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EFFECT OF AUXIN ON MULTIPLE FORMS OF *O*-DIPHENOLASE IN GERMINATING WHEAT EMBRYOS

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Key Word Index—*Triticum aestivum*; Gramineae; wheat embryos; *o*-diphenolase multiple forms; association-dissociation by auxin.

Abstract—The electrophoretic pattern of *o*-diphenolase multiple forms was significantly altered in auxin (2,4-D)-treated embryos, although there was no appreciable change in the total enzyme activity. Quantitative measurements revealed that auxin treatment decreased the activity of the fast migrating peak (D_9–D_{10}) with a concomitant increase in the activity of a relatively slow moving form (D_8). Thus auxin may bring about the molecular rearrangement of multiple forms by the association–dissociation phenomenon. Actinomycin D inhibited the activity of multiple forms (D_8, D_9, D_{10}) in auxin-treated embryos, although the drug proved ineffective in control embryos-[1,2]. The antibiotic also suppressed the auxin-induced callus growth in wheat embryos. This indicated that auxin-mediated callus growth and also the alterations in the multiple forms of *o*-diphenolase are dependent on fresh transcription.

INTRODUCTION

In several plants, polyphenol oxidase is known to exist in multiple forms. In apple [3], mushroom [4], *Neurospora* [5] and potato [6], the multimeric forms of *o*-diphenolase arise by the various degrees of aggregation of monomeric subunits. In grapes, the multiplicity of *o*-diphenolase is attributed to differences in the charge distribution with no appreciable variation in their molecular size [7]. Treatment of grape chloroplasts with acid pH (5.0) or urea (4 M) resulted in an altered electrophoretic pattern of *o*-diphenolase multiple forms [8]. In germinating wheat embryos, the appearance of new *o*-diphenolase multiple forms was strongly inhibited by cycloheximide but not by actinomycin D. This implicated a translational control of *o*-diphenolase multiple forms [1,2,9]. In this communication, we report that auxin alters the electrophoretic pattern of *o*-diphenolase multiple forms by the association–dissociation phenomenon without appreciably affecting the total enzyme activity. Furthermore, the formation of *o*-diphenolase multiple forms in auxin-treated embryos does require fresh transcription.

RESULTS AND DISCUSSION

Effect of auxin on o-*diphenolase multiple forms*

Fractionation of crude extracts of control germinated embryos (48 hr-old) showed ten multiple forms (D_1–D_{10}) of *o*-diphenolase on acrylamide gels. Embryos cultured in a medium containing auxin (2,4-D 10^{-5}, 10^{-4} M) showed significant alteration in the pattern of multiple forms (Fig. 1a) without appreciably affecting the total *o*-diphenolase activity. Supraoptimal concentration of auxin (2,4-D, 10^{-4} M) inhibited the appearance of slow (D_2, D_3, D_4) and fast migrating (D_9, D_{10}) activity bands with a concomitant augmentation of D_8 multiple form (Fig. 1a). A similar response was also observed with

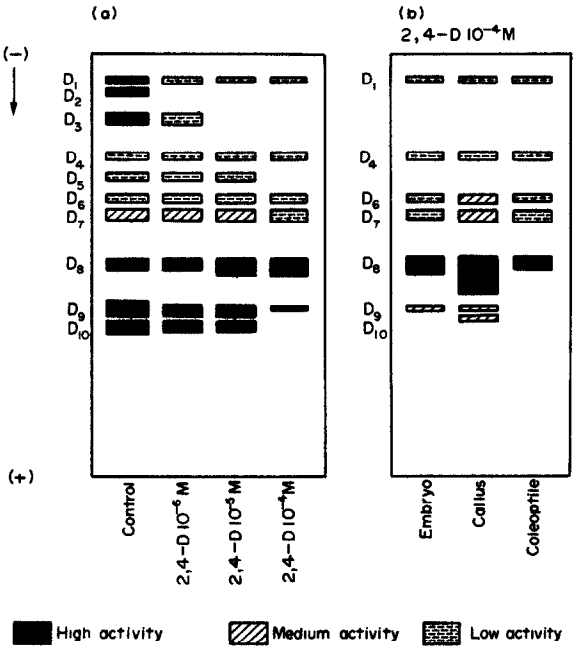

Fig. 1. Diagrammatic representation of *o*-diphenolase multiple forms in auxin-treated wheat embryos. a. Crude extracts prepared from control and auxin-treated embryos (48 hr-old) were fractionated on acrylamide gels (7.5%) and the multiple forms were developed by incubating gels with DL-dopa (1.5 mg/ml) at 37°. b. Crude extracts prepared from whole embryo, callus and coleoptile tissues excised from 2,4-D-treated embryos (48 hr-old) were fractionated on acrylamide gels (7.5%).

IAA 10^{-4} M. Thus auxin application to wheat embryos modulated *o*-diphenolase multiple forms by selective inhibition and simultaneous stimulation of certain

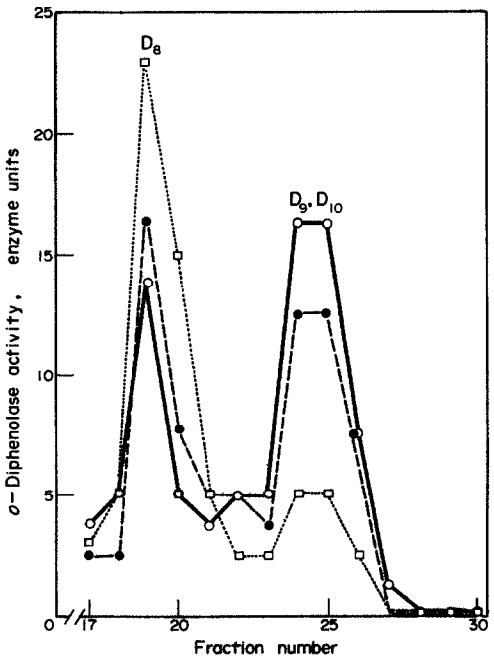

Fig. 2. Effect of 2,4-D on the activity of *o*-diphenolase multiple forms (D_8, D_9, D_{10}) in wheat embryos. Excised wheat embryos were cultured in dark at 25° in the continuous presence of 2,4-D for 48 hr. Crude extracts (400 μg protein/gel column) were fractionated on acrylamide gels (7.5%) for the separation of *o*-diphenolase multiple forms. The gels were sliced (2 mm thick) and eluted with 1.0 ml of Pi buffer. The enzyme activity was measured in eluted fractions using catechol (45 μmol/ml) as a substrate.
○——○ Control; ●– – –● 2,4-D 10^{-5} M; □·····□ 2,4-D 10^{-4} M

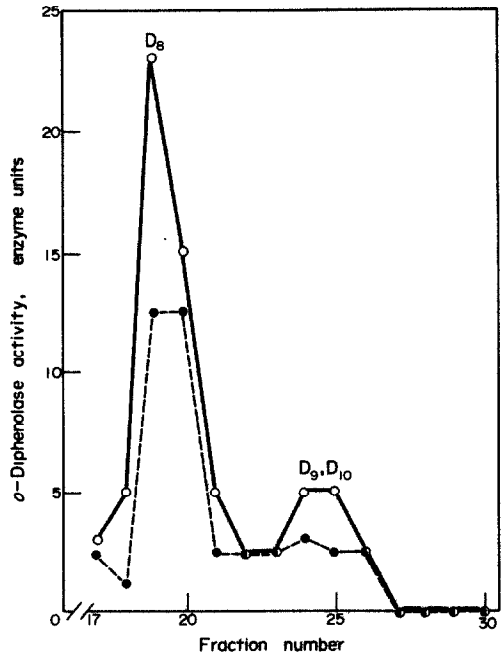

Fig. 3. Effect of actinomycin D on the activity of *o*-diphenolase multiple forms in auxin-treated wheat embryos. Excised embryos were cultured in dark at 25° for 48 hr in a medium containing 2,4-D and 2,4-D + Act D. Crude extracts were fractionated on acrylamide gels (7.5%) and the activity of *o*-diphenolase was measured in eluted fractions as described in legends of Fig. 2.
○——○ 2,4-D 10^{-4} M; ●– – –● 2,4-D 10^{-4} M + Act D 80 μg/ml

activity bands. Auxin is also known to regulate the formation of multiple forms of peroxidase in tobacco [10–12], wheat [13,14], pea [15], carrot [16] and oat [17] and that of IAA oxidase in tobacco pith cultures [18].

An examination of *o*-diphenolase multiple forms in auxin-induced callus tissue excised from auxin-treated wheat embryos, showed a very prominent D_8 activity band along with a marked reduction in the activity of fast migrating bands (D_9, D_{10}) (Fig. 1b). On the other hand, D_8 band was relatively less prominent and the activity of D_9–D_{10} bands was undetectable in coleoptiles excised from 2,4-D-treated embryos (Fig. 1b). This indicated that the bulk of D_8 multiple form resided in the callus zone.

The pattern of *o*-diphenolase multiple forms was not altered by the *in vitro* addition of auxin (2,4-D 10^{-4} M, IAA 10^{-4} M) to crude extracts of control embryos. Also the *in vivo* application of ethrel (10^{-7} M – 10^{-3} M) to germinating wheat embryos showed no change in the electrophoretic pattern of *o*-diphenolase multiple forms, indicating thereby that the auxin response was not an ethylene-mediated phenomenon.

Quantitative effects of auxin on o-diphenolase multiple forms

Since auxin-treated embryos exhibited a marked reduction in the fast migrating bands (D_9–D_{10}) together with the augmentation of a polymeric form (D_8), it was considered desirable to quantify the observed changes in the multiple forms. For this purpose, the crude extracts were fractionated on acrylamice gels and the activity of multiple forms (D_8, D_9, D_{10}) was assayed in fractions eluted from unstained gel slices. As shown in Fig. 2, auxin (2,4-D 10^{-5} M, 10^{-4} M) brought about a quantitative reduction in the fast migrating peak (D_9, D_{10}) together with a proportional increase in the activity of a relatively slow moving peak (D_8). There was nearly 80% stimulation of D_8 activity peak and a concomitant reduction of about 75% in the fast moving peak (D_9, D_{10}) in 2,4-D (10^{-4} M) treated embryos. Auxin possibly brings about a molecular rearrangement of multiple forms through the association–dissociation phenomenon.

Previously we reported [1,2] that the formation of *o*-diphenolase multiple forms and the enhanced enzyme activity in control germinating wheat embryos were insensitive to actinomycin D (Act D 100 μg/ml) treatment. However, in 2,4-D (10^{-4} M)-treated embryos, Act D (80 μg/ml) inhibited (about 40%) the activity of auxin-stimulated D_8 peak. The drug was also responsible for additional inhibition (50%) of the fast moving peak (D_9, D_{10}) in auxin-treated embryos (Fig. 3). Furthermore, Act D (80 μg/ml) partially inhibited (30%) the total *o*-diphenolase activity and simultaneously suppressed the auxin-induced callus growth. It thus appears that there is a requirement of fresh transcription for the formation of *o*-diphenolase multiple forms and the sustenance of callus growth in auxin-treated wheat embryos.

EXPERIMENTAL

Wheat (*Triticum aestivum*, var. Shera) embryos were excised from seeds (presoaked for 10 hr at 4°) and surface sterilized by

dipping in $HgCl_2$ soln (0.02%) for 10 min. The embryos were germinated in dark at 25° on liquid nutrient medium [19] supplemented with White's vitamin soln [20] containing sucrose (2%) and chloramphenicol (50 μg/ml). The germinated embryos were homogenized in 50 mM Pi buffer (pH 6.6). The homogenate was centrifuged at 30000 g for 10 min and the clear supernatant (crude extract) was used for measuring the enzyme activity of *o*-diphenolase as given in ref. [21]. Omission of crude extract from the incubation mixture served as control. Crude extracts (400 μg protein) were fractionated on acrylamide gels as described in ref. [9]. The effect of auxin (2,4-D) and Act D was tested on the quantitative levels of *o*-diphenolase multiple forms in cultured embryos (48 hr-old). Crude extracts (containing 400 μg protein) were layered on each acrylamide gel column. After electrophoresis, the unstained gels were frozen by immersing in liquid N_2 and sliced into 2 mm thick gel pieces. The enzyme fraction was eluted by soaking the gel pieces in one ml of 50 mM Pi buffer (pH 6.6) and allowed to stand for 30 hr at 4° with occasional shaking. The *o*-diphenolase activity was tested in the eluted fractions with 2 ml of catechol soln (45 μmol/ml). The mixture was incubated for 30 min at 37° and the A measured at 430 nm. The eluted fraction from blank gel pieces together with catechol soln served as blank. Protein was estimated by following the procedure of ref. [22]. Actinomycin D was a generous gift from Merck Sharp and Dohme, U.S.A.

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