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PEROXIDASE CHANGES DURING THE CESSATION OF ELONGATION IN *PISUM SATIVUM* STEMS

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Key Word Index—*Pisum sativum*; Leguminosae; pea; peroxidase; isozymes; cell elongation.

Abstract—The cessation of cell elongation in intact *P. sativum* epicotyls is accompanied by an increase in both soluble and cell wall peroxidases. These peroxidases have been resolved by acrylamide gel electrophoresis into four cell wall and 10 cytoplasmic isozymes. The increases in peroxidase activity are due to increases in certain of the isozymes in both cell wall and cytoplasmic fractions rather than to the appearance of new isozymes. The inverse correlation between peroxidase increase and growth rate decrease indicates that peroxidase could play a role in the cessation of cell elongation.

INTRODUCTION

ALTHOUGH peroxidase (E.C. 1. 11. 1. 7) is widespread in plants, its role remains unclear. A possible role in the cessation of cell elongation is suggested by the inverse relation which has been reported[1-3] between the cytoplasmic peroxidase level and the growth rate, and by the increases in peroxidases which are induced by growth inhibitory agents such as ethylene.[4-6] Peroxidase has been implicated in the oxidation of IAA,[7] the polymerization of lignin[8] and the biosynthesis of ethylene.[9] Each of these functions could lead to an inhibition of cell elongation, but data are not available to show whether peroxidase increases before, with or only after the cessation of cell elongation.

Peroxidase is found both in the cell wall and in the cytoplasm;[4,10] in each place more than one isozyme exists.[1,4,10] When total peroxidase increases it could be due to increases in either or both wall and cytoplasmic fractions. Within each fraction the increase could be due to a general increase in all isozymes, to an increase in certain already-existing isozymes, or to the appearance of new isozymes. No data are available to indicate the nature of the changes which accompany the cessation of cell elongation. The following experiments were conducted to obtain this information for *P. sativum* stems.

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RESULTS

Growth of stem segments was monitored by recording final length and fresh weight of sections which had been allowed to grow for 0–72 hr as a part of the intact seedling. Figure 1 shows that elongation growth was nearly linear between 0–18 hr, but abruptly ceased just prior to 24 hr. Growth measured as the increase in fr. wt of the marked segments continued to increase over the entire period but was at its greatest rate between 0–24 hr. These data agree with those obtained by Cleland and Karlsnes[11] and by Chrispeels *et al.*[12]

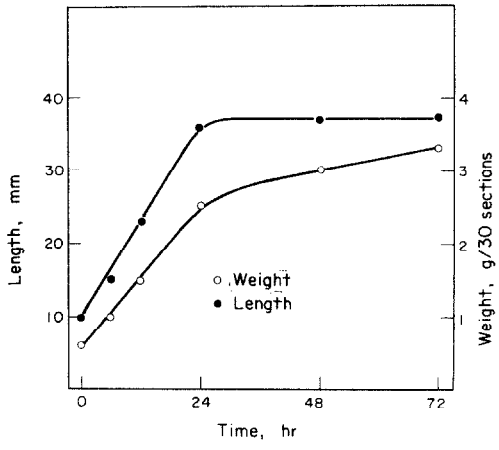

FIG. 1. GROWTH OF INTACT SUBAPICAL INTERNODE SEGMENTS OF ETIOLATED ALASKA PEA STEMS.
Segments 10 mm in length were marked with India ink just below the apical hook and growth was allowed to proceed for up to 72 hr in the dark. At varying times sections were excised between the marks, measured and weighed.

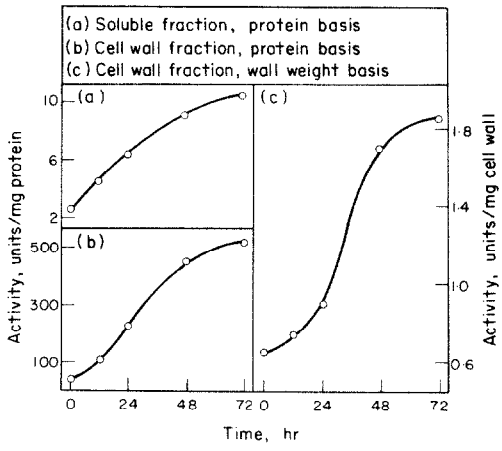

FIG. 2. CHANGES IN PEROXIDASE SPECIFIC ACTIVITY WITH TIME.
Specific activity was calculated from total activity and from determinations of protein in each fraction and dry wt of cell wall residue after extraction.

The specific activity of cytoplasmic peroxidase increased continuously over the whole 72 hr period (Fig. 2a). When expressed on the basis of fr. wt, however, cytoplasmic peroxidase was seen to decrease by about 30% in the first 6 hr and then remain constant over the remainder of the 72 hr (Fig. 3). Similar results have been obtained by Birecka and Galston.[3] In contrast, the sp. act. of cell wall peroxidase increased only after a lag of about 6 hr (Fig. 2b). The lag was more apparent when wall peroxidase was expressed on the basis of cell wall dry wt (Fig. 2c) or section fr. wt (Fig. 3).

Examination of the soluble and cell wall zymograms (Fig. 4) reveals that four of the isozymes to be common to both fractions (A1, A2, C1 and C2). The soluble fraction also contains a number of anionic isozymes unique to that fraction (A3–A8). The relative heights of each peak in the zymogram can be used to determine if the increase in activity is due to one or several of the isozymes. A comparison of the 0, 24 and 48 hr zymograms indicates that the increase in the cell wall fraction is primarily due to increases in the two isozymes, A1 and A2. These same two isozymes do not increase in the cytoplasmic fraction, however. The increase in this fraction is primarily due to the

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isozymes A3, A4, A5, and A6. Isozyme A7, which is initially one of the major bands, diminishes with time. There is also the appearance of isozyme A8, an apparently new but minor band of activity.

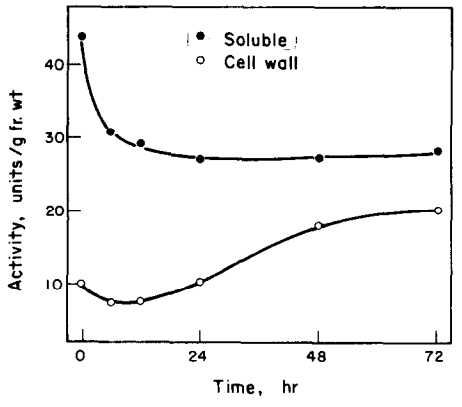

FIG. 3. CHANGES IN PEROXIDASE ACTIVITY EXPRESSED ON A FRESH WEIGHT BASIS.
Activity of peroxidase per g fr. wt of soluble and cell wall fractions.

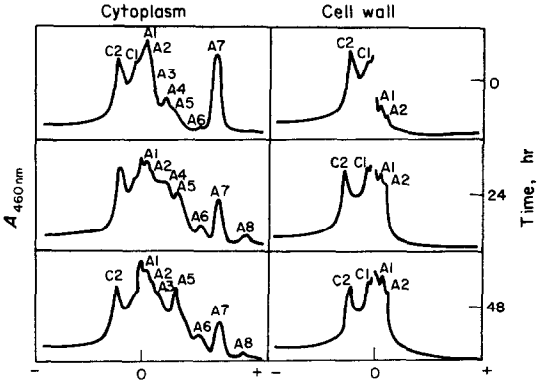

FIG. 4. PEROXIDASE ZYMOGRAMS OF CYTOPLASMIC AND CELL WALL FRACTIONS FROM SECTIONS AGED 0, 24, AND 48 hr *in situ*.
Samples were prepared and run as described in Experimental.

DISCUSSION

The level of peroxidase in plants tends to be inversely related to the growth rate. For example, in *P. sativum* stems the more mature tissues which have the lowest growth rates possess the highest amounts of soluble peroxidase.[1,2,13] Dwarf peas and corn have both a lowered growth rate and an elevated level of soluble peroxidase as compared with the tall varieties.[3,14,15] Treatment of these dwarfs with gibberellin enhances the growth rate and decreases the peroxidase level. Auxin represses the synthesis of specific isozymes of soluble peroxidase in both pea stems and tobacco stem sections while promoting growth.[16,17] Treatment of pea stems with ethylene results in an enhanced peroxidase content and decreased growth rate.[4,18]

While these data suggest that a cause-and-effect relationship may exist between peroxidase and the growth rate, they cannot be considered as conclusive for two reasons. First, with few exceptions,[4,18] only the soluble peroxidases were assayed, yet it has been shown that cells contain wall-associated and wall-bound peroxidase as well.[4,18–21] Changes in soluble peroxidase, then, may simply be due to changes in localization of

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peroxidase rather than to changes in actual amounts of enzyme. Secondly, information as to the timing of the changes in growth rate and peroxidase are invariably lacking, with the result that one cannot determine whether the peroxidase changes precede, are simultaneous with or only follow the changes in the growth rate.

In this study the complete spectrum of peroxidase isozymes has been followed in time and correlated with the cessation of cell elongation. The fact that these changes occur simultaneously indicate that peroxidase could be involved in the cessation of cell elongation. However, at least one other factor shows a similar correlation with the growth rate; this is the cell-wall-bound hydroxyproline-containing protein extensin.[11,12,22] While the role of extensin is by no means established, it has been suggested[23] that these proteins stiffen the wall by crosslinking the polysaccharides. Such crosslinks have now been demonstrated[24] and Sadava and Chrispeels[22] have presented evidence that links the ethylene-induced growth inhibition to extensin synthesis. Thus the cessation of cell elongation may not be under the control of a single factor, but may be influenced by a series of factors including peroxidase-induced lignification, auxin-destruction and estensin-induced wall stiffening.

EXPERIMENTAL

Plant material. Seedlings of *Pisum sativum* L., var. Alaska, were grown for 7 days in the dark at 26° except for brief exposures to dim green light for watering and marking. Plants whose third internode was 25–35 mm in length were selected.

Growth measurements. Two marks, 10 mm apart, were placed on the third internode using India ink. The upper mark was just below the hook. The plants were returned to the dark for 0–72 hrs, and at intervals the distance between the marks was measured and the region between the marks was excized and weighed.

Enzyme preparation. Segments between the marks were excized and homogenized in an all-glass homogenizer with 1 ml 10 mM Tris buffer (pH 8·0) containing 10% sucrose. Cell walls were collected by centrifugation at 1500 *g* for 5 min, reground with 1 ml of the same soln and recentrifuged. The two supernatants were combined, centrifuged at 48 000 *g* for 30 min to remove membranous organelles, and used as the *soluble fraction*. The membranous fraction was discarded since it contained a very low level of peroxidase. Cell walls were further purified by repeated grinding (4 ×) in 50 mM Tris (pH 8·0) containing 0·1% Na-desoxycholate, collecting by centrifugation, and finally washing with H_2O. Walls were then extracted for 1 hr with 1 ml 200 mM $CaCl_2$, the residue was separated by centrifugation and collected on a preweighed filter paper, and the supernatant was dialysed against 1000 vol. of 10 mM Tris, pH 8·0. Preliminary experiments showed that the $CaCl_2$ extraction removed 85–90% of the total cell wall peroxidase. The dialysed $CaCl_2$ extract was called the *wall 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 appearance of oxidized dianisidine was measured at 460 nm and 25°. Reaction rates were calculated from the initial slope; a unit of enzyme activity is defined as the amount of enzyme causing a change of 1 absorbance unit per min. Protein was estimated by the method of Lowry *et al.*[25]

Electrophoresis. Electrophoresis was performed in 5 × 125 mm polyacrylamide gels. The gels contained 7·5% 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 bromphenol 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, and a temp. of about 14°. The separation of anionic isozymes was run until the bromphenol blue marker dye had moved about 90 mm from the origin, but cationic isozymes 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.

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