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Embryonic control of isocitrate lyase activity in cotyledons of germinating soybean

BALWANT SINGH*, P. S. SUKHIJA and I. S. BHATIA
Department of Biochemistry, Punjab Agricultural University, Ludhiana 141 004

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Abstract. The activity of isocitrate lyase (EC 4.1.3.1) in the cotyledons of germinating soybean is controlled by the embryonic axis. Plant growth regulators like gibberellic acid, indole acetic acid and 2,4-dichlorophenoxy acetic acid are able to increase the enzyme activity in cotyledons of whole seedlings but not in dissected cotyledons. The control of induction of the enzyme activity during germination by the embryo could be mediated by the elaboration of kinetin.

Keywords. Isocitrate lyase; soybean; germination.

Introduction

The embryonic axis is known to control many events occurring in the cotyledons of germinating seeds (Penner and Ashton, 1967; Bilderback, 1974; Heimer *et al.*, 1976). However, the role of the embryo in controlling isocitrate lyase (EC 4.1.3.1) activity in cotyledons of germinating seeds is not clear. In castor bean endosperm, pea nut, *Cucumis sativa* and *Cucurbita maxima*, the embryo does not have a controlling function (Tanner and Beevers, 1965; Marcus and Feeley, 1964; Ford *et al.*, 1976), but in the case of squash melon and sunflower, cytokinins from the embryo control the appearance of the lyase activity in cotyledons (Penner and Ashton, 1967; Heimer *et al.*, 1976). A similar role has also been ascribed to gibberellins in wheat aleurone cells (Tavener and Laidman, 1975) and in ponderosa pine seeds (Bilderback, 1974). The present studies were conducted with a view to investigate the role of the embryo in the development of isocitrate lyase activity in germinating soybean seeds.

Materials and methods

Soybean (*Glycine max* L., variety Bragg) seeds were procured from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. Growth regulators such as gibberellic acid (GA_3), indole acetic acid (IAA), kinetin and other chemicals were purchased from Sigma Chemical Co., St. Louis, Missouri, USA.

* Present address: Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, 6701 SZEGED, P.O.B. 521, Hungary.

Abbreviations used: GA_3, Gibberellic acid; IAA, Indole acetic acid; 2,4-D, 2,4-Dichlorophenoxy acetic acid.

Germination

Before germination, the seeds were surface-sterilized with 0.1% mercuric chloride and washed with distilled water. The washed seeds were sown in petri plates (50 mm×20 mm) lined with filter paper saturated with distilled water at 25 ± 1°C in a biological oxygen demand incubator. To study the effect of growth regulators and other chemicals distilled water was replaced with solutions of these compounds. Samples were collected at desired time, washed with distilled water and analysed.

Enzyme assay

The tissues were ground in a chilled mortar, in ice cold 0.05 M phosphate buffer, pH 7.6. The ratio of plant material to buffer was 1:5 (w/v). The slurry was filtered through 4 layers of muslin cloth and the filtrate was centrifuged at 15,000 *g* for 20 min. The clear supernatant was used as the enzyme preparation.

The enzyme was assayed according to the procedure of Gientika and Cherry (1968). The reaction mixture, in a total volume of 1.5 ml, contained—10 Mμ phosphate buffer, pH 7.6; 2.5 μM glutathione; 5 μM $MgSO_4$; 5 μM isocitrate (trisodium salt) and 0.1 ml of enzyme extract. The reaction was started by adding the substrate. After incubation for 15 min at 30°C, the reaction was terminated by adding 0.3 ml of 100% trichloroacetic acid. The denatured protein was removed by centrifugation. To 1 ml of the protein-free supernatant, 0.66 ml of 0.1% 2,4-dinitrophenyl hydrazine in 2 NHCl was added and the reaction mixture was kept at room temperature for 20 min. Then 3.35 ml of 2.5 N sodium hydroxide was added and the absorbance measured at 445 nm.

Protein estimation

Protein content was determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Results and discussion

The data on the effect of the embryo on the appearance of isocitrate lyase activity in cotyledons of germinating soybean are shown in table 1. The enzyme activity in dissected cotyledons was found to be only about 57% of that in cotyledons of whole seedlings germinating under similar conditions. Thus the data indicate that isocitrate lyase activity is partly under the control of the embryonic axis. These findings are contrary to the study of Marcus and Feeley (1964) in pea nut and Ford *et al.* (1976) in *Cucumis sativa* and *Cucurbita maxima* but are in agreement with the studies of Tavener and Laidman (1975), Jones (1972) and Bilderback (1974) in wheat, barley and ponderosa pine seeds respectively. The activity of isocitrate lyase was measured (table 1). The data in table 1 clearly indicate that the removal of the embryo during the first 3 days of germination markedly affects the subsequent appearance of the enzyme activity. But if the embryo was removed after 3 days of germination, it had no effect. It can therefore be concluded that the presence of the embryo is essential during the initial stages of germination and some factor(s) from embryo may be responsible for the observed increase in iso-citrate lyase activity in the cotyledons.

Table 1. Effect of embryo on isocitrate lyase activity in cotyledons of germinating soybean.

Incubation		Specific activity
Intact seedlings (days)	Followed by excision of cotyledons and incubation (days)	μmol of glyoxylate released/mg protein/ 15 min
6 (intact cotyledons)	0	0.98
5	1	0.95
4	2	0.94
3	3	0.70
2	4	0.60
1	5	0.56
0 (dissected cotyledons)	6	0.56

The values are the average of 3 independent determinations.

Isocitrate lyase activity in the cotyledons of seeds germinated for 6 days in the presence of various concentrations of GA_3, IAA, 2,4-D and kinetin is recorded in table 2. All the four growth regulators tested increased the lyase activity in cotyledons. The optimal concentration of GA_3 was 10 μM (which increased the activity by 41 %) while that of kinetin was 100 μM at which a 37% increase in activity was observed. IAA and 2,4-D both had an optimal concentration of 1 μM. Similar effects of plant growth regulators have also been observed in hazel (Pinfield, 1968; Potempta and Galsky, 1973), *Pinus pinea* (Martinez *et al.*, 1975) and ponderosa pine (Bilderback, 1974) seedlings.

Table 2. Effect of growth regulators on isocitrate lyase activity in cotyledons of germinating soybean.

Concentration (μM)	Specific activity [a]			
	GA_3	IAA	Kinetin	2,4-D
Control	1.0	1.0	1.0	1.1
0.1	1.0	1.2	1.1	1.4
1.0	1.2	1.4	1.1	1.5
10	1.5	1.4	1.2	1.3
100	1.2	1.0	1.4	1.2
1000	1.1	1.0	—	—

a μmol of glyoxylate released/mg protein/15 min.

The values are the average of 3 independent observations.

Data on the effect of the interaction of growth regulators on the induction of isocitrate lyase activity are shown in table 3.

Table 3. Effect of interaction of growth regulators on isocitrate lyase activity in cotyledons of germinating soybean.

Growth regulators (μM)	Specific activity (μmol of glyoxylate released/ mg protein/15 min)
Control	1.0
GA_3 (10)	1.4
IAA (1)	1.4
Kinetin (100)	1.4
Kinetin (100) + GA_3 (10)	1.6
Kinetin (100) + IAA (1)	1.4
GA_3 (10) + IAA (1)	1.5

The values are the average of 4 independent determinations.

In an experiment to ascertain the growth regulatory nature of the embryonic factor(s), excised cotyledons were incubated for 6 days in solutions of GA_3, IAA, kinetin and 2,4-D and the isocitrate lyase activity was measured, (table 4). Kinetin increased the lyase activity to a considerable extent but could not completely replace the embryo in this respect. So it appears likely that kinetin may be one of

Table 4. Effect of growth regulators on isocitrate lyase activity in dissected soybean cotyledons.

Growth regulator	Concentration (μM)	Specific activity (μmol of glyoxylate released/ mg protein/15 min)
Control (intact cotyledons)		1.0
Control (excised cotyledons)		0.6
GA_3	10	0.6
IAA	1	0.6
Kinetin	250	0.9
2,4-D	1	0.5

The values are the average of 3 independent determinations.

the factors produced by the embryo. GA_3 could increase the enzyme activity only to a small extent while IAA and 2,4-D failed to do so. This suggests strongly that GA_3, IAA and 2,4-D increase the isocitrate lyase activity in cotyledons of germinating seeds (table 2) in an indirect manner and by themselves are without any effect. In squash cotyledons, benzyl adenine could partially replace the embryonic factors (Penner and Ashton, 1967) while in wheat aleurone cells, GA_3 was effective in increasing isocitrate lyase activity (Tavener and Laidman, 1975).

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