VOLTAMMETRIC MONITORING OF LACCASE-CATALYSED REACTIONS OF DIFFERENT LIGNINS THROUGH OXIDATIVE COUPLING WITH GLUCOSAMINE

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Abstract
The ability of laccase to facilitate grafting hydrophilic compounds, namely glucosamine to lignin in acetone/water mixtures aiming to obtain grafted novel lignin derivatives with new functionalities was assayed by cyclic voltammetry. The oxidation properties registered for the oxidative reactions of syringaldazine lignin model compound with glucosamine evidenced that the reaction scan is direct proportional with the redox potential differences between substrate and laccase. A comparative electrochemical activity of lignins on the GCE in absence and presence of the laccase and glucosamine is presented. From the measurements of the coupling reactions currents it was concluded that the addition of glucosamine can lead to a partial loss of the redox activity of lignin phenolic groups and the co-substrate interaction with the lignin surface groups.

Introduction

Lignin, a highly branched, irregular three-dimensional organic polymer, is the most abundant biopolymer in nature next to cellulose. This natural polymer contains different structures including phenolic and non-phenolic compounds. The applicability of oxidative laccase for both the degradation and the modification of lignin in aqueous media have been intensively studied but limitations still exists due to the low solubility of lignin in media that are compatible with laccase (Cannatelli & Ragauskas, 2016). Organic water-miscible solvents are often required in laccase-catalyzed oxidations because many of the lignin substrates are insoluble in water. Conventionally, the role of fungal laccases in lignin degradation was thought to be limited only to the oxidation of low-redox potential phenolic substructures of the polymer (Johannes & Majcherczyk, 2000).
Due to the low electrochemical reduction potential, laccase can only oxidize the phenolic lignin moiety (<20% of total lignin) and not the non-phenolic aromatic structure (80% of total lignin) (Camarero et al., 1994). Laccase activity on phenols is enhanced by the presence of electron-donating groups at the benzene ring that decrease their electrochemical potentials, thus making them more easily oxidizable. Another factor playing a significant role in the enzymatic catalysis is the pH of the reaction medium and substrate concentration, which affects not only the catalytic activity of laccase, but also the redox potentials of its substrates (Fernández-Sánchez et al., 2002). The purpose of the present study was to evaluate by means of electrochemical techniques the efficiency of laccase in the coupling reactions of four different lignins with glucosamine to obtain modified lignin fractions with potential utilization as biomaterials

Experimental

Lignins

Soda wheat straw lignin (coded SWL) and P1000 soda lignin (coded SGWL) from mixed Sarkanda grass (75%) and wheat straw (25%) were obtained from Greenvalue SA (Lausanne, Switzerland). Organosolv lignin (Alcell) from mixed maple, birch and poplar (hardwoods, coded as OHL) was obtained from Repap Technologies Inc. (Val-ley Forge, PA, USA). Indulin AT, a Kraft lignin from pine (softwood, coded KSL), was obtained from MeadWestvaco

(USA). All lignin samples were previously fractionated by selective extraction at ambient temperature using acetone/water solution of 50% (v/v) acetone.

Laccase

Laccase (Lcc) from *Trametes versicolor* (30.6 U mg^{-1} of solid) were purchased from Sigma-Aldrich (Taufkirchen, Germany. The activity of the laccase was determined by monitoring the oxidation of syringaldazine (Sigma-Aldrich, Taufkirchen, Germany) at 530 nm ($\varepsilon = 65\ mM^{-1}cm^{-1}$) and 25ºC in different acetone: water (v/v) mixtures.

Electrochemical methods

Cyclic voltammetric experiments were performed with a Voltalab 80 PGZ402 potentiostat (Radiometer Analytical, Copenhagen) and controlled by Voltamaster 4 software, version 7.08. All measurements were carried out in a 50-ml thermostated cell, model BEC/EDI, with conventional three electrode configuration. A working glassy carbon electrode (GCE), with a surface diameter of 2.8 mm, was used together with a platinum counter electrode and a saturated calomel reference electrode (SCE), purchased from Radiometer. Before each experiment, the surface of the glassy carbon electrode was polished on a diamond-polishing pad followed by a thorough washing step with distilled water and acetone.

Results and discussion

Cyclic voltammetry of syringaldazine coupling reaction with glucosamine

The oxidation properties registered for the reaction of syringaldazine with glucosamine were demonstrated by cyclic voltammetry, the reaction scan being direct proportional with the redox potential differences between substrate and laccase. The electrochemical oxidation of phenol is known to proceed predominantly via one electron transfer and the formation of polymeric products which lead to electrode fouling (Ivnitski & Atanassov, 2007).

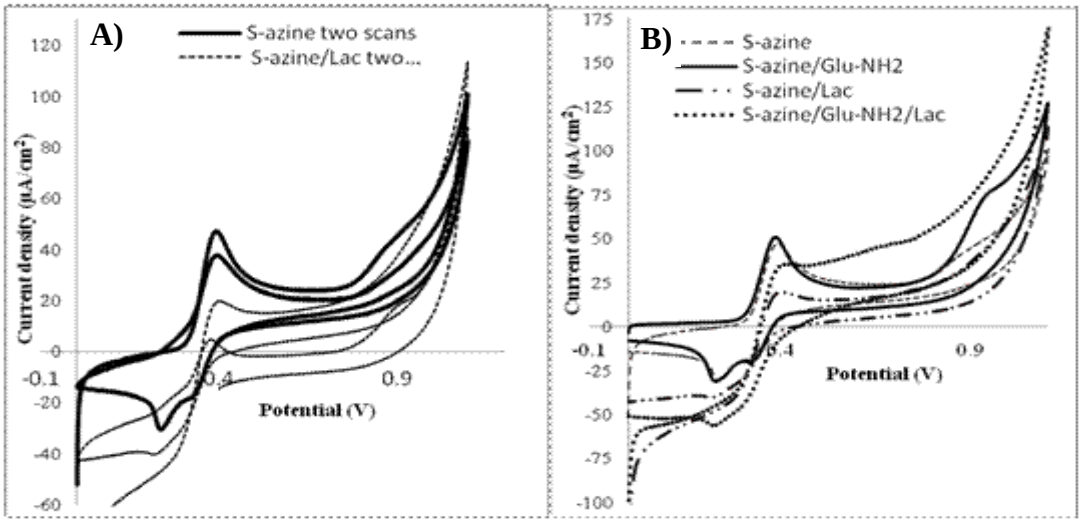

Figure 1. Cyclic voltammograms of A): 0.2 mM syringaldazine (1st and 2nd scan) in acetone/water and 500 mM tartrate buffer (pH 4) in absence (solid line) and presence (dotted line) of T. versicolor laccase immobilized on glassy carbon electrode (GCE); B) 0.2 mM syringaldazine (---), 0.2 mM syringaldazine and glucosamine, 1:2 molar ratio (—), 0.2 mM syringaldazine and laccase immobilised on the GCE (— ··), 0.2 mM syringaldazine, glucosamine (1:2 molar ratio) and laccase immobilised on the GCE (·····). Scan rate: 0.5 mV/s.

In the Figure 1A the two pairs of redox peaks corresponding to syringaldazine reduction and oxidation by laccase two anodic peaks are well observed in the potential area 0.4 V and 0.95 V at a scan rate of 0.5 mV/s. It was assumed (Ivnitski & Atanassov, 2007) that the pair of redox

peaks in low potential area (cathodic peak at 0.32 V and anodic peak at 0.4 V) belongs to the redox process of the T2/T3 center of laccase as being the site for oxygen reduction meanwhile the redox processes at high potentials 0.95 V belongs to T1 copper center. When glucosamine was added in the reaction mixture, the anodic peak decreased more slowly compared with the reaction when only syringaldazine and laccase adsorbed on the electrode surface were present. This could be due to either enzyme inhibition by some products generated in the coupling reaction or by co-substrate inhibition. For the anodic processes the peak potential, E_{a1} is approximatively constant for all the reactions, meanwhile the current peak i_{pa1} decreased for the reactions with laccase being more obvious when no glucosamine was added. In the absence of laccase, the electrochemical parameters corresponding to the first cathodic peak have approximatively the same values, ip_{c1} decreased for laccase mediated reactions the cathodic peak being more evident in the coupling reaction of syringaldazine with glucosamine.

Cyclic voltammetry of lignins coupling reaction with glucosamine

The ability of laccase to facilitate grafting hydrophilic compounds, namely glucosamine to lignin in acetone/water mixtures aiming to obtain grafted novel lignin derivatives with new functionalities was assayed by cyclic voltammetry. Four technical lignins obtained by different isolation technology previously extracted with 50% (v/v) acetone-water mixture were used. To find the compromise conditions at which the substrate is soluble while the enzyme remains active, the reaction was carried out in an 50/50 (v/v) aqueous–acetone mixture with laccase directly adsorbed on the surface of the (glassy carbon electrode) GCE.

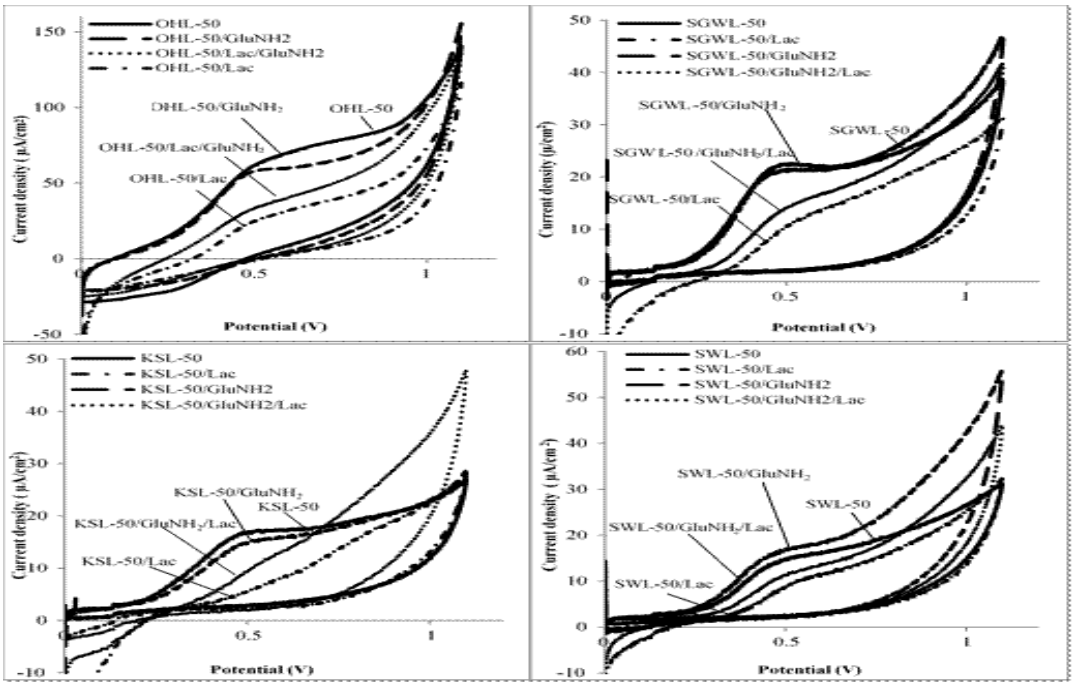

Figure 2. Cyclic voltammograms of coupling reactions of OHL, SGWL, KSL and SWL lignin fractions with glucosamine (1:2.8 total OH_{Ar} : $GluNH_2$ molar ratio) in 50 % (v/v) acetone/water using *T. versicolor* laccase immobilized on glassy carbon electrode (GCE). Scan rate: 0.5 mV/s.

A comparative electrochemical activity of lignins on the GCE in absence and presence of the laccase and glucosamine will be presented in the following. The reactions were performed with the laccase adsorbed on the graphite electrode surface and a remarkable decrease in the redox peak was observed for all studied lignins (Figure 2). Observation of the catalytic activity of laccase confirmed that the in vivo function was retained throughout the immobilization process. All studied lignins presented a redox potential at 480 mV that remain unchanged after the enzymatic treatment. As was expected the intensity of the anodic peak decreased when the GCE

laccase modified electrode was immersed in the reaction mixture as the result of the lignins phenolic group oxidation, the current density being two times smaller than those registered without laccase treatment. With the addition of glucosamine in solution the anodic peak recorded a less pronounced decrease compared with the reaction when only lignin and laccase was present and can be attributed to the partial loss of the redox activity of lignins in presence of glucosamine and the glucosamine interaction with the lignin surface groups.

Conclusion

The absence of the cathodic peaks that were well-defined for syringaldazine reactions are indicating an oxidation process followed by a chemical reaction that quickly removes the generated products. When laccase is adsorbed on graphite, bioelectrocatalytic reduction of oxygen occurs and is observed as a reduction current caused by direct electron transfer from the electrode to the immobilized laccase and then further to molecular oxygen in solution. Small changes in the anodic currents were observed for all the studied lignin when glucosamine was added (Lignin/GluNH2) before catalytic initiation of the reaction with laccase. The studied lignins behave differently in oxidative coupling reactions due to their origin, different extraction methods and the phenolic content. In all cases the redox currents registered for lignin with laccase system in comparison to the reactions without catalyst are significantly reduced.

References

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