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Peroxidase-based oxidative polymerization of monolignols

Polymérisation oxydative des monolignols par les peroxydases

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ABSTRACT

Oxidative polymerization of the monolignols (sinapyl alcohol [SA] and coniferyl alcohol [CA]) has been performed using enzyme-based biocatalysts. The oxidation of SA, CA, or an SA/CA mixture has been carried out using peroxidase enzyme–assisted H_2O_2/*t*-BHP (oxidation reagent). The reaction provided radicals with high reactivity, in turn yielding a variety of polymeric structures. The efficiency of the oxidative polymerization system has been evaluated in terms of substrate conversion. Also, the polymeric products were characterized with the gel permeation chromatography technique (GPC). Accordingly, optimum experimental parameters have been set up (e.g. temperature, type of peroxidase enzyme, and oxidation reagent). Under optimum conditions, a maximum of 90% of the SA was transformed to polymeric products with MW = 3188 Da, Mn = 1115 Da, and PD = 2.8.

RÉSUMÉ

Mots-clés:
Monolignol
Enzyme peroxydase
Oxy-polymérisation
Lignine

L'oxy polymérisation de monolignols (SA, alcool de sinapyle, et CA, alcool de coniféryle) a été réalisée en utilisant un biocatalyseur enzymatique. Ainsi, SA, CA et un mélange de ces deux composés ont été oxydés par une peroxydase utilisant H_2O_2/*t*-BHP comme agent oxydant. La réaction fournit des produits radicalaires de haute réactivité, qui conduisent finalement à une structure polymérique. L'efficacité de ce système d'oxy-polymérisation a été évaluée en terme de niveau de conversion. Les polymères produits sont caractérisés par analyse en CG. Une optimisation a été conduite en faisant varier des paramètres tels que la température, le type de peroxydase et la nature de l'agent oxydant. Dans les conditions optimales, un maximum de 90% de SA est transformé en polymère de MW = 3188 Da, Mn = 1115 Da et DP = 2.8.

1. Introduction

Lignin is the second most plentiful renewable natural polymer after cellulose, accounting for 15%–30% (wt) of the lignocellulosic biomass and almost 30% of all non-fossil–based carbon sources [1]. It has a highly branched three-dimensional optically inactive structure comprising three phenyl propane units (monolignols), for example, sinapyl alcohol (SA), coniferyl alcohol (CA), and *p*-coumaryl alcohol. The monolignols are cross-linked together by C–C (5-5 and β-1 linkages) and C–O–C (α-O-4 and β-O-4

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linkages) bonds leading to a complex structure with a large variety of functional groups (e.g. hydroxyl −OH, carbonyl −CHO, and carboxyl −COOH moieties) [2].

The ratio of monolignols in lignin composition entails classification of lignin into three major groups: (1) softwood lignin consisting mainly of CA and traces of SA units, (2) hardwood lignin with comparable ratios of SA and CA, and (3) grass lignin with major amounts of *p*-coumaryl alcohol [3]. In addition, softwood lignin has around 56 ether linkages, whereas hardwood lignin has around 72% [4]. This functionalized aromatic architecture recommends lignin as an energy supplier and aromatic precursor for fuel applications [5,6]. Consequently, its industrial exploitation is receiving tremendous attention nowadays.

The pulp and paper industry is an important provider of lignin as a major waste byproduct [7]. Unfortunately, only 2% of this waste is commercially exploited. The main reason is related to the high degree of physicochemical heterogeneity of lignin (e.g. built-in variety of aromatic units, interunit linkages, functional groups, and molecular size) [8].

However, lignin has several additional properties that can enhance its potential value. For example, lignin has adhesive properties (e.g. useful as rubber intensifier, polyolefin and rubber packing, and filler and comonomer for unsaturated polyester and vinyl ester materials). It can also be used as binder for glass wool building insulation, fiber nutritional source for pet and human food, dispensing agent, flocculent, and thickener or auxiliary agent in coatings and paints [3,9].

Another scenario for achieving proper exploitation of lignin may involve the development of derivatization methods of the lignin structures via chemical, enzymatic, and/or chemoenzymatic pathways. The enzymatic methods may even mimic the natural biosynthesis of lignin. Thus, peroxidase and laccase enzymes can provide oxidation of lignin building blocks leading to reactive species (radicals) with a multitude of coupling possibilities. Following this strategy, Dordick et al. [10] used horseradish peroxidase to incorporate phenols into lignin leading to the production of reactive polymers with properties of phenolic resins. Also, laccase alone or in combination with mediators was used to introduce phenolic-based “building block” functionalities onto lignocellulosic materials [11]. Similar strategies were developed for different comonomers such as guaiacol sulfonate, vanillylamine, or acrylamide grafted onto lignin [12,13]. However, achieving effective control over the heterogeneity of lignin structure is still an unsolved problem and constitutes an important drawback for industrial applications.

In this context, we propose the construction of polymers starting from the monolignols found in lignin, for example, SA and CA, leading to products similar to natural lignins. The oxidative polymerization (oxi-polymerization) of monolignol(s) was performed in the presence of hydrogen peroxide (H_2O_2) or *tert*-butyl hydroperoxide (*t*-BHP). The reaction was catalyzed by peroxidase enzyme leading to products with less heterogeneous polymeric structures. The polymeric products have been characterized using the gel permeation chromatography (GPC) technique. The versatility of the developed method has been proved by testing monolignol mixtures with different SA/CA molar ratios (oxi-copolymerization).

2. Experimental section

2.1. Chemicals and solutions

Enzyme screening for the investigated oxi-polymerization process was performed with three peroxidase enzymes from two sources, for example, PaDa-I (AaeUPO1 variant, unspecific peroxidase produced in *Saccharomyces cerevisiae*, 25.82 U mL^{-1} enzyme activity), R4, and 2-1B (versatile peroxidase originally from *Pleurotus eryngii*, expressed in *S. cerevisiae*, 12.95 U mL^{-1} enzyme activity) [14,15]. PaDa-I, R4, and 2-1B mutants were provided by Dr. Miguel Alcalde (Institute of Catalysis, CSIC, Madrid, Spain).

Stock solution of 1 M phosphate-buffered saline (PBS) was prepared by dissolving 8 g NaCl, 0.2 g KCl, 1.43 g $Na_2HPO_4 \cdot 2H_2O$, and 0.34 g KH_2PO_4 in 1 L distilled water. Dilution of the stock solution led to 10 mM PBS (pH = 7.4) used as aqueous buffer solution. CA and SA were of analytic purity and were purchased from Sigma–Aldrich together with the other reagents/solvents (30 wt % solution of H_2O_2, *t*-BHP, and THF).

Stock solution of monolignols (e.g. 10 mg mL^{-1} CA or SA) was prepared in methanol, that is, 10 mg of each monolignol was dissolved in 1 mL of methanol. These solutions were used further for the preparation of the reaction mixtures for the oxi-(co)polymerization process.

2.2. Oxi-(co)polymerization of monolignols

Oxi-(co)polymerization of monolignols (e.g. CA, SA, or CA/SA mixture) was performed using a peroxidase-based biocatalytic system. One hundred microliters of monolignol(s) stock solution was mixed with 300 μL PBS (10 mM, pH 7.4). The oxidation reagent (H_2O_2 or *t*-BHP) and peroxidase enzymes (PaDa-I, R4, and 2-1B) were then added to the solution to reach a final concentration of 0.6% and 2.582 U mL^{-1}, respectively, and a final concentration of 2 mg mL^{-1} monolignol used for the oxi-(co)polymerization reaction. The reaction mixture was incubated for 2 h under controlled temperature (e.g. 25, 40, 50, and 60 °C) and gentle stirring (100 rpm).

2.3. Analysis of the reacted mixture

The reacted mixture was treated as indicated in Scheme 1. First, the mixture was separated into two phases (liquid and solid) by centrifugation. The liquid phase contained buffer salts, unreacted monolignol(s), and an excess of oxidation reagent, whereas the enzyme and polymeric products were deposited as a solid phase. For the liquid phase, the monolignol content was analyzed based on spectrophotometry (UV–vis; Specord 250, Analytic Jena), that is, the sample absorbance was read at 280 nm. The conversion (C, %) of monolignol has been calculated from the difference between the concentrations of monolignol before and after the reaction.