*Journal of Biochemistry and Molecular Biology*, Vol. 33, No. 3, May 2000, pp. 256-262

JOURNAL OF
Biochemistry
and
Molecular Biology



# Leaf Senescence in a Stay-Green Mutant of *Arabidopsis thaliana*: Disassembly Process of Photosystem I and II during Dark-Incubation

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*Received 14 March 2000, Accepted 12 April 2000*

**In this study the disassembly process of chlorophyll (Chl)-protein complexes of a stay-green mutant *(ore10* of *Arabidopsis thaliana)* was investigated during the dark incubation of detached leaves. During this dark-induced senescence (DIS), the Chl loss was delayed in the mutant, while the photochemical efficiency of photosystem II (PSII) or Fv/Fm was accelerated when compared with the wild type (WT) leaves. This indicates that the decrease in Fv/Fm is a separate process and not causally-linked to the degradation of Chl during DIS of *Arabidopsis* leaves. In the native green gel electrophoresis of the Chl-protein complexes, which was combined with an additional two-dimensional SDS-PAGE analysis, the delayed senescence of this mutant was characterized by the appearance of an aggregate at 1 d or 2 d, as well as very stable light harvesting complex II (LHCII) trimers until 5 d after the start of DIS. The polypeptide composition of the aggregates varied during the whole DIS at 5 d. D1 protein appeared to be missing in the aggregates. This result supports the idea of a faster depletion of functional PSII in the mutants compared with WT, as suggested by the earlier reduction of Fv/Fm and the stable Chl a/b ratio in the mutants. At 5 d, the WT leaves also often showed aggregates, but the polypeptide composition was different from those of *ore10*. The results presented suggest that the formation of aggregates, or stable LHCII trimers in the stay-green mutants, is a way to structurally protect Chl-protein complexes from serious proteolytic degradation. Detailed disassembly processes of Chl-protein complexes in WT and *ore10* mutants are discussed.**

**Keywords:** Aggregate, Arabidopsis thaliana, Chlorophyll-protein complex, Delayed dark-induced senescence, Stay-green mutant

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## Introduction

During leaf senescence, a gradual loss of chlorophyll (Chl) is normally accompanied by a decrease in photosynthetic activities, as well as the degradation of structural components of the photosynthetic apparatus. However, in some non-functional stay-green mutants, the Chl content is retained while other physiological functions are nearly the same as wild-type (WT).

One of the most comprehensively studied non-functional stay-green mutants is Bf993. It is a genotype of the pasture grass *Festuca pratensis,* in which the chloroplast disassembly during senescence is partially disabled (Thomas and Howarth, 2000). A mutation of a genetic locus *sid* in pasture grass renders the leaves permanently green. It is also expressed in the persistence of a number of Chl-binding proteins. The *sid* gene codes for or regulates phaeophorbide a oxygenase, an enzyme in the macrocycle-opening step of the Chl catabolism (Vicentini *et al.*, 1995).

The assembly process of the Chl-protein complexes is a sequential process (Dreyfuss and Thornber, 1994; McCormac, 1996). Therefore, the disassembly process is expected to be the same. We investigated the disassembly process during the dark-incubation of detached leaves of the WT *Arabidopsis thaliana* (Oh and Lee, 1996). During this artificial dark-induced senescence (DIS), a light-harvesting complex (LHC) was degraded first in photosystem I (PSI), while the LHC was degraded last in the photosystem II (PSII). In the case of Bf993, the retained Chl was related to LHCII. The D1 protein was also unusually stable, while extrinsic proteins were labile as in the case of WT (Hilditch *et al.*, 1989).

For the study of the disassembly process in the model plant, *A. thaliana*, several mutant lines are available. Among them, a non-functional stay-green mutant, *ore11,* is reported by Oh *et al.* (1997) to be an allele of another non-functional stay-green mutant, *ore10,* (personal communication).

In this study, we investigated the disassembly process of the Chl-protein complexes in the non-functional stay-green mutant, *ore10,* during DIS in comparison with the process in WT leaves. In *ore10* mutants, LHCII was also a stable Chl-

protein complex form. In addition, this mutant could be characterized by the gradual appearance of aggregates in the process of DIS. The detailed disassembly process of the *ore10* mutants is described comparing it with the process in WT.

## Materials and Methods

**Plant materials and growth** Seeds of the *Arabidopsis thaliana* ecotype Columbia, as well as mutant lines, were grown on a compound soil mix (vermiculite/peat moss/perlite = 1 : 1 : 1) in a growth chamber with a cycle of 16h light /8h dark and a temperature cycle of 23°C day/18°C night. The non-yellowing mutant lines, *ore10* and *ore11*, screened from seed pools mutagenized with ethylmethane-sulfonate, were kindly donated by Dr. Hong Gil Nam (Pohang Univ. of Science and Technology, Kyungbuk, Korea).

For the dark treatment, the third or fourth foliar leaves that were excised from about 21 day-old leaves just prior to bolting were floated on 3 mM 2-[N-morpholino]-ethanesulfonic acid (MES) buffer (pH 5.8) and then incubated in permanent darkness.

**Measurement of Chl content and Chl Fluorescence** Leaves were ground in 2 ml of 80% acetone with a glass homogenizer. The Chl content was calculated on a fresh tissue weight basis according to the method of Arnon (1949). The Chl content was presented as a relative value to that of a non-senesced control leave.

The Chl fluorescence of foliar leaves was measured using a portable PAM-2000 fluorometer (Walz, Effeltrich, Germany) after dark-adaptation for 10 min at room temperature. The fluorometer was connected to a leaf-clip holder (2030-B, Walz) with a trifurcated fiber optic (2010-F, Walz) and to a computer with data acquisition software (DA-2000, Walz). This provided an on-line analysis of the fluorescence data. The photochemical efficiency of PSII, or photosynthetic efficiency in short, was deduced from the maximal efficiency of PSII photochemistry, Fv/Fm = (Fm-Fo)/Fm (Kitajima and Butler, 1975). The minimal fluorescence level (Fo) for all open PSII reaction centers was measured by the measuring modulated light, which was sufficiently low (<0.1 μmol $m^{-2}s^{-1}$) in order not to induce any significant variable fluorescence. The maximal fluorescence level (Fm), with all of the PSII reaction centers closed, was determined by a 0.8 s saturating pulse at 8000 μmol $m^{-2}s^{-1}$ in dark-adapted leaves.

**Green gels and two-dimensional gels of thylakoid membrane proteins** Thylakoid membranes were prepared as described in the preceding paper (Eu *et al.*, 1996; Oh and Lee, 1996). The isolated thylakoid membrane pellets were washed twice in 2 mM Tris maleate (pH 7.0) and resuspended in a solubilization buffer (0.45% octyl glucoside, 0.45% decyl maltoside, 0.1% lithium dodecyl sulfate, 10% glycerol and 2 mM Tris maleate, pH 7.0) to yield a ratio of total nonionic detergent to chlorophyll of 20 : 1 (w/w). In order to achieve a better solubilization of thylakoid membranes from WT leaves that were dark-incubated for 5 d, the amount of solubilization buffer added was doubled to yield a ratio of total nonionic detergent to chlorophyll of 40 : 1 (w/w). The native Chl-protein complexes were separated as described by Allen and Staehelin (1991). An aliquot of the solubilized thylakoid membranes, which contained 13.5 μg of Chl per lane, was loaded per lane of 1.5 mm thick gel. Samples were electrophoresed at 4°C and constant 6 mA.

For the second dimensional SDS-PAGE analysis, 1.5 mm thick strips were excised from green gel lanes and incubated for 30 min at room temperature in a denaturing buffer containing 1 × stacking gel buffer, 2% sodium dodecyl sulfate, 2% β-mercaptoethanol, and 10% glycerol. The fully denatured gel strips were laid directly on the stacking gel of 1.5 mm thickness using the buffer system of Laemmli (1970). Electrophoresis was done at 30 mA constant current per gel using a vertical electrophoresis unit (LKB 2001, LKB, Sweden). Gels were stained with silver nitrate (Sambrook *et al.*, 1989).

## Results

**Morphological characterization of *ore10* mutants** The leaves of *ore10* mutants are smaller and wrinkled compared with those of WT plants at a similar age (Fig. 1A). The flowering time of this mutant, measured as the time of the visual appearance of the floral inflorescence, was similar to that of WT plants. Figure 1B also shows that the heights of inflorescence in both *ore10* and WT are quite similar. In WT leaves, the progress of natural senescence is noticeable by the yellowing, which signals the loss of Chl. However, the senescing leaves of *ore10* mutants started to lose rigidity without yellowing (Fig. 1C). The senescence shown in the 3rd and 4th foliar leaves after bolting generally started earlier in the mutant than in WT (pictures not shown). Mutant leaves stayed green even after most of the cells were dead. This is noticeable from the dry leaves with green color (Fig. 1C).

**Changes in Chl contents and Fv/Fm during DIS in WT and mutants *ore10* and *ore11* leaves** After the start of DIS in WT *Arabidopsis* leaves, the Chl content decreased gradually during the whole period of DIS for 5 d. The rate of

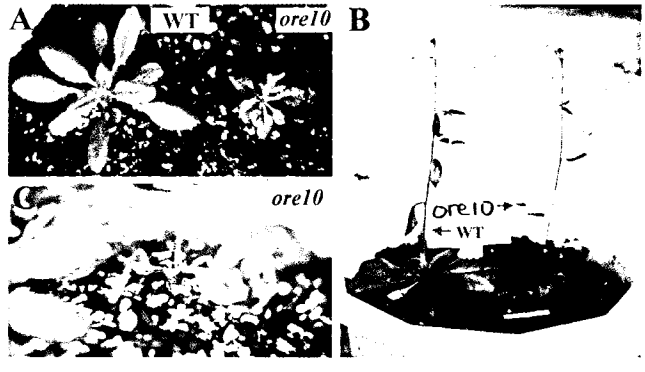


**Fig. 1.** Morphological characterization of a WT *Arabidopsis* plant and a non-functional stay- green mutant *ore10*. A. Phenotypes of a whole WT plant (left) and an *ore10* mutant (right) prior to bolting. B. The floral inflorescence of WT (left) and an *ore10* mutant (right) at the same age. C. Wilted-but-green leaves of a *ore10* mutant during its natural senescence.

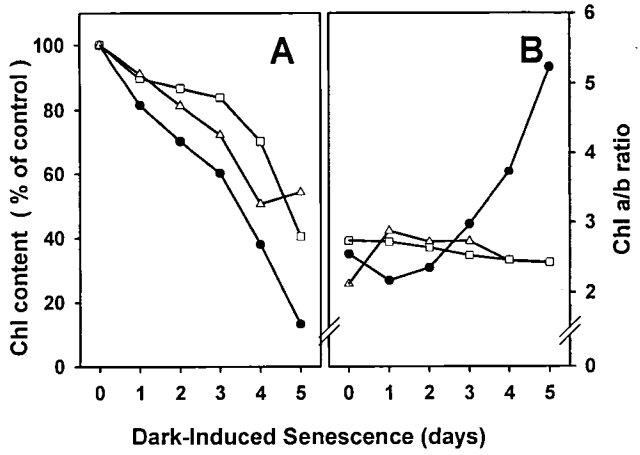


**Fig. 2.** Changes of total Chl content (A) and Chl a/b ratio (B) during dark-induced senescence of a WT (●), an *ore10* mutant (△) and an *ore11* mutant (❑). Chl content was measured at the given times after incubating detached leaves in darkness and was expressed as a relative value in percentage to that of a non-senesced control leave.

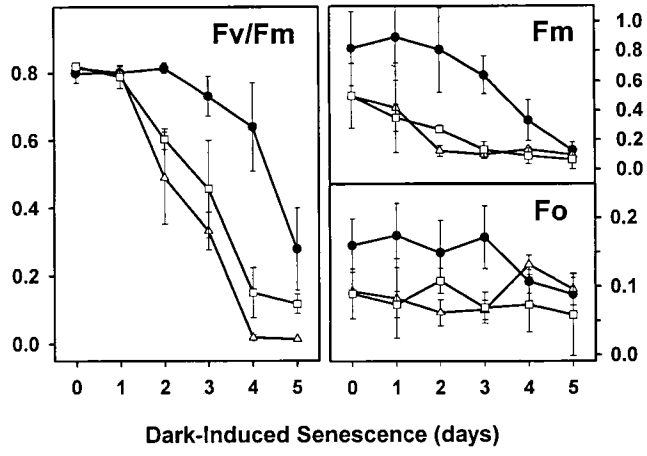


**Fig. 3.** Changes in Fv/Fm, Fm and Fo during dark-induced senescence of a WT (●), an *ore10* mutant (△) and an *ore11* mutant (❑). Chl fluorescence of foliar leaves of non-senesced plants was measured after dark-adaptation for 10 min at room temperature.

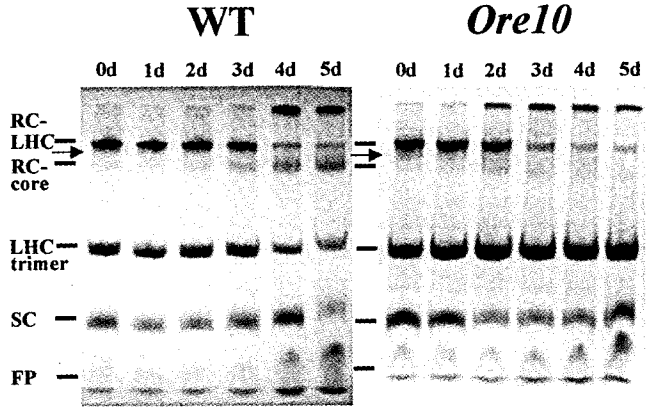


**Fig. 4.** Non-denaturing green gel fractionation of Chl-protein complexes from WT and *ore10* mutant leaves during dark-induced senescence. Each lane is loaded with solubilized thylakoids containing 13.5 μg Chl. Detailed methods and labeling of Chl-protein complexes are described in the text. The gels were pictured without staining.

Chl degradation was slower both in *ore10* and in *ore11* (Fig. 2A). In both mutants, a significant amount of Chl remained at 5 d. However, the photosynthetic efficiency, or Fv/Fm, started to decrease after 2 d in WT leaves, while it began to drop rapidly after 1 d in both *ore10* and *ore11* (Fig. 3). Although Oh *et al.* (1997) described these mutants as non-functional stay-green mutants, the phenotype of the mutants was not only a delayed loss of Chl content, but also the DIS in the functional sense proceeded faster in these mutants than in WT (shown in Fig. 3.) As noticed from the term non-functional stay-green mutants, the decrease in Fv/Fm is a separate process not-causally-linked to the degradation of Chl during DIS in *Arabidopsis*.

**Changes of Chl a/b ratio, Fo and Fm during DIS in WT and mutants *ore10* and *ore11*** During DIS, the Chl a/b ratio increased significantly after 3 d in WT as we reported previously (Oh and Lee, 1996). However, the Chl a/b ratios in *ore10* and *ore11* mutants did not increase at all during DIS for 5 d (Fig. 2B). The increase in the Chl a/b ratio in WT was due to a rapid disappearance of Chl b (data not shown) that was bound mostly to the proteins in LHCs. Therefore, the degradation of the Chl b seemed to be partly blocked in mutant leaves during DIS. This suggests that the degradation of LHCs is delayed in the mutants.

The decrease in Fv/Fm in WT, shown in Fig. 3, was due to the decrease both in Fm and in Fo. However, such a noticeable drop in Fo was not observed in the two mutants. The size of error bars for both Fm and Fo are relatively large compared to that of Fv/Fm, because both Fm and Fo are roughly proportional to the Chl content. Therefore, the individual differences in the absolute Chl content are cancelled out in Fv/Fm. A decrease in energy coupling between LHCII and the PSII traps leads to an increased fluorescence emission from LHCII itself, which results in an increase of Fo. Because the Chl content drops significantly during DIS (shown in Fig. 1), the slow changes in Fo shown in the mutants during DIS support our idea of a preferential delay in the degradation of LHCII during the disassembly of photosystems.

**Changes of Chl-protein complexes during DIS in WT and *ore10* mutants** The non-functional stay green mutant of *Arabidopsis* retains its photosynthetic pigments without functional delay during DIS (Figs 1 and 3). Most of the Chls integrate as Chl-protein complexes by non-covalent binding with proteins (Markwell *et al.*, 1979). Therefore, we examined the changes in band patterns of the Chl-protein complexes during DIS (Fig. 4).

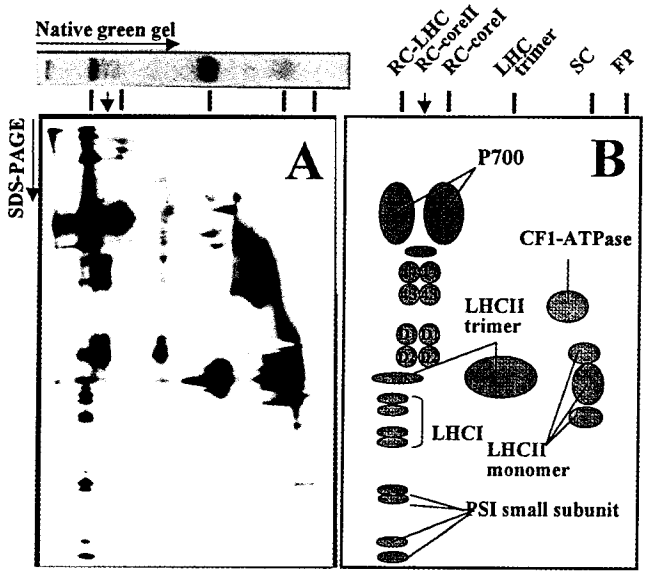


**Fig. 5.** A typical two-dimensional SDS-PAGE profile of non-senesced *Arabidopsis* plant leaves (A) and description of the resolved spots (B). Detailed methods and labeling of Chl-protein complexes and spots are described in the text.

The nomenclature of the separated green gel bands was made according to Allen and Staehelin (1991), which could be confirmed according to the polypeptide composition of each band as we previously reported (Oh and Lee, 1996; also referred to in the next section). Briefly, the green gel pattern shown in non-senescing leaves is divided into five zones (Fig. 4): (1) RC-LHC, containing PSI-LHCI and PSII-LHCII complexes; (2) RC-Core, containing smaller PSII and PSI reaction-center core complexes; (3) LHCII, containing trimeric LHCII complexes; (4) SC, or small complexes, containing a number of small PSII-related complexes as well as some monomeric LHCII complexes; (5) FP (free-pigment zone), containing free pigments composed largely of carotenoids.

The green gel band pattern of non-senescing leaves of WT was almost the same as that of the *ore10* mutant (Fig. 4). However, we did notice three major differences in the disassembly process of the Chl-protein complexes between WT and the *ore10* mutant during DIS. The changes in the band pattern for WT are in good agreement with our previous report (Oh and Lee, 1996).

(1) The intensities of both the RC-LHC and RC-Core band decreased during DIS in the *ore10* mutants, while the decrease in RC-LHC in WT was matched by an increase in RC-Core. In *ore10* mutants, bands for both RC-LHC and RC-Core became fainter after 3 d without any additional noticeable changes in other regions.

(2) The trimeric form of LHCII is stable in the mutants. This was expected since the Chl a/b ratio (Fig. 2B) and Fo values (Fig. 3) are stable. However, the band intensity of this form in WT decreased significantly at 4 d and 5 d.

(3) In *ore10* mutants, a band that could not run through the stacking gel during electrophoresis was observed at 1 d or 2 d thereafter. Its band intensity was roughly the same from 2 d to 4 d and decreased at 5 d. A similar band could be observed only at 4 d and 5 d in the case of WT. However, it should be kept in mind that the amount of Chl in each aliquot loaded was equally adjusted for each lane, and the Chl content for each leaf was very low at 4 d and 5 d in the case of WT. Interestingly, the accumulation of aggregates in the *ore10* mutants is consistently matched with the loss of a band in the RC-LHC region and a band in the SC region.

**Disassembly of Chl-protein complexes in *ore10 mutants***

Detailed changes in the Chl-protein complexes can be examined by a two-dimensional (2D) electrophoresis with a native green gel in the first dimension and fully denaturing SDS-PAGE in the second. Therefore, the polypeptides composing each green band are shown as spots in a vertical lane corresponding to the band as depicted in Figure 5. The region labeled as RC-LHC in the first dimension contains a number of large PSI and PSII complexes that are attached with antennas. The PSII complexes can be identified in the 2D pattern by the presence of the apoproteins CP47, CP43, D1 and D2. Most of the P700 apoproteins are shown as pigmented CPI on the second-dimensional gel, and the positions of the other PSI subunits were in the range of 27-8.5 kDa as described by Allen and Staehelin (1994). However, we did notice the existence of two bands (marked with an arrow in Figs 4 and 5) composed of PSII core complexes without P700 apoproteins between the major RC-LHC band and the major RC-Core band. Although these two bands are included in the RC-LHC region by Allen and Staehelin (1994), we think they are RC-Core bands, since they are without LHCs. Therefore, we include them in a new RC-CoreII region, and the existing RC-Core region is named as a RC-CoreI region.

The major differences in the 2D gel profiles between WT and *ore10* mutants (Fig. 6) are as follows:

(1) In WT, we have shown in Fig. 4 that a decrease in the RC-LHC was matched by an increase in the RC-Core. During this process, there is a noticeable increase in the P700 apoproteins without the small PSI subunits, including the LHCI polypeptides in the RC-CoreI region. This confirms our previous report (Oh and Lee, 1996). However, in the *ore10* mutants we observed no sign of an increase in the band intensities in the RC-CoreI regions. Instead, the P700 apoproteins and LHCI proteins in the RC-LHC region and P700 apoproteins in the RC-CoreI region seemed to be degraded faster in the *ore10* mutants than in WT. In addition, in the *ore10* mutants a new band containing PSII core complexes with LHCII was observed at 2 d. These migrate slower than the RC-LHC band. After 2 d, the PSII core complexes in this new band, as well as those in the RC-CoreII region, disappeared more rapidly than PSI subunits.

(2) In *ore10* mutants, the disappearing PSII core complexes may be located in the aggregates, which increased while RC-

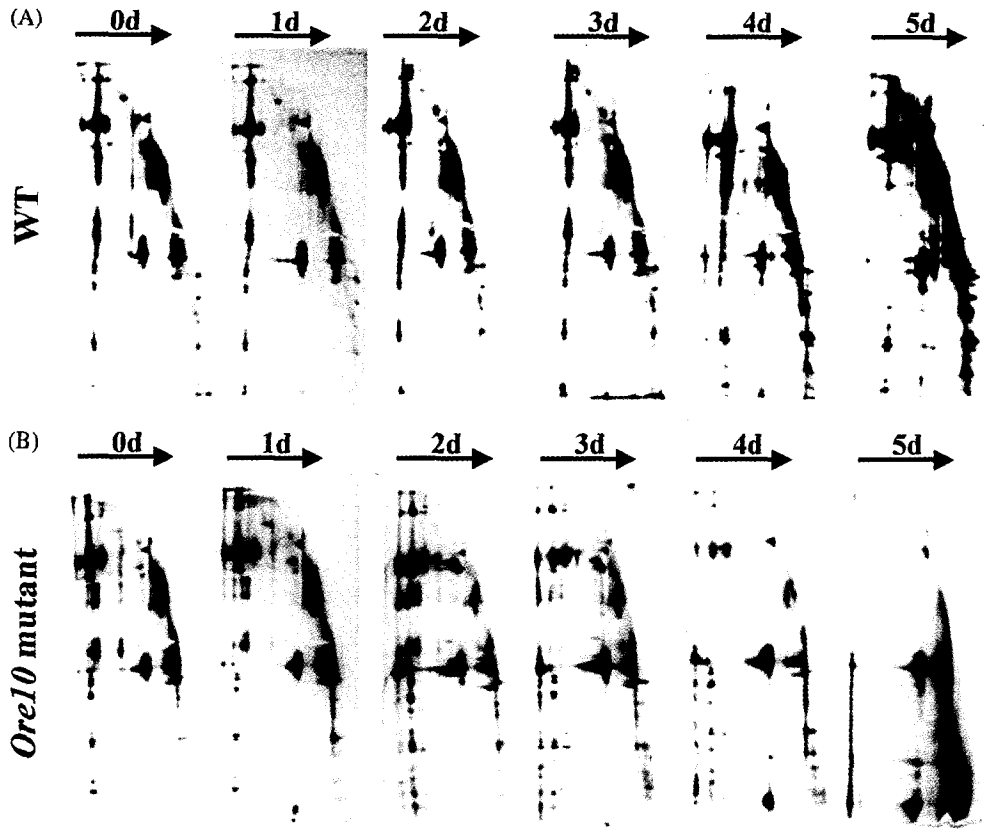


**Fig. 6.** Two-dimensional SDS-PAGE analysis of polypeptides composition of Chl-protein complexes during dark-induced senescence of WT (A) and *ore10* mutant (B) leaves. See Figure 5 for the identification of individual protein spots.

LHC and RC-Core bands decreased. These aggregates are different than the ones that are often located on top of the stacking gel in non-senescing leaves of WT and mutants, which contain only a small amount of the P700 apoproteins. The aggregates that were observed in the *ore10* mutants at 2 d contained P700 apoproteins, PSII core complexes and LHCII. The increase in LHCII in the aggregates was roughly matched by a decrease in the LHCII monomers in the SC region. After 2 d, aggregates showed PSI subunits, including LHCI, in addition to the P700 apoproteins, which had a significant decrease in the bands in the RC-LHC region. The polypeptide composition of the aggregates at 3 d in the mutants was similar to the composition of the band in the RC-LHC region. After this stage, PSI/II core proteins were significantly degraded in the aggregates, which had a concurrent accumulation of small polypeptides.

The polypeptide composition of aggregates (shown in WT at 4 d and 5 d) was different than those in the mutants, which consisted of P700 apoproteins, LHCII proteins and a small amount of PSI small subunits.

(3) As shown in Figures 4 and 6, the LHCII trimer bands in the green gel, and the corresponding spots in the 2D gel, were quite stable during DIS in the *ore10* mutants (Fig. 6). At 5 d in senescing *ore10* mutant leaves, most of the Chls were bound with LHCII trimers, LHCII monomers and aggregates, and all of the other Chl-protein complexes seemed to be degraded. After 5 d, only the LHCII trimer proteins were left even after all of the other proteins disappeared in the 2D gel (pictures not shown).

## Discussion

During DIS, we compared the disassembly process of the photosynthetic apparatus among WT, *ore10* and *ore11* mutants. These mutants were characterized by their 'stay-green' leaves, but the senescence was rather accelerated in a functional sense, especially in the photosynthetic machinery.

**Relationship of the changes in the photosynthetic efficiency or Fv/Fm with the changes in Chl contents** In WT leaves, high (>0.8) photosynthetic activity was retained for 2 d, although the gradual degradation of Chls during DIS resulted in the reduction of the amount down to 70% of the control (Figs 2A and 3). When Fv/Fm decreased less than 10% at 3 d, the Chl content decreased to 60% of the control value. This result suggests that the function of each existing photosynthetic apparatus was not hindered during DIS for 2 d, but the number of functional apparatus was decreased. At 3 d and thereafter, some functional units might be damaged, resulting in a reduction in the average value of Fv/Fm. A

similar phenomena was demonstrated in the leaves of young spinach grown in a Mg- and S-deficient medium (Dannehl *et al.*, 1995).

However, Fv/Fm dropped rapidly even after 1 d in the mutants, while the degradation of Chl was delayed. This result indicates that the photosynthetic apparatus of the mutants are more vulnerable to damages in the functional sense, but Chls in some Chl-protein complexes seemed to be protected against degradation. The stable Chl-protein complexes were found to be LHCIIs in this report.

**Relationship of LHCII, Chl a/b ratio with Fo** Because the Chl a/b ratio of LHCII is normally near 1 or below 2, and this value is much lower than the values of other Chl-protein complexes (Thornber, 1995), the stability of LHCII shown in the green gel patterns, as well as their 2D gel pattern, can explain the stable Chl a/b ratio in the mutants during DIS for 5 d (Fig. 2B).

If all of the Chl-protein complexes were degraded, except LHCII, the excitation energy coupling between LHCII and PSII core complexes would be blocked. This would result in an increase in Fo. The time points for the changes during DIS in these three parameters are roughly similar (shown in Figs 2, 3 and 4.)

An example for the relationship of LHCII with Fo can be found in Zucchelli *et al.* (1988). They showed an increase in Fo when LHCII was partially uncoupled from the PSII reaction centers at low cation concentrations. Ottander *et al.* (1995) also suggested that the increase in Fo in a winter stressed leaf indicated damaged photochemistry. This resulted from the detachment of LHCII from PSII reaction centers upon the decrease in D1 content of the reaction centers. In this report, the PSII core complexes, indicated by an arrow in between the RC-LHC region and the RC-Core region of the 2D gel (Fig. 6), decreased significantly at 3 d. A significant increase in Fo is a well known phenomena in plant leaves under high temperature stress (Renger and Schreiber, 1986).

**Disassembly process and stability of Chl-protein complexes in stay-green mutant** During DIS, the disassembly of photosystems is probably a sequential process, as is their assembly process (Dreyfuss and Thornber, 1994; McCormac, 1996). In detached leaves of *A. thaliana*, the disassembly process for PSII was different than that of PSI. Oh and Lee (1996) described the detailed processes for both PSI and PSII. As described in this report, more detailed information about the disassembly process could be obtained by a comparison of the WT with mutants that are defective in the disassembly process.

In mutants *ore10* and *ore11*, the rapid decrease in Fv/Fm, shown at 2 d and thereafter (Fig. 3), is probably due to the blockage of energy transfer from LHCII to the reaction centers in CCII. Although the increase in Fo, or the stable Chl a/b ratio as supporting evidence for this assumption, could be observed clearly at 3 d (Figs 2B and 3) we expect a mature PSII is separated into CCII and LHCII as an early process after the start of DIS. According to Ghanotakis *et al.* (1987), Barbato *et al.* (1989) and Camm and Green (1989), the treatment with octyl-β-D-glucoside in high-salt conditions specially removes LHCII and CP24, while CP29 and CP26 are enriched with CCs, and a more complete treatment with detergent results in CCII being attached to CP29 only. Therefore, monomeric LHCIIs, including CP26 and CP29, are expected to be bound with CCII, leaving LHCII mainly in its trimeric form, or together with a monomeric CP24.

The stability of the Chls in mutants is probably related with the early appearance of aggregates and the very stable LHCII trimers (shown in Figure 6.) The formation of the aggregates can explain most of the characteristic differences of the mutants in the disassembly process compared with the process in WT. This includes the disappearance of PSI in the RC-LHC region, without the increase of P700 apoprotein in the RC-CoreI region, the disappearance of LHCII monomers in the SC region at 2 d, etc.

The appearance of aggregates that could not run through the stacking gel during electrophoresis was also reported in Ottander *et al.* (1995) in winter leaves of *Pinus sylvestris*. After the completion of the Chl loss, the aggregates were formed during winter by the reorganization of the remaining Chl and Chl-protein complexes from PSII and PSI. The aggregates in pine leaves were devoid of D1 protein and efficiently quenched excitation energy through non-radiative dissipation. Although the aggregates formed in the mutants during DIS at 1 d or 2 d seemed to be also devoid of D1 proteins, their polypeptide compositions differed from those reported by Ottander *et al.* (1995) and also from the composition of the aggregates shown in WT (Fig. 6). The polypeptide composition of the aggregates was not static, but kept changing during DIS as described earlier. Funk *et al.* (1994) also observed similar aggregates on top of the stacking gel when the Chl-protein complexes were separated from spinach using octyl-thioglucopyranoside. Although we did not use the same detergent as Ottander *et al.* (1995), the formation of aggregates is nevertheless probably due to the hydrophobic attractions of the Chl-protein complexes, which are modified during DIS by proteases.

As mentioned earlier, the aggregates formed in the mutants are probably devoid of the D1 protein. However, the D1 protein could be observed in WT even at 4 d in the RC-LHC region, but no D1 proteins were observed in the *ore10* mutants even in the RC-LHC region (Fig. 6). This result supports the idea of a faster depletion of functional PSII in the mutants compared with WT, as suggested by the earlier reduction of Fv/Fm and the stable Chl a/b ratio in the mutants (Figs 2B and 3).

The results presented suggest that the formation of aggregates, or stable LHCII trimers in the stay-green mutants, is a way of protecting Chl-protein complexes from serious proteolytic degradation. However, the protection cannot cover the functionally important proteins, such as D1 proteins,

although the depletion of D1 protein in the aggregates needs to be confirmed. The factors causing the formation of the aggregates are under investigation. Finally, the results presented provide experimental evidence showing that the protection of protein degradation, as in the *ore10* or *ore11* mutants, is an important way of delayed senescence in addition to the mere blockage of Chl catabolism.

**Acknowledgments** The authors wish to acknowledge the financial support of the Korean Research Foundation made in the program year of 1997.

## References

Allen, K. D. and Staehelin, L. A. (1991) Resolution of 16 to 20 chlorophyll-protein complexes using a low ionic strength native green gel system. *Anal. Biochem.* **194**, 214-222.

Allen, K. D. and Staehelin, L. A. (1994) Polypeptide composition, assembly and phosphorylation patterns of the photosystem II antenna system of *Chlamydomonas reinhardtii*. *Planta* **194**, 42-54.

Arnon, D. I. (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant. Physiol.* **24**, 1-15.

Barbato, R, Rigoni, F, Giardi, M. T. and Giacometti, G. M. (1989) The minor antenna complexes of an oxygen evolving photosystem II preparation: purification and stoichiometry. *FEBS Lett.* **251**, 147-154.

Bassi, R, Soen. S. Y, Frank, G, Zuber, H. and Rochaix, J. D. (1992) Characterization of chlorophyll-a/b proteins of photosystem-I from *Chlamydomonas reinhardtii*. *J. Biol. Chem.* **267**, 25714-25721.

Bassi, R. and Dainese, P. (1992) A supramolecular light-harvesting complex from chloroplast photosystem-II membranes. *Eur. J. Biochem.* **204**, 317-326.

Camm, E. L. and Green, B. R. (1989) The chlorophyll a/b complex, CP29, is associated with the photosystem II reaction center core. *Biochim. Biophys. Acta* **974**, 180-184.

Dannehl, H, Herbik, A. and Godde, D. (1995) Stress-induced degradation of the photosynthetic apparatus is accompanied by changes in thylakoid protein turnover and phosphorylation. *Physiol. Plant.* **93**, 179-186.

Dreyfuss, B. W. and Thornber, J. P. (1994) Assembly of the light-harvesting complexes (LHCs) of photosystem II. *Plant Physiol.* **106**, 829-839.

Eu, Y-J, Ha, S-.B. and Lee, C-.H. (1996) Effects of chilling injury in the light on chlorophyll fluorescence and D1 protein turnover in cucumber and pea leaves. *J. Biochem. Mol. Biol.* **29**, 398-404.

Funk, C, Schroder, W. P, Green B. R, Renger, G. and Andersson, B. (1994) The intrinsic 22kDa protein is a chlorophyll-binding subunit of photosystem II. *FEBS Lett.* **342**, 261-266.

Gapstain, S. (1988) Photosynthesis; in *Senescence and Aging in Plants*, Nooden, L. D. and Leopold, A. C. (eds), pp. 85-109, Academic Press, London.

Ghanotakis, D. F, Demetriou, D. M. and Yokum, C. F. (1987) Isolation and characterization of an oxygen-evolving photosystem II reaction center core preparation and a 28 kD Chl-a-binding protein. *Biochim. Biophys. Acta* **891**, 15-21.

Hilditch, P, Thomas, H, Thomas, B. J. and Rogers, L. J. (1989) Leaf senescence in a non-yellowing mutant of *Festuca pratensis*: Proteins of photosystem II. *Planta* **177**, 265-272.

Kitajima, M. and Butler, W. L. (1975) Quenching of chlorophyll florescence and primary photochemistry in the chloroplasts by dibromothymoquinone. *Biochimica. Biophysica. Acta.* **376**, 105-115.

Laemmli, U. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Markwell, J. P, Thornber, J. P and Boggs, R. T. (1979) Higher plant chloroplasts: Evidence that all the chlorophyll exists as chlorophyll-protein complexes. *Proc. Natl. Acad. Sci. USA* **76**, 1233-1235.

McCormac, D. J, Marwood, C. A, Bruce, D. and Greenberg, B. M. (1996) Assembly of photosystem I and II during the early phases of light-induced development of chloroplasts from proplastids in spirodela oligorrhiza. *Photochem. Photobiol.* **63**, 837-845.

Oh, M-.H. and Lee, C-.H. (1996) Disassembly of chlorophyll-protein complexes in *Arabidopsis thaliana* during Dark-Induced Foliar Senescence. *J. Plant Biol.* **39**, 301-307.

Oh, S. A, Park, J. H, Lee, G. I, Paek, K. H, Park, S. K. and Nam, H. G. (1997) Identification of three genetic loci controlling leaf senescence in *Arabidopsis thaliana*. *Plant J.* **12**, 527-535.

Ottander, C, Campbell, D. and Oquist, G. (1995) Seasonal changes in photosystem II organization and pigment composition in *Pinus sylvestris*. *Planta* **197**, 176-183.

Renger, G. and Schreiber, U. (1986) Practical Applications of Fluorometric Methods to Algae and Higher Plant Research; in *Light Emission by Plants and Bacteria*, Govindjee, J, Amesz, D. C. and Fork (eds), Vol 2, pp. 587-619, Academic Press, London.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular cloning, a laboratory manual.* 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Thomas, H. and Smart, C. M. (2000) Five ways to stay green. *J. Exp. Botany* **51**, 329-337.

Thornber, J. P. (1995) Thirty years of fun with antenna pigment-proteins and photochemical reaction centers: A tribute to the people who have influenced my career. *Photosynth. Res.* **44**, 3-22.

Vicentini, F, Hortensteiner, S, Schellenberg, M, Thomas, H. and Matile, P. (1995) Chlorophyll breakdown in senescent leaves: identification of the biochemical lesion in a stay-green genotype of *Festuca pratensis* Huds. *New phytol.* **129**, 247-252.

Zuchelli, G, Farlashi, F. M. and Jennings, R. C. (1988) Influence of electrostatic screening by cations on energy coupling between photosystem II reaction centers and the light-harvesting chlorophyll a/b protein complex II. *Biochim. Biophys. Acta* **934**, 144-150.