

Comparison of lignins formed *in vitro* by Peroxidases type I and II

Kiyota, E., Sawaya, A.C.H.F., Cesarino, I., Mazzafera, P.

Departamento de Biologia Vegetal, Instituto de Biologia, Unicamp, Brazil

Lignins are polymeric phenolic structures that are found in secondary thickened plant cell walls, composed mainly of three monolignol units of (G) guaiacyl, (S) syringyl and (H) p- hydroxyphenyl which are derived from the three phenylpropanoid alcohols (coniferyl, sinapyl and p-coumaroyl respectively). Different from other plant polymers, such as cellulose and proteins, its structure is not linear, making its structural elucidation far more complex. The polymerization process is initiated by enzymes that oxidize the monolignols, resulting in electron-delocalized radical units, with unpaired electron density at their 1-, 3- O-4-, 5 and 8 positions, favoring coupling at these positions. The objective of this study was to determine types of lignins formed by the addition of the individual monolignols, and mixtures of these, to peroxidases type I and II, with a view to compare these structures with lignins found in sugar cane and other plants. The individual or mixtures of monolignols were dissolved in water with cetyl-trimethyl-ammonium sulfate and 3.5 units of peroxydase (Type I or II) were added followed by hydrogen peroxide solution, this mixture was kept at 30°C for 5 minutes and 1 hour. The reaction was ended using a 5% sodium thiosulfate solution, and the lignins extracted from the medium with ethyl acetate. The organic phase was collected, washed with a saturated saline solution, and then evaporated. The resulting lignins were dissolved in acetonitrile, diluted in purified water, and analyzed by liquid chromatography-mass spectrometry. The main peaks in the chromatogram were selected for fragmentation and the resulting spectra were analyzed to determine their composition. Smaller polymers (mainly dilignols and low concentration of trilignols) were observed with the use of peroxydase I. With peroxydase II, mainly trilignols and tetralignols were observed. Over a dozen compounds have been detected with *m/z* compatible with lignins, and their structures are being determined.

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