Korean Journal of Botany
Vol. 27, No. 1, 1~6(1984)

Cell Biological Studies on the Mechanism of Development and Differentiation VIII

1. Participation of Ornithine Decarboxylase in the Putrescine Biosynthesis in Corn Embryo.

Young Dong Cho*, Young Hee Kang, Sun Hi Lee, and Byoung Sik Pyo

(Department of Biochemistry* and Department of Biology, College of Science, Yonsei University, Seoul)

생체발생 및 분화기구의 세포생물학적 연구 VIII

1. 옥수수 배에서 Putrescine 합성에 관여하는 Ornithine Decarboxylase.

趙暎東*·康榮熹·李舜熙·表炳植

(연세대학교 이과대학 생화학과*, 생물학과)

ABSTRACT

Kinetin (4.7×10^{-5}M) and 6-benzylaminopurine (2.22×10^{-5}M) were found to increase ca. 1.5-fold putrescine content in corn grown in medium containing kinetin and 6-benzylaminopurine (6-BAP) for 3 days whereas kinetin was found to decrease ca. 30% spermidine and spermine, respectively. KCl (3×10^{-3}M) was found to decrease more than 50% putrescine content. After germination, ornithine decarboxylase activity was observed to increase constantly whereas arginine decarboxylase activity remained constant, suggesting involvement in putrescine biosynthesis. 6-benzylaminopurine was shown to increase more activities of arginine and ornithine decarboxylase than kinetin when they were added to medium.

INTRODUCTION

Polyamines are widely distributed in plants, microorganisms, and animal tissues, but their precise role in cellular processes is not fully understood. They are known to associated with cell proliferation, tissue regeneration, malignancy (Bachrach, 1970; Goyns, 1979; Heald, 1979; Prouty, 1976; Russell, 1973). Most of the information on the biosynthetic pathway of putrescine, its regulation and possible site of action has been obtained from

본 연구는 1983년도 문교부 기초과학육성 연구비 지원에 의한 것의 일부임.

studies with microorganisms and mammalian cells (Bachrach, 1970). Such information is lacking for plant system. Several reports (Cocucci and Bagni, 1968; Heimer and Mizrahi, 1982; Montague and Armstrong, 1979; Montague and Koppenbrink, 1978; Smith, 1970, 1975; Suresh *et al.*, 1978; Wang, 1972), however, describe the prescence of various polyamines and the occurrence of enzyme involved in polyamine biosynthesis, ornithine decarboxylase (EC 4. 1. 1. 17) and arginine decarboxylase (EC 4. 1. 1. 19). It has been commonly accepted that arginine decarboxylase is the enzyme responsible for the production of putrescine in plants and that ornithine decarboxylase is of lesser importance (Cho *et al.*, 1982, 1983; Montague and Amstrong, 1979; Montague and Koppenbrink, 1978; Smith, 1963, 1970, 1975; Suresh *et al.*, 1978; Srivenugopal and Adiga, 1981; Yanagisawa and Suzuki, 1981). In addition to them, 6-benzyladenine or kinetin which is known to reverse inhibition of germination by the naturally occurring inhibitors, coumarin and xanthatin (Khan, 1971) is shown to increase arginine decarboxylase activity (Suresh *et al.*, 1978). But participation of ornithine decarboxylase in putrescine biosynthesis is shown in a few plants (Altman *et al.*, 1982; Cohen *et al.*, 1982a, 1982b; Heimer and Mizrahi, 1982; Kaur-Sawhney *et al.*, 1982). In the present paper, we provide additional support for our claim that ornithine decarboxylase is surely participated in putrescine biosynthesis in corn embryo in view of the contradictory results regarding the activity and importance of arginine decarbxylase in plants. For this purpose, we examined activities of both arginine and ornithine decarboxylases, and the effect of cytokinins on both enzymes and level of polyamines.

MATERIALS AND METHODS

Plant material. Seeds of corn (*Zea mays* L.) were germinated in running water at 25°C for 1 day in the dark. Seedlings were transferred to Toyoroshi No. 3 filter paper containing Hoagland solution, which contained kinetin ranging from 0 to 4.7×10^{-5}M and 6-BAP ranging from 0 to 2.22×10^{-5}M as described elsewhere (Cho, 1983). The harvested embryo was washed with deionized H_2O and used for the experiment.

Chemicals. Polyamines were obtained from Sigma Chemical Co., U.S.A.. Dansyl Chloride was purchased from Wako Chemical Co., Japan. L-[1-^{14}C]-ornithine hydrochloride (324 mCi/m mole) and L-[^{14}C-(U)] -arginine hydrochloride (53 mCi/m mole) were purchased from New England Nuclear, U.S.A.. Other chemicals used were reagent grade and purified if necessary.

Extraction and assay of polyamines. 5g of fresh sample was homogenized as described elsewhere (Cho *et al.*, 1982). The amount of polyamines were also measured as described elsewhere (Cho *et al.*, 1982).

Extraction and assay of arginine and ornithine decarboxylases. Embryo was harvested, weighed, and ground in a prechilled mortar with a pestle (2 g fresh weight/2 ml medium).

The rest of procedures of extraction and assay was same as previous report (Altman *et al.*, 1982).

Protein determination. Protein in the crude extract was determined by using the Lowry's method.

RESULTS AND DISCUSSION

The putrescine level in the corn embryo of control increased steeply constantly after germination. As shown in Fig. 1, cytokinins such as kinetin and 6-benzylaminopurine were found to increase the putrescine content of corn embryo when both of them were added to medium and grown for 3 days, but kinetin reduce the spermidine and spermine levels (Fig. 2 and 3). 6-benzylaminopurine has no remarkable effect on the spermidine and spermine levels. KCl reduced the putrsecine and spermine but not spermidine. Polyamine level was dependent on the amount of kinetin, 6-benzylaminopurine and KCl respectively.

Activities of ornithine and arginine decarboxylase on time course is depicted in Fig. 4. One day after germination, arginine decarboxylase activity remains to be higher than ornithine decarboxylase whereas ornithine decarboxylase activity increase steadily. Arginine and ornithine decarboxylase activities are shown in Table 1 and 2 of corn grown in medium containing kinetin, 6-benzylaminopurine and KCl, respectively. Clearly, 6-benzylaminopurine treatment increased arginine decarboxylase activity but kinetin had slight effect. 6-benylaminopurine also had effect on increase in ornithine decarboxylase activity but kinetin. Interestingly, KCl tends to increase ornitine decarboxylase activity but arginine decarboxylase activity. Increase in the putrescine amount in corn embryo by kinetin is similar to previous report in which kinetin treatment increase the putrescine level in excised cucumber cotyledons (Suresh *et al.*, 1978). Such results suggest that kinetin might

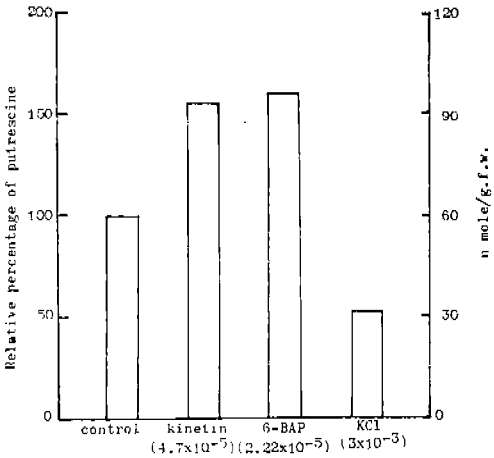

Fig. 1. Dependence of putrescine formation on kinetin, 6-BAP, and KCl.

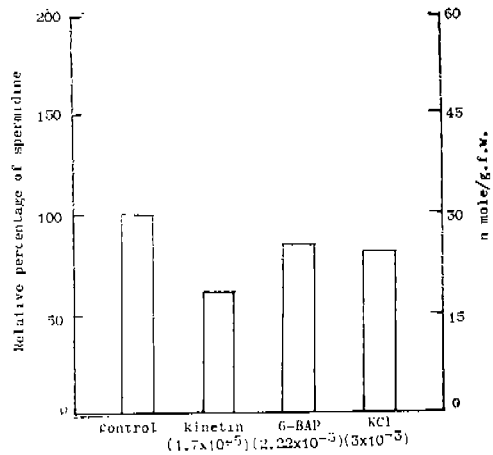

Fig. 2. Dependence of spermidine formation on kinetin, 6-BAP, and KCl.

Table 1. Arginine decarboxylase (EC 4. 1. 1.19) activity in 3 days old corn embryo. L-[^{14}C-(U)]-arginine was used as a substrate and released $^{14}CO_2$ was measured

Treatment	ADC activity (DPM/mg protein)
Control	2070
Kinetin*	2300
6-BAP**	3000
KCl***	2160

control; distilled water *; 4.7×10^{-5}M
; 2.22×10^{-5}M *; 3×10^{-3}M

Table 2. Ornithine decarboxylase (EC 4. 1. 1. 17) activity in 3 days old corn embryo. L-[1-^{14}C]-ornithine was used as a substrate and released $^{14}CO_2$ was measured

Treatment	ODC activity (DPM/mg protein)
Control	2680
Kinetin*	2620
6-BAP**	3320
KCl***	2940

control; distilled water *; 4.7×10^{-5}M
; 2.22×10^{-5}M *; 3×10^{-3}M

enhance putrescine biosynthesis or possibly inhibit the conversion from putrescine to spermidine, increasing the putrescine amount. Such assumption is supported with result that kinetin treatment reduces spermidine and spermine levels (Fig. 2 and 3), and increase in arginine decarboxylase activity by kinetin (Suresh *et al.*, 1978). On the other hand, the putrescine level seems to be closely related to potassium deficiency in many higher plants. The explanations of polyamine accumulation caused by the deficiency of inorganic cations are contradictory. But as far as KCl (3×10^{-3}M) seems to be so sufficient that there might be a decrease in the putrescine amount. 6-benzylaminopurine is shown to increase the putrescine amount as much as kinetin. However, 6-benzylaminopurine seems to have a different mechanism for polyamine since 6-benzylaminopurine has no remarkable effect on spermidine and spermine amounts whereas kinetin reduce both of them, suggesting that kinetin might inhibit the conversion of putrescine to spermidine

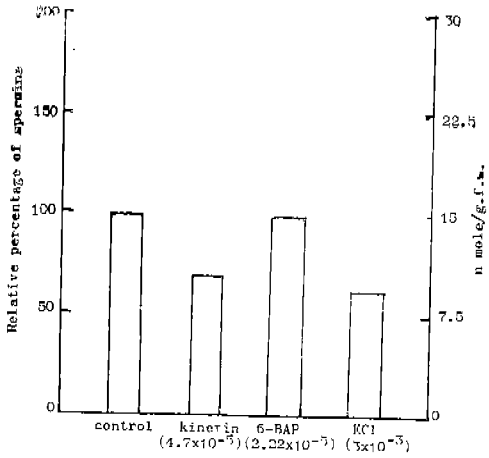

Fig. 3. Dependence of Spermine formation on kinetin, 6-BAP, and KCl.

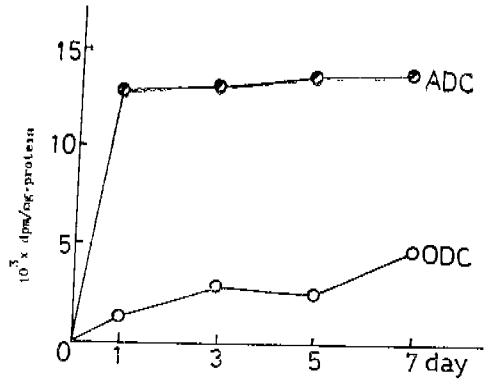

Fig. 4. Activities of ornithine decarboxylase and arginine decarboxylase in corn on the time course.

and spermine, and 6-benzylaminopurine enhance biosynthesis of putrescine mainly. The putrescine amount is believed to be dependent on arginine decarboxylase activity (Montague and Amstrong, 1979; Montague and Koppenbrink; 1978; Smith, 1963; Smith, 1975; Suresh *et al.*, 1978; Srivenugopal and Adiga, 1981; Yanagisawa and Suzuki, 1981). Accordingly, the enzymatic activity and the rate of conversion from putrescine to spermidine and spermine are key factors for putrescine amount under normal conditions such as appropriate amount of K^+ ion. Arginine decarboxylase activity can play a major role of the putrescine biosynthesis. However, the constant increase in ornithine decarboxylase activity suggests that the enzyme may contribute to the putrescine biosynthesis as age even if the activity of ornithine decarboxylase is lower than that of arginine decarboxylase. Such suggestions are in agreement with previous reports (Altman *et al.*, 1982; Cohen *et al.*, 1982a and 1982b; Heimer and Mizrahi, 1982; Kaur-Sawhney *et al.*, 1982). Higher activity of arginine and ornithine decarboxylases by 6-benzylaminopurine that kinetin increase in the same smount of putrescine by kinetin and 6-benzylaminopurine, respectively also provides support for assumption that kinetin may inhibit the conversion of putrescine to spermidine and spermine whereas 6-benzylaminopurine enhance putrescine biosynthesis. Cytokinins also have a different mechanism for the both enzymes considering difference in activity. Higher activity of arginine and ornithine decarboxylases by 6-benzylaminopurine than kinetin, and increase in the same amount of putrescine by 6-benzylaminopurine, respectively also provide support for assumption that kinetin may inhibit the conversion of putrescine to spermidine and spermine whereas 6-benzylaminopurine enhance putrescine biosynthesis through higher enzymatic activities of arginine and ornithine decarboxylase. And 6-benzylaminopurine and kinetin also seem to have different effect on both enzymes.

摘 要

Kinetin과 6-benzylaminopurine을 각각 함유하는 배지에서 3일간 생육시킨 옥수수에서 kinetin (4.7×10^{-5}M)과 6-benzylaminopurine (2.22×10^{-5}M)은 각각 약 1.5배의 putrescine 함량을 증가시킴을 보았다. 반면에 kinetin은 spermidine과 spermine 함량을 약 30% 감소시킴을 관찰하였다. KCl (3×10^{-3}M)은 putrescine 함량을 50%까지 감소시킴을 보았다. Ornithine decarboxylase 활성은 발아후 계속하여 증가하나 arginine decarboxylase 활성은 거의 일정하다. 이는 ornithine decarboxylase가 putrescine 합성에 관여함을 암시한다. 6-benzylaminopurine과 kinetin을 배지에 첨가시, 두 효소의 활성이 6-benzylaminopurine을 첨가할 때 kinetin보다 높게 나타남을 관찰하였다.

REFERENCES

Altman, A., R. Friedman and N. Levin. 1982. Arginine and ornithine decarboxylase, the polyamine biosynthetic enzymes, of mung bean seedlings. *Plant Physiol.* 69 : 876~879.

Bachrach, U. 1970. Metabolism and function of spermine and related polyamines. *Ann. Rev. Micro-*

biol. **24** : 109~134.

Cho, S. C. 1983. Effect of cytokinin and several inorganic cations on the polyamine content of lettuce cotyledons. *Plant Cell Physiol.* **24** : 27~32.

Cho, Y. D., B. G. Cho, S. H. Lee and Y. H. Kang. 1982. Cell biological studies on the mechanism of development and differentiation IV. *Korean Biochem. J.* **15** : 125 ~133.

_____, Y. H. Kang, M. J. Cho, S. K. Kim and S. H. Lee. 1983. Cell biological studies on the mechanism of development and differentiation VI. *Korean Biochem. J.* **16** : 151~159.

Cocucci, S. M. and M. Bagni 1968. Polyamine induced activation of protein synthesis in ribosomal preparation from *Helianthus tuberosus* tissue. *Life Sci.* **7** : 113~120.

Cohen, E., S. Arad, Y. M. Heimer and Y. Mizrahi. 1982a. Participation of ornithine decarboxylase in early stages of tomato fruit development. *Plant Physiol.* **70** : 540~543.

_____, Y. M. Heimer and Y. Mizrahi. 1982b. Ornithine decarboxylase activities in meristematic tissues of tomato and potato plants. *Plant Physiol.* **70** : 544~546.

Goyns, M. H. 1979. Changes in the nuclear polyamine content of chick erythrocytes during embryonic development. *Biochem. J.* **184** : 607~612.

Heald, P. J. 1979. Changes in ornithine decarboxylase during early implantation in the rat. *Biol. Repord.* **20** : 1195~1201.

Heimer, Y. M. and Y. Mizrahi. 1982. Characterization of ornithine decarboxylase of tobacco cells and tomato ovaries. *Biochem. J.* **201** : 373~376.

Kaur-Sawhney, R., L. M. Shin and A. W. Galston. 1982. Relation of polyamine biosynthesis to the initiation of sprouting in potato tubers. *Plant Physiol.* **69** : 411~415.

Khan, A. A. 1971. Cytokinins: permissive role in seed germination. *Science* **171** : 853~859.

Montague, M. J., T. A. Armstrong and E. G. Jaworski. 1979. Polyamine metabolism in embryogenic cells of *Daucus carota.* II. Changes in ADC activity. *Plant Physiol.* **63** : 341~345.

_____ and J. W. Koppenbrink. 1978. Polyamine metabolism embryogenic cells of *Daucus carota.* I. Changes in intracellular content and rates of synthesis. *Plant Physiol.* **62** : 430~433.

Prouty, W. F. 1976. Ornithine decarboxylase inactivation in Hela cells. *J. Cell. Physiol.* **89** : 65~96.

Russell, D. H. 1973. The role of the polyamines, putrescine, spermidine and spermine in normal and malignant tissues. *Life Sci.* **13** : 1635~1647.

Smith, T. A. 1963. L-arginine decarboxylase in higher plants and its relation to potassium nutrition. *Phytochemistry* **2** : 241~252.

_____. 1970. Putrescine, spermidine and spermine in higher plants. *Phytochemistry* **9** : 1479~1486.

_____. 1975. Recent advances in the biochemistry of plant amines. *Phytochemistry* **14** : 865~890.

Srivenugopal, K. S. and P. R. Adiga. 1981. Enzymic conversion of agmatine to putrescine in *Lathyrus sativus* seedlings. *J. Biol. Chem.* **256** : 9532~9541.

Suresh, R. M., S. Ramakrishna and P. R. Adiga. 1978. Regulation of arginine decarboxylase and putrescine levels in *Cucumis sativus* cotyledon. *Phytochemistry* **17** : 57~63.

Wang, L. C. 1972. Polyamines in soybeans. *Plant Physiol.* **50** : 152~156.

Yanagisawa, H. and Y. Suzuki. 1981. Corn agmatine iminohydrolase. *Plant Physiol.* **67** : 697~700.

(Received March 20, 1984)