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SHORT COMMUNICATION

THE EFFECT OF SUBSTRATE CONCENTRATION ON THE VISUALIZATION OF ISOPEROXIDASES IN DISC ELECTROPHORESIS

ANTON NOVACKY and RAYMOND E. HAMPTON*

Department of Plant Pathology, University of Kentucky, Lexington, Kentucky

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THE HETEROGENEITY of plant peroxidase has been the subject of several recent reports. The spectrum of isoperoxidases, as revealed by electrophoresis in starch gels or polyacrylamide gels is constant for a given species or tissue under specified conditions and is apparently influenced by species or varieties,[1–6] age,[7,11,13] growth-regulating substances,[8] and disease.[9–14] In several cases, isoperoxidases which were not detected in young healthy tissue were detected in plant tissues upon aging or after infection by various pathogens. Such changes have been attributed to biosynthesis of new proteins under these conditions. However, we recently reported that the appearance of these "new" isoperoxidases may simply represent an increase in activity of isoperoxidases normally present at low activity or concentration in young healthy tissue.[15] The failure to detect them in young healthy tissues appeared to result from substrate or product inhibition. We have, therefore, investigated the effect of substrate concentration on the visualization of individual isoperoxidases in polyacrylamide gels.

RESULTS AND DISCUSSION

Extracts of *Phaseolus aureus* Roxb. were subjected to disc electrophoresis in polyacrylamide gels and bands of peroxidase activity were detected by placing the gels in solutions

*Research Associate and Associate Professor respectively. Present address of senior author: Institute of Phytopathology and Entomology, Ivanka pri Dunaji, Czechoslovakia.

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containing various concentrations of H_2O_2 (0·0003 to 0·03 percent) as substrate and benzidene dihydrochloride as an electron donor.

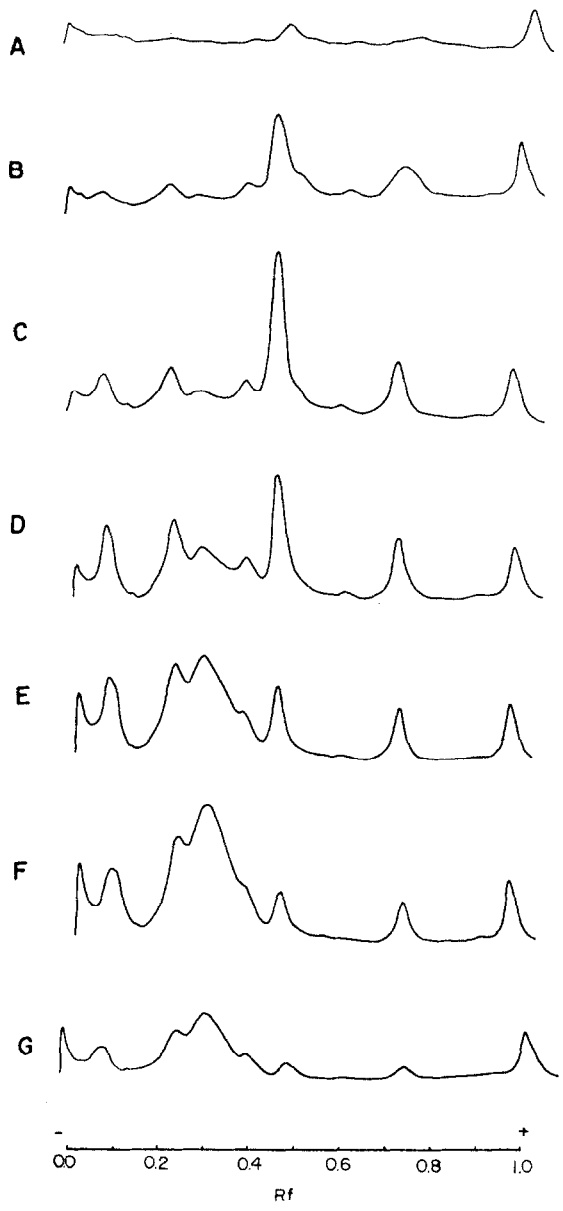

FIG. 1. DENSITOMETER RECORDINGS OF ISOPEROXIDASES BANDS IN POLYACRYLAMIDE GELS USING DIFFERENT SUBSTRATE CONCENTRATIONS.

A, 0·0003 per cent H_2O_2; B, 0·00075 per cent H_2O_2; C, 0·0015 per cent H_2O_2; D, 0·003 per cent H_2O_2; E, 0·0075 per cent H_2O_2; F, 0·015 per cent H_2O_2; G, 0·03 per cent H_2O_2.

Densitometric recordings of the gels (Fig. 1) illustrate the effect of substrate concentration on the number and intensity of bands of activity. Based on response to

substrate concentration, two groups of isoperoxidases may be distinguished. The more slowly migrating isoperoxidases (R_f 0·15–0·40) stained more intensely at higher substrate concentration (0·0075 per cent) than the faster migrating group for which the optimum substrate concentration was 0·0015 per cent (Fig. 1). For example, the band at R_f 0·46 had an optimum substrate concentration of 0·0015 per cent while the band at R_f 0·27 had an optimum substrate concentration of 0·0015 per cent. A substrate concentration of 0·03 per cent resulted in reduced intensity of all bands and some bands were very faint or invisible at this concentration. The lowest substrate concentration used, 0·0003 per cent, resulted in the appearance of only very faint bands. The optimum substrate concentration for visualization of all bands was 0·003 per cent. Enzyme concentration was much less critical than substrate concentration. The effect of lowering or increasing the amount of tissue extract over a 2- to 3-fold range only increased or decreased the intensity of staining of all bands but had no effect on the number of bands detected.

It is evident from these data that the visualization of isoperoxidases in polyacrylamide gels is strongly influenced by the substrate concentration used in the "staining" procedure. The use of a relatively high substrate concentration may actually mask some of the sites of enzyme activity, probably because of substrate or product inhibition. The need for establishing optimum enzyme and substrate concentrations is apparent since the use of only one substrate concentration can yield misleading results.[15,16] The same experiment was conducted using tissue from *Zea mays* L. and *Phaseolus vulgaris* L. In both cases a similar substrate concentration effect was shown.

EXPERIMENTAL

The first four leaves of 14-day-old plants of *Phaseoleus aureus* grown at 26° under 18 hr photoperiod at a light intensity 1200 ft-c were used as a source of peroxidases. The leaf tissues was homogenized in a chilled mortar containing acid-washed sand and an extraction medium described previously.[15] The homogenate was centrifuged at 20,000 × *g* for 1 hr at 4° and the supernatant was used immediately for electrophoresis.

Disc electrophoresis[17] was run on polyacrylamide gels as previously described,[15] with samples of 0·2 mg protein per tube. Isoperoxidases were detected in the gels after electrophoresis by a previously described modification of the method of Ornstein.[15,18] Hydrogen peroxide concentration in the staining medium was varied between 0·0003 per cent and 0·03 per cent. Development of bands was stopped after 30 min by washing the gel columns and fixing in 7% acetic acid. They were then photographed and recorded using a Photovolt densitometer.

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