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Effect of an Extracellular Laccase of *Rigidoporus lignosus* on *Hevea brasiliensis* Lignin

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ABSTRACT

The abilities of white-rotting fungi to depolymerize lignin and to excrete laccases (*p*-diphenoloxidases), though brown-rotting fungi do not present these two biological properties, are the main differences between these two types of rotting fungi. Therefore it was assumed that the lignin scission was a result of the laccase reaction. Nevertheless, *in vivo*, this enzyme may play other major roles such as detoxifying of the medium by oxidation or condensing fungal growth-inhibiting phenolics. As *R. lignosus* (causing a white rot on Hevea roots) secretes two laccases, our purpose was to determine whether these enzymes are able to depolymerize the lignin macromolecule or not. This was realized by showing the effect of the *R. lignosus* purified laccase L1 on the lignin polymerization degree by using Sephadex G100, G50, or G25 gel filtration.

The laccase substrate was a lignin extracted from healthy Hevea root tissues by thioglycolic acid. This thioglycolic lignin (TGL) is hydrosoluble and is characterized by a differential ionization spectrum identical to that of a native lignin. This preparation is heterogeneous with regard to molecular weight (mw): 3000 daltons up to very high mw (excluded with the void volume of the G100 column), with a major fraction at nearly 10,000 daltons.

The validity of the gel filtration method as for TGL molecular weight determination, was proved by chromatographing two TGL fractions that differ by their mw: a fraction A of high mw and a fraction B of low mw that were isolated by fractionation of crude TGL on a PM 10 Amicon Ultrafilter. The resulting elution patterns show a "normal" distribution for both fraction A and B. Moreover, repeating rechromatographies of three TGL fractions, differing from each other by their mw, demonstrated the high reproducibility of the gel filtration method.

After incubation of TGL with the laccase L1, several related events could be observed (incubation in and column elution with phosphate 0.05*M* pH 6 buffer):

1. A quick browning of the solution and an increase (up to 50%) of the OD at 280 nm.
2. A progressive modification of the differential spectrum:

   disappearance of the maximum at 300 nm
   decrease of the maximum at 260 nm
   increase of the maximum at 365 nm

   which reflects the decrease of the ionizable phenolic hydroxyl groups and the increase of the number of α-carbonyl groups of the phenylpropane side chain.

3. The modification of the elution patterns on G100, G50, and G25, namely:

   A shift of the major "10,000 dalton peak" to a region where molecules of higher mw (50,000 daltons) are eluted.
   The appearance of several peaks corresponding to low mw molecules. This is especially clear when the reaction medium is filtrated on G50 or G25. The differential spectrum of one of those fractions shows a maximum at 335 nm, indicating most probably the presence of phenylcoumarone derivatives.

These results show that the enzymatic activity of the laccase L1 on TGL results in a modification of the polymerization degree of the macromolecule leading to both condensation and depolymerization. Most probably an equilibrium between those reactions does exist. Nevertheless, it can also be assumed that the chemical bonds that are involved, respectively, in condensing and in splitting reactions, differ from each other.