Phytochemistry, 1971, Vol. 10, pp. 2609 to 2613. Pergamon Press. Printed in England.

APPLE EMBRYOS PEROXIDASES

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(*Received* 12 *March* 1971)

Abstract—Several peroxidase fractions were separated by disc electrophoresis of extract of apple seed embryos. Three fractions of slowest electrophoretic mobility showed little substrate specificity, did not change during seed development and were not influenced by plant hormones. The appearance of the faster moving peroxidases was stimulated by gibberellin and benzyladenine, inhibited by abscisic acid and coumarin and unaffected by indolylacetic acid. The stimulation of those peroxidases by gibberellin, strong in dormant apple embryos, diminished during seed after-ripening.

INTRODUCTION

PEROXIDASE may play an important role in the regulation of plant growth and development[1-3] although its precise function is as yet not understood. Electrophoretic studies indicated that plant tissues contain several peroxidases differing in substrate specificity.[4-8]

Many genetic dwarfs reveal abnormally high peroxidase activity.[9] Gibberellin treatment relieves dwarfism and causes a decrease in peroxidase activity.[10]

Apple seedlings cultured from seeds without after-ripening reveal abnormal development.[11] Treatment with gibberellin does not restore their normal growth.[12,13] We decided therefore to study the effect of gibberellin and some other plant growth regulators on the activity of peroxidases during the after-ripening of apple seeds.

RESULTS

The polyacrylamide-gel electrophoresis of extracts from acetone powder[14] was used to separate the peroxidases during the after-ripening of apple seeds. Peroxidase activity on the gel was developed by the method of Macko *et al.*[15] The peroxidase activity could be resolved into a number of bands differing in substrate specificity. A large variety of electron donors, phenols present in apple tissue such as—phloridzin, catechin, caffeic and chlorogenic acids and other compounds—guaiacol, pyrogallol, catechol, quercetin and benzidine were

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used. The localization of these fractions on electrophoretograms, and their number depending on the electron donor used is presented in Fig. 1.

The incubation of electrophoretograms in electron donor solution before addition of H_2O_2 made it possible to distinguish the bands of phenoloxidase from those having peroxidase activity only. The phenoloxidase fraction bands (designated by an asterisk) were observed only when chlorogenic and caffeic acids and catechin were used as electron donors.

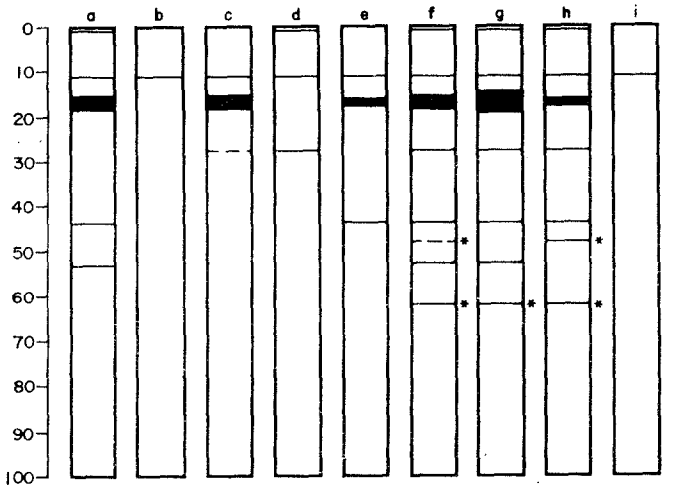

FIG. 1. ELECTROPHORETOGRAMS OF THE PEROXIDASES OF APPLE SEED EMBRYOS.
Electron donors: (a) benzidine, (b) guaiacol, (c) pyrogallol, (d) phloridzin, (e) catechol, (f) chlorogenic acid, (g) caffeic acid, (h) catechin, (i) quercetin. (*) phenoloxidase bands.

Only one peroxidase band is developed on the gel after incubation in guaiacol or quercetin solutions. Three bands—in the presence of pyrogallol or phloridzin, five—with benzidine and seven as the result of incubation with catechin, caffeic or chlorogenic acids.

The peroxidases represented by the three slowest moving bands exhibited little substrate specificity, the second slowest appearing with all the substrates used. A considerable number of the faster moving enzyme bands appeared when the electrophoretograms were incubated with benzidine, catechin, chlorogenic and caffeic acids. Benzidine was chosen for further studies because of its clear, blue oxidation products, convenient for semi-quantitative estimations. Another advantage of benzidine was that under the conditions used, it did not appear to give a reaction with phenolase.

In the extracts from the embryos isolated from dormant apple seeds after soaking in water (controls) only three peroxidase fractions, characterized by a low electrophoretic mobility and the lack of substrate specificity, were observed. During the culture of the embryos two new peroxidase bands appeared. They were visible after 48 hr and measurable after 72 hr of culture (Fig. 2).

The formation of the new bands was influenced by the presence of growth regulators in the culture medium. The presence of gibberellin A_3 (5×10^{-5} M) and benzyladenine (5×10^{-5} M) stimulated the appearance of two faster moving peroxidases. Abscisic acid (1×10^{-5} M) and coumarin (1×10^{-3} M) inhibited their formation and indolylacetic acid (5×10^{-6} M) had no effect (Fig. 2). It should be pointed out, that the presence of all the growth regulators studied did not affect the activity of the three peroxidase fractions

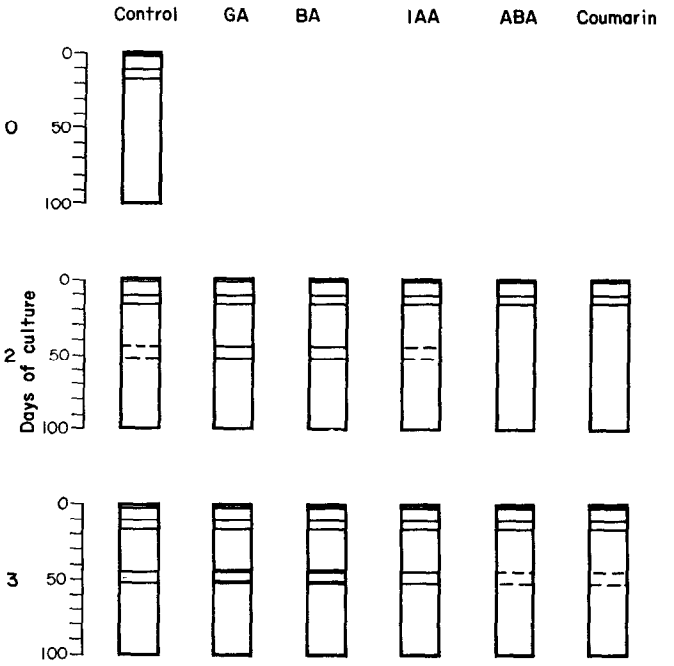

FIG. 2. ELECTROPHORETOGRAMS OF THE PEROXIDASES OF NON-STRATIFIED APPLE SEED EMBRYOS CULTURED IN THE PRESENCE OF WATER (CONTROL) OR SOME PLANT GROWTH REGULATOR SOLUTIONS.

GA = gibberellin A_3, IAA = indolylacetic acid,
BA = benzyladenine, ABA = abscisic acid.

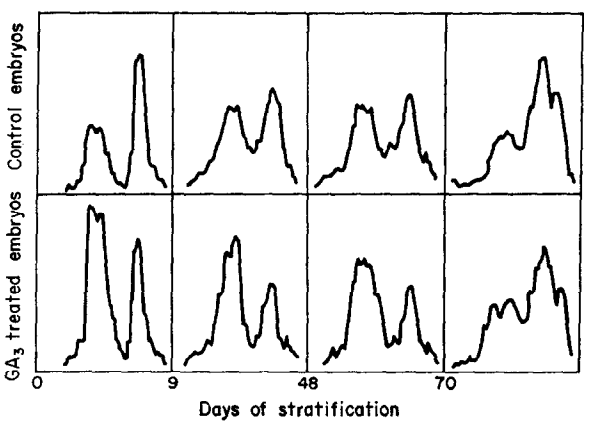

FIG. 3. DENSITOMETER TRACINGS OF THE ELECTROPHORETOGRAMS DEVELOPED FOR PEROXIDASE ACTIVITY.

The embryos were isolated from apple seeds in different stages of after-ripening and cultured for 3 days on water (control) or gibberellin solution.

present in dormant embryos. Gibberellin A_3 was used throughout the experiments as the effect of native apple seeds gibberellins A_4 and A_7 was the same.

Embryos isolated from apple seeds at different stages of after-ripening were cultured for three days in the presence of gibberellin or water (control) and peroxidase activity was assayed in the respective extracts by the method described in the Experimental section. The stimulation of peroxidase activity by gibberellin varied, depending on the length of seeds after-ripening. Thus, in the non-stratified seed embryos it was 110 per cent higher than in the control, 50 per cent higher after 10 days of stratification and only 20–30 per cent higher after 32 days. Towards the end of stratification no stimulatory effect of gibberellin was noted. Those data indicate the sensitivity of apple embryos peroxidase to gibberellin to be dependent on the stage of seed development. The proof of the variable stimulation of peroxidase by gibberellin is presented in Fig. 3. Comparing the densitograms of peroxidases from the embryos grown on water (upper graphs) with the corresponding densitograms from gibberellin treated embryos (lower graphs) it is evident that the differences in peroxidase patterns between control and treated are limited to two bands of higher electrophoretic mobility (starting point of electrophoresis is on the right side of each densitogram). These differences, due to the stimulation of the two peroxidases, are very pronounced in dormant embryos (0 day), distinct after 9 days of stratification, still visible after 48 days but insignificant after 70 days of after-ripening.

DISCUSSION

Our results have revealed the heterogeneity of apple seed peroxidases and their substrate specificity towards various electron donors. We have shown that certain regulars of plant growth and development have a differential effect on the activity of some of the peroxidases. Therefore the different peroxidases may be assumed to play a different role in the biological oxidation processes occurring during seeds after-ripening.

According to Galston and Davies,[16] the regulation of the auxin level may involve the inhibition of peroxidase activity (which destroys auxin) by gibberellin. Our results with gibberellin-induced stimulation of some apple embryo peroxidases agree with this suggestion. Dwarfism of seedlings grown from non-stratified apple seeds may be due to the high activity of peroxidases and the consequent failure to maintain a high auxin concentration. During the process of after-ripening the influence of gibberellin on the peroxidase activity decreases gradually to almost nothing at the end of stratification. During stratification of apple seeds endogenous GA_4 level reaches its maximum on the 30th day.[17] Therefore, changes in the endogenous gibberellin level are not correlated in time with the changes in the sensitivity to gibberellin of the peroxidase synthetizing and/or activating system. We observed that these changes were accompanied by different sensitivity to benzyladenine. Consequently, the variations in the sensitivity of the system responsible for the appearance of peroxidase activity to exogenous gibberellin cannot be explained by the shift in the equilibrium between the various endogenous growth and development regulators during after-ripening.

Therefore, it seems probable that the changes in the sensitivity to hormonal stimulus of the system producing and/or activating the enzyme protein are one of the regulatory factors in seed development.

The regulation of auxin level by gibberellin-mediated peroxidase activity proposed by Galston[16] and probably acting during the apple seed development cannot be the only one

[16] A. W. GALSTON and P. J. DAVIES, *Science* **163**, 1288 (1969).

responsible for the normal growth of the apple seedling. This mechanism does not occur in the later stages of development as gibberellin does not restore the normal growth of dwarf seedlings.

The similar character of the influence on the peroxidases activity of both cytokinins and gibberellins and the lack of influence of auxin (IAA) cannot be discussed without elucidation of the role of those hormones in the process of after-ripening. The effect of the seed germination inhibitors abscisic acid and coumarin in inhibiting the development of peroxidase activity seems to indicate that hormone-sensitive peroxidases may also play some part in the process of development of apple embryos.

EXPERIMENTAL

Material. Apple seeds, cv. Antonówka were obtained from the Experimental Station of the Institute of Pomology at Sinołęka, 1968 and 1969 collection. The seeds were stratified under the conditions described earlier.[18]

The embryos were isolated from dormant seeds or from seeds removed from stratification. The isolated embryos were grown for 72 hr, or as indicated, in lots of 30 on Petri dishes on filter paper moistened with 5 ml of the solution of plant growth regulator or 5 ml of water (control). The embryo culture was effected in light and temperature controlled conditions: day length—12 hr, day temperature—24°, light intensity 10^4 lx (fluorescent lamps), night temperature—20°.

Methods. From batches of 90 seeds the acetone powder was prepared and the enzyme extracted in Tris-HCl buffer, pH 7·2.[19] The polyacrylamide-gel electrophoresis of this extract was carried out at pH 8·4 in conditions described earlier.[19]

After electrophoresis the gels were incubated in the appropriate substrate solution (0·01 M) in 0·05 M acetate buffer at pH 4·0 and 4° for 30 min. Then they were soaked in 0·1% H_2O_2 for 2–3 min.[15] The relative mobilities of the coloured bands were estimated and the intensity of the bands were recorded using a densitometer (Zeiss, Jena, GDR).

Peroxidase activity in the extracts was estimated with benzidine as electron donor under the following conditions: the reaction mixture contained 2 mM benzidine solution—2 ml, 0·2 M acetate buffer, pH 5·0–1 ml, 0·2 M H_2O_2—0·5 ml, enzyme extract—0·1 ml and water to the volume of 6·2 ml. The changes in absorbancy at 590 nm were recorded after 20–30 sec.

Acknowledgements—This work was partly supported by Grant F.G.-Po-236 from U.S. Department of Agriculture, Agricultural Research Service, according to Public Law 480.

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