생명과학회지
Korean J. Life Science, 7(4), 297-308, 1997

Role of Plant Hormones in the Senescing Detached Leaves of *Phaseolus vulgaris*

Dong-Hee Lee, Young-Hee Seo and Young-Sang Kim†

Dept. of Biology, College of Natural Sciences, Pusan National University, Pusan 609-735, Korea

Abstract

Role of plant hormones on the leaf senescence of *Phaseolus vulgaris* were investigated by measuring the disassembly of chlorophyll-protein complexes in detached leaves treated with NAA, GA_3 or BA. The loss of chlorophyll that was characteristic of leaf senescence induced disassembly of chlorophyll-protein complexes. During dark-induced senescence, PSI complex was rapidly degraded after the early stage, whereas RC-Core3 was slightly increased until the middle stage and slowly decreased thereafter. And gradual degradation of trimeric LHCII progressed after the late stage of senescence. Exogenous applications of NAA and GA_3 had little or no effect in protecting disassembly of chlorophyll-protein complexes during leaf senescence compared to control. However exogenous BA application strongly delayed the disassembly of chlorophyll-protein complexes, particularly RC-Core2, RC-Core3 and SC-1 in senescing leaves. In the simultaneous treatment of plant hormones and light, BA application under illumination of light was most effective in the stability of chlorophyll-protein complexes, particularly PSI, LHCII, RC-Core2, RC-Core3 and SC-1. These results suggest, therefore, that simultaneous application of BA and light induced synergistic effect on the stability of chlorophyll-protein complexes during leaf senescence.

Key words : *senescence, chlorophyll-protein complexes, Phaseolus vulgaris, NAA, GA_3, BA*

Introduction

One of the most conspicuous changes shown in senescing leaves is the degradation of chlorophyll and consequent yellowing. The loss of chlorophyll during leaf senescence induces the decrease of photosynthesis rate as well as the disassembly of chloroplast[1,2,3,4]. The disassembly of chloroplast may be associated with the structural changes of thylakoid membranes induced by leaf senescence. Of particular interest is the fact that the synthesis of the majority of thylakoid-associated proteins in bean leaves, such as the 68 kD apoprotein of PSI, ATPase, light-harvesting chlorophyll-binding protein, and cytochrome *f* and *b6*, is given rise to about two and four-fold decrease in senescing leaves, whereas the synthesis of the D1 protein of PSII is not affected by senescence[5,6]. Also recent studies suggest that leaf senescence causes the degradation of PSII proteins extrinsic to thylakoid membranes, such as the 33 kD protein of oxygen-evolving complex in the leaves of *Festuca pratensis*[7]. In a normal cultivar and a nonyellowing mutant of *Festuca pratensis* turnover of various proteins and chlorophyll-

† Corresponding author

binding apoproteins associated with thylakoid membranes is induced by leaf senescence[8,9,10,11]. However, the studies on the disassembly of chlorophyll-protein complexes during leaf senescence are not fully understood, though there are many studies on the structural changes of thylakoid membranes of senescing leaves.

Factors influencing the initiation of senescence include plant hormones, light, nutrients, environmental stress, such as extremes of temperature and water, and invasion by pathogen[12,13]. Particularly application of plant hormones may influence leaf senescence by affecting the transport of available nutrients and photosynthetic products, or alternatively may exhibit more direct effect on chlorophyll turnover[14,15]. There are two types of evidence for the role of cytokinin in the control of senescence. One is external application of cytokinin which causes dramatic senescence retardation in the detached leaves[16,17]. The other evidence is that the decrease of endogenous cytokinin levels may induce foliar senescence [18,19]. Gibberellic acid has also been proposed to delay leaf yellowing in cut flowering branches of *Alstroemeria pelegrina*[17,20].

However, very little information has been published on the disassembly of chlorophyll-protein complexes affected by plant hormones, such as, auxin, gibberellin and cytokinin. Also, the overlapping role of plant hormones and light during leaf senescence gives rise to the interesting questions of whether plant hormones and light act independently to affect senescence or whether plant hormones are correlated with light in senescence progress. Therefore, in order to clarify the effect of plant hormones under dark or light condition on the disassembly of chlorophyll-protein complexes in the detached leaves of *Phaseolus vulgaris*, we describe the physiological changes of chlorophylls and the disassembly of chlorophyll-protein complexes in the detached leaves of bean plants treated with plant hormones under dark condition or light condition.

MATERIALS AND METHODS

Plant material

The bean seeds (*Phaseolus vulgaris* L.) were soaked in running tap water for 4 h and planted on the pot, and grown in growth chamber at 25℃/18℃ (light/dark) temperature cycle under 18 h of light /6 h of dark regime condition with 70% humidity for 3 weeks. And the leaves of 21 day-old plants were excised by razor blades and the detached leaves, grown in hydroponically in 3 mM MES (2-(N-morpholine) ethanesulfonic acid) buffer (pH 5.6) with or without plant hormones under dark condition or white light condition for 12 days, were used as the experimental materials. Experimental system in which detached leaves are used will offer excellent results because of the relative ease of manipulating the experimental protocol compared to the complexity using attached leaves, and there is a remarkable variability in experimental results in attached leaves because of the uncertainty of the exact time of onset senescence[10,21].

The concentrations of NAA (napthaleneacetic acid), GA_3 (gibberellic acid) and BA (benzyladenine) were ascertained to be 5×10^{-3}, 5×10^{-2} and 5 μM, respectively, which were chosen for optimal concentration from the previous report described in Lee *et al.* (1995)[22].

Thylakoid membrane isolation

For the isolation of thylakoid membranes, the detached leaves were homogenized with Waring blender in homogenization buffer consisted of 50 mM HEPES (pH 7.6), 0.3 M sorbitol, 10 mM NaCl and 5 mM $MgCl_2$. To remove cellular debris the homogenate was filtered through four layers of cheesecloth, and the filterate was centrifuged at 350 g for 10 min. The supernatant was pelleted at 5,000 g for 10 min. The membrane pellet was washed twice in washing buffer consisted of 50 mM HEPES (pH 7.6), 0.1 M sorbitol, 10 mM NaCl and 5 mM $MgCl_2$. The resulting pellet was resuspended in

a small volume of the same buffer with 10% glycerol, aliquoted and frozen at −80℃. All procedures of thylakoid membrane isolation were performed at −4℃.

Thylakoid membranes solubilization

For native green gel electrophoresis thylakoid membranes was washed twice in 2 mM Tris-maleate (pH 7.0), and the washed pellet was resuspended in solubilization buffer containing 2 mM Tris-maleate (pH 7.0), 10% glycerol, 0.45% octyl glucoside, 0.45% decyl maltoside, and 0.1% LDS (lithium dodecyl sulfate). Solubilization buffer was added to give a ratio of total nonionic detergent to chlorophyll of 20 : 1 (w/w). Samples were incubated on ice for 30 min and centrifuged at 15,000 g for 10 min to remove insoluble materials.

Native green gel electrophoresis

Native green gel was consisted of a stacking gel containing 5% acrylamide, 25 mM Tris-HCl (pH 6.3), 50 mM glycine and 10% glycerol, and a resolving gel containing 8% acrylamide, 25 mM Tris-HCl (pH 8.3), 50 mM glycine and 10% glycerol. The acrylamide/bisacrylamide ratio was 100 : 1. The gel was polymerized by adding 0.05% TEMED (tetramethylethylenediamine). The electrode buffer contained 25 mM Tris (pH 8.3), 192 mM glycine and 0.1% SDS (sodium dodecyl sulfate) as described by Allen and Staehelin (1991)[23]. After the gel was prerun at 10 mA for 1 h in cold chambers, samples (about 13.5 μg chlorophyll) were loaded onto the gel. The amount of chlorophyll was determined with spectrophotometer (Shimadzu, UV 240, Japan) according to the method of Lichtenthaler (1987)[25]. The gel was electrophorized at 10 mM constant current, 4℃ for 3 h.

Denaturing SDS-PAGE

For two-dimensional gels, gel slices were excised from native green gel lanes, incubated for 15 min at 55℃ in solubilization solution containing 25 mM Tris-HCl (pH 6.3), 50 mM glycine, 2% SDS, 2% β-mercaptoethanol, and 10% glycerol. Gel slices treated with the solution were loaded directly onto the gel of 12% polyacrylamide. PAGE (polyacrylamide gel electrophoresis) in the presence of SDS was performed as described by Laemmli (1970)[25].

Densitometry scanning

Gel lanes of a native green gel were scanned using a TLC scanner (Shimadzu, CS-930, Japan). The measuring wavelength of the densitometer was 675 nm. Peak areas for each green band in the lane was measured and represented as a percentage of the total chlorophyll in chlorophyll-protein complexes excluding free pigment. The values for each green band were the average of independent three experiments.

RESULTS

Changes in the contents of chlorophyll

To characterize the senescence process of the detached leaves we determined the changes of chlorophyll contents under different experimental conditions. Changes in the contents of chlorophyll from the detached leaves of *Pheseolus vulgaris* which were kept in the dark or light condition during leaf senescence were shown in Fig. 1 (see Lee *et al.*, 1997)[26]. Control was defined as the leaves of bean plants grown hydroponically in 3 mM MES solution without plant hormones under the darkness or light. Leaves of bean plants that were placed in the darkness showed gradual loss of chlorophyll along with senescence period, thus the decrease in chlorophyll content of the control under darkness on 12 d was 66% of the initial value. The illumination of light was significantly effect in the suppressing of chlorophyll loss during leaf senescence when compared to the dark. To examine whether or not plant hormone is regulatory factor during leaf senescence, chlorophyll contents of the leaves, which had been treated with plant hormones for

12 d, were investigated. The changes of chlorophyll contents in the NAA-treated leaves during leaf senescence was similar to those in the control (Fig. 1 and Table 1). The chlorophyll content of GA_3-treated leaves was decreased during leaf senescence when compared to those in the control (Fig. 1 and Table 2). However, exogenous BA application was more effective in protecting the degradation of chlorophyll of senescing leaves than the control, particularly BA application under illumination of light strongly retarded the chlorophyll breakdown of senescing leaves (Fig. 1 and Table 3). These results suggest that exogenous BA application induces synergistic effect on the suppression of chlorophyll breakdown, particularly the reciprocal effect of BA and light may strongly retard the degradation of chlorophyll during leaf senescence.

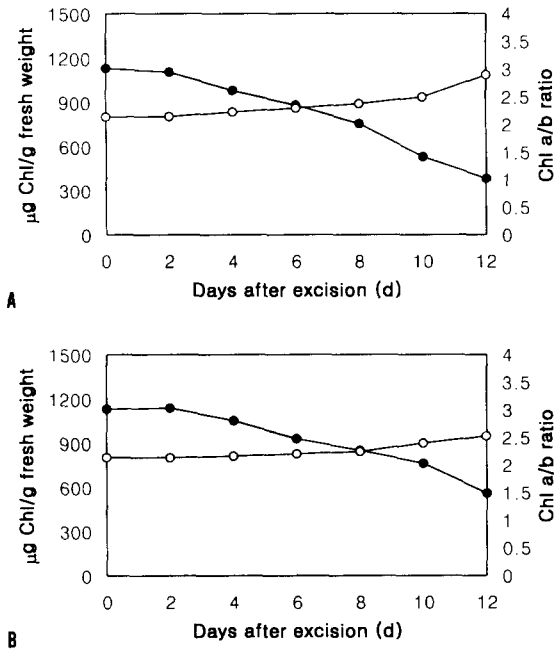

Fig. 1. Changes in the contents of chlorophyll and Chl a/b ratios from the detached leaves of *Phaseolus vulgris* which were kept in the dark (A) or light condition (B) for 12 d. The values are the average of three independent expreiments. —●—, total Chl ; —○—, Chl a/b ratio.

Chl a/b ratio of the control which was kept in the dark did not changes significantly during 8 d of dark-induced senescence, and then the leaves showed remarkable increase of Chl a/b ratio upon prolonged incubation in the dark. Chl a/b ratio of leaves illuminated with light was not significantly altered during leaf senescence when compared to those which were kept in the dark (Fig. 1). Exogenous BA application caused the stability of Chl a/b ratio during leaf senescence than control, NAA and GA_3 (Fig. 1 and Tables 1~3). The stability of Chl a/b ratio during leaf senescence may be due to the retardation in the breakdown of light-harvesting chlorophyll complexes containing both Chl a and Chl b.

Effect of light on the disassembly of chlorophyll-protein complexes

The changes of chlorophyll contents and Chl a/b ratios may induce the structural changes of thylakoid components. The following experiments, therefore, conducted to elucidate how plant hormones and light regulate the disassembly of chlorophyll-protein complexes during leaf senescence. The bean thylakoids solubilized with nonionic detergent/chlorophyll weight of 20 : 1 were separated to ten main pigmented bands in the native green gel system to be nomenclatured as follow . two bands of RC(reaction center)-LHC, three bands of RC-Core (RC-Core1, 2, and 3), LHCII, three bands of SC (small complexes, two bands of SC-1 and SC-2) and FP (free pigment). In present study, PSI complex was main band in two bands of RC-LHC in the green gel, therefore we designated RC-LHC complex as PSI complex. The band pattern was obtained in the previous study described in Lee *et al.* (1997)[26]. We could observe 4 major changes in the relative distribution of chlorophyll-protein complexes during dark induced senescence (Fig. 2) : (1) PSI complex was remarkably decreased after 2 d, (2) RC-Core1 and 2 was rapidly degraded at the early stage of leaf senescence, (3) RC-Core 3 was slightly

Table 1. Changes in the contents of chlorophyll and Chl a/b ratios from the detached leaves of *Phaseolus vulgris* treated with NAA under dark or light condition

μg Chl/g fresh weight

days after excision (d)		chlorophyll a	chlorophyll b	chlorophyll a/b	total chlorophyll
Dark	0	771.5[a)]	360.5	2.14	1132
	2	764.2	353.8	2.16	1118
	4	687.9	303.1	2.27	991
	6	609.3	262.7	2.32	872
	8	494.1	205.9	2.40	700
	10	406.6	153.4	2.65	560
	12	300.7	97.3	3.09	398
Light	0	771.5	360.5	2.14	1132
	2	769.2	357.8	2.15	1127
	4	747.9	343.1	2.18	1091
	6	648.8	296.2	2.19	945
	8	582.6	254.4	2.29	837
	10	546.5	233.5	2.34	780
	12	404.5	160.5	2.52	565

[a)]The values are the average of three independent experiments.

Table 2. Changes in the contents of chlorophyll and Chl a/b ratios from the detached leaves of *Phaseolus vulgris* treated with GA_3 under dark or light condition

μg Chl/g fresh weight

days after excision (d)		Chl a	Chl b	Chl a/g	Total Chl
Dark	0	771.5[a)]	360.5	2.14	1132
	2	741.4	341.6	2.17	1083
	4	636.5	275.5	2.31	912
	6	570.4	238.6	2.39	809
	8	463.7	189.3	2.45	653
	10	348.4	123.6	2.82	472
	12	224.9	68.1	3.30	293
Light	0	771.5	360.5	2.14	1132
	2	722.8	336.2	2.15	1059
	4	681.9	303.1	2.25	985
	6	621.7	270.3	2.30	892
	8	554.9	233.1	2.38	788
	10	494.7	200.3	2.47	695
	12	377.0	130.0	2.90	507

[a)]The values are the average of three independent experiments.

Table 3. Changes in the contents of chlorophyll and Chl a/b ratios from the detached leaves of *Phaseolus vulgris* treated with BA under dark or light condition

μg Chl/g fresh weight

days after excision (d)		Chl a	Chl b	Chl	Total Chl
Dark	0	771.5[a)]	360.5	2.14	1132
	2	770.1	359.9	2.14	1130
	4	689.6	313.4	2.20	1003
	6	630.8	284.2	2.22	915
	8	561.7	245.3	2.29	807
	10	442.4	180.6	2.45	623
	12	365.4	141.6	2.58	507
Light	0	771.5	360.5	2.14	1132
	2	775.6	362.4	2.14	1138
	4	758.9	348.5	2.18	1107
	6	676.2	308.8	2.19	985
	8	615.0	277.0	2.22	892
	10	559.8	240.2	2.33	800
	12	538.4	221.6	2.43	760

[a)]The values are the average of three independent experiments.

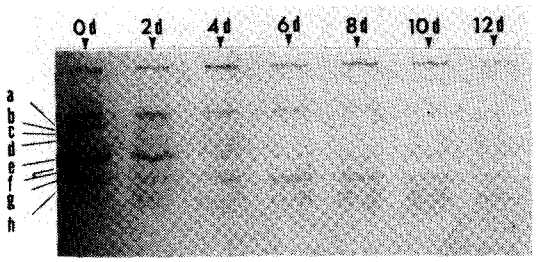

Fig. 2. Changes in the disassembly of chlorophyll-protein complexes from *Phaseolus vulgris* which were kept in the dark for 12 d. Thylakoid membranes were solubilized with octyl glucoside, decyl matoside and lithium dodecyl sulfate. Letters on the left-hand side indicate the designation of chlorophyll-protein complexes resolved : a, RC-LHC ; b, RC-Core1 ; c, RC-Core2 ; d, RC-Core3 ; e, LHCII ; f, SC-1 ; g, SC-2 ; h, FP.

increased until 4 d and slowly decreased thereafter, and (4) as the gradual decrease of LHCII progressed at the late stage of senescence, there was a steady increase in the amount of SC-2. White light was generally effect in the structural stability of chlorophyll-protein complexes of senescing leaves when compared to the darkness. Particularly the degradation of PSI and RC-Core3 was suppressed by the illumination of light. These results agree with the notion using the leaves of *Lolium tumelentum*[27].

Effect of plant hormones on the disassembly of chlorophyll-protein complexes

To elucidate effect of plant hormones on the disassembly of chlorophyll-protein complexes during leaf senescence, the changes of relative distribution of chlorophyll-protein complexes in the detached leaves treated with plant hormones for 12 d were investigated (Tables 5~7). The leaves treated with NAA under dark condition was little changes in the relative distribution of chlorophyll-protein complexes of senescing leaves when compared to control which was kept in the dark (Tables 4 and 5). The disassembly of chlorophyll-protein complexes in the detached leaves treated with GA_3 was faster to some extent than control (Tables 4 and 6). Particularly, the leaves treated with GA_3 showed decr-

Table 4. Changes in the disassembly of chlorophyll-protein complexes from *Phaseolus vulgris* which were kept in the dark or light condition for 12 d

days after excision (d)		Relative amount of chlorophyll in chlorophyll-protein complexes						
		PSI	RC-Core1	RC-Core2	RC-Core3	LHCII	SC-1	SC-2
Dark	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	8.6	–	5.7	5.3	39.6	17.6	23.2
	4	3.3	–	–	9.8	34.5	1.6	50.3
	6	1.8	–	–	8.6	33.9	0.6	55.1
	8	–	–	–	1.6	32.9	–	65.5
	10	–	–	–	2.0	35.4	–	62.6
	12	–	–	–	–	34.0	–	66.0
Light	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	11.8	1.2	4.9	3.5	45.2	15.2	18.2
	4	8.5	–	7.1	4.8	47.6	4.8	27.2
	6	6.0	–	6.5	16.4	40.4	4.3	26.4
	8	3.9	–	–	2.2	50.9	3.9	39.1
	10	2.0	–	–	2.9	29.7	3.7	61.7
	12	–	–	–	–	37.5	–	62.5

Table 5. Changes in the disassembly of chlorophyll-protein comlexes from *Phaseolus vugris* treated with NAA under dark or light condition for 12 d

days after excision (d)		Relative amount of chlorophyll in chlorophyll-protein complexes						
		PSI	RC-Core1	RC-Core2	RC-Core3	LHCII	SC-1	SC-2
Dark	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	13.2	–	6.5	4.5	39.6	17.7	18.5
	4	3.5	–	–	9.2	35.2	1.8	50.3
	6	1.5	–	–	9.2	33.9	1.6	53.8
	8	–	–	–	4.2	35.2	–	60.6
	10	–	–	–	1.3	37.2	–	61.5
	12	–	–	–	–	32.8	–	67.2
Light	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	12.0	1.3	4.5	4.2	46.8	13.2	18.0
	4	9.7	–	8.2	4.5	43.0	6.8	27.8
	6	5.9	–	6.4	16.6	41.2	3.9	26.0
	8	3.5	–	–	2.8	48.6	3.9	41.2
	10	2.1	–	–	2.4	32.8	2.9	59.8
	12	–	–	–	–	39.2	–	60.8

Table 6. Changes in the disassembly of chlorophyll-protein complexes from *Phaseolus vulgris* treated with GA_3 under dark or light condition for 12 d

days after excision (d)		Relative amount of chlorophyll in chlorophyll-protein complexes						
		PSI	RC-Core1	RC-Core2	RC-Core3	LHCII	SC-1	SC-2
Dark	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	6.2	–	4.5	6.2	40.3	13.4	29.4
	4	0.8	–	–	8.3	41.2	4.1	45.6
	6	–	–	–	0.8	39.2	0.3	59.7
	8	–	–	–	–	35.4	–	64.6
	10	–	–	–	–	33.2	–	66.8
	12	–	–	–	–	31.8	–	68.2
Light	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	10.8	0.7	5.0	4.2	44.1	13.3	21.9
	4	5.2	–	6.8	5.3	43.8	5.2	33.7
	6	3.1	–	5.7	11.3	40.8	5.2	33.9
	8	1.8	–	–	5.1	43.2	2.8	47.1
	10	–	–	–	–	35.7	–	64.3
	12	–	–	–	–	30.2	–	69.8

Table 7. Changes in the disassembly of chlorophyll-protein complexes from *Phaseolus vulgris* treated with BA under dark or light condition for 12 d

days after excision (d)		Relative amount of chlorophyll in chlorophyll-protein complexes						
		PSI	RC-Core1	RC-Core2	RC-Core3	LHCII	SC-1	SC-2
Dark	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	13.1	–	6.5	4.0	42.3	16.2	17.9
	4	3.3	–	4.0	9.2	40.8	7.5	35.2
	6	1.5	–	1.8	8.2	40.4	3.2	45.9
	8	–	–	–	2.8	39.5	2.7	55.0
	10	–	–	–	2.3	38.5	1.3	57.9
	12	–	–	–	–	37.2	0.8	62.0
Light	0	26.4	3.5	3.8	4.2	35.7	12.0	14.4
	2	20.2	1.8	4.5	2.9	40.2	13.8	16.6
	4	13.2	1.2	6.2	3.9	38.9	5.3	31.3
	6	10.9	0.7	5.4	10.3	40.5	5.2	34.0
	8	9.5	–	4.2	4.3	43.8	6.7	31.5
	10	5.7	–	–	4.2	45.8	3.9	40.4
	12	5.2	–	–	–	47.2	3.5	44.1

eased levels of chlorophylls associated with PSI and RC-Core3. On the other hand, exogenous BA application caused the structural stability of chlorophyll-protein complexes during leaf senescence when compared to control placed in the darkness (Tables 4 and 7). Particularly, BA was significantly effect in retarding the disassembly of RC-Core2, RC-Core3 and SC-1 in senescent leaves. These results suggest that BA causes a suppressing effect on the disassembly of chlorophyll-protein complexes during leaf senescence while NAA and GA_3 had little effect. The stability of chlorophyll-protein complexes by exogenous BA application may be attributed to the proposal that cytokinin induces the stability of grana in chloroplast, and promotes the synthesis of chloroplast proteins[28,29]. In the simultaneous treatment of plant hormones and light, BA application illuminated light was most effective in the stability of chlorophyll-protein complexes in senescing leaves (Tables 4~7). BA application collaborated with light strongly suppressed not only the degradation of and PSI and LHCII but also that of RC-Core2, RC-Core3 and SC-1.

DISCUSSION

Chlorophyll loss during dark-induced senescence was a good representative in the senescing leaves. The illumination of light caused significantly suppressing effect in the breakdown of chlorophyll when compared to darkness (Fig. 1), these results are consistent to the observations with many plant species[27,30]. To elucidate effect of plant hormones during leaf senescence, chlorophyll contents in the detached leaves of bean plants, which had been treated with plant hormones for 12 d, were investigated. Exogenous application of NAA and GA_3 had little effect in the retardation of chlorophyll degradation during leaf senescence but exogenous application BA significantly caused suppressing effect in the chlorophyll breakdown, when compared to the control

(Fig. 1, Tables 1~3). However, Horton and Bourguoin (1992) suggested that gibberellic acid retarded loss of chlorophyll in the juvenile ivy[31], and some reports also showed the same effect of GA_3 on the retardation of chlorophyll breakdown[20,32]. On the other hand, Wittenbach (1977) had proposed that GA_3 had little or no effect on the senescence rate of chlorophyll loss in the wheat seedlings[33], and these results were similar to present experimental results. The contrary effect of GA_3 on the breakdown of chlorophyll may be attributed to the different degrees of delaying export of nutrients from the leaves of source organs to the other sink organs during senescence or the regulatory degrees of chlorophyll turnover. Cytokinin has been known to play an important role for the retardation of chlorophyll loss in a large number of species[17,19]. These results suggest that cytokinin is very effect in retardation the loss of ALA (aminolevulinic acid) dehydratase and PBG (porphobilinogen) deaminase, two enzymes was involved in chlorophyll biosynthesis[11].

Chl a/b ratio of the control which was kept in the dark did not changes significantly until the middle stage of dark-induced senescence, and then was increased in the late stage of senescence (Fig. 1). The increase of Chl a/b ratio may be attributed to the degradation of light-harvesting chlorophyll complexes containing Chl a and Chl b. To better understand the changes in the chlorophyll-binding proteins during leaf senescence, we studied the disassembly of chlorophyll-protein complex in the detached leaves during senescence using native green gel system. The bands, which were separated in native green gel, were nomenclatured in order of increasing mobility as follows ; PSI, three bands of RC-Core (RC-Core1, 2 and 3), LHCII, the three bands of SC (two bands of SC-1 and SC-2) and FP (Fig. 2) as shown in previous study[26]. The protein components of the band was showed in a fully denaturing SDS-PAGE as follows : (1) PSI complexes contained P700 apoprotein, a number of LHCI apoprotein and the smaller PSI subunits, (2) RC-Core complexes contained a number of reaction center and core complexes associated with PSI and PSII, (3) LHCII was preserved as the trimeric form of the main light harvesting antenna of PSII, and (4) SC complexes were composed of partially disassociated PSII components, particularly SC-2 contained a number of LHCII monomer[26]. As shown in Fig. 2, the disassembly of LHCII was suppressed until the middle stage of dark-induced senescence, and the LHCII was significantly degraded thereafter. The disassembly of LHCII may be induced by the increase of Chl a/b ratio, and these results are consistent with the notion that the degradation of LHCII apoprotein was retarded until the late stage of leaf senescence in *Lolium tumelentum*[27]. However, it is not clear whether disassembly of chlorophyll-protein complexes is caused by breakdown of chlorophyll, or by proteolytic digestion of the protein moiety. Therefore further studies are required to elucidate proteolytic digestion of the protein moiety during leaf senescence. Illumination of light, in general, offered the structural stability of chlorophyll-protein complexes of the detached leaves during senescence when compared to the darkness, particularly was significant effect in protecting PSI and RC-Core3 degradation (Table 4). The effect of light on the disassembly of chlorophyll-protein complexes could be observed in the previous study that white light retards the degradation of chlorophyll-binding proteins, particularly suppresses strongly the breakdown of CPI and LHCII apopreteins[30,34].

Plant hormones such as auxin, gibberellin and cytokinin are known to regulate loss of chlorophyll of leaves in a large number of plant species during senescence[32,35,36]. However, there are little informations on the disassembly of chlorophyll-protein complexes of senescing leaves. In the present study, we have studied the effect of plant hormones on the changes of chlorophyll-protein complexes during senescence. Exogenous applications of NAA and GA_3 were little or no effect on the changes of relative distribution in chlorophyll-protein complexes

of senescing leaves when compared to the control (Tables 4~6). The fact suggested the possibility that chlorophyll-protein complexes are degraded with kinetics similar to that of disappearance of chlorophyll. Exogenous BA application caused the suppressing of the disassembly of chlorophyll-protein complexes, particularity RC-Core2, RC-Core3 and SC-1, comparing to the control (Tables 4 and 7). The stability of chlorophyll-protein complexes by exogenous BA application may be induced by the proposal that cytokinin induced the stability of grana in chloroplast, and promotes the synthesis of chloroplast proteins[28,29]. Huang *et al.* (1990) also have reported the similar results with wheat seedlings[37]. On the other hand, in the simultaneous treatment of plant hormones, BA application collaborated with light was most effective in protecting the disassembly of chlorophyll-protein complexes, particularly PSI, RC-Core2, RC-Core3 and SC-1 in senescing leaves (Table 7). The question of whether light and cytokinin act independently to affect leaf senescence or whether plant hormones are involved in the senescing responses induced by physiologically photoreceptors is still an open one. Mohr (1984) suggested a simple additive interaction when cytokinin and light were applied simultaneously and concluded that two factor act independently on the chlorophyll accumulation[38]. In contrast, Flores and Tobin (1986) suggested that red light or cytokinin was each more effective on the *cab* and *rbcS* expression in the presence of the other than when administered alone[39]. In present study, it could be suggested that simultaneous application of light and BA induces synergistic effect on the retardation of the disassembly of chlorophyll-protein complexes during leaf senescence when compared to light or BA application alone, and light may play an important role for the retardation of the disassembly of chlorophyll-protein complexes in senescing leaves when compared to BA. Further studies are needed to elucidate proteolytic digestion of chlorophyll-binding proteins and molecular mechanism that allows the structural changes of chloroplast by simultaneous application of plant hormones and light.

REFFERENCE

1. Gut, H., Rutz, C., Matile, P. and Thomas, N. : Leaf senescence in a non-yellowing mutant of *Festuca pratensis* : Degradation of carotenoid. *Physiol. Plant.*, **70**, 659-663(1987).
2. Thomson, W. W. and Platt-Aloia, K. A. : Ultrastructure and senescence in plants. *Plant Senescence : Its Biochemistry and Physiology*, Thomson, W. W., Nathanael, E. A. and Huffier. R. C. eds., pp. 20~30, American Society of Plant physiologists, Rockville (1987).
3. Nooden, L. D. : Whole plant senescence. *Senescence and Aging in Plant*, Nooden, L. D. and Leopoid, A. C. eds, pp. 391~439, Academic press, San Diego (1988).
4. Tobias, D. J., Ikemeto, A. and Nishimura, T. : Leaf senescence patterns and photosynthesis in four leaf flushes of two deciduous oak (*Quercus*) species. *Photosynthetica*, **31**, 231-239(1995).
5. Robert, D. R., Thompson, J. E., Dumbroff, E. B., Gepstein, S. and Mattoo, A. K. : Differential changes in the synthesis and steady-state levels of thylakoid proteins during bean leaf senescence. *Plant Mol. Biol.*, **9**, 343-353(1987).
6. Droillard, M. J., Bate, N. J., Rutstein, S. J. and Thompson, J. E. : Active translation of the D-1 protein of photosystem II in senescing leaves. *Plant physiol.*, **99**, 589-594(1992).
7. Emyr-Davies, T. G., Thomas, H., Thomas, B. J. and Rogers, L. J. : Leaf senescence in a nonyellowing mutant of *Festuca pratensis. Plant Physiol.*, **93**, 588-595(1990).
8. Davies, T. G. E., Thomas, H., Thomas, B. J. and Rogers, L. J. : Leaf senescence of a nonyellowing mutant of *Festuca pratensis*. Metabolism of cytochrome *f. Plnat Physiol.*, **93**, 588-595(1990).
9. Nock, L. P., Rogers, L. J. and Thomas, H. : Metabolism of protein and chlorophyll in leaf tissue of *Festuca pratensis* during chloroplast assembly and senescence. *Phytochemistry*, **31**, 1465-1470(1992).
10. Thomas, H., Oughamx, H. J. and Davies, T./ G. E. : Leaf senescence in a non-yellowing mutants of *Festuca pratensis*. Transcripts and translation products. *J. Plant Physiol.*, **139**, 403-412(1992).

11. Hukmani, P. and Tripathy, B. C. : Chlorophyll biosynthetic reactions during senescence of excised barley (*Hordeum vulgare* L. cv IB 65) leaves, *Plant Physiol.*, **105**, 1295-1300(1994).
12. Thomas, H. and Stoddart, J. L. : Leaf senescence. *Annu. Rev. Plant physiol.*, **31**, 83-111(1980).
13. Roberts, J. A., and Hooley, R. : Leaf, flower and fruit development. *Plant Growth Regulators*, pp. 143~133, Blackie, London(1988).
14. Patrick, J. W. and Mulligan, D. R. : Gibberellic acid promoted transport of assimilates in stems *Phaseolus vulgaris*. Effect on assimilate accumulation by the sink. *Ann. Bot.*, **63**, 581-587(1989).
15. Jordi, W., Dekhuijzen, H. M., Stoopen, G. M. and Overbeek, J. H. M. : Role of other plant organs in gibberellic acid-induced delay of leaf senescence in *Alstroemeria* cut flowers. *Physiol. Plant.*, **87**, 426-432(1993).
16. Stoddart, J. L. and Thomas, H. : Leaf senescence. *Encyclopedia of Plant Physiology*, Boulter, D. and Pitman, M. G. eds., Vol. 14, pp. 592~636, Springer-Verlag, Berlin(1982).
17. Singh S., Palni, L. M. S, and Letham, D. S. : Cytokinin biochemsitry in relation to leaf senescence V. Endogenous cytokinin levels and metabolism of zeatin riboside in leaf discs from green and senescent tobacco (*Nicotiana rustica*) leaves. *J. Plant Physiol.*, **139**, 279-283(1992).
18. Colbert, K. A. and Beever, J. E. : Effect of disbudding on root cytokinin export and leaf senescence in tomato and tobacco. *J. Exp. Bot.*, **32**, 121-127 (1981).
19. Van Staden, J., Cook, E. L. and Nooden, L. D. : Cytokinins and senescence. *Senescence and Aging in Plants*, Nooden, L. D. and Leopold, A. C. eds., pp. 281~328, Academic Press, San Diego(1988).
20. Van Doorn, Hibma, W. G., J. and de Wit, J. : Effect of exogenous hormones on leaf yellowing in cut flowering branches of *Alstroemeric pelegrina* L. *Plant Growth Regul.*, **11**, 59-62(1992).
21. Thomas, H. : Leaf development in *Lollium temulentum* : protein metabolism during development and senescence of attached and excised leaf tissue. *J. Plant physiol.*, **136**, 45-50(1990).
22. Lee, D. H., Hong, J. H. and Kim, Y. S. : The effect of plant hormones and light quality on the invertase activity in maize (*Zea mays* L.) and mung bean (*Pgaseolus radiatus* L.). *J. Korean Environ. Sci. Soc.*, **4**, 323-333(1995).
23. Allen, K. D. and Staehelin, L. A. : Resolution of 16 to 20 chlorophyll-protein complexes using a low ionic strength native green gel system. *Anal. Biochem.*, **194**, 214-222(1991).
24. Lichententhaler, H. K. : Chlorophyll and carotenoids : Pigments of photosynthetic biomembrane, *Methods in Enzymology*, Lester Packer and Roland Doncd eds., 148, pp. 350~367, Academic Press, New York(1987).
25. Laemmli, U. K. : Cleavage of structural proteins during the assembly of the head proteins of bacteriophage T_4, *Nature*, **227**, 680-685(1970).
26. Lee, D. H., Hong, J. H. and Kim, Y. S. : Effect of Light on Disassembly of Chloroplast during Senescence of Detached Leaves in *Phaseolus vulgris, J. Korean Environ. Sci. Soc.*, in press(1997).
27. Mae, T., Howard, T., Alan, G. P., Amane, M. and Jun, H. : Leaf development in *Lolium temulentum* : Photosynthesis and Photosynthetic Proteins in Leaves Senescing under Different Irradiances, *Plant Cell Physiol.*, **34**(3), 391-399(1993).
28. Dalling, M. J. and Nettleton, A. M. : Chloroplast senescence and proteolytic enzyme. *Plant Proteolytic Enzymes*, Darring, M. J. eds., Vol. II, pp. 125~153, CRC press, Florida(1986).
29. Stabel, P. A. S. and Engstrom, S. : Cytokinin treatment of embryos inhibits the synthesis of chloroplast proteins in Norway spruce, *Planta*, **183**, 520-527(1991).
30. Okada. K., Inoue, Y., Satoh, K. and Katoh, S. : Effect of Light Degradation of Chlorophyll and Protecting during Senescence of Detached Rice Leaves, *Plant Cell Physiol.*, **33**(8), 1183-1191(1992).
31. Horton, R. F and Bourguoin, N. : Leaf senescence in juvenile ivy, *Plant Physiol. Biochem.*, **30**, 119-122 (1992).
32. Hicklenton, P. R. : GA_3 and benzylaminopurine delay leaf yellowing in cut *Alstroemeria* stems, *Hortscience*, **26**, 1198-1199(1991).
33. Wittenbach, V. A. : Induced senescence of intact wheat seedlings and its reversibility. *Plant Physiol.*, **59**, 1039-1042(1977).
34. Hilditch, P. I., Thomas, H., Thomas, B. J. and Rogers, L. J. : Leaf senescence in a non-yellowing mutants of *Festuca pratensis* : Proteins of photosystem II, Planta, **177**, 265-272(1989).
35. Thiamann, K. V. : The senescence of leaves, *Senescence in Plants*, Thiamann, K. V. eds., pp. 5803~5805(1980).

36. Dai, J. W. and Paul. R. E. : Postharvest handling of alstroemeria, *Hortscience*, 26, 314(1991).
37. Huang, H., Yang, S. Y. and Tang, T. W. : A functional cytokinin-binding protein in photochemical feactions of chloroplast, *Plant Growth Substance*, Pharis, R. P. and Rood, S. B. eds, pp. 173~178, Springer-Verlag(1990).
38. Mohr, H. : Phytochrome and chloroplast development. *Chloroplast Biogenesis,* Baker, N. R. and Barber, J. eds, pp. 305~347, Elsevier, Amsterdam(1984).
39. Flores S. and Tobin, E. : Benzyladenine modulation of the expression of two genes for nuclear-encoded chloroplast proteins in *Lemma gibba* : apparent post-transcriptional regulation, *Planta*, 168, 340~349 (1986).

초록 : 강낭콩 잎의 노화에 있어서 식물 호르몬의 역할

이동희 · 서영희 · 김영상†
(부산대학교 자연과학대학 생물학과)

NAA, GA_3 및 BA등의 식물 호르몬이 노화중인 강낭콩 잎의 엽록소-단백질 복합체의 분해에 미치는 효과를 조사하였다. 노화의 대표적인 특징인 엽록소의 소실은 엽록소-단백질 복합체의 분해를 수반하였다. 암유도 노화과정동안, PSI 복합체는 노화초기에 급격히 감소한 반면 RC-Core3은 오히려 노화 중기까지 조금씩 증가하다가 이후 서서히 분해되었다. 그리고 LHCII는 노화 과정 후기부터 점진적으로 분해되었다. NAA와 GA_3는 노화동안 엽록소-단백질 복합체의 분해를 억제하는데 거의 영향을 미치지 못 하였다. 그러나 BA는 노화 과정동안 엽록소-단백질 복합체, 특히 RC-Core1, RC-Core2와 SC-1의 분해를 억제하는데 매우 효과적이었다. 한편 식물 호르몬과 광선의 동시 처리에 있어서, BA와 광선의 동시 처리는 노화 과정동안 엽록소-단백질 복합체, 특히 PSI, LHCII, RC-Core2, RC-Core3과 SC-1의 분해를 억제하는데 가장 효과적이었다. 이와 같은 결과에서 노화 과정동안 엽록소-단백질 복합체는 BA 혹은 광선의 단독 처리보다 BA와 광선의 동시 처리에 의하여 보다 높은 안정성을 가질 것으로 사료된다.