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THE STRUCTURE OF THE PRIMARY CELL-WALL OF HIGHER PLANTS, AND CONTROL OF WALL DEGRADATION BY PATHOGEN SECRETED ENZYMES.

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Cell walls isolated from suspension-cultured sycamore (Acer pseudoplatanus) cells consist of 26% cellulose, 21% xyloglucan, 19% arabinogalactan, 15% pectic polysaccharides and 19% hydroxyproline-rich glycoprotein with its associated arabinosyl tetrasaccharides. These five interconnected polymers account for essentially the entire cell wall.

The following description of cell wall structure is based on the evidence available at this time. Some of the details of this model may be altered as the results of future experiments are obtained. The elementary cellulose fibrils are encapsulated by xyloglucan chains. The xyloglucan chains apparently are hydrogen-bonded along their length to the outer surface of the cellulose fibrils. And, the xyloglucan chains are covalently attached, through their reducing ends, to the arabinogalactan side chains of the pectic polysaccharides. The pectic polysaccharides are rhamnogalacturonan chains in which there are regions of uninterrupted α-1,4-linked galacturonosyl residues, and regions in which rhamnosyl residues are concentrated. In the latter regions, galacturonic acid is linked at carbon 2 of rhamnose, and rhamnose is linked at carbon 4 of galacturonic acid. Arabinogalactan chains form a bridge between the xyloglucan-cellulose complex and the pectic chains. The arabinogalactan polymers are glycosidically bonded to the pectic chains through carbon-4 of the rhamnosyl residues. The pectic chains appear to be covalently attached through their reducing ends to the hydroxyproline-rich protein. Our best evidence suggests that the pectic chains are joined to the protein through a 1,3-linked galactan bridge. This galactan, with its arabinose side chains, is probably glycosidically bonded to the hydroxyl groups of seryl residues of the hydroxyproline-rich protein. The hydroxyproline residues have glycosidically attached arabinose tetrasaccharides. We have no evidence that other sugars are connected to these arabinosyl residues.

Details of the structure of the individual polymers will be presented, as will evidence that this proposed structure for sycamore cell walls may be considered as a general structural model for the primary cell walls of a variety of higher plants.

Our studies of plant cell wall structure have been facilitated by the use of purified degradative enzymes. The well-defined fractions thus liberated were analyzed by a variety of techniques including combined gas chromatographic-mass spectrometric analysis of the methylated alditol acetates derived from the polymeric constituents. These volatile derivatives of the polysaccharides or of the oligosaccharide fragments are synthesized by permethylation using the method of Hakomori; the permethylated polymers are hydrolyzed in trifluoroacetic acid, reduced with sodium borohydride, and the resulting partially methylated alditols acetylated with acetic anhydride. Computer assisted data reduction has been used to analyze the outputs of the gas chromatographs and of the mass spectrometer.

DISCLAIMER

The only enzyme which, by itself, has been found to efficiently attack native cell walls is an endopolygalacturonase. The endopolygalacturonase hydrolyzes the homogalacturonan regions of the pectic polymers to tri-, di-, and monogalacturonic acid. The enzyme also releases galacturonic acid containing fragments rich in rhamnose, galactose and arabinose. Some of these pectic fragments have small pieces of xyloglucan covalently attached.

The endopolygalacturonase used in these studies is secreted by the plant pathogen Colletotrichum lindemuthianum; the enzyme has been purified to homogeneity. This is the first polysaccharide-degrading enzyme to be secreted when this fungus is grown in culture on isolated cell walls as the sole carbon source. Isolated cell walls which have been treated with the endopolygalacturonase have greatly increased susceptibility to subsequent degradation by cellulolytic and proteolytic enzymes. However, that portion of the wall released by the action of cellulase originates from the xyloglucan rather than from cellulose. These results have demonstrated that efficient enzymatic hydrolysis of plant cell walls to yield their monomeric constituents requires the *sequential* action of a series of glycanases and glycosidases.

A variety of plant tissues, including cultured sycamore cells, possess proteins capable of inhibiting the endopolygalacturonases secreted by several plant pathogens. The inhibitor can be extracted from the plant's cell wall with buffered 0.5 M salt solutions, and, therefore, these proteins are not covalently attached to the structural matrix of the cell wall.

The inhibitor of the C. lindemuthianum endopolygalacturonase has been purified about 600-fold from Red Kidney bean hypocotyl extracts. This purified inhibitor is 40 times as effective an inhibitor of the C. lindemuthianum endopolygalacturonase as of a Fusarium oxysporum polygalacturonase, and the inhibitor does not demonstrably affect the activity of a Sclerotium rolfsii polygalacturonase. A crude Red Kidney bean hypocotyl extract that completely inhibits these three polygalacturonases does not inhibit C. lindemuthianum-secreted cellulase, xylanase, α-galactosidase, α-arabinofuranosidase, or exopolygalacturonase. The purified bean hypocotyl protein combines with the C. lindemuthianum endopolygalacturonase to form a complex with a dissociation constant of 10^{-9} or less. Indeed, it appears that it may require only a single molecule of inhibitor to inactivate completely a single molecule of endopolygalacturonase.

Thus, plants possess, in their cell walls, proteins which are highly efficient inhibitors of pathogen-secreted endopolygalacturonases, the enzymes which can initiate degradation of plant cell walls. These inhibitors are not only efficient but they are highly specific, for the inhibitors distinguish between endopolygalacturonases and other degradative enzymes - enzymes which require the prior action of endopolygalacturonases before they can attack their cell wall substrates. Moreover, these inhibitors distinguish between the polygalacturonases secreted by different species of pathogenic fungi. These results suggest that such inhibitors participate in disease resistance.

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