem I (PSI), such as CPI, A1, or A2, which contain the greatest amount of long-wavelength absorbing pigments (Staehelin and Arntzen, 1983). The relative ratios of shoulder/peak in the red region of Thornber and MARS extract of testa thylakoids were higher than those of leaf, this being due to the enrichment of Chl b in testa.

In water-immersed mungbean testa, 24 h treatment with intense illumination seems to accelerate the degradation of Chl, whereas darkness or dim light delays it (Yang et al., 1994). This was confirmed by repetition (Figure 3). Imbibed testa, however, treated in darkness

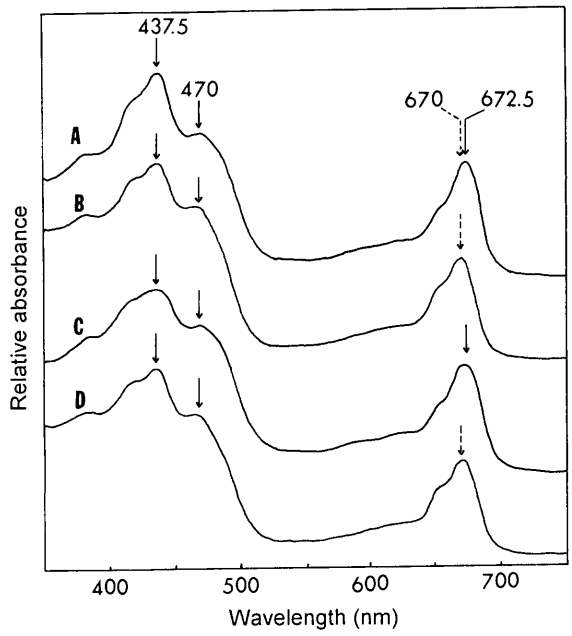

Figure 2. Room temperature absorption spectra of Thornber and MARS extracts of thylakoid membranes isolated from mungbean leaf and testa. Identification: **A** and **B**, Thornber extract of leaf and testa, respectively; **C** and **D**, MARS extract of leaf and testa, respectively.

or dim light for 8 or 16 h had lower Chl and protein contents of thylakoid membranes than that treated for a corresponding period with intense illumination (Figures 3 and 4). The Chl level of mungbean testa under a light intensity of 400 μmol m^{-2} s^{-1} declines as incubation time increases, whereas that of testa in dark or dim light for the corresponding period increases. With or without

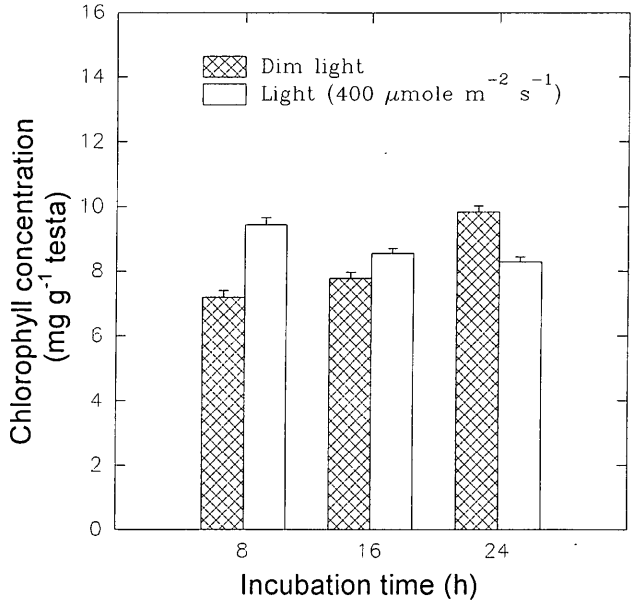

Figure 3. Influence of high light illumination (400 μmol $m^{-2}s^{-1}$) on chlorophyll concentration of mungbean testa. Error bars indicate the standard deviation for triplicate determinations.

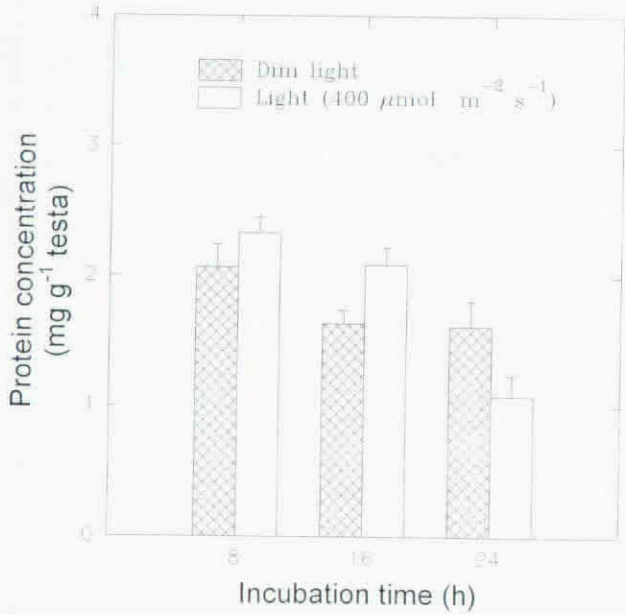

Figure 4. Influence of intense illumination (400 μmol $m^{-2}s^{-1}$) on the concentration of protein in thylakoid membranes isolated from mungbean testa. Error bars indicate the standard deviation for triplicate determinations.

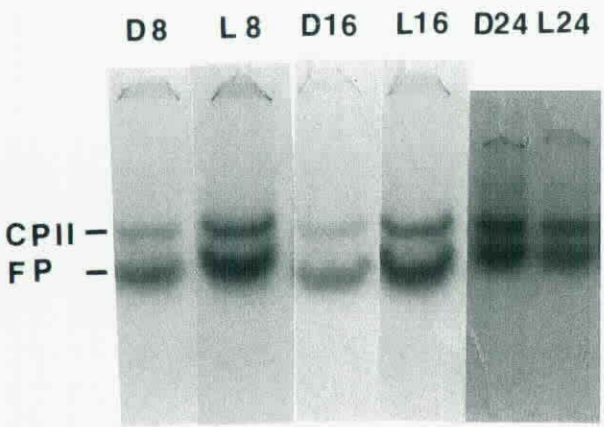

Figure 5. Influence of intenset illumination (400 μmol $m^{-2}s^{-1}$) on the pigment-protein complexes of mungbean testa fractionated by Thornber's electrophoretic system. Identification: **D**, dim light; **L**, intense light.

illumination, the protein level in the thylakoid of mungbean testa decreases as incubation time increases.

All Chl are non-covalently linked to polypeptides to form pigment-protein complexes, which are distributed in the thylakoid membranes in higher plants (Markwell et al., 1979), and therefore the pigment-protein complexes of imbibed mungbean testa treated with intense illumination (400 μmol m^{-2} s^{-1}) and with dim light (<15 μmol m^{-2} s^{-1}) for 8, 16, or 24 h were fractionated by Thornber's electrophoretic gel system (Figure 5). Testa intensely-illuminated for 8 or 16 h contained approximately 3 times as much pigment-protein complex CPII as did the dim-light-treated testa. In contrast, testa treated for 24 h in dim light had more pigment-protein complex CPII than did the corresponding intensely illuminated testa. The levels of Chl, protein, and pigment-protein complex CPII were consistent with each other (Figures 3, 4, and 5). Pigment-protein complexes isolated from the thylakoid of mungbean testa immersed in water for more than 24 h produced smears on the Thornber and MARS gel systems (data not shown), indicating that the mung bean testa thylakoid becomes fragile after 24 h of imbibition, with or without illumination.

It has been reported that imbibition of seeds can restore respiration, cell division, DNA replication, protein and RNA synthesis, membrane integrity, and lipid metabolism, of the embryo (Osborne, 1980). There have been no reports, however, on the physiological changes in the imbibed testa during the course of seed germination. The present investigation shows that the thylakoid structure of mungbean testa is very different from that of leaf, and that degradation of the pigment-protein complexes in imbibed mungbean testa is delayed by intense illumination for less than16 h, and by darkness or dim illumination for more than 16 h. The simultaneous delay or acceleration of the degradation of pigment-protein complexes in mungbean testa reflects mutual protection of pigments and their associated polypeptides. One possible explanation of the discrepancy between delay and acceleration of pigment-protein complex CPII is that during imbibition for less than 16 h the mungbean testa is still able to synthesize a small amount of Chl in the presence of illumination to compensate for the degradation of Chl, leading to delayed degradation of pigment-protein complexes, whereas after 16 h, the mungbean testa no longer synthesizes Chl because of a depletion of metabolites; continued illumination with intense light then leads to photo-oxidation of pigments or pigment-protein complexes. More research is being conducted.

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光線延緩浸水綠豆種皮色素蛋白複合體的崩解

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本研究檢驗綠豆葉和種皮的類囊膜之吸收光譜的差異，和探討光線對浸水綠豆種皮類囊膜上的葉綠素、蛋白質和色素蛋白複合體之影響。種皮類囊膜的吸光自 679.5 和 437 nm (爲植物葉類囊膜的吸光區) 藍移到 663.5 和 432.5 nm。以 Thornber 和 MARS 方法分離的色素蛋白複合體亦自 672.5 nm 藍移到 670 nm。在光度 400 μmol m^{-1} s^{-1}，8 和 16 小時的種皮類囊膜比在光度低於 15 μmol m^{-1} s^{-1} 同時間含有較高濃度的葉綠素、蛋白質和色素蛋白複合體。但照光 24 小時則完全相反。綠豆種皮類囊膜的色素蛋白複合體之組成結構與葉子者顯著不同。強光照射 16 小時延緩，而超過 16 小時則加速綠豆種皮色素蛋白複合體的崩解。

關鍵詞: 葉綠素；綠豆；色素蛋白複合體；種皮；類囊膜。