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REGULATION OF AGMATINE IMINOHYDROLASE ACTIVITY IN GERMINATING GROUNDNUT SEEDS

R. K. SINDHU* and H. V. DESAI

Biochemistry Department, M.S. University, Baroda, India

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Key Word Index—*Arachis hypogea*; Leguminosae; groundnut; agmatine iminohydrolase; auxins; gibberellic acid; kinetin; ethrel; ethylene chlorohydrin; polyamines; inhibition.

Abstract—Activity of agmatine iminohydrolase present in the embryo of germinating groundnut seeds was reduced by removal of both of the cotyledons after soaking of the seeds, but removal of one cotyledon had no effect. The enzyme activity in the cotyledons and the embryo was decreased by feeding ethrel and ethylene chlorohydrin to the seedlings. The enzyme activity was inhibited when polyamines were added to the assay mixture but feeding these amines to the seedlings had no effect on the enzyme activity.

INTRODUCTION

Agmatine iminohydrolase, which converts agmatine to *N*-carbamylputrescine and ammonia, has been reported to be present in extracts of tobacco plants [1] and in the leaves and seeds of maize and sunflower seedlings [2]. We have described earlier the purification and properties of this enzyme from groundnut cotlyedons [3]. The groundnut enzyme has a pH optimum in the range 5.5–8.5 and the K_m for agmatine is $7.6\ (\pm 0.8) \times 10^{-4}$ M. In this communication, we report the results of some studies on the regulation of agmatine iminohydrolase activity in germinating groundnut seeds.

RESULTS AND DISCUSSION

Control of embryo agmatine iminohydrolase activity by the cotyledons

Removal of the embryo before or after soaking the seeds had no effect on agmatine iminohydrolase activity of the cotyledons. However, the presence of the cotyledons was essential for the formation of embryo enzyme since removal of both cotyledons resulted in a considerable decrease in the enzyme activity (Table 1). Removal of one cotyledon, however, had no effect on the enzyme activity.

Effect of hormones on agmatine iminohydrolase activity

Groundnut seeds were soaked for 16 hr in various concentrations of hormones and then allowed to germinate. IAA inhibited agmatine iminohydrolase activity in the cotyledons as well as in the embryo during the early period of germination and the enzyme activity tended to return to the control level with further germination (Table 2). A similar pattern was obtained in the presence of 2,4-D, GA and kinetin.

Effect of ethrel and ethylene chlorohydrin on agmatine iminohydrolase activity

The auxins and cytokinins have been shown to enhance the synthesis of ethylene in tissues [4, 5] and ethylene may therefore control this enzyme. To investigate this, groundnut seeds were germinated after soaking for 16 hr in ethrel (2-chloroethyl phosphonic acid) and ethylene chlorohydrin (2-chloroethanol), substances known to form ethylene *in vivo* [4]. Agmatine iminohydrolase activity was decreased in the cotyledons and the embryo with increasing concentrations of ethrel during early stages of germination (Table 3) and the enzyme activity tended to return to the control level with further germination. A similar pattern was obtained when seeds were soaked and germinated in the presence of ethylene chlorohydrin.

Effect of polyamines on agmatine iminohydrolase activity

The effect of hormones or ethylene-producing substances on agmatine iminohydrolase activity may be due to the accumulation of polyamines in tissues. To investigate this, groundnut seeds were soaked in the presence of 0.1% putrescine, spermidine or spermine and then allowed to germinate. No effect on the enzyme activity was observed in either the cotyledons or the embryo. However, the enzyme activity in the embryo was inhibited when polyamines were added to the assay mixture (Table 4). The enzyme activity in the cotyledons was inhibited only slightly by putrescine and spermidine but spermine inhibited the enzyme activity significantly.

The polyamine metabolism in groundnut seeds thus appears to be controlled in the following manner. The auxins and cytokinins [4, 5] as well as GA [6] enhance the production of ethylene in the tissue which inhibits

* Present address: Biochemistry Department, Postgraduate Institute of Medical Education and Research, Chandigarh-160 012, India.

Table 1. Effect of the presence of cotyledons on groundnut embryo agmatine iminohydrolase activity

Period of germination (days)	Agmatine iminohydrolase (nkat/g fr. tissue)		
	Embryos from whole seeds	Embryos from seeds where one cotyledon was removed after soaking	Embryos from seeds where both cotyledons were removed after soaking
0	0.72	0.72	0.72
2	1.32	1.25	1.02
4	1.45	1.38	0.64
6	1.22	1.10	0.20

Table 2. Effect of IAA on groundnut cotyledons and embryo agmatine iminohydrolase activity

Tissue	Concentration (ppm)	Agmatine iminohydrolase (nkat/g fr. tissue) on day			
		0	2	4	6
Cotyledons	0	0.60	1.26	1.88	1.98
	50	0.58	0.82	1.75	1.82
	200	0.56	0.70	1.08	1.46
Embryo	0	0.73	1.30	1.41	1.21
	50	0.72	1.08	1.20	1.05
	200	0.70	0.88	0.95	0.94

Table 3. Effect of ethrel on groundnut cotyledons and embryo agmatine iminohydrolase activity

Tissue	Concentration (mM)	Agmatine iminohydrolase (nkat/g fr. tissue) on day			
		0	2	4	6
Cotyledons	0	0.60	1.26	1.88	1.98
	5.0	0.53	0.73	1.24	1.95
	12.5	0.52	0.62	0.94	1.42
Embryo	0	0.73	1.30	1.41	1.21
	5.0	0.64	0.79	1.20	1.21
	12.5	0.58	0.60	0.98	1.08

Table 4. Effect of polyamines on groundnut agmatine iminohydrolase activity *in vitro*

Concentration (mM)	Agmatine iminohydrolase units with added polyamine*					
	Putrescine		Spermidine		Spermine	
	Cotyledons	Embryo	Cotyledons	Embryo	Cotyledons	Embryo
0	0.13	0.13	0.13	0.13	0.13	0.13
2.5	0.13	0.13	0.12	0.12	0.11	0.05
10.0	0.12	0.13	0.11	0.09	0.07	0.03
25.0	0.11	0.08	0.10	0.05	0.06	0.02

*Polyamines were added to the assay mixture. Groundnut seeds germinated for 2 days were used for this study.

the synthesis of diamine oxidase [7] and results in the accumulation of polyamines. Increased polyamine levels were observed by feeding hormones to groundnut seedlings (Sindhu, R. K., unpublished observations). The increased polyamine concentration then controls agmatine iminohydrolase activity by end-product inhibition.

EXPERIMENTAL

Plant material. Groundnut seeds (*Arachis hypogea* L. cv Punjab-1) were soaked and germinated as described in ref. [3]. In cases where the cotyledons were removed after soaking, the embryos were kept in Petri dishes on moist filter paper for the same period. The time when seeds or embryos were placed in Petri dishes for germination, after soaking for 16 hr in H_2O or other test material, was considered as zero day of germination.

Enzyme activity was determined by estimating *N*-carbamylputrescine by the method of ref. [8]. The reaction mixture contained 100 μmol Pi buffer, pH 7.5, 5 μmol agmatine and enzyme (1 ml) in a total vol. of 4 ml. After incubation at 37° for 1 hr, the reaction was terminated by adding 0.5 ml of 10% TCA. The supernatant, after removal of proteins by centrifugation, was used for the estimation of *N*-carbamylputrescine. Diamine oxidase, which has a broad substrate specificity, has been shown [9, 10] to act on agmatine and *N*-carbamylputrescine in addition to several other amines. In the present study, however, no increase in agmatine iminohydrolase activity was observed on introducing semicarbazide, an inhibitor of diamine oxidase, in the assay system. Semicarbazide (10 mM) completely inhibited the diamine oxidase activity but agmatine iminohydrolase activity was not affected.

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REFERENCES

1. Yoshida, D. (1969) *Plant Cell Physiol. (Tokyo)* **10,** 923.
2. Smith T. A. (1969) *Phytochemistry* **8,** 2111.
3. Sindhu, R. K. and Desai, H. V. (1979) *Phytochemistry* **18,** 1937.
4. Abeles, F. B. (1973) *Ethylene in Plant Biology.* Academic Press, New York.
5. Burg, S. P. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70,** 591.
6. Ketring, D. L. and Morgan, P. W. (1970) *Plant Physiol.* **45,** 268.
7. Sindhu, R. K. (1977) Doctoral Thesis, M.S. University, Baroda, India.
8. Archibald, R. M. (1944) *J. Biol. Chem.* **156,** 121.
9. Kenten, R. H. and Mann, P. J. G. (1952) *Biochem. J.* **50,** 360.
10. Rangarajan, S. M., Ramakrishna, S. and Adiga, P. R. (1976) *Phytochemistry* **15,** 483.