Substrate Specificity of Peroxidase Isoenzymes for Hydrogen Donors

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Abstract. The investigation of the substrate specificity of the anionic peroxidase isoenzymes, isolated from the zone of differentiation of the primary roots of Zea mays, for some representatives of phenolic compounds and aromatic amines, as hydrogen donors, is reported. The investigation was carried out electrophoretically with peroxidase isoenzymes partially purified by a combination of gel filtration by Sephadex G-25 and Sephadex G-100. A difference in the substrate specificity of the individual isoenzymes is observed. It was established that the anionic peroxidase isoenzymes showed a similarity in total number and relative activity on staining with bivalent phenols and difference on staining with trivalent phenols, as hydrogen donors. A greater number of isoenzymes was stained with benzidine and o-dianisidine and a lesser number with o- and p-phenylenediamine. The substrate specificity of the peroxidase isoenzymes was compared for guaiacol and benzidine. The substrate specificity of peroxidase isoenzymes was discussed as regards their diverse role in the plant metabolism.

The multiple molecular forms of the enzymes, catalyzing one and the same chemical reaction, could differ significantly between themselves by a number of physico-chemical properties: molecular weight, pH and temperature optimum, substrate specificity. The investigations show that the multiple molecular forms of the peroxidase — the isoenzymes differ as regards their preferences to diverse hydrogen donors (FARKAS and STAHLANN 1966, MACKO and NOVACKY 1966, MARKLUND et al. 1974). It is assumed that these specific substrates and other characteristics of the isoenzymes of the peroxidase can explain the polyfunctionality and the flexibility in the behaviour of the enzyme. Our aim in the investigation is to compare the substrate specificity of the anionic peroxidase isoenzymes for several hydrogen donors, representatives of the phenolic compounds and the aromatic amine group, which are the most frequently used in investigations with peroxidase.

MATERIAL AND METHODS

We carried out the investigations with enzyme extract from the zone of differentiation of a primary root of 64-h seedlings of Zea mays cv. Kn-2L-611. The seeds were germinated on moist filter rolls in the dark, in a growth
The separation of peroxidase isoenzymes was accomplished by disc electrophoresis on polyacrylamide gel, by the method of Davis (cf. MAURER 1971). The electrophoresis was carried out for 1.30 h at 2 mA per tube. The staining of the isoenzymes was achieved by means of colour reactions with the following hydrogen donors, at suitable conditions: phenolic compounds — guaiacol, catechol, pyrogallol and phloroglucinol in concentration 0.08 M and final concentration of $H_2O_2$ 0.05%, pH 6.0 of the incubation medium; aromatic amines — benzidine, o-dianizidine, o-tolidine in a concentration of 0.004 M and final concentration of $H_2O_2$ 0.003%, pH 5.0 of the incubation medium; o-phenylendiamine, p-phenylendiamine in a concentration of 0.5% and final concentration of $H_2O_2$ 0.03%, pH 6.0 of the incubation medium. The conditions for resolution and staining of the isoenzymes, the amounts of protein applied and the substrate concentrations were chosen after preliminary assays with a view to staining the greatest possible number of isoenzymes, well differentiated in the gel. The stained isoenzyme patterns were recorded densitometrically. We judged of the differences and the similarities in the substrate specificity of the individual isoenzymes for the different hydrogen donors by their number and values of the relative electrophoretic mobility ($R_m$). The data for $R_m$ were processed statistically by Student-Fisher method (cf. ROKITSKIĬ 1967).

RESULTS

Densitometric scans of the anionic isoenzymes of the peroxidase stained with phenolic compounds as hydrogen donors (Fig. 1) show the similarity in the total number and the relative activity of the individual isoenzymes with guaiacol (A) and catechol (B) and the difference in their activity with pyrogallol (C) and phloroglucinol (D). Five isoenzymes are stained with guaiacol and catechol. The similarity between them is confirmed by the statistical analysis of $R_m$ data (Table 1), which show only isoenzyme-3 to differ. Four isoenzymes are stained with pyrogallol and phloroglucinol. Comparing the isoenzyme spectra stained with pyrogallol and phloroglucinol and guaiacol, we can see that isoenzyme-3 does not stain with pyrogallol and