Pergamon

PII: S0031-9422(98)00338-0

Phytochemistry Vol. 49, No. 8, pp. 2255–2259, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

PHOTOINDUCTION OF ARGININE DECARBOXYLASE ACTIVITY IN LEAVES OF *PHARBITIS NIL*

IZUMI YOSHIDA and EIJI HIRASAWA*

Department of Biology, Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka, 558, Japan

(*Received* 9 *March* 1998)

Key Word Index—*Pharbitis nil*; Convolvulaceae; morning glory; photoinduction; arginine decarboxylase; polyamines.

Abstract—The activity of arginine decarboxylase (ADC) in leaves of *Pharbitis nil* was induced by light. The ADC activity increased to a maximum 1 h after illumination, followed by a gradual decrease. This suggested light either induced synthesis of ADC protein *de novo* or was involved in its activation. Cycloheximide inhibited the photoinduction of ADC activity, and the half life of ADC in leaves was 30–40 min. The temperature and relative humidity in darkness before illumination had no effect on the photoinduction of ADC activity, contrary to the photoresponse of *S*-adenosylmethionine decarboxylase (SAMDC) activity where the conditions of darkness before lights-on have a marked effect. The light response of the polyamine (PA)-biosynthetic enzyme activity produced transient accumulation of PA. The level of spermidine increased in leaves in which activities of both SAMDC and ADC increased after illumination, while the level of putrescine increased in leaves in which the activity of ADC increased but that of SAMDC did not.

INTRODUCTION

Polyamines (PAs) are found in a wide range of higher plants and it has been proposed that they play important roles in the regulation of plant growth and development [1, 2]. Several studies have indicated that PAs are implicated in such varied processes as response to stress [3, 4], regulation of the cell cycle [5, 6], floral initiation [7–9] and gene regulation [10–12].

The PA biosynthetic pathway is relatively well established in living organisms [13]. The biosynthesis of PAs involves two steps. The first step is the biosynthesis of putrescine (Put). In animals, Put is formed only by decarboxylation of ornithine, via ornithine decarboxylase (ODC). In plants and bacteria, two pathways lead to Put. One is the ODC reaction and the other involves decarboxylation of arginine by arginine decarboxylase (ADC). The second step is the biosynthesis of spermidine (Spd) from Put and the aminopropyl moiety of decarboxylated *S*-adenosylmethionine (SAM), and the biosynthesis of spermine (Spm) from Spd and the aminopropyl moiety. *S*-Adenosylmethionine decarboxylase (SAMDC) is considered to be a key enzyme in the biosynthesis of both Spd and Spm [14]. Some reports indicate that in plants ADC activities are more correlated with the synthesis of Put than are ODC activities. ADC activity increases have been reported for cell growth and embryogenesis [15], synthesis DNA [5] and stress responses [1, 16–18]. On the other hand, ODC has been reported to be associated with proliferative growth [19, 20] and fruit development [21].

There is considerable current interest in the mechanisms that regulate ADC activity in plants, since this activity is modulated by light and by plant hormones [22]. We reported previously that SAMDC activity in leaves of *Pharbitis nil* was induced by light [23], and that the photoinduction was influenced by temperature and relative humidity during the dark period before lights-on [24]. In this report, we show that ADC activity in leaves of *Pharbitis* is also induced by light. In addition, we examine the levels of PAs in the leaves in which ADC and/or SAMDC activities are induced.

RESULTS AND DISCUSSION

In plants Put is produced via alternative pathways that involve ADC or ODC [14]. However, why these two biosynthetic pathways exist or which

*Author to whom correspondence should be addressed.

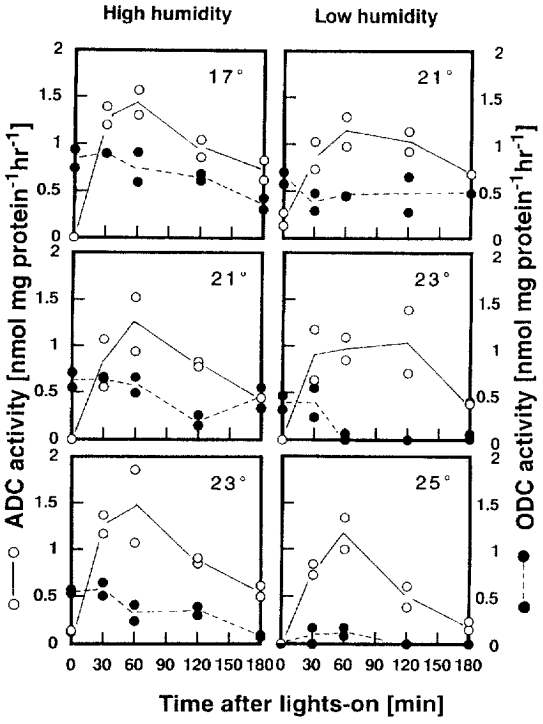

Fig. 1. Effects of temperature and humidity during the dark period on the photoinduction of ADC and ODC activities in leaves of *Pharbitis nil*. Five-day-old seedlings were incubated in a high- or low-humidity incubator in darkness for 16 h at various temperatures. After the darkness, the seedlings were transferred into light. In this experiment, unlabeled L-arginine or L-ornithine was added to the assay medium to give a final concentration of 5 mM, for the purpose of measuring ADC and ODC activities under substrate-saturated conditions. A symbol indicates a determination representing 3 plants. The duplicate zero time values for ADC activity in most cases are coincident.

factors determine the relative flux rate through the respective pathways is not known. In a previous study, we showed that SAMDC activity in leaves of *Pharbitis nil* was induced by light [23]. In this study, we examined whether ADC and/or ODC activities for the synthesis of Put in leaves of *Pharbitis nil* were induced by light. Figure 1 shows that ADC activity increased after illumination; the greatest activity was found 1 h after illumination and then ADC activity gradually decreased, while ODC activity did not increase after illumination under any conditions. A few studies have examined the relationship between light and ADC activity [25–27]. However, a rapid photoresponse of ADC activity, as found in this study, has not previously been described.

The temperature and relative humidity during the dark period before lights-on have a remarkable effect on the photoinduction of SAMDC activity in leaves of *Pharbitis nil* [24]. When the temperature and relative humidity are high in the dark period before lights-on, less SAMDC activity is induced. However, these conditions had no effect on the induction of ADC activity after illumination (Fig. 1).

To examine whether the photoinduction of ADC activity in leaves of *Pharbitis nil* is due to synthesis *de novo*, the plants were treated with cycloheximide (CH) which blocks protein synthesis. Seedlings, which had been grown under continuous light followed by a dark treatment for 16 h at 21°C and low humidity, had their roots excised 10 min before lights-on and then incubated with 50 μM CH by the same method as that described previously [23]. As a control, seedlings which had their roots excised were incubated with distilled water instead of the CH-solution. Figure 2(A) shows that CH inhibited the light induction of ADC activity. These results

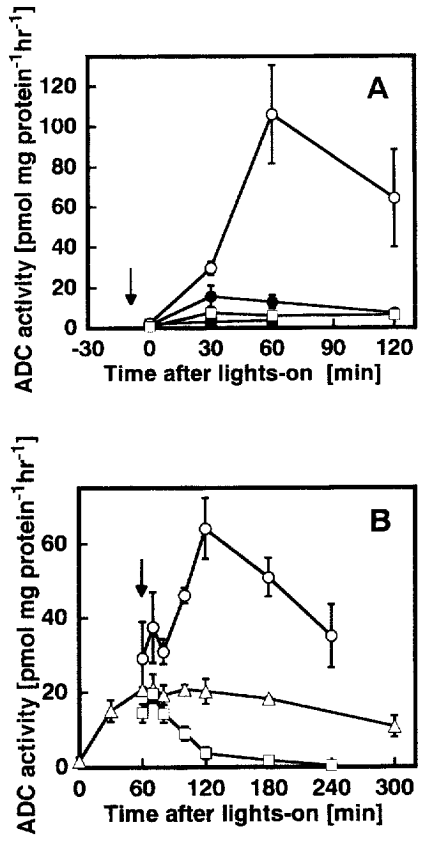

Fig. 2. Effect of CH on the photoinduction of ADC activity in leaves of *Pharbitis nil*. The roots of 7-day-old seedlings were cut off 10 min before lights-on (A) or 1 h after lights-on (B), and then the cut seedlings were put into a 50 μM solution of CH (□). Distilled water instead of the CH-solution was used as a control (○). Seven-day-old seedlings without cutting the roots were used as another control (△). The same treatment was conducted in darkness. The cut seedlings were put into CH-solution (■) or distilled water (●), and kept in darkness. The time of root-excision in each case is indicated by an arrow. Values are the means of 3 or 4 replicates, each with 2 plants. In this experiment, unlabeled L-arginine was not added to the assay medium, only labeled substrate was used. Vertical bars represent standard errors when the errors are larger than the symbols.

suggested that photoinduction of ADC activity may have resulted through *de novo* protein synthesis or through post-translational modification of preexisting ADC protein to the active form. A treatment with actinomycin D, which blocks RNA synthesis, did not inhibit the photoinduced increase of ADC activity (data not shown), suggesting that the photoinduction of ADC activity might not be at a transcriptional level. Some reports indicated that ADC is under translational and post-translational control [4, 28–30].

Root excision might cause osmotic stress by absorbing distilled water through the stem. In order to examine the effect of the root-cut stress and osmotic stress on ADC activity in this experiment, the same experiment was conducted in darkness. Several studies indicate ADC activity in response to environmental stress; e.g. acid [16], inorganic ions [4], osmosis [17, 30] and oxygen [3]. Though ADC activity was induced by a root-cut treatment in darkness, light was more effective in the induction of ADC activity than the stress (Fig. 2).

We determined the half life of ADC activity in leaves of *Pharbitis nil* upon a treatment with CH 1 h after illumination. Figure 2(B) showed that the half life of ADC activity was 30–40 min, being as short as that of SAMDC activity [23]. The rapid turnover of both ADC and SAMDC in leaves of *Pharbitis nil* might be necessary for the strict regulation of levels of PAs. Recently, ADC was reported to be localized in chloroplasts associated with the thylakoid membrane [31]. A plant-specific Put biosynthetic pathway via ADC was shown to be located in active photosynthetic tissues. In this study, we showed that only ADC activity of the alternative Put biosynthetic pathways was induced by light. These results indicate a possible involvement of ADC activity in photosynthetic function. There also exist several data indicating a possible relationship between photosynthetic activity and PAs. PAs, especially Spd and Spm, were shown to stabilize the molecular composition of thylakoid membranes [32, 33]. It was suggested that the mechanism might involve direct binding of PAs to membranes, thereby preventing lipid peroxidation and proteolytic attack. In addition, Rubisco protein was stabilized by PAs. Earlier, Margosiak *et al.* [34] reported that protein-conjugated PAs might be involved in photosynthetic functions, since increased TCA-insoluble PAs were found after light exposure. They also suggested that Rubisco function might be affected by PA binding. Del Duca *et al.* [35] showed that the activity of transglutaminase, which is Ca^{2+}- and light-stimulated, catalyzed the incorporation of PAs into thylakoid and stromal proteins, and suggested that PAs might be involved in the light-harvesting function.

We examined the changes in levels of PAs in leaves of *Pharbitis nil* under the conditions that yielded different photoresponses of ADC and SAMDC activities (Fig. 3). The increase of Put levels in leaves occurred from 3 to 6 h after illumination following 16 h of darkness at 25°C and high humidity. Under these conditions, the activity of ADC in leaves increased after illumination, while that of SAMDC did not [24]. On the other hand, after 16 h of darkness at 21°C and low humidity, the level of Spd increased from 1 h after illumination and peaked at 3 h after illumination. Under these conditions, the activities of both ADC and SAMDC [24] increased after illumination. Under both sets of conditions, the level of Spm remained lower than those of Put and Spd throughout the time course. The increase in activities of both ADC and SAMDC caused transient accumulation of Spd rather than Put, whereas the only increase of ADC activity produced transient accumulation of Put. These results accorded with the fact that ADC and SAMDC have regulatory roles in the PA biosynthetic pathway.

EXPERIMENTAL

Plant materials

Plants were grown as described in Ref. [24]. Seeds of *Pharbitis nil* (strain Violet) were allowed to

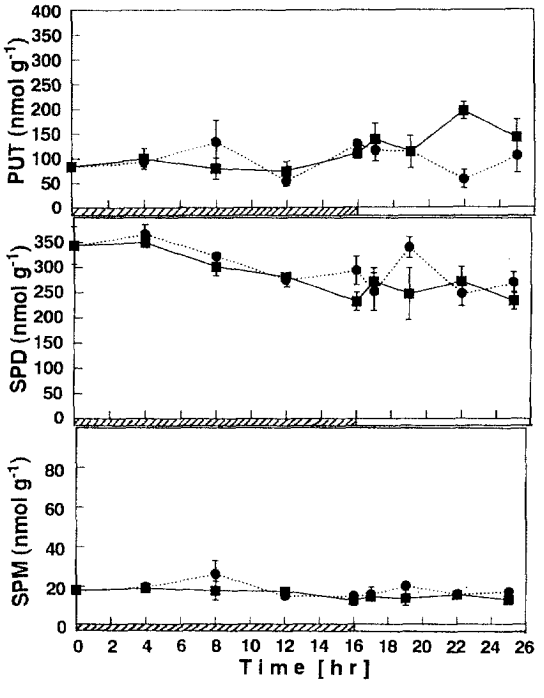

Fig. 3. Levels of PAs in leaves of *Pharbitis nil* during dark incubation for 16 h and the following 9 h light period. The conditions in darkness before lights-on were set at 21°C and low humidity (●) or 25°C and high humidity (■). Values are the means of 3 replicates, each with 2 plants. Vertical bars represent standard errors when the errors are larger than the symbols.

germinate in vermiculite under darkness at 25°C for 2 days. Then the plants were placed in a growth chamber at 22°C for 3 or 4 days under continuous light from fluorescent lamps at a photosynthetic photon flux density, at the top of the plants, of 100 μmol m^{-2} s^{-1}.

Light treatment

The 5-or 6-day-old seedlings of *Pharbitis nil* were transferred to incubators with low humidity (relative humidity of 30% to 40%) at 21, 23 and 25°C respectively, or with high humidity (relative humidity of 80 to 90%) at 17, 21 and 23°C respectively in darkness for 16 h. After the dark incubation, the seedlings of *Pharbitis* were subjected to white light by fluorescent lamps at a photosynthetic photon flux density, at the top of the plants, of 100 μmol m^{-2} s^{-1}.

In the case of treatments with CH, roots were cut from seedlings and the cut seedlings were incubated in 50 μM solution of CH or H_2O as controls.

Preparation of crude extracts

About 1 g of cotyledons from 2 or 3 seedlings of *Pharbitis nil* was crushed briefly in a chilled mortar and then homogenized in a Polytron homogenizer with 5 vol (v/w) of 0.1 M Tris–HCl buffer (pH 7.5) that contained 15 mM 2-mercaptoethanol, 0.1 mM EDTA and 0.01 g ml^{-1} PVP (insoluble). The homogenate was centrifuged at 10,000*g* for 3 min at 4°C and the supernatant was assayed for various enzymatic activities.

Assays for ADC and ODC

Assays were carried out in 1.5-ml Eppendorf tubes by a modified version of the methods described in Ref. [24]. A glass-fibre filter was cut to give a disc of 7 mm in diameter and it was impregnated with 20 μl of Solvable. It was fixed on the inside of the cap of a vial to trap liberated $^{14}CO_2$. ADC activity was determined by measuring the amount of $^{14}CO_2$ formed from L-[1-^{14}C] arginine. The reaction mixture consisted of 200 μl of the crude extract, 200 μl of 250 mM MES-NaOH buffer (pH 6.5), which contained 15 mM 2-mercaptoethanol, 0.1 mM EDTA and 45 μM PLP, and 50 μl of a solution of L-[1-^{14}C] arginine (2.25 μmol; 18.5 kBq, in Fig. 1, 9.09 nmol; 18.5 kBq, in Fig. 2). The reaction mixture was incubated at 37°C for 30 min and the reaction terminated by the addition of 200 μl of 1 M KH_2PO_4. ODC activity was measured with the same reaction mixture as that used for ADC except that L-[1-^{14}C] ornithine (2.25 μmol; 18.5 kBq) was used as the substrate and the pH of the MES-NaOH buffer was 6.0 instead of 6.5. Boiled preparations of crude extracts were used as controls.

Protein concentrations were determined by a dye-binding assay [36] with a Protein-Assay Kit and bovine serum albumin as the standard.

Analysis of PAs

PAs were extracted and analyzed by the procedure of Ref. [37] with modifications. Dansylated PAs were analyzed by HPLC. Samples were injected onto a column (4.6 mm i.d. × 250 mm) of Shimpack CLC-ODS(M), eluted with a mobile phase of MeOH–CH_3CN–H_2O (77:5:18) and detected with a fluorescence detector, with an excitation wavelength of 365 nm and an emission wavelength of 510 nm.

REFERENCES

1. Evans, P. T. and Malmberg, R. L., *Annual Review of Plant Physiology and Plant Molecular Biology*, 1989, **40,** 235.
2. Galston, A. W. and Kaur-Sawhney, R., *Plant Physiology*, 1990, **94,** 406.
3. Reggiani, R., Hochkoeppler, A. and Bertani, A., *Plant Physiology*, 1989, **91,** 1197.
4. Watson, M. B. and Malmberg, R. L., *Plant Physiology*, 1996, **111,** 1077.
5. Maki, H., Ando, S., Kodama, H. and Komamine, A., *Plant Physiology*, 1991, **96,** 1008.
6. Altamura, M. M., Capitani, F., Cerchia, R., Falasca, G. and Bagni, N., *Protoplasma*, 1993, **175,** 9.
7. Kaur-Sawhney, R., Kandpal, G., McGonigle, B. and Galston, A. W., *Planta*, 1990, **181,** 212.
8. Caffaro, S. V. and Vicente, C., *Journal of Plant Physiology*, 1995, **145,** 756.
9. Havelange, A., Lejeune, P., Bernier, G., Kaur-Sawhney, R. and Galston, A. W., *Physiologia Plantarum*, 1996, **98,** 439.
10. Bueno, M., Garrido, D. and Matilla, A., *Physiologia Plantarum*, 1993, **87,** 381.
11. Van den Broek, D., Van der Straeten, D., Van Montagu, M. and Caplan, A., *Plant Physiology*, 1994, **106,** 559.
12. Fowler, M. R., Kirby, M. J., Scott, N. W., Slater, A. and Elliott, M. C., *Physiologia Plantarum*, 1996, **98,** 439.
13. Tabor, C. W. and Tabor, H., *Annual Review of Biochemistry*, 1984, **53,** 749.
14. Slocum, R. D., in *Biochemistry and Physiology of Polyamines in Plants*, ed. R. D. Slocum and H. E. Flores. CRC Press, London, 1991, p. 23.
15. Feirer, R. P., Mignon, G. and Litvay, J. D., *Science*, 1984, **223,** 1433.
16. Young, N. D. and Galston, A. W., *Plant Physiology*, 1983, **71,** 767.
17. Flores, H. E. and Galston, A. W., *Plant Physiology*, 1984, **75,** 102.
18. Lee, T. M. and Chu, C., *Plant Physiology*, 1992, **100,** 238.

19. Hiatt, A. C., McIndoo, J. and Malmberg, R. L., *Journal of Biological Chemistry*, 1986, **261,** 1293.
20. Slocum, R. D. and Galston, A. W., *Plant Physiology*, 1985, **79,** 336.
21. Cohen, E., Malis-Arad, S., Heimer, Y. and Mizrahi, Y., *Plant Physiology*, 1982, **70,** 540.
22. Rastogi, R. and Davies, P. J., in *Biochemistry and Physiology of Polyamines in Plants*, ed. R. D. Slocum and H. E. Flores. CRC Press, London, 1991, p. 187.
23. Hirasawa, E. and Shimada, A., *Plant Cell Physiology*, 1994, **35,** 505.
24. Kamachi, S. and Hirasawa, E., *Plant Cell Physiology*, 1995, **36,** 915.
25. Dai, Y. R. and Galston, A. W., *Plant Physiology*, 1981, **67,** 266.
26. Kaur-Sawhney, R., Shih, L., Flores, H. E. and Galston, A. W., *Plant Physiology*, 1982, **69,** 405.
27. Turano, F. J. and Kramer, G. F., *Phytochemistry*, 1993, **34,** 959.
28. Rastogi, R., Dulson, J. and Rothstein, S. J., *Plant Physiology*, 1993, **103,** 829.
29. Malmberg, R. L. and Cellino, M. L., *Journal of Biological Chemistry*, 1994, **94,** 2703.
30. Borrell, A., Besford, R. T., Altabella, T., Masgrau, C. and Tiburcio, A. F., *Physiologia Plantarum*, 1996, **98,** 105.
31. Borrell, A., Culianez-Macia, F. A., Altabella, T., Besford, R. T., Flores, D. and Tiburcio, A. F., *Plant Physiology*, 1995, **109,** 771.
32. Besford, R. T., Richardson, C. M., Campos, J. L. and Tiburcio, A. F., *Planta*, 1993, **189,** 201.
33. Tiburcio, A. F., Besford, R. T., Capell, T., Borrell, A., Testillano, P. S. and Risueno, M. C., *Journal of Experimental Botany*, 1994, **45,** 1789.
34. Margosiak, S. A., Dharma, A., Bruce-Carver, M. R., Gonzales, A. P., Louie, D. and Kuehn, G. D., *Plant Physiology*, 1990, **92,** 88.
35. Del Duca, S., Tidu, V., Bassi, R., Esposito, C. and Serafini-Fracassini, D., *Planta*, 1994, **193,** 283.
36. Bradford, B. B., *Analytical Biochemistry*, 1976, **72,** 248.
37. Smith, M. A. and Davies, P., *Plant Physiology*, 1985, **78,** 89.