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REGULATION OF *O*-DIPHENOLASE ACTIVITY BY AUXIN AND ABSCISIC ACID IN GERMINATING WHEAT EMBRYOS

SHANT R. TANEJA and R. C. SACHAR
Division of Biochemistry, Indian Agricultural Research Institute, New Delhi-110012, India

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Abstract—During germination *o*-diphenolase activity increased several fold (×13) in wheat embryos. The enzyme activity and the number of multiple forms were reduced by the continuous presence of abscisic acid (ABA) *in vivo*. The *in vitro* addition of ABA to *crude extracts* failed to inhibit the *o*-diphenolase activity. Auxin (2,4-D) substantially alleviated the inhibitory action of ABA on *o*-diphenolase activity, although 2,4-D alone failed to bring about any appreciable stimulation of enzyme activity.

INTRODUCTION

A variety of physical and chemical agents have been implicated in the regulation of *o*-diphenolase activity in plant systems. Treatment of intact chloroplasts and partially purified enzyme preparations of grapes with acid pH (5) and 4M urea resulted in the activation of *o*-diphenolase activity [3]. The latent form of *o*-diphenolase was also activated by treating leaf chloroplasts with a peptide preparation of autolysed trypsin [4]. In wheat leaf chloroplasts, low intensity red light brought about the activation of *o*-diphenolase [4]. Exposure of tissues to ethylene resulted in a two-fold increase of *o*-diphenolase activity in sweet potato [5, 6] and a 4-fold increase in white potato [5]. A 5-fold stimulation of *o*-diphenolase activity was observed in tobacco pith cultures by supplementing the medium with IAA. The stimulatory response of auxin was counteracted by kinetin [7, 8]. In embryo-free wheat endosperm, the stimulation (two-fold) of monophenolase activity by GA_3 was completely counteracted by ABA [9]. In the present communication, we discuss the role of auxin and ABA in the regulation of *o*-diphenolase activity in germinating wheat embryos.

RESULTS AND DISCUSSION

Excised wheat embryos were germinated in presence of different plant growth substances to test their effect on *o*-diphenolase activity. Gibberellic acid (GA_3, 10^{-7}M, 10^{-5}M), kinetin (10^{-7}M, 10^{-6}M) and ethrel (10^{-6}M, 10^{-5}M, 10^{-4}M) failed to alter the enzyme activity and the multiple forms of *o*-diphenolase. However, the regulation of *o*-diphenolase activity was witnessed in embryos cultured in presence of ABA and auxin.

Effect of ABA and auxin on embryo germination

ABA (5×10^{-5}M) inhibited the germination of excised wheat embryos. The germination response of embryos cultured in low concentration of auxin (2,4-D, 10^{-6}M) was comparable to that of controls. A slight inhibition of coleoptile and root growth was found at 10^{-5}M of 2,4-D. This was accompanied by the formation of a callus tissue at the base of the coleoptile. At supra-optimal concentration (2,4-D, 10^{-4}M), there was severe inhibition of root and coleoptile growth; instead, 2,4-D supported the growth of a loose proliferating callus tissue. The auxin-induced callus growth was strongly suppresed by ABA (5×10^{-5}M) and actinomycin D (Act D) (80 μg/ml). However, the inhibitory action of auxin on coleoptile and root growth was not reversed by ABA and Act D.

Effect of ABA on o-diphenolase activity and its multiple forms

About 50% inhibition of *o*-diphenolase activity was observed in embryos cultured (48 hr old) in the presence of ABA (5×10^{-5}M). Further increase in the concentration of ABA (5×10^{-4}M) exhibited 65% inhibition of enzyme activity (Table 1). The continuous presence of ABA was necessary for the inhibition of enzyme activity, since transfer of embryos from ABA (10^{-4}M) to control medium abolished its inhibitory response. Addition of

Table 1. Effect of abscisic acid on *o*-diphenolase activity in germinating wheat embryos

Additions	Enzyme units/mg protein	% Inhibition
Control	610	—
ABA:		
10^{-6}M	592	3
10^{-5}M	450	26
5×10^{-5}M	312	49
10^{-4}M	259	58
5×10^{-4}M	213	65

Excised wheat embryos were germinated in dark at 25° for 48 hr in the continuous presence of ABA. The enzyme activity was determined in crude extracts.

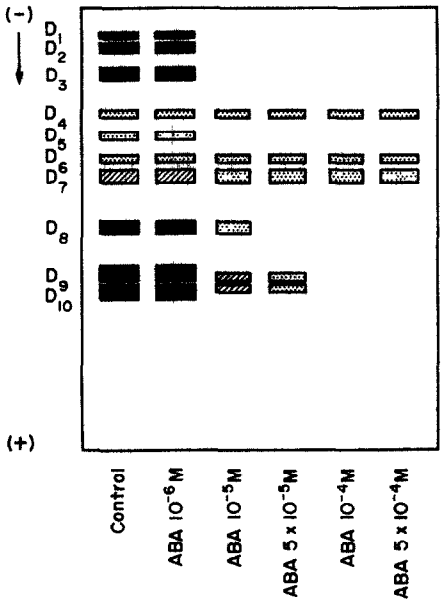

Fig. 1. Effect of abscisic acid on multiple forms of *o*-diphenolase in germinating wheat embryos. Excised embryos were germinated in dark at 25° in the continuous presence of different concentrations of ABA for 48 hr. Crude extracts were fractionated on anionic (pH 8.3) acrylamide gels (7.5%). The multiple forms of *o*-diphenolase were developed by incubating gels with DL-dopa (1.5 mg/ml) for 30 min at 37°. ■ High activity, ▨ Medium activity, ⊠ Low activity.

Act D (80 μg/ml) along with ABA (5×10^{-5} M) to wheat embryos showed 52% inhibition of *o*-diphenolase activity which was almost identical to the inhibition (54%) observed with ABA (5×10^{-5} M) alone. This ruled out the requirements of fresh transcription for the inhibitory effect of ABA. ABA did not appear to inactivate the preformed enzyme molecules since the *in vitro* addition of ABA (10^{-4} M) to *crude extracts* or during homogenization of control embryos (48 hr old) was completely ineffective in retarding *o*-diphenolase activity. Similarly *crude extracts* of control embryos when mixed with extracts of ABA-treated embryos showed no decrease in the enzyme activity. Also, the *in vivo*-induced inhibition (50%) of *o*-diphenolase activity by ABA (5×10^{-5} M) was not relieved following exhaustive dialysis of *crude extracts*. This suggested that the action of ABA on *o*-diphenolase activity was not mediated by some inhibitory factor.

Fractionation of *crude extracts* of control embryos (zero hr germination) revealed 4 multiple forms of *o*-diphenolase (D_4, D_6, D_7, D_8) on acrylamide gels. 6 new activity bands (D_1, D_2, D_3, D_5 D_9, D_{10}) appeared in 48 hr-old germinated embryos. Thus in all, 10 multiple forms were consistently observed in control embryos (48 hr old). The inhibitory effect of ABA on multiple forms of *o*-diphenolase is shown in Fig. 1. Abscisic acid (ABA, 10^{-4} M) completely abolished the appearance of 6 newly formed activity bands and could be responsible for the inhibition of enzyme activity.

In cotton embryos, ABA inhibited protease and isocitratase activities by interfering with the translation of their conserved messengers. The effect of ABA was alleviated by Act D, suggesting that the growth retardant inhibited enzyme activities through the production of RNA [10, 11]. In barley aleurone layers, ABA inhibited the GA_3-induced amylase activity by operating at the translational level. Cordycepin counteracted the inhibitory response of ABA, thus implicating the action of ABA through fresh transcription [12, 13]. In wheat embryos, we have earlier reported that the stimulation of *o*-diphenolase activity was supported by its conserved message [1,2]. Conceivably, ABA inhibited the *o*-diphenolase activity by operating at some post-transcriptional step. Inhibition of protein synthesis by ABA has been reported in germinating wheat embryos [14]. Since Act D failed to relieve the ABA-induced inhibition of *o*-diphenolase activity, the action of ABA did not seem to require fresh transcription.

Effect of auxin on o-diphenolase activity

Embryos cultured in presence of auxin showed no appreciable stimulation of *o*-diphenolase activity. Low concentration of 2,4-D (10^{-6} M) exhibited a 12% increase in enzyme activity, while supraoptimal levels (10^{-4} M) showed a 10% decrease in *o*-diphenolase activity. Auxin, however, proved substantially effective in counteracting the ABA-induced inhibition of *o*-diphenolase activity. 2,4-D at 10^{-5} M could overcome 20–37% inhibition of enzyme activity, while at 10^{-4} M, it alleviated the inhibitory effect of ABA by 51–74% (Table 2). It is likely that the normal stimulation of enzyme activity in control embryos is supported by the endogenous pool of auxin.

Table 2. Inhibitory effect of abscisic acid on *o*-diphenolase activity and its counteraction by auxin in germinating wheat embryos

Additions	*o*-Diphenolase activity					
	Expt I			Expt II		
	Enzyme units/mg protein	% Inhibition	% Inhibition overcome by 2,4-D	Enzyme units/mg protein	% Inhibition	% Inhibition overcome by 2,4-D
Control	620	—	—	760	—	—
ABA 5×10^{-5} M	290	53	—	390	49	—
ABA 5×10^{-5} M + 2,4-D 10^{-5} M	360	42	20	530	31	37
ABA 5×10^{-5} M + 2,4-D 10^{-4} M	460	26	51	660	13	74

Excised embryos were cultured in dark at 25° for 48 hr. ABA and 2,4-D were present throughout the period of germination. *o*-Diphenolase activity was assayed in crude extracts.

EXPERIMENTAL

Wheat embryos (*Triticum aestivum*, var. Shera) were scooped from grains (soaked for 10 hr at 4°) and surface sterilized with 0.02% $HgCl_2$ soln for 10 min. The excised embryos were cultured in dark at 25° on aseptic liquid nutrient medium [15] fortified with vitamin soln [16], sucrose (2%) and to which chloramphenicol (50 μg/ml) was added. The effect of plant growth substances (GA_3, kinetin, auxin, ethrel, ABA) was tested on *o*-diphenolase activity in germinating embryos. The embryos were homogenized in 50 mM Pi buffer (pH 6.6) and centrifuged at 30000*g* for 10 min. The clear supernatant (*crude extract*) was used for measuring *o*-diphenolase activity by following a slightly modified procedure of ref. [17]. Omission of *crude extract* from the incubation mixture served as control. The multiple forms of *o*-diphenolase were fractionated on acrylamide gels by the procedure described earlier [18]. Protein was estimated as given in ref. [19]. Actinomycin D was a generous gift from Merck Sharp and Dohme, U.S.A.

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