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Effect of ABA on Disassembly of Chloroplast during Senescence in Detached Leaves of *Zea mays*

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The effect of ABA on the chloroplast disassembly of Zea mays was investigated by measuring the changes in the relative distribution of chlorophyll(Chl) between the Chl-protein complexes in ABA treated and untreated senescing leaves. The reaction center(RC)-light harvesting complex(LHC) regions were rapidly disassembled in the late stage of dark-induced senescence. Plus, during dark-induced senescence, the disassembly of a reaction center of P700 apoproteins containing mainly Chl a was faster than that of a reaction center of LHCI apoproteins containing both Chl a and Chl b. The increase in the relative distribution of Chl-protein complexes in the RC-Core2 in the late stage of senescence was due to the accumulation of core complexes such as CP47/43 and reaction centers including D1/D2 apoproteins disassembled from the RC-Core1 containing the dimer of D1/D2 apoproteins. The LHCII region was more stable than the other Chl-protein complexes throughout leaf senescence. Accordingly, it is suggested that the preferential breakdown of Chl a gives rise to the disassembly of Chl a-binding proteins, particularly reaction centers and core complexes during dark-induced senescence, plus the primary target of the photosynthetic apparatus in senescing leaves would seem to be Chl a along with the proteins associated with Chl a. The application of ABA promoted the disassembly of the P700 apoproteins in the PSI reaction center and the dimer of D1/D2 apoproteins, and the conversion of the trimeric LHCII apoprotein to the monomeric LHCII apoprotein during the middle stage of leaf senescence, thereby suggesting that ABA accelerates the disassembly of both Chl a-binding and Chl a+b-binding proteins, particularly Chl a-binding proteins during the middle stage of leaf senescence.

Key words : senescence, chlorophyll-protein complex, Zea mays, ABA

1. Introduction

Leaf senescence in plants, leading to such diverse processes as cell breakdown and death through the decline of cellular components and biological activities, is a complex degradative event(Gan and Amasino, 1997 ; Nooden *et al.*, 1997). It can be initiated by a wide variety of external factors including nitrogen deficiency, temperature, light limitation, drought, and pathogen infection, as well as by various internal factors such as plant growth regulators and reproduction(Thomas and Stoddart, 1980). As leaf senescence progresses, the photosynthetic apparatus is disassembled and the leaf comes to serve as sink organ of mobilized nutrients instead of a source organ. This is due to the yellowing of the leaf as a result of the preferential degradation of Chl rather than carotenoid(Thomas and Stoddart, 1975 ; Fisher and Feller, 1994). The rapid breakdown of Chl during leaf senescence can induce changes in the chloroplast internal structure(Blank and McKeun, 1991). Of particular interest is the fact that, along with Chl, thylakoid proteins can also be disassembled during leaf senescence. From previous studies using maize leaves, the majority of thylakoid-associated proteins, such as the 68 kD apoprotein of PSI, ATPase, light-harvesting Chl-binding proteins, and cytochrome

f and b6, all exhibit a two- to four-fold decrease in senescing leaves compared to the control(Robert *et al.*, 1987), whereas the D1 protein of PSII is unaffected by leaf senescence(Droillard *et al.*, 1992). It has also been suggested that PSII proteins extrinsic to the thylakoid membrane, such as the 33 kD protein of an oxygen-evolving complex, degrade in the senescing leaves of *Festuca pratensis* (Davies *et al.*, 1990). Furthermore, Hukamani and Baishnab(1994) have proposed that in the normal cultivar and non-yellowing mutant of *Festuca pratensis*, a turnover of various Chl-binding proteins associated with the thylakoid membrane is induced by leaf senescence(Nock *et al.*, 1992 ; Thomas *et al.*, 1992). However, very little detail is known about the disassembly of Chl-protein complexes during leaf senescence, even though there has been some research on the structural and physiological changes of the photosynthetic apparatus in senescing leaves(Siffel *et al.*, 1991 ; Pancaldi *et al.*, 1996).

The native green gel system, which can fractionate chloroplast proteins, may be a very valuable tool for evaluating the composition, organization, and degradation of the thylakoid membrane (Thornber, 1986). Based on the native green gel system using only a small lease of free pigment, Chl-protein complexes can be classified as follows : multiple PSI-LHCI complexes, multiple PSII-LHCII complexes, trimeric LHCII, several core complexes, and a number of small complexes(Allen and Staehelin, 1991 ; Lee *et al.*, 1997).

Leaf senescence can be controlled by the application of plant hormones that affect the transport of available nutrients and photosynthetic products or alternatively by the induction of a direct effect on the turnover of Chl(Patrick and Muligan, 1989). Abscisic acid(ABA) and ethylene are known to stimulate or accelerate leaf senescence(Gold thwaite, 1987). Particularly, ABA strongly promotes the senescence of detached maize leaves grown in dark conditions(Hung and Kao, 1997). Gespstein and Thimann(1980) also reported that ABA is an endogenous and exogenous factor in stimulating the senescence of oat leaves. Recently, many investigators have studied the effect, action, and signalling of ABA in senescing leaves(Giraudat *et al.*, 1994). The relative importance of the effect of ABA, as an essential mediator in triggering plant responses to various environmental stresses, on the disassembly of Chl-protein complexes versus senescence processes has not yet been addressed. The main objectives of the present study were (a) to identify the changes in the Chl-protein complexes during the senescence of detached *Zea mays* leaves, and (b) to investigate the effect of ABA on the changes in the Chl-protein complexes in the senescing leaves.

2. Materials and Methods

2.1. Plant material and growth conditions

The maize(*Zea mays* L.) was grown in a pot containing a mixture of commercial soil and vermiculite(1 : 1, v/v) for 10 days as described in Lee *et al.*(1997). The environmental conditions in the growth chamber were a 25/18 ℃(day/night) temperature, 70 % humidity, and light intensity of 200 μ mol m^{-2} sec^{-1} with an 18 h photoperiod. The detached leaves of 10 day-old seedlings were floated in a 3 mM MES(2-(N-morpholine) ethanesulfonic acid) buffer(pH 5.8) with or without ABA and then kept in dark conditions for 7 days. The control was defined as detached maize leaves incubated in a 3 mM MES solution(pH 5.8) without plant hormones in dark conditions. The ABA concentration was determined as 3 μ M, which was selected as the optimal concentration based on the results on the Chl contents of detached leaves treated with various concentrations of ABA for 3 days(Table 1).

2.2. Thylakoid membrane isolation

The detached leaves were homogenized with a Waring blender in a 50 mM HEPES buffer(pH 7.6) containing 0.3 M sorbitol, 10 mM NaCl, and 5 mM $MgCl_2$. The homogenate was strained through four layers of cheesecloth and centrifuged at 350 g for 10 min. The supernatant was centrifuged at 5,000 g for 10 min. The pellet of thylakoid membrane was washed two times in a 50 mM HEPES buffer(pH 7.6) containing 0.1 M sorbitol, 10 mM NaCl, and 5 mM $MgCl_2$. The washed membrane was then resuspended in a small volume of the same buffer with 10 % glycerol, aliquoted, and frozen at -80 ℃. All operations were performed at 4 ℃.

Table 1. Changes of chlorophyll contents in detached maize leaves treated with various concentrations(1, 3 and 9 μM) of ABA for 3 days (μg Chl/g fr. wt.)

Days after excision	ABA concentration		
	1 μM	3 μM	9 μM
0	705.4	705.4	705.4
1	696.0	685.8	694.4
2	560.4	520.6	587.1
3	521.0	474.1	527.5

2.3. Thylakoid membrane solubilization

The Thylakoid membrane solubilization was based on the method developed by Allen and The Staehelin(1991). Thylakoid membrane was washed two times in a 2 mM Tris-maleate buffer(pH 7.0), then the washed membrane was resuspended in a solubilization buffer(2 mM Tris-maleate, pH 7.0, 10 % glycerol, 0.45 % octylglucoside, 0.45 % decylmaltoside, and 0.1% lithium dodecyl sulfate(LDS)). The ratio of total nonionic detergent to Chl was 20 : 1(w/w). The solubilized membranes were incubated on ice for 30 min and centrifuged at 15,000g for 10 min to remove any insoluble materials.

2.4. Native green gel electrophoresis

Equal amounts(13.5 μg) of Chl from the detached leaves were subjected to discontinuous polyacrylamide gel electrophoresis(PAGE) under nondenaturing and nonreducing conditions, essentially as described by Allen and Staehelin(1991). The amount of Chl was determined with an 80 % acetone solution according to the method of Lichtenthaler(1987). The native green gel supported by 10 % glycerol consisted of a stacking gel containing 5 % acrylamide, 25 mM Tris-HCl(pH 6.3), and 50 mM glycine, along with a separating gel containing 8 % acrylamide, 25 mM Tris-HCl(pH 8.3), and 50 mM glycine. The electrode buffer consisted of 25 mM Tris-HCl(pH 8.3), 192 mM glycine, and 0.1 % sodium dodecyl sulfate(SDS). The electrophoretic separation was performed at 4 ℃ for 3 h with a constant current of 10 mA per gel. After completing the electrophoresis the gels were scanned using a TLC scanner(Shimadzu, CS-930, Japan). The measuring wavelength of the densitometer was 676 mm. The peak areas for each green band in the lane were measured and represented as a percentage of the total Chls in the Chl-protein complexes. The values for each green band were the average of three independent experiments.

2.5. Denaturing SDS-PAGE

For a two-dimensional gel electrophoresis, gel slices were excised from the native green gel lanes, and equilibrated in a solution of 25 mM Tris-HCl, pH 6.3, 50 mM glycine, 2% SDS, 2% β-mercaptoethanol, and 10% glycerol for 15 min at 55℃. The gel slices equilibrated in the solution were then loaded directly onto a 12% SDS-PAGE gel. The electrophoretic separation was performed as described by Laemmli(1970). After completing the electrophoresis, the gels were stained with silver salts.

3. RESULTS

3.1. Biochemical changes of chlorophylls during leaf senescence

The total Chl content changes in detached maize leaves kept in dark conditions for 7 days are shown in Fig. 1A. There was a gradual decrease in the Chl content during the senescence period. The total Chl content after 7 days decreased by about 70% of the initial value. To examine whether or not ABA is a negative regulator during leaf senescence, the Chl contents of leaves treated with ABA for 7 days were investigated. The exogenous application of ABA induced a rapid decline in the Chl content after 4 days of dark-induced senescence compared to the control, accordingly, ABA would seem to accelerate Chl degradation during the middle stage of dark-induced senescence.

An investigation of the Chl a/b ratios during leaf senescence offers fundamental information on the structural changes of thylakoids. As the detached leaves of 10 day-old maize plants progressed through senescence, the Chl a/b ratio gradually decreased from 3.97 to 2.45(Fig. 1B). The application of ABA caused a significant decrease in the Chl a/b ratio after 4 days of dark-induced senescence when compared to the control.

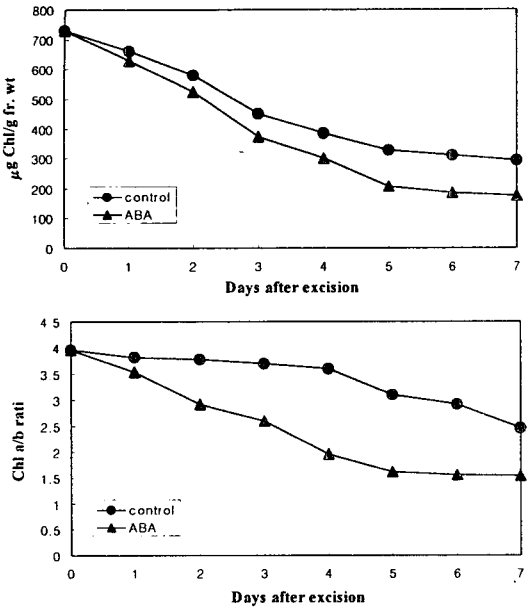

Fig. 1. Changes in Chl contents (A) and Chl a/b ratio (B) from maize leaves which were kept in the dark under dark 7 days

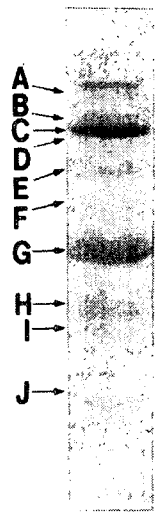

Fig. 2. Native green gel after electrophoresis of Chl-protein complexes from maize leaves. Letters on the left hand side indicate the designation of Chl-protein complexes resolved : A, RC-LHC1 ; B, RC-LHC2 ; C, RC-LHC3 ; D, CCII ; E, RC-Core1 ; F, RC-Core2 ; G, LHCII ; H, SC-1 ; I, SC-2 ; J, FP.

3.2. Relative distribution of chlorophyll-protein complexes

The main pigmented bands separated in the green gel system were classified as follows : three bands of RC-LHC(RC-LHC1, RC-LHC2, and RC-LHC3), a core complex of photosystem II (CCII), two bands of RC-Core(RC-Core1 and RC-Core2), LHCII, two bands of a small complex(SC, SC-1 and SC-2), and a free pigment(FP) (Fig. 2). The relative amount of each complex in the detached leaves were 7.71% of RC-LHC1, 9.09% of RC-LHC2, 25.10% of RC-LHC3, 7.75% of CCII, 6.97% of RC-Core1, 5.47% of RC-Core2, 17.67% of LHCII, 10.26% of SC-1, 5.44% of SC-2, and 4.54% of FP. As exhibited by the two-dimensional denaturing gel(Fig. 3), RC-LHC3, known as the main PSI complex, consisted of P700 and LHCI apoproteins, CCII contained CP47/43 and D1/D2 apoproteins, RC-Core2 contained a number of CP47/43 and D1/2 apoproteins, RC-Core1 consisted of mainly a PSII reaction center, LHCII was shown as a trimetic form of the light-harvesting antenna of PSII, the SC-1 region contained a number of small complexes of PSII, and SC-2 mainly consisted of monomeric LHCII apoproteins.

3.3. Disassembly of chlorophyll-protein complexes during dark-induced senescence

The data in Fig. 4 illustrate the changes in the relative distribution of the Chl-protein complexes in the detached maize plant leaves incubated in the dark for 7 days. Of the main PSI complexes, RC-LHC3 gradually decreased for 5 days, thereafter, there was a rapid decline in the late stage of the dark-induced senescence. The pattern of RC-LHC1 disassembly was similar to that of RC-LHC3 disassembly. CCII remained in the native green gel until late in the dark-induced senescence. RC-Core1 remained in the native green gel for 5 days of senescence, thereafter, very little was detected in the gel. In contrast, RC-Core2 increased in the relative distribution of Chl-protein complexes in the late stage of senescence. LHCII gradually decreased during the dark-induced senescence, whereas SC-2 showed a steady increase. The pattern of SC-1 disassembly was similar to that of LHCII disassembly. For a detailed analysis of the thylakoid polypeptide composition, the green gel

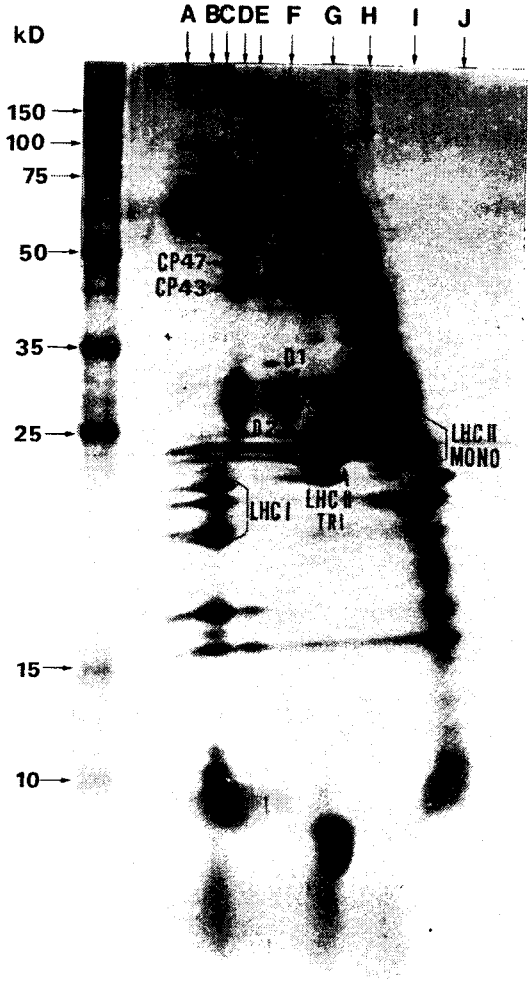

Fig. 3. Two dimensional gel in which maize Chl-protein complexes have been separated on a native green gel in the first dimension, and on a fully denaturing SDS-PAGE gel in the second dimension. Letters on the top indicate the designation of Chl-protein complexes resolved. A, RC-LHC1 ; B, RC-LHC2 ; C, RC-LHC3 ; D, CCII ; E, RC-Core1 ; F, RC-Core2 ; G, LHCII ; H, SC-1 ; I, SC-2 ; J, FP. Silver-stained gel. P700, P700 apoproteins; CP43/CP47, internal Chl a-binding of PSII ; D1/D2, core apoproteins of PSII ; LHCII, apoproteins of LHCII.

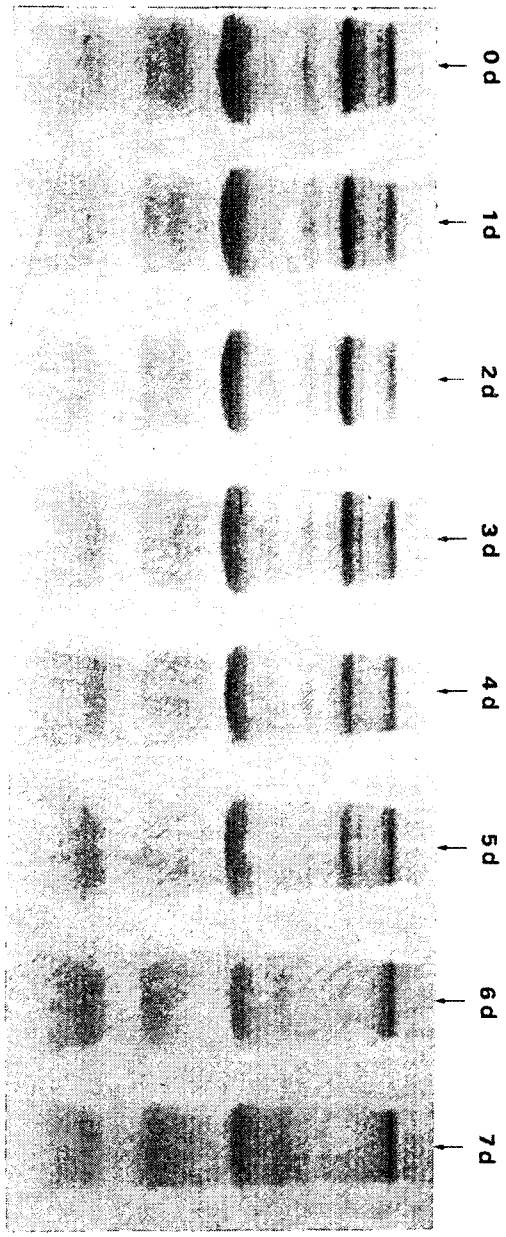

Fig. 4. Changes in the disassembly of Chl-protein complexes from maize leaves which were kept in the dark for 7 days. Thylakoid membranes were solubilized with octyl glucoside, decyl maltoside and lithium dodecyl sulfate (LDS).

lanes produced by the native green gel system were incubated in an SDS-solubilization buffer and applied to a fully denaturing gel(Figs. 5~6). Among the RC-LHC groups, the disassembly of

the reaction center including P700 apoproteins was faster than that including LHCI apoproteins during darkinduced senescence. The accumulation of core complexes such as the CP47/43 and D1/2 apoproteins disassembled from RC-Core1 during leaf senescence induced an increase of RC-Core2 in the relative distribution of Chl-protein complexes. When compared to RC-Core2, the fast disassembly of RC-Core1 was due to the decomposition of the reaction center. The disassembly of D1/D2 apoproteins was faster than that of CP47/43 apoproteins in the senescing leaves. As the gradual disassembly of trimeric LHCII progressed during the leaf senescence, there was also a steady increase in the amount of SC-2 containing monomeric LHCII.

3.4. Effect of ABA on disassembly of chlorophyll-protein complexes

As shown in the results on the Chl content and Chl a/b ratio changes, ABA was a negative regulator in the stability of Chl during leaf senescence. Accordingly, the following experiments were conducted to investigate how ABA regulates the

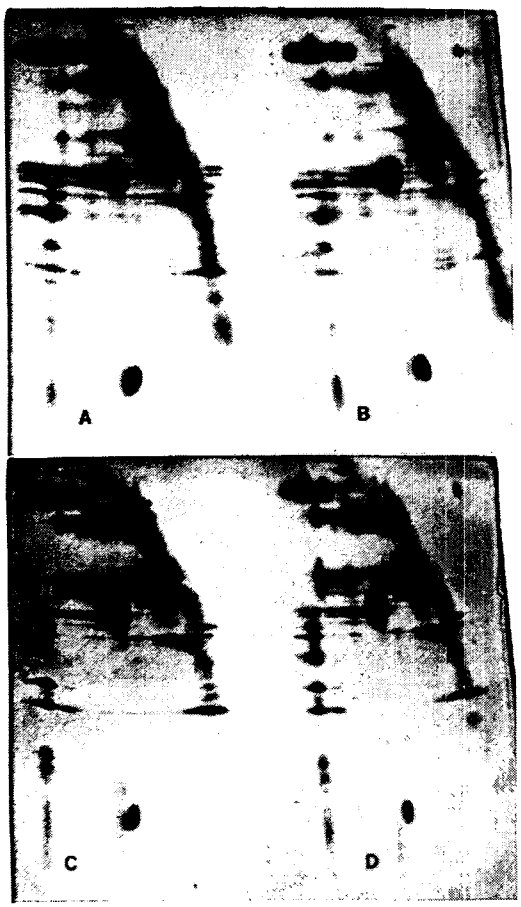

Fig. 5. Changes in the polypeptide composition of Chl-protein complexes from the detached leaves of maize plants which were kept in the dark for 0 (A), 1 (B), 2 (C) and 3 (D) days.

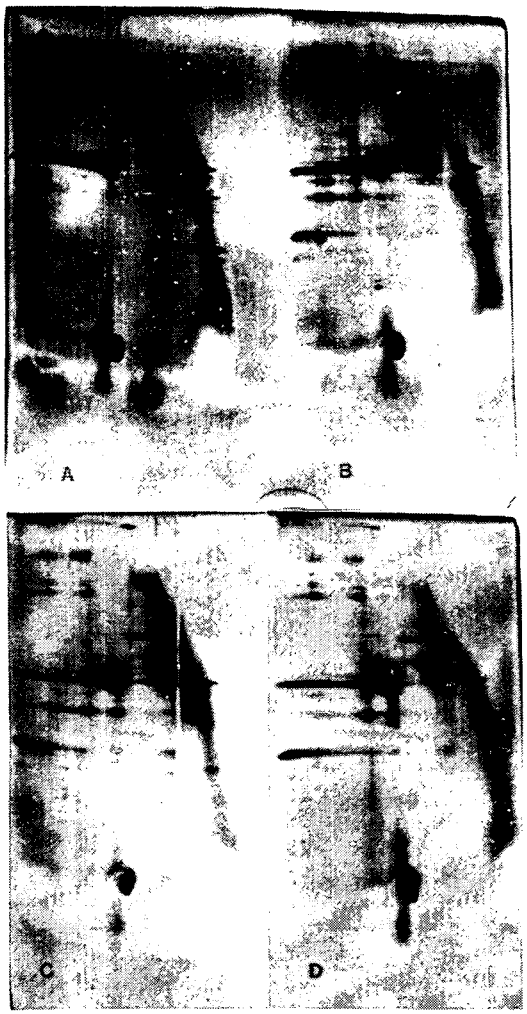

Fig. 6. Changes in the polypeptide composition of Chl-protein complexes from the detached leaves of maize plants which were kept in the dark for 4 (A), 5 (B), 6 (C) and 7 (D) days.

disassembly of Chl-protein complexes in senescing leaves(Figs. 7～9). RC-LHC3, the reaction center of PSI, gradually decreased for 3 days and, thereafter, decreased rapidly in the middle stage of the dark-induced senescence. RC-Core1 remained in the native green gel for 4 days of senescence, thereafter, very little was detected in the gel. RC-Core2 increased in the relative distribution of Chl-protein complexes in the late stage of senescence. As shown in the results of the control, LHCII gradually decreased during dark-induced senescence, whereas SC-2 showed a steady increase. Yet the levels of SC-2 accumulation in the ABA-treated maize plants were higher than those of the control plants during leaf senescence.

4. Discussion

In previous studies, Chl loss in senescing leaves has been closely connected with a decrease in the photosynthesis rate in the photosynthetic apparatus(Camp *et al.*, 1992) and this decrease in the photosynthesis rate is induced in the initial stage of leaf senescence(Wollhouse, 1984). Consistent with this hypothesis, this study has presented biochemical evidence that a good indication of leaf senescence in maize plants is the yellowing induced by the preferential degradation of Chl over carotenoid. In this experiment, the leaf yellowing started all over the senescing leaves, although in natural senescence yellowing starts at the tip of the leaf (Lohman *et al.*, 1994). These results are consistent with observations of many plant species(Lee *et al.*, 1997). To elucidate the effect of ABA during leaf senescence, the Chl contents in the detached leaves of maize plants treated with ABA for 7 days were examined. Exogenous ABA application had a considerable effect in accelerating Chl degradation in the middle stage of dark-induced senescence(Fig. 1A). A closer investigation of the foliar Chl levels in terms of the Chl a/b ratio revealed further differences in the photosynthetic apparatus during dark-induced senescence. The Chl a/b ratio of the control, which was kept in the dark, gradually reduced until the middle stage of leaf senescence, thereafter, it declined rapidly(Fig. 1B). Previous reports have found that the Chl a/b ratio steadily increases with the progress of senescence in some plant species(Lee *et al.*, 1997 ; Oh and Lee, 1996). The results of this study indicate a preferential retention of Chl b over Chl a in the photosynthetic apparatus during dark-induced senescence. Several proteins in the core PSI and PSII complexes bind Chl a, yet Chl b is only found in the peripheral LHCs. Therefore, these results suggest that the reaction center and core complexes, which mainly consist of Chl a, are considerably disassembled in the senescing leaves of maize plants, whereas LHCs, which are rich in Chl b, are hardly degraded. These results are similar to those found with *Lolium temulentum* and soybeans (Guiamet *et al.*, 1991 ; Mae *et al.*, 1993). The changes in the Chl a/b ratio during leaf senescence appear to be the adaptive response of the leaves to a changing environment. In contrast, ABA caused a significant decrease in the Chl a/b ratio in the middle stage of dark-induced senescence(Fig. 1), suggesting that ABA is effective in promoting the degradation of the Chl a-binding proteins of PSI and PSII including the reaction center and core complexes.

The biochemical changes of macromolecules such as Chls during leaf senescence can induce chloroplast dismantling, particularly, the changes in the relative distribution of Chl between Chl-protein complexes in senescent leaves. The relative amount of each complex in the detached leaves of 10 day-old maize plants according to the fractionation of the Chl-protein complexes using the native green gel system were 7.71% of RC-LHC1, 9.09% of RC-LHC2, 25.10% of RC-LHC3, 7.75% of CCII, 6.97% of RC-Core1, 5.47% of RC-Core2, 17.67% of LHCII, 10.26% of SC-1, 5.44% of SC-2, and 4.54% of FP(Fig. 2). The green band patterns in the native gels were similar to those previously reported by other researchers using *Arabidoposis, Chlamidomonas,* and *Phaseolus vulgaris*(Allen and Staehelin, 1991 ; Peter and Thornber, 1991 ; Lee *et al.*, 1997). From a detailed analysis of the Chl-protein complex polypeptide composition in the fully denaturing gel system(Fig. 3), the RC-LHC regions appeared to be associated with PSI, whereas the RC-Core groups, CCII, LHCII, and SC groups appeared to be related to PSII. The P700 apoprotein in the reaction center of PSI and LHCI apoprotein were the main components of RC-LHC. The CP47/43 apoproteins of the PSII core and D1/D2 apoproteins of the PSII reaction

center were also represented in the RC-Core and CCII regions. RC-Core1 appeared to consist of mainly the dimer of the D1/D2 apoproteins. Based on the native green gel results in the present study, LHCII, represented as the trimeric form of the light-harvesting antenna of PSII, was composed of only one type of LHCII, although the trimeric LHCII complex is known consist of at least three types of LHCII(Allen and Stahelin, 1991). These results are consistent with observations using barley and oat seedlings(Dreyfuss and Thornber, 1994 ; Lee *et al.*, 1996). A number of small complexes which appeared to be partially dissociated PSII components such as oxygen evolving complexes were easily seen in the SC-1 region, whereas monomeric LHCII apoproteins were the main component of SC-2.

The disassembly status of the various Chl-protein complexes was easily tracked throughout the dark-induced senescence of maize plants during 7 days(Figs. 4～6). RC-LHC3 as the main PSI complex gradually declined for 5 days and, thereafter, declined rapidly in the late stage of dark-induced senescence. There was a gradual disassembly of RC-LHC1 throughout leaf senescence as with RC-LHC3. As shown in Figs. 5 and 6, during dark-induced senescence, the disassembly of the RC-LHC group reaction centers including P700 apoproteins with mainly Chl a were faster than the reaction centers including LHCI apoproteins with both Chl a and Chl b. When compared to RC-Core2, the fast disassembly of RC-Core1 was due to the decomposition of the PSII reaction center. The increase of RC-Core2 in the relative distribution of Chl-protein complexes in the late stage of senescence appeared to be due to the accumulation of core complexes such as CP47/43, and reaction centers including D1/D2 apoproteins disassembled from RC-Core1 containing the dimer of D1/D2 apoproteins. Accordingly, it would appear that the disassembly of the reaction centers may be faster than that of the core complexes during leaf senescence. The results on the relative increase of RC-Core2 during the middle stage of leaf senescence are similar to other observations using oat and barley seedlings where the levels of the core complexes associated with PSII increase during the early stage of greening and gradually decrease thereafter(Dreyfuss and Thornber, 1994 ; Lee *et al.*, 1996). The LHCII region was stable throughout leaf senescence compared to the other Chl-protein complexes which also conforms with previous reports that state that the disassembly of the LHCII apoprotein is delayed until later in the leaf senescence of *Lolium tumelentum*(Mae *et al.*, 1993). In contrast, the monomeric LHCII accumulation during leaf senescence appeared to be due to the disassembly of trimeric LHCII. Accordingly, it would appear that the LHCII trimer is reorganized into a pigmented monomeric LHCII in the thylakoid membranes as a stable form during dark-induced senescence. Based on the changes observed in the Chl a/b ratio and the disassembly of Chl-protein complexes during dark-induced senescence, it is suggested that during dark-induced senescence the preferential breakdown of Chl a gives rise to the disassembly of Chl a-binding proteins, particularly, the reaction centers and core complexes, plus the primary target of the photosynthetic apparatus in senescing leaves seems to be Chl a and the proteins associated with Chl a.

The plant hormone ABA, which indigenously increases when tissue dehydrates, is an important signal for physiological and molecular responses to water-limiting stress, such as senescence, desiccation, and environmental stress(Hetherington and Quatrano, 1991 ; Moons *et al.*, 1997). As shown in the results of the Chl content and Chl a/b ratio changes, ABA was a negative regulator in the stability of Chl during leaf senescence, as previously established(Thiamann, 1980). ABA can play an important role in the dismantling of chloroplast, and investigations on the effect of ABA on chloroplast dismantling can reveal the regulatory mechanism of senescing leaves for the structural changes in the photosynthetic apparatus. As a result, the effect of ABA on the changes in the Chl-protein complexes during dark-induced senescence was studied(Figs. 7～9). A gradual disassembly of RC-LHC3 occurred for 3 days, thereafter, a rapid disassembly occurred during the middle stage of dark-induced senescence. The level of RC-Core1 was hardly changed during the first 4 days of senescence, thereafter it decreased rapidly. These results indicate that, when compared to the control, ABA application may accelerate the disassembly of the P700 apoproteins in the PSI reaction center and the dimer of the D1/D2 apoproteins during

the middle stage of leaf senescence. The increase of the RC-Core2 region in the relative distribution of Chl-protein complexes in the late stage of senescence appeared to be due to the decomposition of the PSII reaction center as shown in the results of the control plants. The levels of SC-2 accumulation in the ABA-treated maize plants were higher than those of the control plants during leaf senescence, suggesting that ABA accelerates the conversion of the trimeric LHCII apoprotein to the monomeric LHCII apoprotein during dark-induced senescence. Accordingly, these results indicate that ABA was effective in promoting the disassembly of the Chl a-binding and Chl a+b-binding proteins, and particularly the Chl a-binding proteins during the middle stage of leaf senescence. Further studies are needed to elucidate the molecular mechanism that facilitates the structural changes of chloroplast during dark-induced senescence.

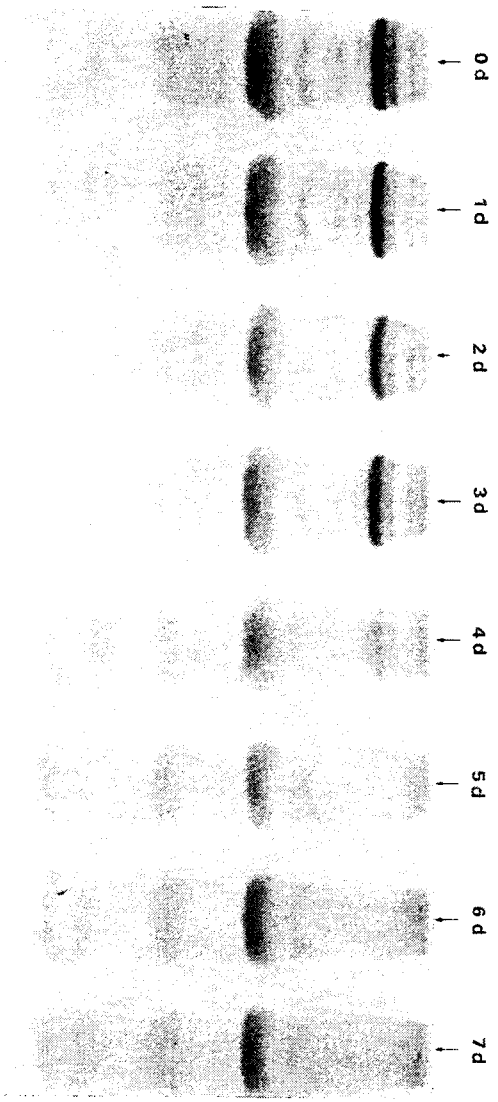

Fig. 7. Changes in the disassembly of Chl-protein complexes from the detached leaves of ABA-treated maize plants which were kept in the dark for 7 days. Thylakoid membranes were solubilized with octyl glucoside, decyl maltoside and lithium dodecyl sulfate(LDS).

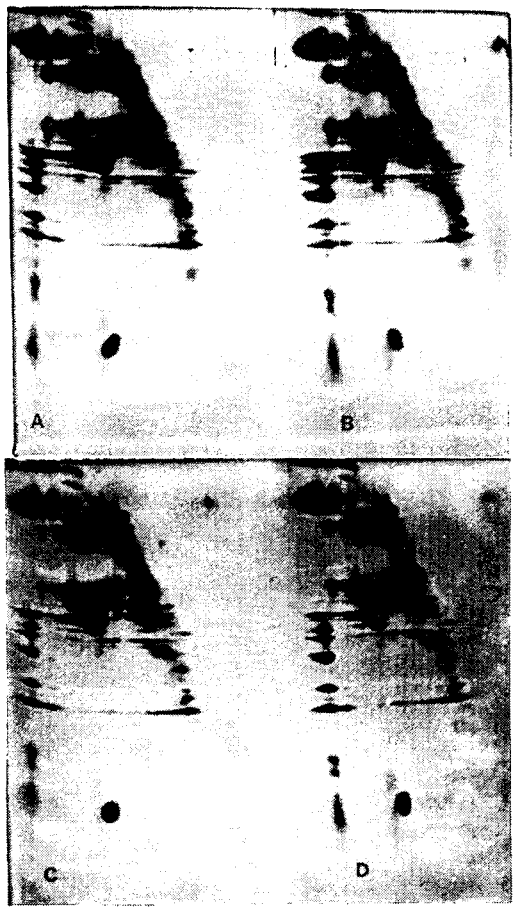

Fig. 8. Changes in the polypeptide composition of Chl-protein complexes from the detached leaves of ABA-treated maize plants which were kept in the dark for 0 (A), 1 (B), 2 (C) and 3 (D) days.

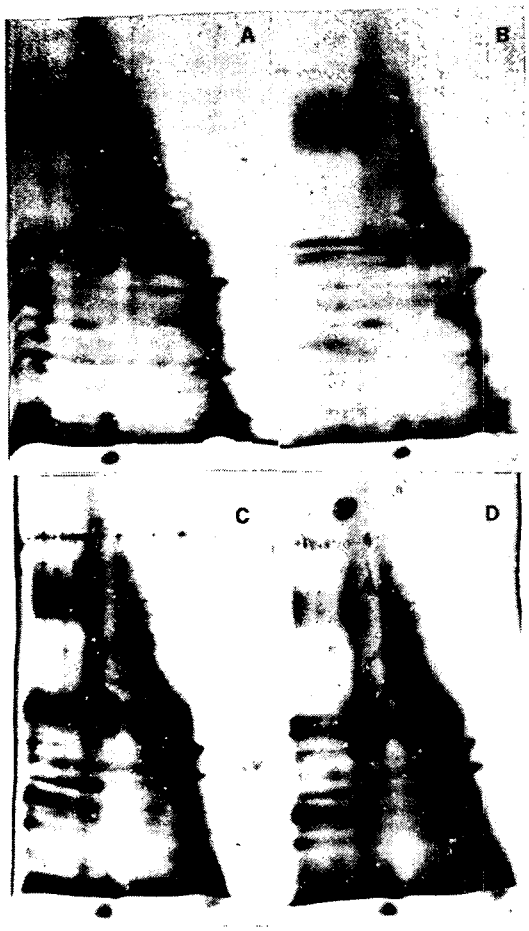

Fig. 9. Changes in the polypeptide composition of Chl-protein complexes from the detached leaves of ABA-treated maize plants which were kept in the dark for 4 (A), 5 (B), 6 (C) and 7 (D) days.

Acknowledgements

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