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COMPARISON OF TRYPTIC PEPTIDE MAPS OF EIGHT ISOPEROXIDASES FROM TOBACCO TISSUE CULTURES

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; isoperoxidase; peptide mapping; tissue culture.

Abstract—Eight isoperoxidases from tobacco suspension culture WR-132 (termed C_n, C_3, C_4, A_c, and A_f) and tobacco callus culture W-38 (termed A_1, A_2, and A_3) have been subjected to trypsin digestion followed by peptide mapping. The peptide maps of isoperoxidases A_f and A_3 are identical. All other isoperoxidases do not appear to be dramatically dissimilar in certain portions of their sequence, since many matching peptides have been found when various isoperoxidases are cross-compared. However, only two, and possibly three, highly homologous peptides are present in all of the isoperoxidases.

INTRODUCTION

The existence of multiple forms of peroxidase has been reported for many plants [1]. Studies in our laboratories and by other workers on individual isoperoxidases (peroxidase isoenzymes) from many different plants indicate that physical and kinetic properties and substrate preferences of these isoperoxidases from a single source may vary significantly [2–6]. Although much is known about physicochemical and catalytic properties, research concerning structural differences among the multiple forms of peroxidases is more limited. Shih *et al.* [7], on the basis of tryptic peptide map experiments, showed that 5 horseradish isoperoxidases can be segregated into at least three distinct groups. They further indicated that there may be many points of difference in the primary structure among the three groups. Welinder and Mazza [8] studied in detail the tryptic peptide patterns of 5 isoperoxidases from turnip and horseradish. Their study showed that turnip isoperoxidases, which they called P_1 and P_2, are almost identical, whereas the maps of other isoperoxidases are very different. They further reported that there are two highly homologous sequences containing histidine in all 5 isoperoxidases examined. More recently, Welinder and Mazza [9] have compared the sequence around the histidine. They observed that substitution of residues is rare close to these histidines but is more abundant with greater distance.

In the present study, we have attempted to clarify the structural relationships of these isoperoxidases, by peptide mapping of trypsin digests of 8 isoperoxidases from tobacco suspension cultures WR-132 (termed C_n, C_3, C_4, A_c, and A_f) and tobacco callus culture W-38 (termed A_1, A_2, and A_3). Of particular interest is the comparison of A_f (which appears in significant amounts only when WR-132 is grown in darkness) with A_3 from W-38, since we have shown that these two enzymes appear to be the same when MWs, kinetic properties, and substrate preferences are compared [10].

RESULTS AND DISCUSSION

Among the results obtained from the tryptic peptide mapping, the most striking aspects are illustrated in Figs. 1 and 2. A close examination of these two maps shows that isoperoxidase A_f obtained when WR-132 is grown in total darkness [10], and isoperoxidase A_3 obtained from W-38 [11] appear to be indistinguishable. All major peptides and medium intensity peptides of A_f are also found in the fingerprint of A_3. This information coupled with the MW, kinetic, and substrate specificity data strongly supports the hypothesis that A_f and A_3 are the same enzyme. Why WR-132 tobacco tissue suspension culture, which normally has only a few isoperoxidases, would begin, when grown in darkness, to elaborate significant amounts of an isoperoxidase that appears in W-38, which itself has a wide variety of isoperoxidases, is unknown.

In addition, tryptic peptide chromatographic maps have been obtained for C_n, C_3, C_4, A_1, A_2, and A_c. Matching or overlapping peptides were counted after drawing a series of horizontal and vertical lines 1 cm apart, beginning at the origin of each chromatograph. Two tryptic peptides from different isoperoxidases falling within the resulting 1 cm squares (or at least touching each other) were defined as matching or overlapping peptides. Table 1 compares the number of overlapping tryptic peptides from the 8 isoperoxidases studied. Among the 3 cathodic isoperoxidases, C_3 and C_4 appear to be more closely related structurally than either C_n and C_3 or C_n and C_4. This might be expected since C_3 and C_4 migrate very close together upon electrophoresis. *Ca* 15 different tryptic peptides of C_n overlapped with those of C_3 and C_4. Of these, *ca* 9–10 homologous tryptic peptides are present in all 3 cathodic isoperoxidases.

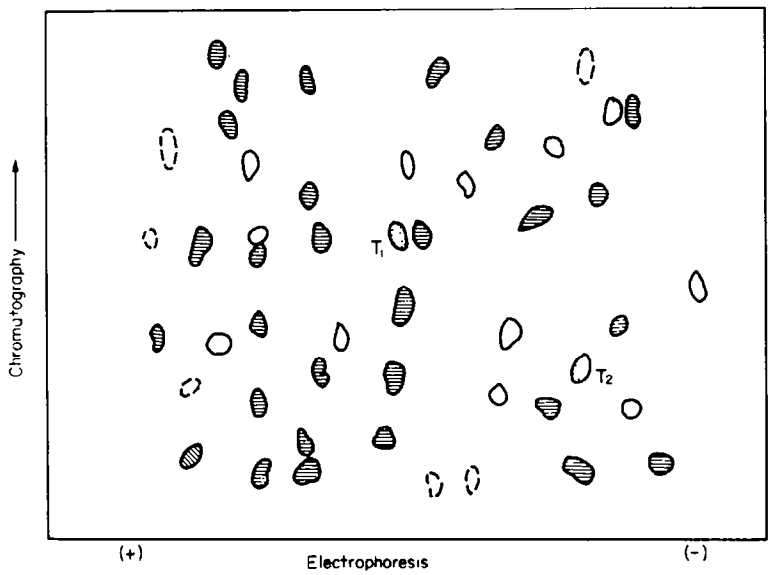

Fig. 1. A trypsin peptide pattern of isoperoxidase A_f. ⊖, Major ninhydrin spots; O, medium intensity ninhydrin spots; ○, weak intensity ninhydrin spots; ⊙, origin.

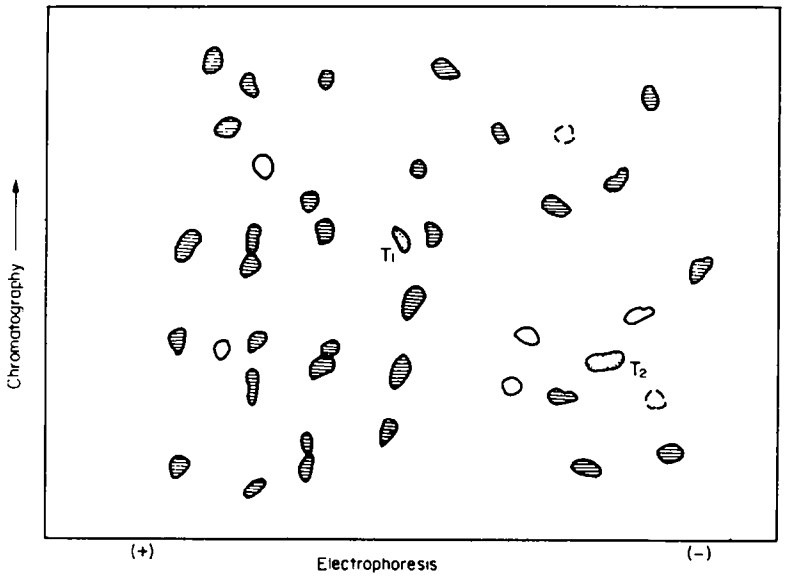

Fig. 2. A trypsin peptide pattern of isoperoxidase A_3. ⊖, Major ninhydrin spots; O, medium intensity ninhydrin spots; ○, weak intensity ninhydrin spots; ⊙, origin.

As far as anodic isoperoxidases are concerned, the peptide patterns of A_1 and A_2 from W-38 callus were very similar (Table 1), even though the MWs and subunit structures of the two isoperoxidases are dissimilar [5]. *Ca* half of the peptides of both isoperoxidases overlapped. The peptide maps of A_c and A_f/A_3 indicated some similarities to both A_1 and A_2 with A_c having more overlapping peptides than A_f (Table 1). Many overlapping peptides are also present in a comparison of A_c and A_f/A_3. *Ca* 50% of the A_f tryptic peptides match those of A_c. When all the anodic isoperoxidases are compared, 8 homologous tryptic peptides appear to be present.

A comparison of the peptide maps derived from the cathodic anodic isoperoxidases studied showed that although none of the isoperoxidases (except A_f and A_3) are identical, all other isoperoxidases (C_n, C_4, C_3, A_1, A_2, and A_c) from tobacco tissue cultures are not dramatically dissimilar in certain portions of the sequence, since many matching peptides have been found among these isoperoxidases despite their different electrophoretic movements. In particular, portions of the peptide maps of isoperoxidases A_1, A_2, and A_c were similar to portions of the maps of isoperoxidases C_3 and C_4. The peptide maps of isoperoxidase A_f/A_3 showed the least similarities to those of the cathodic

Table 1. Comparison of overlapping tryptic peptides of various isoperoxidases

Isoperoxidases	$C_n(35)$	$C_4(45)$	$C_3(52)$	$A_1^*(63)$	$A_2^*(63)$	$A_c(49)$	$A_f(43)$
$C_n(35)$	—	15†	15	16	20	17	15
$C_4(45)$	15	—	22	18	23	20	10
$C_3(52)$	15	22	—	24	23	20	18
$A_1^*(63)$	16	18	24	—	35	24	18
$A_2^*(63)$	20	23	23	35	—	22	17
$A_c(49)$	17	20	20	24	22	—	22
$A_f(43)$	15	10	18	18	17	22	—

*Isoperoxidases from W-38 tobacco tissue culture.

†Possible overlapping tryptic peptides among different isoperoxidases. Overlapping peptides were counted by overlapping origin of each tryptic peptide pattern after drawing 1 cm square lines from the origin.

(): Number of tryptic peptides of isoperoxidases. The figures represent major and medium intensity peptides.

Note: Isoperoxidase A_3 was not included here because it was identical to A_f.

isoperoxidases. In contrast to the other anodic isoperoxidases, however, isoperoxidase A_f/A_3 appeared to be more closely related to isoperoxidases C_n and C_3 than to isoperoxidase C_4. In their studies, Welinder and Mazza [8] found two highly homologous sequences containing histidine in all 5 of the isoperoxidases they studied. They suggested that two histidine sequences are essential for peroxidase activity. A composite tryptic peptide map of all the tobacco tissue culture isoperoxidases examined revealed that two (and possibly three) highly homologous peptides are present in all of the isoperoxidases. Although it is premature to conclude, without detailed analysis of the peptides, that the two homologous peptides are analogous to the homologous sequences proposed by Welinder and Mazza [8], it is probable that some portion of the peptides which include active sites has been conserved among all isoperoxidases from tobacco tissue cultures.

EXPERIMENTAL

Isoperoxidases A_f, A_c, and C_n were purified from WR-132 by the method of ref. [10]. Isoperoxidases C_3 and C_4 from the same source were purified by a slight modification of the method of ref. [5]. C_4 was eluted from a carboxymethylcellulose column with 20 mM NaPi buffer (pH 6), while C_3 was obtained by subsequent elution with 45 mM NaPi buffer (pH 6). Isoperoxidases A_1 and A_2 from W-38 were gifts from Dr. Bernard L. Powell. Isoperoxidase A_3 from W-38 was isolated and purified by the method of ref. [11]. All of the isoperoxidases were further purified by subjecting each isoperoxidase preparation to gel filtration on a Sephadex G-150 column (90 × 2.5 cm) using 40 mM NaPi buffer (pH 6) as the eluant.

Two-dimensional chromatography and high voltage electrophoresis. These procedures were performed according to a slight modification of the methods of ref. [12]. Descending chromatography was done using a solvent system of *sec*BuOH–HCO_2H(90%)–H_2O (7:1:2) for *ca* 15 hr with methyl red as the indicator. High voltage paper electrophoresis was performed for 1 hr at 2.4 kV. The solvent system consisted of Py–HOAc–H_2O water (1:10:300), pH 3.5. Quinine sulfate was used as an indicator. The chromatogram was developed by spraying with 0.5% ninhydrin in Me_2CO followed by heating at 100° for 20 min.

For the prepn of samples, 5 mg of each isoperoxidase was dissolved in 1 ml of 50 mM ammonium carbonate buffer, pH 8.3. Vials containing each isoperoxidase were heated at 100° for 30 sec. Trypsin (Sigma; 80 mg) in 0.5 ml of the above buffer was added to each sample, and the sample was then incubated at 30° for 1.5 hr. The reaction mixture was heated at 100° for 5 min to stop the proteolytic digestion and then centrifuged at 27000 g for 15 min. The resulting supernatant was collected and lyophilized. The lyophilized sample was dissolved in 0.1 ml of deionized H_2O and spotted on Whatman 3 MM filter paper.

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