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SHORT COMMUNICATION

GEL ELECTROPHORETIC COMPARISON OF LIGHT-INDUCED ASCORBIC ACID OXIDASE FROM MUSTARD SEEDLINGS AND FROM PUMPKIN TISSUE

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Abstract—Ascorbic acid oxidase activity was identified in extracts of *Sinapis alba* after separation by polyacrylamide gel electrophoresis. No isoenzymes could be detected. In comparison to dark-grown controls, seedlings irradiated continuously with far-red light contain the same enzyme but in greater amount.

INTRODUCTION

AN EARLIER paper[1] showed that continuous irradiation with standard far-red light causes a fourfold increase of ascorbic acid oxidase activity in the mustard seedlings (*Sinapis alba* L.) when compared with dark-grown controls. This induction is regulated by the phytochrome system. The far-red irradiation maintains a low but stationary concentration of the active phytochrome (P_{fr}) in the seedlings.[2,3] In order to understand more closely the regulatory mechanism of enzyme induction by phytochrome, it is important to know whether dark- and light-induced enzyme are of the same molecular type. We therefore decided to investigate the enzyme from both dark- and light-grown seedlings by discontinuous electrophoresis on polyacrylamide gels.

A procedure for the study of ascorbic acid oxidase from *Cucurbita pepo* in such systems has been reported recently.[4] It is, however, unsuited for the study of the enzyme from *Sinapis* seedlings. We therefore describe the results obtained by the application of our own method to both *Sinapis* and *Cucurbita* extracts.

RESULTS

The distinct electrophoretic behaviours of *Sinapis* and *Cucurbita* enzyme is presented in Fig. 1. The *Cucurbita* enzyme yields multiple bands only after anodic migration at pH 9·5 (gels 3 and 4). After cathodic migration at pH 6·6, however, the enzyme is lost (gel 5 and 6). The enzyme from the organs of *Sinapis* seedlings, by contrast, gives one coloured zone after electrophoresis at pH 6·6 (gel 1) and does not move far from the start at pH 9·5 (gel 2).

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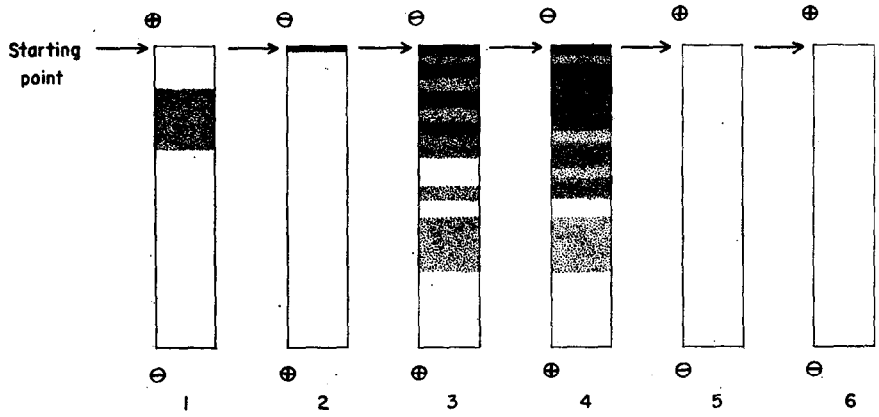

FIG. 1. COMPARATIVE ACRYLAMIDE GEL ELECTROPHORESIS OF EXTRACTS FROM MUSTARD SEEDLINGS (GEL 1, 2) AND PUMPKIN FRUIT PEEL (GEL 3–6) WITH ASCORBIC ACID OXIDASE ACTIVITY.

Coloration of active zones by incubation of gels with 10^{-3} M solution of ascorbic acid and $0{\cdot}5 \times 10^{-3}$ M solution of 2,6-dichlorophenolindophenol successively. Gel 1, 2, 3 and 5 with extracts obtained at pH 5·0; gel 4 and 6 with extracts obtained at pH 7·0. Electrophoresis at pH 6·6 (gel 1, 5 and 6) and at pH 9·5 (gel 2, 3 and 4).

Figure 2 shows the zone of ascorbic acid oxidase activity from hypocotyls and cotyledons of either dark- or light-grown *Sinapis* seedlings. Far-red irradiation of the seedlings causes an intensification of the enzymatically active zone in the gel (gel 7 and 9). This is in agreement

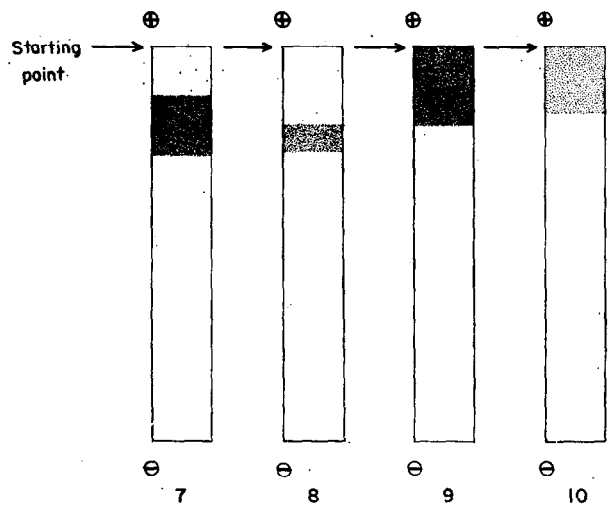

FIG. 2. ZYMOGRAMS FROM ORGANS OF DARK- AND LIGHT-GROWN MUSTARD SEEDLINGS.

Gel 7 and 8, light- and dark-grown cotyledons respectively. Gel 9 and 10, light- and dark-grown hypocotyls respectively. Extractions at pH 5·0, electrophoresis at pH 6·6.

with the results of the kinetic induction analysis.[1] To ascertain that the active zones effectively contained ascorbic acid oxidase, they were excised and extracted in the original buffer of the crude extract. After centrifugation, about 65% of the enzyme activity was recovered manometrically.

Relative to the migration front, the cotyledon enzyme runs somewhat faster than that of the hypocotyl; possibly the respective enzymes are slightly different.

It is obvious that ascorbic acid oxidase from *Cucurbita* and *Sinapis* behave very differently. The electrophoretic properties of the enzyme from *Cucurbita* seeds at germination are very similar to those of the enzyme in the fruit. In the organs of *Sinapis* the enzyme is homogenous and does not dissociate under our experimental conditions. Differences in electrophoretic pattern between dark- and light-induced *Sinapis* enzyme were not detected.

We assume that increased activity after far-red irradiation is based on enzyme accumulation. Our results suggest that ascorbic acid oxidase synthesis in the light is qualitatively identical to that occurring in the dark-grown seedlings.

EXPERIMENTAL

Cultivation and irradiation of the *Sinapis* seedlings was described.[5] Light-grown seedlings: 36 hr dark + 36 hr far-red; dark controls: 72 hr dark. Maceration of forty hypocotyls or forty pairs of cotyledons with quartz sand in 2·5 ml citric acid (0·1 M)–Na_2HPO_4 (0·2 M) buffer (pH 5·0) containing 12% sucrose; centrifugation during 20 min at 17,000 *g*. For comparison, ascorbic acid oxidase was also extracted from peel of freshly harvested *Cucurbita pepo*, cv. condensa: extraction as for *Sinapis* seedlings or simplified after the method for *Cucurbita*[4] (i.e. without dialysis and high-speed centrifugation). The *Cucurbita* enzyme was found to be more active in neutral than in acid media, whereas the *Sinapis* enzyme has its optimum of activity at pH 5·0.

Electrophoretic separation of supernatant fluids on polyacrylamide gels was performed at pH 9·5[6] and at pH 6·6.[7] In both cases the upper gel was omitted and the concentration of persulfate in the lower gel was reduced to 1/3. Each column received 0·02 ml extract and was run at 4° and 2 mA for about 1·5 hr. Extruded gels were incubated for 20 min at room temp. in citric acid (0·1 M)-Na_2HPO_4 (0·2 M) buffer (pH 5·0) or in phosphate buffer (0·05 M), pH 7·0, containing 10^{-3} M ascorbic acid correspondingly neutralized. Subsequently the gels were washed twice in distilled water and transferred to a $0{\cdot}5 \times 10^{-3}$ M solution of 2·6-dichlorophenolindophenol. Coloration of active zones was optimal after about 15 min.

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