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REGULATION OF DIAMINE OXIDASE ACTIVITY IN GERMINATING PEA SEEDS

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Key Word Index—*Pisum sativum*; Leguminoae; pea; diamine oxidase; auxins: cycloheximide; 5-fluorouracil; induction.

Abstract—Diamine oxidase present in the cotyledons of germinating pea seeds is induced by putrescine, spermidine and ornithine. Auxins inhibit enzyme synthesis in cotyledons only in the presence of embryo. Cycloheximide inhibits the synthesis of the cotyledon enzyme but has no effect on the embryo enzyme. 5-Fluorouracil inhibits the synthesis of both cotyledon and embryo enzymes.

INTRODUCTION

Amine oxidases, which may be involved in the regulation of intracellular polyamine concentration, are widely distributed [1] and function by a variety of mechanisms [2]. The pea diamine oxidase has a broad specificity and oxidizes many primary amines [2]. This enzyme has been extensively purified and characterized from pea seedlings [3–7]. The present communication reports the results of studies on the regulation of diamine oxidase from germinating pea seeds.

RESULTS AND DISCUSSION

Effect of period of germination on diamine oxidase activity

Pea diamine oxidase is not detected in resting seeds. In earlier studies [8,9] it was said to appear 3–4 days after germination and to reach a maximum on the 6th–12th day. In the present study (Table 1) diamine oxidase in cotyledons was detected 62 hr after germination, increased up to 110 hr and was constant to 182 hr. In the embryo, the pattern is similar but some activity appeared even 38 hr after germination. Similar results have recently been reported by Smith and Wilshire [10].

Table 1. Diamine oxidase activity in the cotyledon and embryo of pea seedlings germinated in dark

Period of germination (hr)	Diamine oxidase (nkat/g fr. tissue)	
	Cotyledon	Embryo
14	<0.08	0.2
38	0.2	3.7
62	10.9	12.2
86	12.7	13.5
110	14.2	14.7
135	13.5	15.0
158	13.4	13.4
182	14.2	15.0

Control of cotyledon diamine oxidase activity by the embryo

Removal of the embryo before soaking of seeds completely abolishes the cotyledon enzyme confirming the results of Werle *et al.* [11]. However, if the embryo was removed from seeds after soaking for 14 hr, the cotyledons showed full activity 48 hr later. A time course study on the induction of cotyledon enzyme in seeds soaked for 14 hr and allowed to germinate with embryo, and in seeds where the embryo was removed after soaking for 14 hr showed that in the former group activity appears only 28 hr later whereas in the latter group it appears within 24 hr. The enzyme activity was however, not significantly different in the two groups after 32 hr. These results suggest that the embryo, apart from its initial inducing action, may inhibit the appearance of enzyme in the cotyledons if it is present during the later stages of germination.

To investigate this further, mature fresh green pea seeds were removed from the pods and divided into two groups. One group containing embryo was dried, in the other, cotyledon and embryo were removed and dried separately. The enzyme activity was assayed 62 hr after germination. The results showed that fresh seeds have practically no enzyme activity, but when the whole seed is dried and germinated for 62 hr, diamine oxidase appears in cotyledon. However, the dried cotyledons alone when treated in the same manner do not show any activity. The embryo alone under similar conditions shows activity. If the cotyledons were allowed to soak in 30% embryo extract in water and assayed after 62 hr, about 50% of the whole seed activity was obtained, indicating the presence of some factor in the embryo responsible for the induction of cotyledon diamine oxidase. The factor was heat-stable and dialysable. The total amine fraction obtained from the embryo by cellulose phosphate column chromatography subsitituted for the factor present in embryo. In another experiment dried cotyledons without embryo were soaked in the presence of 0.1% ornithine, citrulline, arginine, agma-

Table 2. Effect of 2,4-D on pea cotyledon and embryo diamine oxidase activity

Concentration (ppm)	Diamine oxidase (nkat/g fr. tissue)		
	Cotyledon		Embryo
	Seeds soaked and germinated with embryo	Embryo removed from seeds after soaking	
0	10.5	10.7	12.0
2.5	<0.08	11.0	9.2
5.0	<0.08	9.7	6.2
7.5	<0.08	9.7	5.3
10.0	<0.08	10.5	3.5

Enzyme activity was determined after 62 hr from the commencement of treatment. The seeds were soaked in 2,4-D for 14 hr.

tine, putrescine, cadaverine, spermidine, or spermine and the activity was determined 62 hr after germination. Out of the various compounds tested putrescine, spermidine and ornithine induced cotyledon enzyme. The embryo enzyme was however, not affected by these compounds. A dose response study showed that a minimum of about 400 ppm putrescine during soaking is needed for induction of the cotyledon enzyme.

Effect of hormones on diamine oxidase activity

To investigate whether hormones have any effect on diamine oxidase activity, seeds were soaked in different hormones and then allowed to germinate for 62 hr. 2,4-D and IAA inhibited enzyme activity whereas GA showed activation. Kinetin, however, had no effect. In a separate experiment where different concentrations of 2,4-D were used (Table 2) the enzyme activity was completely abolished at 2.5 ppm in the whole seeds where the embryo was present during soaking and germination. However, if the embryo was removed after soaking for 14 hr, 48 hr later the activity in the cotyledons was not reduced even by a concentration of 2,4-D which was four times higher. Activity of the embryo enzyme was reduced, but to a lesser extent. These results indicate that 2,4-D does not directly control the cotyledon enzyme but acts through the embryo. Similar results were obtained with IAA. The inhibitory effect of 2,4-D could be abolished by allowing the germination to proceed for a longer period (Table 3).

Table 3. Reversal of 2,4-D inhibition of pea cotyledon diamine oxidase activity

Concentration (ppm)	Diamine oxidase (nkat/g fr. tissue)			
	at hr			
	62	86	110	158
0	12.9	15.9	15.0	14.9
1.0	4.7	14.7	13.7	14.7
2.5	0.3	10.4	12.0	13.9
5.0	<0.08	7.5	12.4	14.2
7.5	<0.08	6.5	12.0	13.6
10.0	<0.08	<0.08	12.9	14.7

Enzyme activity was determined at different hr from the commencement of treatment. The seeds were soaked with 2,4-D for 14 hr.

Table 4. Effect of gibberellic acid on pea cotyledon diamine oxidase activity

Concentration (ppm)	Diamine oxidase (nkat/g fr. tissue)	
	Seeds soaked and germinated with embryo	Embryo removed from seeds after soaking
0	<0.08	2.7
25	2.2	4.3
50	3.5	4.0
75	4.2	4.2
100	4.5	4.3

Enzyme activity was determined after 38 hr from the commencement of treatment. The seeds were soaked in GA for 14 hr.

GA induces the cotyledon enzyme (Table 4) 38 hr after germination and the effect is enhanced if the embryo is removed after soaking of seeds. Embryo enzyme, however, was not affected by GA.

Pea diamine oxidase has been considered to be involved in the formation of IAA from tryptamine in plants [12,13] though recently this was thought unlikely [14]. Results of the present study however, show that auxins control cotyledon diamine oxidase activity by inhibiting its synthesis, and this control is mediated through the embryo. GA has an activating effect which would ensure a balanced synthesis of enzyme in cotyledon. These results are of significance since IAA, whose synthesis may be controlled by diamine oxidase, seems to inhibit its own synthesis in a feed-back manner. The decreased synthesis of diamine oxidase in presence of high levels of IAA would result in increased levels of di and polyamines which would again induce the synthesis of the enzyme.

Effect of inhibitors of protein synthesis on diamine oxidase activity

The data reported in Tables 5 and 6 show the effect of cycloheximide and 5-fluorouracil on cotyledon and

Table 5. Effect of cycloheximide on pea cotyledon and embryo diamine oxidase activity

Concentration (ppm)	Diamine oxidase (nkat/g fr. tissue)	
	Cotyledon	Embryo
0	10.2	13.2
0.5	2.5	13.7
1.0	2.0	14.4
1.5	1.7	14.4
2.0	1.3	11.5
5.0	<0.08	12.4
10.0	<0.08	12.4

Enzyme activity was determined after 62 hr from the commencement of treatment. The seeds were soaked in cycloheximide for 14 hr.

Table 6. Effect of 5-fluorouracil on pea cotyledon and embryo diamine oxidase activity

Concentration (ppm)	Diamine oxidase (nkat/g fr. tissue)	
	Cotyledon	Embryo
0	10.4	13.7
50	2.7	6.9
100	2.2	6.2
150	2.2	3.8
200	1.0	3.2
300	0.3	2.3
400	<0.08	1.5
500	<0.08	0.8

Enzyme activity was determined after 62 hr from the commencement of treatment. The seeds were soaked in 5-fluorouracil for 14 hr.

embryo diamine oxidase activity. Cycloheximide completely blocks the synthesis of cotyledon diamine oxidase at 5 ppm but has no effect on the embryo enzyme even at 10 ppm. 5-Fluorouracil however, affects both cotyledon and embryo enzyme but requires a concentration three times greater than for the cotyledons to abolish the embryo enzyme activity completely. Putrescine or spermidine could not induce the cotyledon enzyme in the presence of cycloheximide.

EXPERIMENTAL

Plant material. Pea seeds (*Pisum sativum*) were surface sterilized and thoroughly washed with H_2O and then soaked for 14 hr in H_2O or other test material as specified. The seeds were then kept for germination at 22° in dark in Petri dishes on moist filter papers. In cases where the embryo was removed before or after soaking, the cotyledons were kept in Petri dishes on moist filter papers for the same period of time. The time of the commencement of treatment or soaking of the seeds or cotyledons was considered as zero time.

Enzyme extract. At specified periods cotyledon and embryo were separated and a 10% extract of the tissue was prepared by grinding in a chilled pestle–mortar using 60 mM Pi buffer, pH 7. The extract after passing through 2 layers of cheese cloth was used for enzyme assays.

Enzyme activity. This was determined according to the method of ref. [15]. The assay system consisted of 50 μmol 0.1 M Pi buffer, pH 7.5, 5 μmol putrescine and enzyme (0.2 ml) in a total vol of 4 ml after incubation at 37° for 30 min the reaction was terminated by adding 0.5 ml of 10% TCA followed by 0.1 ml (10 mg/ml) *o*-aminobenzaldehyde. The coloured complex, after removal of proteins by centrifugation, was read at 430 nm ($E_0 = 1.86 \times 10^3$/mol/cm [15]). Using manometric technique for the assay of this enzyme other workers [3–6] have incorporated catalase in the assay system but its addition in the present assay system showed no increase in enzyme activity.

Separation of pea seedling amines. This was according to the method of ref. [16]. A known amount of tissue was extracted with 10% cold TCA and the supernatant obtained after centrifugation was adjusted to pH 6 and 4 vol of Pi buffer 10 mM, pH 6, was added. This was put on a 3 ml column of cellulose phosphate washed and activated according to the method of ref. [17]. The column was washed with 20 ml of 10 mM Pi buffer, pH 6 and then with 30 ml of 0.02 M HCl. The amines were eluted with 50 ml of 0.4 M HCl. The eluate was evaporated at 100° and the dried sample was made up in 10 ml H_2O and used for soaking the cotyledons.

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