Herbicide Metabolism in Plants: Specificity of Peroxidases for Aniline Substrates

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Studies on the metabolism in plants (4, 5, 6, 8) and in soil (1, 2) of the herbicide propanil (3,4-di-chloropropionanilide) have shown that the parent compound is rapidly hydrolyzed to release 3,4-di-chloroaniline (DCA). In plants, the aniline moiety is recovered in several compounds including sugar and lignin conjugates (6, 7), while in soil the major product is an azo compound identified as 3,3′,4,4′-tetrachloroazobenzene (TCAB) (1). This unusual conversion, although catalyzed by soil peroxidases and by crystalline horseradish peroxidase, could not be detected in plants (6, 7). We have compared the peroxidase activities in crude extracts of barnyard grass, Echinocloa crus-galli, (a plant which is readily killed by propanil and might be expected to contribute to the peroxidase activity of the soil), fresh horseradish root, rice, and the commercially purified crystalline horseradish enzyme. We infer that the failure of some plants to accomplish the aniline to azo conversion (DCA → TCAB) may be due to differences in substrate specificity among the plant peroxidases.

Peroxidase activity was measured essentially as described by De Jong et al. (3). The assay mixture contained 0.2 ml of 1 M p-anisidine in methanol, 1 ml of 0.2 M acetate buffer pH 5, 0.3 ml of a 0.1 % (v/v) H_2O_2 solution, in a final volume of 3 ml. The reaction was started by the addition of 0.1 ml of enzyme preparation. Activity was measured as the change in optical density at 460 nm determined on a Beckman DU spectrophotometer equipped with a Gilford Model 220 optical density converter and a potentiometric recorder. The cuvette compartment was maintained at 25°C. There was no detectable activity in the absence of enzyme.

A crude enzyme preparation from barnyard grass was prepared from 100 g of grass at the 3-leaf stage, grown in the greenhouse. Whole plants were washed carefully and ground in a cold mortar and pestle. The expressed juice was filtered through 8 layers of cheesecloth and the solid residue discarded. The green supernatant was centrifuged at 12,000g for 10 min to obtain an amber supernatant fraction which was then used as the source of enzyme for peroxidase assays.

Distilled water solutions of horseradish peroxidase, EC 1.11.1.7 (Sigma Chemical Company, Horseradish Peroxidase type 11, 135 purpurogallin units per milligram) and barnyard grass extract were diluted so as to produce an optical density change equivalent to 5.8 OD units/min in the p-anisidine assay system. This was equivalent to a crystalline horseradish peroxidase concentration of 35 μg/ml. Enzymatically catalyzed reaction products of mono and dichloroanilines were produced by mixing 1.5 ml of freshly prepared enzyme solution (either barnyard grass supernatant as prepared above, or horseradish peroxidase) with 1.0 ml of 0.2 M acetate buffer pH 5.0, 0.3 ml of a 0.1 % H_2O_2 solution, and 0.2 ml of substrate (an aniline) which was 1 M in methanol. All anilines were purified by recrystallization from ligroin, redistillation and/or filtration through activated charcoal. The purity of all substrates was verified by thin layer chromatography. At the end of 30 min, the reaction mixture was extracted with chloroform, concentrated, and chromatographed on silica gel thin layer plates (5 × 10 cm Quanta Gram precoated TLC plates) with chloroform as the solvent. The plates were examined under room lighting and under U.V. light. By the streaking technique for sample application 10 μg of TCAB is easily detected on the developed plate. By concentrating the material in a single spot the detection limit is about 1 μg.

The reaction products obtained with the different anilines are shown in Fig. 1 and the results are summarized in table I. The horseradish peroxidase preparation gave products with all anilines except the 2,5 and 2,6-dichloroanilines. The barnyard grass preparation, however, showed a markedly different behavior, giving products only with aniline, 4-chloroaniline, 2-chloroaniline, and 2,3-dichloroaniline substrates. Crude extracts of rice plants showed activities similar to those

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Fig. 1. TLC comparison of products formed by crystalline horseradish peroxidase (H) and an extract of barnyard grass (B). (1) Aniline, (2) 2-chloroaniline, (3) 3-chloroaniline, (4) 4-chloroaniline, (5) 2,3-DCA, (6) 2,4-DCA, (7) 2,5-DCA, (8) 2,6-DCA, (9) 3,4-DCA, (10) 3,5-DCA. On plates 5-B, 5-H, and 10-H the product is not easily seen but appear on the original plates at the position indicated by P.

of barnyard grass, while similar extracts of fresh horseradish had substrate specifications identical to the crystalline horseradish enzyme.

We have treated intact plants of both rice and barnyard grass with the free aniline (DCA) so that both the roots and leaves were thoroughly permeated, and still failed to detect TCAB in extracts using both thin layer and gas chromatography (flame ionization, detection limit 0.1 µg).

It thus seems clearly possible that the failures to detect TCAB in rice plants and the rapid accumulation of this compound in soil might be attributable to the surprising specificity of peroxidases for their hydrogen donor substrate. We suggest that a more complete knowledge of the substrate range of plant and soil peroxidases may permit predictions (and therefore the possibility of exclusion) of undesirable transformation products of aniline-base pesticides applied at different points in the environment.

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Literature Cited