PEGYLATION OF LACCASE FROM TRAMETES VERSICOLOR: EFFECT ON ENZYME ACTIVITY AND SEPARATION

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Introduction. Laccase is a multicopper blue oxidase that catalyzes the oxidation of phenolic compounds and aromatic amines using molecular oxygen as an electron acceptor. It has been extensively studied due to its potential [1]. Recently, the anti-proliferative activity of laccase towards tumor cells has been reported [2]. In this sense, special attention must be directed to enhance the physico-chemical properties of the enzyme. PEGylation is the covalent attachment of one or more molecules of polyethylene-glycol (PEG) to a protein. This strategy has been mainly exploited in the pharmaceutical area with the aim of increasing the molecular weight, thus improving therapeutic treatments [3].
The objective of this work is to study the amino N-terminal PEGylation of the laccase from *T. versicolor,* its effect on the enzyme's activity and to achieve the separation of the resulting bioconjugates.

Methods. PEGylation reaction was performed with 20 kDa methoxy-PEG propionaldehyde. Chromatographic techniques were employed for the separation of the products. The characterization of PEGylated conjugates and its native form was carried out using electrophoresis with silver staining and I_2–$BaCl_2$ for PEG detection. Additionally, enzymatic activity was measured throughout the reaction using ABTS.

Results. PEGylation was carried out using amino N-terminal reaction. The typical molar ratio reported is 1:4 (protein:PEG). However, different molar ratios were employed to evaluate their effect on the total enzymatic activity (see Table 1). It was possible to observe an effect when the mass of each compound was changed. Namely, the enzymatic activity increases with higher concentrations of protein and PEG. In this sense, it is important to minimize losses of activity, which are common after PEGylation. PEGylation reaction produces three PEGylated proteins, as shown in Fig. 1. That was confirmed with SDS-PAGE. The product of interest is the monoPEGylated protein, so it is important to determine if it exists as one bioconjugate or isomers bioconjugates are present.

Table 1. Recovery of total activity of laccase using different molar ratios and ABTS.

Molar Ratio (laccase:PEG)	Laccase (mg/mL)	mPEG (mg/mL)	% Recovery of total activity (ABTS)
1:12	6	22.5	52
1:8	6	15	56
1:4	6	7.5	44
1:4	12	15	67

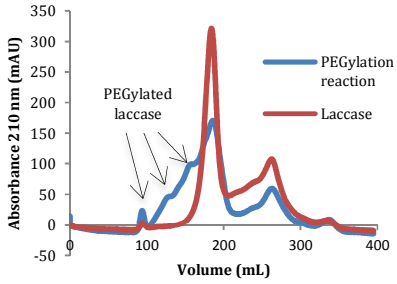

Fig.1 Size Exclusion Chromatographic profile of the PEGylation reaction of laccase.

Laccase activity is being evaluated with other substrates, including 2,6-DMP, syringaldazine and guaiacol, to compare affinity changes resulting from PEGylation.

Conclusions.

- The recovery of total activity is higher using 12 mg/mL of laccase and 15 mg/mL of PEG, with a molar ratio of 1:4.
- No activity is lost during the PEGylation process, when compared to the control.
- At least three bioconjugates were detected.

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