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Involvement of Polyamines in the Control of Senescence in *Lemna gibba* G3 Fronds

Yi, Ho Chul, Jeong Hoe Kim and Bin G. Kang

(Department of Biology, Yonsei University, Seoul)

Lemna gibba G3의 노화조절에 대한 폴리아민의 관여

李 浩 哲·金 政 會·姜 濱 求

(延世大學校 理科大學 生物學科)

ABSTRACT

Exogenously applied spermine, spermidine and putrescine caused a delay of senescence in fronds *Lemna gibba* G3 under continuous illumination. When the proximal half of a frond containing the meristematic "pockets" was removed, endogenous spermidine level in the distal half (half frond) increased initially to a miximal level, which was followed by a decline during a period of 10 days of incubation in light. No appreciable changes were observed with putrescine or spermine levels. Treatment of fronds with α-difluromethylarginine (DFMA) resulted in both reduced level of spermidine and enhancement of chlorophyll loss in half fronds. α-difluoromethylornithine (DFMO) was found to be virtually ineffective in either parameter. Results of experiments with ABA and kinetin indicate that there is a close correlation between the progress of senescence and spermidine level in *Lemna* fronds under illumination. It is suggsted that endogenous level of spermidine is associated, at least in part, with frond senescence in this aquatic plant.

INTRODUCTION

Polyamines (PAs) are simple aliphatic compounds ubiquitous in all living organisms (Tabor and Tabor, 1984). In plants, PAs are implicated in the regulation of a variety of developmental processes (Evans and Malmberg, 1989). Many senescence-related processes in plants are known to be inhibited by PAs (Altman, 1982; Smith, 1985; Galston and Kaur-Sawhney, 1987; Carbonell and Navarro, 1989). However, problems of endogenous polyamines playing a regulatory role in such celleular processes need to be carefully scrutinized. In greening cucumber cotyledons, for instance, cytokinin increases both chlorophyll and putrescine levels, but inhibition of putrescine biosynthesis do not affect chlorophyll levels, and moreover, applied putrescine is inhibitory to greening, indicating that endogenous putrescine is not related to the greening processes (Walker *et al.*, 1988).

The present work was undertaken to investigate actions and possible regulatory roles of polyamines in the senescence of fronds in *Lemna gibba* G3. This aquatic plant presents an interesting, and in some respects a unique, model system that has been established to study plant senescence (Kang and Cleland, 1990).

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MATERIALS AND METHODS

Plant Material. Fronds of *Lemna gibba* L., strain G3 were used for all experiments. All cultures were grown in 125-m*l* Erlenmeyer flasks with 50 m*l* of E medium (Cleland, 1979). Four-frond colonies were selected from stock cultures and grown on short days (9 h light/15 h dark) for the production of A-1-1 colonies (Cleland, 1979). This procedure produces uniform starting material and guarantees that the A-1-1 frond of the A-1-1 colony is the first daughter frond of the A-1 frond. This is important especially for senescence work because late formed daughter fronds are smaller with a shorter life span and may produce fewer daughter fronds (Wangermann, 1965; Claus 1972).

Either six A-1-1 fronds (intact fronds) or distal halves of these fronds where the proximal parts containing the meristems have been excised (half fronds) were placed in each flask to start the experiment. In the case of intact fronds, successively produced daughter fronds were removed from the flask as they separated from the parent A-1-1 frond (Kang and Cleland, 1990).

Application of PAs, Hormones and Other Chemicals. Since the fronds were grown in asceptic culture, PAs, hormmones and the inhibitors were added to an auotclaved medium by membrane filtration (pore size, 0.45 μm) at the start of the experiment.

Determination of Polyamines. For estimation of ploymine titers, the method developed by Flores and Galston (1982) was slightly modified. Six fronds collected at appropriate times during the culture period were homogenized with 300 μl of 5% perchloric acid at 4 C with a mortar and pestle. The homogenates were spun at 15,000 rpm for 20 min with a microcentrifuge. To the supernatant (200 μl), 400 μl of dansyl chloride (5 mg/m*l* acetone) and 200 μl of saturated sodium carbonate were added, and the mixture was incubated in the dark at room temperature for 16 h. At the end of the 16 h period, 100 μl of proline (100 mg/m*l*) was added and incubated in the dark for another 30 min after which the dansyl derivatives were extracted with benzene. The extract (200 μl) was loaded on a silica gel plate and TLC was performed for 75 min with chloroform: triethylamine (25: 2,v/v) solvent system. The dansylated amine band was scraped and eluted with ethyl acetate and its fluorescence was determined with a spectrophotofluorimeter (Perkin-Elmer, model LS-5; excitation at 350 nm, emission at 495 nm).

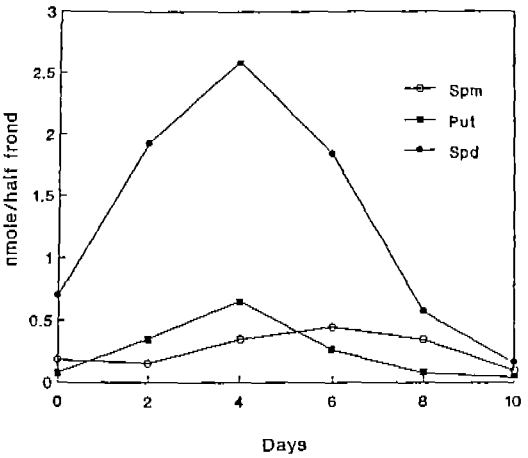

Fig. 1. Time course for changes in PA contents of *Lemna* half fronds under continuous light.

Chlorophyll Measurements. Six fronds were homogenized with 2 m*l* ethanol, and the homogenates centrifuged at 11,000 g for 20 min. The pellets were washed once more with 2 m*l* ethanol, and the pooled supernatants (4 m*l*) were used for measurements of absorbance at 665 nm with a spectrophotometer (Hitachi, model 200-20).

Chemicals. Polyamines (putrescine, spermine, and spermidine), hormones (abscisic acid and kinetin) and other fine chemicals were purchased from Sigma (St. Louis, MO, USA). The inhibitors of polyamine biosynthesis, α-difluoromethylarginine (DFMA) and α-difluoromethylornithine (DFMO) were kindly provided by Dr. E. H. W. Bohme of Merrell Dow Research Institute, Cincinnati, OH, USA.

RESULTS AND DISCUSSION

Exogenously applied putrescine, spermidine, and spermine all retarded chlorophyll loss in *Lemna* fronds under continuous illumination (Table 1). Unlike in excised leaf segments where senescence is known to be delayed in light compared to the dark control (Thimann, 1987), senescence in *Lemna* fronds is characterized by light-accelerated chlorophyll loss (Kang and Cleland, 1990). Although none of the three PAs tested could retard the chlorophyll loss completely, the PA effect seems to be significant, spermidine being slightly more active than putrescine or spermine.

Table 1. Effect of putrescine, spermidine and spermine at various concentrations on chlorophyll contents in senescing fronds under continuous illumination. The initial level of chlorophyll at time zero had an absorbance value of 0.332 (100%), and the control value without treatment at 15 days of incubation when all frond samples were taken for chlorophyll measurements had an absorbance value of 0.190 (57%), respectively. Values in parentheses represent percent of the initial value.

PA Conc. (mM)	Chlorophyll (A 665)		
	Spermine	Spermidine	Putrescine
0.001	0.191 (58)	0.158 (56)	0.191 (58)
0.01	0.196 (59)	0.232 (70)	0.212 (58)
0.1	0.210 (63)	0.225 (68)	0.214 (64)
0.2	0.234 (70)	—	—
0.6	0.274 (83)	—	—
1.0	—	0.289 (87)	0.246 (74)

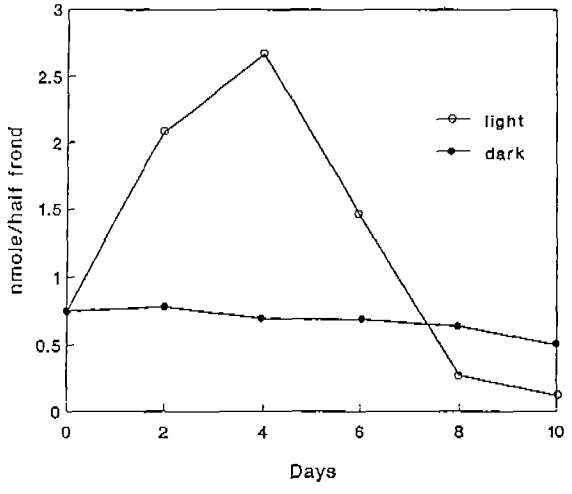

Fig. 2. Time course for changes in spermidine level of half fronds under continuous light or in complete darkness.

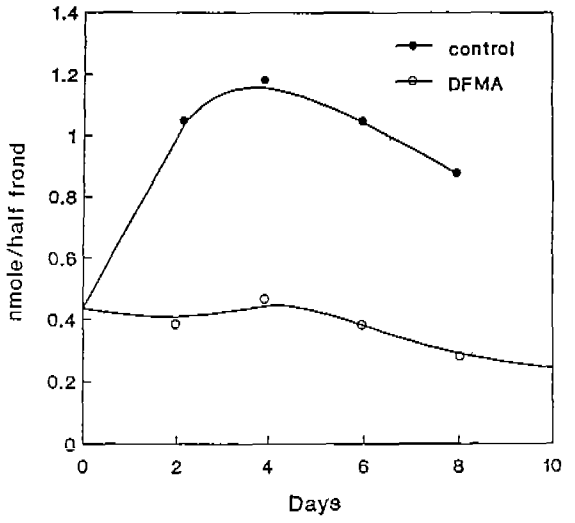

Fig. 3. Time course for changes in spermidine level of half fronds treated with or without DFMA (1 mM) under continuous light.

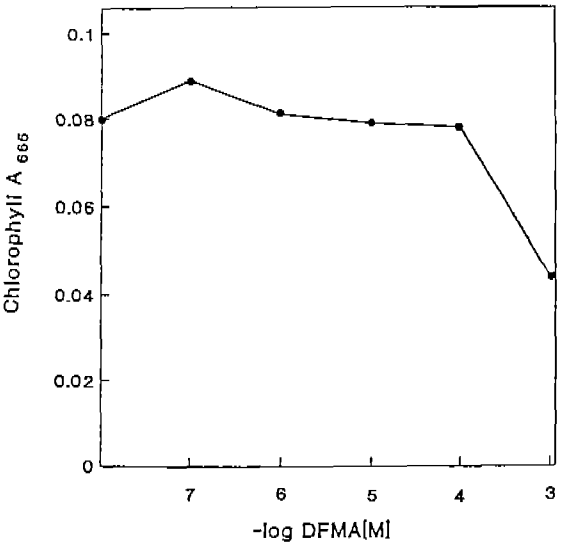

Fig. 4. Effect of DFMA at various concentrations on the chlorophyll level in senescing half fonds incubated for 10 days under continuous light.

In half fronds which are devoid of the mersitem, chlorophyll loss both in light and in darkness is prematurely induced compared with intact fronds, but responses to light and senescence-affecting hormones (ABA and kinetin) are essentially unaltered (Kang and Cleland, 1990). Levels of endogenous PAS in *Lemna* half fronds were measured with regard to the progress of senescence. Results shown in Fig. 1 indicate that spermidine levels increased initially during the first 4 days of incubation under continuous illumination followed by a rapid decline. The onset of the decline in spermidine levels roughly coincides with that of chlorophyll loss under comparable conditions (Kang and Cleland, 1990). Moreover, the content of spermidine, which appears more active than the other two PAs (Table 1), was higher than the others, and showed pronounced changes with time. The initial rise and the subsequent fall in the spermidine level observed in il-

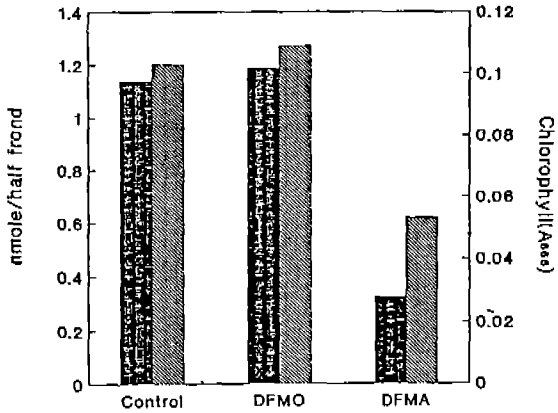

Fig. 5. Spermidine (dark bars on the left) and chlorophyll (light bars on the right) levels of senescing half fornds treated with DFMO and DFMA for 4 days under continuouls light. The initial values at time zero were 0.412 nmole/half frond for the spermidine and 0.321 (A665) for the chlorophyll levels, respectively.

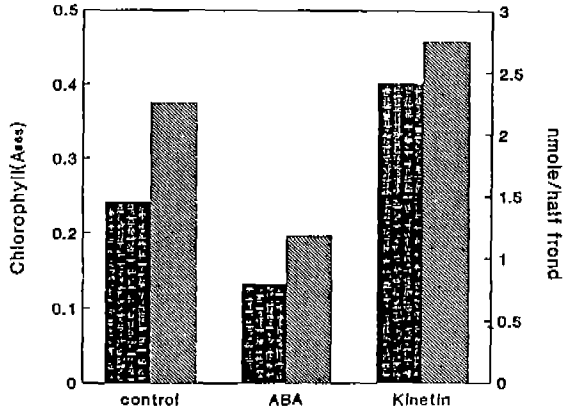

Fig. 6. Chlorophyll (dark bars on the left) an spermidine (light bars on the right) levels of senescing half fronds treated with 1μM ABA or kinetin for 4 days under continuous light.

luminated tissue were found to be completely abolished in the dark (Fig. 2). Elevated polyamine levels by illumination was also reported for cucumber cotyledons during the light-induced greening (Walker *et al.*, 1988).

In an effort to study the possibility of endogenous polyamines playing a regulatory role in the control of frond senescence in *Lemna*, effects of DFMA and DFMO, specific enzyme-activated irreversible inhibitors of arginine decarboxylase and ornithine decarboxylase, respectively (Birecka *et al.*, 1988), on both spermidnine titer and chlorophyll loss were investigated. The light-induced increases in the spermidine level was blocked by a high concentration (1 mM) of DFMA (Fig. 3), at which the chlorophyll loss was effectively inhibited by approximately 50% (Fig. 4 and 5). The data in Fig. 5 indicate, however, that DFMO is totally ineffective to alter either spermidine or chlorophyll levels.

The progress of senescence as expressed by reduction of chlorophyll was compared with the level of spermidine in half fronds treated with agents either promoting or inhibiting *Lemna* senescence. Treatment of fronds with kinetin, which is known to delay senescence in this plant (Kang and Cleland, 1990), also resulted in a slight elevation of the spermidine level in the frond tissue as shown in Fig. 6. It is also clear that ABA both promoted the chlorophyll loss and reduced the spermidine level in the tissue (Fig. 6). The data presented indicate that there exists a close correlation between the progress of senescence and the level of spermidine in *Lemna* fronds.

Although a positive correlation could be established clearly between the senescence and endogenous spermidine levels in *Lemna* under illumination, the possibility of the polyamine playing any role in the frond senescence in the dark seems non-existent. First, the spermidine level in the frond did not change significantly during the dark incubation of half fronds (Fig. 2), whereas the chlorophyll level was reduced considerably in the same tissue under the same conditions within this period (Kang and Cleland, 1990). Secondly, DFMA which promoted frond senescence in light (Figs. 4 and 5) did not have any effect on the chlorophyll loss in the dark (data not shown). The nature of senescence in *Lemna* fronds in the dark where it is rather delayed as opposed to isolated leaf segments in which senescence is promoted (Thimann, 1987) awaits an explanation from further studies.

적 요

Lemna gibba G3의 frond에서 spermine, spermidine 및 putrescine의 처리는 광조건하에서 노화의 지연을 초래하였다. 분열조직이 있는 부분을 제거한 half frond를 광조건하에서 10일 동안 배양하는 동안 초기에 spermidine 함량이 증가한 후 곧 감소하였다. Putrescine이나 spermine의 경우에는 이러한 변화가 나타나지 아니하였

다. DFMA를 처리한 frond에서는 spermidine 함량의 감소와 동시에 엽록소 분해의 촉진이 관찰되었다. 반면 DFMO는 이러한 효과가 없었다. ABA와 kinetin에 의하여 노화가 각각 촉진되고 억제된 frond에서 spermidine의 함량이 역시 ABA에 의하여 감소되었고 kinetin에 의하여 증가된 결과를 얻으므로서 노화의 진전과 spermidine 함량간에 밀접한 상관관계가 있음이 확인되었다. 이러한 실험결과는 frond의 내생적 spermidine 함량이 노화와 연관되어 있다는 것을 시사하고 있다.

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