Hormonal Control of Isoperoxidases in Lentil Embryonic Axis

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ABSTRACT

Detachment of the cotyledons from the lentil (Lens culinaris Med.) embryonic axis causes in the latter an increase in total peroxidase activity which is shown to be due to enhancement of specific cathodic isoperoxidases. Kinetin treatment of attached or detached axes promotes activity of essentially the same cathodic isoperoxidases. In addition kinetin enhances the activity of two anodic peroxidases and represents specifically that of a cathodic one. Abscisic acid inhibits the production of all isoenzymes in the presence or absence of kinetin. Cytokinins and abscisic acid actions are discussed in relation to the nature of a wounded hormone and the role of natural inhibitors in cotyledons during germination. Indoleacetic acid stimulates the activity of certain isoenzymes which are also stimulated by kinetin, whereas in others the effects of the two hormones are different. Specific inverse effects of indoleacetic acid and kinetin are demonstrated on the two most cathodic isoperoxidases. Indoleacetic acid-kinetin interactions on the cathodic isoperoxidases have been found in the literature and are discussed as a possible mechanism for explaining interactions of the two regulators on growth and other physiological processes.

The level or the balance of growth-promoting hormones and natural growth inhibitors seems to control many plant growth processes (1, 8, 13, 15). Cytokinins have been shown to oppose the actions of abscisic acid (2, 3, 6, 7, 13–15, 23, 24, 27, 28, 30). They are also known to oppose the action of auxins in different physiological processes, notably apical dominance (29) and root growth (5, 12). Earlier work from our laboratories showed that kinetin influences the growth of lentil seedlings through its action on the peroxidase-mediated auxin metabolism (5, 11). It has been possible to resolve the lentil peroxidases into several isoenzymes by starch gel electrophoresis (4, 10, 16, 17). Recently it was demonstrated that a number of isoperoxidases are synthesized de novo in the lentil embryonic axis and that an enhancement of peroxidase activity takes place as a result of detachment of the axis from the cotyledons (10, 16, 17). We discuss here the control of isoperoxidases in the two types of hormonal interactions mentioned above. The influence of hormones on isoperoxidases is described in the embryonic axes grown in situ (attached to the cotyledons) and in vitro after excision (detached from cotyledons).

MATERIALS AND METHODS

Biological Material and Treatment. The seeds of Lens culinaris Med. (Vicia lens) were soaked for 5 min in 1% sodium hypochlorite solution, washed, and then stirred for 2 hr in distilled water. Embryos were obtained by gently squeezing them out of the seedcoats. Thirty embryos or axes (obtained by detaching the two cotyledons) per series were grown on 50 ml of 0.75% bacto-agar in a Petri dish (13 cm diameter, dark, 26°C) containing one or two of the following substances: 6-furfurylamino- purine (kinetin from Fluka) or ABA (Hoffmann-La Roche), or IAA (Sigma).

Peroxidase Activity. Crude enzymic extracts were prepared by grinding 20 embryonic axes or the same weight of axes with 1 ml of 0.2 M phosphate buffer, pH 7.8, at 2°C. After 30 min the breis were centrifuged (25,000 g; 10 min), and the supernatant was used as the enzyme. Guaiacol-peroxidase activity (incubation mixtures: 8 ml of phosphate buffer, pH 6.1, +1 ml of H2O2, 0.2 volume, +1 ml of 1% guaiacol + 0.1 ml of enzyme) was determined spectrophotometrically by measuring the increase of absorbancy at 420 nm (11). Protein was assayed by the method of Lowry et al. (21). Starch gel electrophoresis was adapted from Scopes (25) and described in detail elsewhere (4). Electrophoretic runs were made for 3 to 4 hr at 300 v at 0°C. The gels were stained for isoperoxidases using o-dianisidine (sometimes guaiacol) as a substrate (4). The curves are composed of average data from at least three concordant assays.

RESULTS

Peroxidase Activity and Isoperoxidase Pattern in Lentil Embryonic Axes Grown in vitro and in situ. Two types of differences were observed (Fig. 1). First, in the case of detached axes, some of the peroxidases appear with a lower intensity (A, C, C, C) or after a delay (A, A). Second, in the same axes, other peroxidases (mainly A, C, C, C) appear more intense (Fig. 5). A parallel investigation on the evolution of total peroxidase activity in growing attached and detached axes shows an increase in the enzyme activity in the latter (Fig. 2). Thus the negative effect on some enzymes of removing the cotyledons is overcome and even superseded by a considerably higher promotive action on others.

Peroxidases in Attached and Detached Embryonic Axes Treated with Kinetin and ABA, Alone and in Combination. Peroxidase level was affected differently by kinetin in attached and detached axes (Fig. 2). In detached axes during the first 20 to 24 hr of growth, kinetin did not influence the enzyme level to any marked degree. After this time the peroxidase level in the kinetin-treated detached axes increased over that

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ABA decreased the intensity of almost all peroxidases. In presence of kinetin, the inhibitory action of ABA on these isoenzymes was less or not apparent.

**Peroxidases in Attached and Detached Embryonic Axes Treated with Kinetin and IAA, Alone and in Combination.** Figure 4 compares the production of total peroxidase activity in kinetin and IAA-treated attached and detached axes. In both axes, IAA enhanced the peroxidase activity over that produced by kinetin. The effect was considerably more pronounced in the case of attached axes. A notable feature was that IAA enhancement of the enzyme activity was detectable from the very beginning of the growth. The effect of IAA was depressed by kinetin during approximately the first 20 hr of growth. Thereafter, the pattern changed and IAA plus kinetin of the control and continued to increase until 42 hr. In the attached axes on the other hand, there was no significant difference in peroxidase level between the water and kinetin treatments. ABA greatly decreased the peroxidase synthesis in both axes. In the presence of kinetin the ABA inhibition was less or not apparent.

The effects of kinetin and ABA, alone or in combination, on the ontogeny of isoperoxidases in detached axes are shown in Figs. 3 and 5. In the presence of kinetin several isoperoxidases (C1, C2, C3, A1, A2, A3, A4) appeared with greater intensity than their counterpart in the untreated axis, while the intensity of one isoenzyme (C2) was diminished. The effects were essentially the same on attached axes (data not shown).

**Fig. 1.** Isoenzymatic composition of peroxidase in lentil embryonic axis when grown attached or detached from the cotyledons for 2, 12, 24, 48, or 72 hr on water. Same numbers of axes are compared.

**Fig. 2.** Changes in total peroxidase activity at different stages of growth of attached and detached lentil embryonic axes with and without KIN (10 μM), ABA (10 μM), and KIN + ABA.

**Fig. 3.** Effect of ABA (10 μM), KIN (10 μM), and ABA (10 μM) + KIN (10 μM) on isoperoxidase patterns of cultured (12, 24, and 48 hr) detached axes of lentil. Same weights of axes are compared.

**Fig. 4.** Changes in total peroxidase activity at different stages of growth of attached and detached lentil embryonic axes with and without KIN (10 μM), IAA (10 μM), and KIN + IAA.
treatment showed more activity than the IAA treatment alone. This was true for both attached and detached axes.

The zymograms of IAA-treated attached and detached axes can be seen in Figure 5 in comparison with those treated with kinetin. In both types of axes, IAA stimulated isoperoxidases C₁, A₁, A₂, A₃, A₄. In attached axes IAA, in addition, intensified C₅, C₆, C₇, A₈, whereas in detached ones C₈ was more intense. In contrast to these promotive effects, IAA diminished the intensity of C₈ in both axes. In attached axes, IAA and kinetin, thus, had opposite effects on C₈ and C₉.

When kinetin and IAA were given simultaneously, interesting changes were observed. On C₁ and C₉, the negative effect of one hormone compensated the promotive effect of the other. Furthermore, IAA reduced the stimulating effect of kinetin on A₈. In detached axes, IAA which had no effect alone enhanced considerably the effect of kinetin on C₈ and A₈.

**DISCUSSION**

Detachment of axis from the cotyledons leads to a lower intensity or to a delay in the appearance of some (mainly two anodic) isoperoxidases, whereas some of the cathodic (mainly two) isoperoxidases appear more intense. The first effect could possibly be attributed to the lack of action of some factors which are made available to the axis only when the axis is attached to the cotyledons. The second could be related either to an absence of an inhibiting substance migrating from the cotyledons to the axis or to a hormone appearing in the axis as a result of injury caused by the detachment (8, 17, 18, 22).

It is interesting that kinetin intensifies several isoenzymes (C₉, C₈, C₆, A₆) which are similar to those positively affected as a result of detachment. From this it would appear that the effects of kinetin and detachment are essentially similar and additive. This similarity further suggests the possibility that a wounding hormone is released in the axis as a result of detachment of the cotyledons and that this hormone might exhibit cytokinin properties. That this hormone may resemble a cytokinin, and is not present in any substantial quantity in the attached axes, is indicated by the fact that kinetin treatment enhances in these axes the production of essentially the same isoperoxidases that are enhanced as a result of detachment.

There is an objection to this hypothesis, however: the inverse effect of detachment and kinetin on the isoperoxidases A₈ and A₆ which would imply the participation of one or more additional factors in the wounding complex or a participation of one cytokinin having a wider range of effects than that produced by kinetin.

The suppression of kinetin effects by ABA in a manner somewhat similar to that caused by the presence of cotyledons lead us to suggest that ABA or other inhibitors from the cotyledons may exert their effect on the peroxidase patterns during growth of the axis. However, this inhibitor-promotor interaction does not seem to be specific to any particular isoenzyme, as revealed by the ABA effect of zymograms.

Results obtained by IAA treatments appear to be extremely significant in two ways. First, different responses of the detached or attached axes to IAA could suggest that this hormone is also a component of the wounding complex, since IAA and kinetin produce a synergistic effect on some isoenzymes (C₆, C₆) which are also influenced by the detachment. The other feature of the response to IAA is that, in comparison to kinetin, it has inverse effects on C₆ and C₉. Kinetin enhances C₆ and diminishes C₉, whereas the opposite is true for IAA. A slight decrease in the total peroxidase in the presence of kinetin (Figs. 2 and 4) could be related to this specific effect of kinetin and has also been observed elsewhere (18, 20, 22, 26). These opposite effects of IAA and kinetin on our C₆ peroxidase are very similar to those obtained by Lavee and Galston (18) and Galston et al. (9) in geranium and tobacco pith tissues. They have shown that auxin represses and kinetin promotes the synthesis of one cathodic isoenzyme which appear after excision of the tissues. The two regulators interact in the control of cathodic peroxidase as they do in the control of growth (4, 8) and other physiological processes (29). This regulatory process thus could be fundamental.

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**Fig. 5.** Effect of KIN (10 μM), IAA (10 μM), and KIN (10 μM) + IAA (10 μM) on isoperoxidase patterns on cultured (24 and 48 hr) attached and detached axes of lentil. Same weights of axes are compared.

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**Table:**

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**24 HOURS**

**48 HOURS**
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