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PEROXIDASE ISOENZYMES OF THE *AVENA* COLEOPTILE

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Abstract—The bulk of the peroxidases of *Avena* coleoptile sections exist in soluble and salt-extractable, wall-associated fractions with lesser amounts in membranous and wall-bound fractions. In the presence of auxin the peroxidase levels remain nearly constant while in the absence of auxin the peroxidase of each fraction increases 2-to 6-fold in 22 hr. There are qualitative and quantitative changes in the isoenzyme patterns with time, but these changes are independent of auxin. It is concluded that the peroxidase changes are induced by isolation of the tissues from the coleoptile and are unrelated to the growth rate.

INTRODUCTION

THE POSSIBILITY that the enzyme peroxidase (E.C.1.11.1.7) is involved in the control of cell elongation is suggested by the inverse relation between peroxidase levels and the growth rate.[1-3] The correlation between peroxidase levels and the cessation of growth during stem maturation is striking.[4] What is less clear is whether peroxidase is correlated with the growth rate of tissues in which elongation can stop and start; i.e. in tissues whose elongation is stimulated by auxin.[5,6]

Much of our knowledge concerning auxin-induced cell elongation comes from studies involving *Avena* coleoptile sections,[7] but little is known about the peroxidases of coleoptiles or the effects of auxin on them.[5,8,9] The objective of this study was to determine the changes in the amounts and types of peroxidase which occur in *Avena* coleoptile sections in the presence and absence of auxin.

RESULTS

Distribution of peroxidase

The distribution of peroxidase in the various fractions and washes of freshly excised *Avena* coleoptile sections is given in Table 1. The bulk of the activity (67%) is soluble and can be washed out of the crude cell wall residue with buffer. Upon further extraction of the washed cell walls with 200mM $CaCl_2$ most of the remaining peroxidase activity (22%)

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TABLE 1. PEROXIDASE CONTENT OF VARIOUS FRACTIONS AND WASHES OF FRESHLY EXCISED COLEOPTILES

Fraction*	Peroxidase activity* (units/50 sections)	% Total activity
Soluble	43·5	40
1st Wash	27	25
2nd Wash	2	1·8
3rd Wash	0	0
Salt extractable cell wall 1st	20	18
Salt extractable cell wall 2nd	5	4·5
Salt extractable cell wall 3rd	0	0
Enzyme-extractable cell wall	2·5	2·3
Cell Wall Residue	4·5	4·1
Membrane	5·5	5·0
Total	110·0	100·7

* Fractionation and assay as described in Experimental.

is removed, leaving a residual cell wall activity (6%) some of which can be solubilized after treatment of the cell wall matrix with hydrolytic enzymes.

Changes in peroxidase during growth

Auxin-treated sections extend at a rate which is at least 8-fold greater than that of sections incubated without auxin. In the presence of sucrose the growth rates both with and without auxin are nearly constant over at least a 22 hr period.

The changes in peroxidase levels which occur in each fraction during a 22 hr incubation with or without auxin are shown in Fig. 1. In the absence of auxin the peroxidase levels

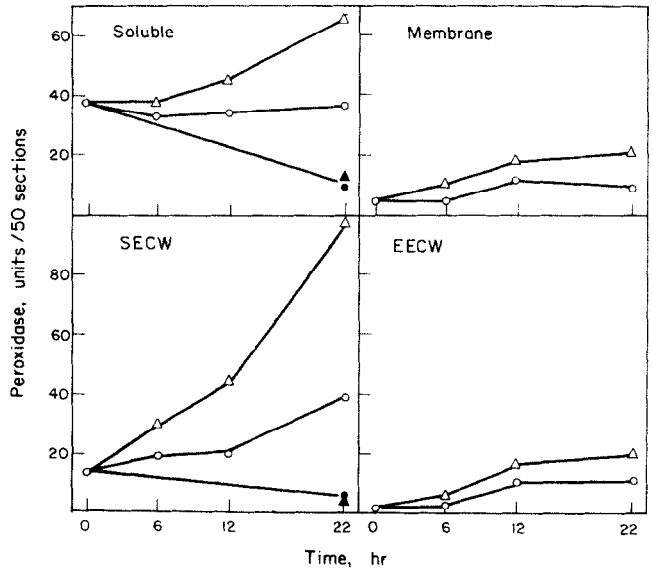

FIG. 1. CHANGE IN PEROXIDASE CONTENT OF COLEOPTILE FRACTIONS AGED IN THE PRESENCE OR ABSENCE OF AUXIN.

Sections were aged in the presence of auxin (—○—), absence of auxin (—△—), with auxin and cycloheximide (—●—), or without auxin but with cycloheximide (—▲—). Fractionation and enzyme assay procedures were as described in Experimental.

in each fraction increase over the whole period. The greatest increase is found in the salt-extractable cell wall (SECW) fraction where the level increases 6-fold in 22 hr. In the presence of auxin the peroxidase level either remains nearly constant (soluble) or increases only slightly (SECW). Even under these conditions considerable synthesis of peroxidase must be occurring, since inhibition of protein synthesis with cycloheximide results in a marked decrease in the peroxidase level. Similar results are obtained if the peroxidase is expresssed on a specific activity or on a cell wall weight basis (data not shown).

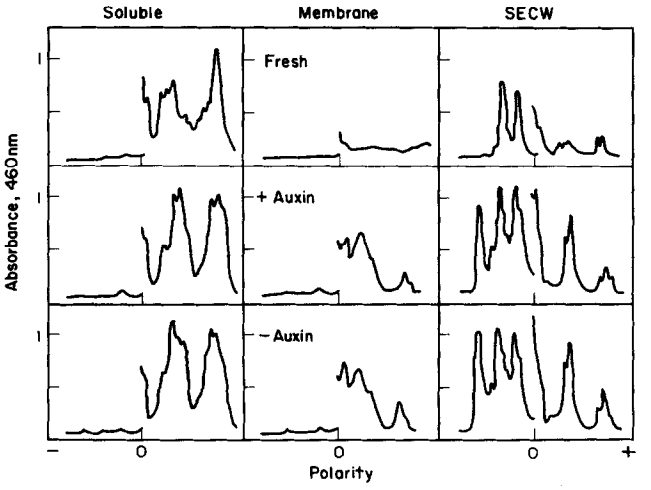

FIG. 2. ZYMOGRAM OF SOLUBLE, MEMBRANE, AND SALT-EXTRACTABLE CELL WALL (SECW) PEROXIDASES OF FRESH AND AGED SECTIONS.
Sections were aged for 0 or 12 hr with or without (+/−) auxin, and electrophoresis, staining, and densitometry was performed as described in Experimental.

Isoenzyme patterns

The changes which occur in the peroxidase isoenzymes in the soluble, membranous and SECW fractions during an incubation with and without auxin are shown in Fig. 2. In the soluble fraction almost all of the peroxidase is found as anionic isoenzymes. During a 12 hr incubation, with or without auxin, only quantitative changes were found in the isoenzyme pattern. In the membranous fraction no distinct isoenzymes can be separated from freshly-cut sections, but both auxin-treated and buffer-treated sections develop similar anionic isoenzyme patterns during the 12 hr incubation. In contrast, the SECW fraction of freshly-cut sections contains both anionic and cationic isoenzymes. During an incubation with or without auxin there are similar quantitative changes in the anionic isoenzymes while there is the appearance in both cases of a new major cationic isoenzyme. In no fraction is there any obvious effect of auxin on any of the isoenzyme patterns.

DISCUSSION

In the coleoptiles of oats, as in those of wheat,[5,8,9] the bulk of the peroxidase is located in the soluble and the salt-extractable cell wall-associated fractions. Only a small portion is bound to the cell wall. Of interest is the peroxidase found in the membranous fraction, which from the isoenzyme pattern would appear to be distinct from the soluble fraction.

As in wheat coleoptiles[5] the peroxidase levels increase in sections during an incubation without auxin while remaining nearly constant in the presence of auxin.

The inverse relation between peroxidase and the rate of cell elongation has been frequently noted.[1–6] The present results appear to suggest that such a relation also exists for *Avena* coleoptile tissues since the peroxidase increases continuously in slowly-growing tissues while remaining constant in rapidly-growing ones. However, it must be noted that 1 hr after the start of an incubation with and without auxin, when the auxin-treated sections are growing at least 6 times more rapidly than the controls, there is still little difference in the amount of peroxidase between the two sets of tissues. It is not known how much peroxidase might be required to decrease growth if, in fact, it is affecting growth. It might be that deposition of peroxidase in the cell wall is not directly related to growth but related to an auxin-regulated molecular differentiation of the cell wall that is only indirectly related to the decrease in growth.

In pea stem[10] and tobacco pith tissues[11] auxin has been shown to repress the synthesis of specific peroxidase isoenzymes. In *Avena* coleoptiles, on the other hand, the synthesis of all the isoenzymes appears to be suppressed to a similar degree. Some changes in peroxidase isoenzymes do occur with time during incubations of coleoptile sections. However, these qualitative changes occur in both auxin-treated and non-treated sections and do not reflect an effect of auxin on the tissues. The observed changes in isoenzyme composition may be in response to some other factor common to both treated and non-treated sections alike, possibly the excision or aging of the coleoptile tissue.

EXPERIMENTAL

Plant material consisted of 10 mm sections cut from 28–32 mm coleoptiles of *Avena sativa*, cv. Victory. Methods for growing seedlings and harvesting sections are described elsewhere.[12] Sections were floated on H_2O until used (not more than 30 min).

Incubations. Groups of 50 sections were incubated for 0–22 hr in beakers with 10 ml buffer (K-maleate, 2·5 mM, pH 4·7) containing 2% sucrose, 0·1 mM penicillin G, and with or without IAA (5 μg/ml) and cycloheximide (4 μg/ml). Beakers were rotated at about 1 rps; incubations and all manipulations were carried out under dim red light. At the end of the incubation the sections were measured, washed in H_2O and ground in an all-glass homogenizer with 0·5 ml 10 mM Tris–HCl, pH 8·8, containing 10% sucrose.

Preparation of fractions. The crude homogenate was centrifuged for 15 min at 1450 *g* to give a crude cell wall and supernatant fraction. The supernatant was then centrifuged for 60 min at 4×10^4 *g*. The resulting pellet was washed once by resuspension in the same medium, dissolved in 1 ml of 10 mM Tris–HCl, pH 8·8, containing 1% Na-deoxycholate and used as the *membranous fraction*. The crude cell wall fraction was washed 5 × by regrinding in 0·05 M Tris–HCl, pH 8·8, and recentrifuging, and the supernatant and wash solns were combined to form the *soluble fraction*. On extraction for 1 hr at 4° with 0·5 ml 200 mM $CaCl_2$ and centrifugation, the supernatant was dialyzed overnight against 10 mM Tris-HCl, pH 8·8, and used as the *salt-extractable cell wall* (SECW) *fraction*. The pellet was subjected to enzymatic digestion by a mixture of 1 unit/ml Worthington Cellulase-I and 1 unit/ml Sigma pectinase for 4 hr at room temp. After centrifugation the supernatant was assayed as the *enzyme-extractable cell wall* (EECW) *fraction* while the pellet formed the cell wall residue fraction.

Assays. The peroxidase assay mixture contained 0·3 mM *o*-dianisidine, 3·3 mM H_2O_2 and 50 mM K-acetate (pH 5·4) in 3 ml. The reaction was started by the addition of 20 μl enzyme extract, and the appearance of oxidized dianisidine was measured at 460 nm and 25°. Reaction rates were calculated from the initial slope; a unit of enzyme activity being defined as the amount of enzyme causing a change of one absorbance unit per min. Protein was estimated by the method of Lowry *et al.*[13]

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Electrophoresis was performed in 5 × 125 mm polyacrylamide gels. The gels contained 7–1/2% polyacrylamide with 5% cross-linking and 370 mM Tris buffer, pH 8·8. Polymerization was accomplished using 0·1% ammonium persulfate and TMEDA. Samples were made to 10% in sucrose and 0·001% in bromophenol blue and were layered directly onto the upper surface of the gel. Separations were run at a constant current of 3 mA per gel using 50 mM Na-borate electrode buffers, pH 8·8, at about 14°. The separation of anionic isoenzymes was run until the bromophenol blue marker dye had moved about 90 mm from the origin, but cationic isoenzymes were run for 6 hr because of their low mobility at this pH. Following electrophoresis the gels were removed and stained directly in the peroxidase assay mixture for 30 min followed by densitometer recording of the stained bands at 460 nm.

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