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Study of laccase activity and stability in the presence of ionic and non-ionic surfactants and the bioconversion of indole in laccase-TX-100 system

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ABSTRACT

The aim of this study was to characterize the stability and activity of laccase from *Trametes versicolor* in the presence of three different surfactants, namely sodium di-2-ethylhexylsulfosuccinate (AOT), Triton X-100 (TX-100), and cetyltrimethylammonium bromide (CTAB). The kinetic parameters (such as K_m, k_{cat}, k_{cat}/K_m ratio), optimal pH and temperature and the thermostability of the enzyme at different temperatures were determined and compared in the absence and presence of the three surfactants. Results revealed that the catalytic activity of the enzyme was greatly improved in the presence of low concentrations of AOT, whereas the activity declined in the presence of TX-100 and CTAB inactivated it almost completely. Results also depicted that, in general, the presence of the surfactants affected the enzyme optimum pH and temperature. In terms of stability, TX-100-induced stabilization and AOT and CTAB-mediated destabilization of the enzyme were observed. Laccase-mediated bioconversion of indole to 2,2-bis(3′-indolyl)-indoxyl in the presence of TX-100 as the effective stabilizing surfactant and TEMPO as the enzyme mediator was also investigated.

1. Introduction

Mixed enzyme–surfactant systems are receiving increasing interest due to their enormous importance in many applied technologies such as the food and beverage industries, pharmaceuticals, detergents, cosmetics, coating processes, and oil recovery [1,2]. Many researchers have recently focused on improving enzyme stability in an attempt to use biocatalysts in organic synthesis through modifying with high molecular weight synthetic polymers or surfactants [3]. Ionic and non-ionic surfactants with different properties, due to their polar and hydrophobic moieties, can modulate the hydrophobic and electrostatic interactions related to the charge of the enzyme and promote the stability and activity of enzymes in reaction conditions [4–6]. Many studies have reported an increase in the activity of various enzymes such as α-chymotrypsin [7,8], tyrosinase [9], and lipases in the presence of surfactants [4,10,11]. At critical micelle concentration (CMC), surfactant molecules associate with their hydrophobic chains to form micelles with a generally hydrophilic water-exposed exterior and a hydrophobic interior [12]. At concentrations below the CMC, enzymes undergo conformational changes as they bind to increasing numbers of surfactant monomers. Anionic surfactants bind to cationic side chains that include amino acids such as lysine, arginine, and histidine, whereas cationic surfactants bind to anionic side chains with amino acids such as glutamine and asparagine [13]. At higher concentrations, the surfactants saturate the initial binding sites, and this in turn results in the formation of clusters that lead to protein unfolding [13]. In addition, surfactants at concentrations above CMC act as emulsifiers that enhance the solubility of organic compounds in water by incorporating them into the micelle core, solubilized in the bulk solvent [14]. Although many workers have described in the literature that higher

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surfactant concentrations (above CMC) are not beneficial for enzymes activity, however, there are some investigations carried out at over CMC concentrations which indicate the positive effects of some surfactants on the enzyme catalytic characteristics [15–19].

Laccases belong to the multicopper-containing oxidoreductases which are broadly distributed in bacteria, fungi, and higher plants and catalyze the oxidation of a wide range of aromatic substrates by using the O_2 and producing H_2O as a by-product [20]. Laccases, due to their unique properties, have found a wide range of applications in bioremediation, paper and pulp processing, pharmaceutical industry, biosensors, and organic synthesis [21].

In the present study, the effect of three types of surfactants, namely sodium di-2-ethylhexylsulfosuccinate (AOT; an anionic surfactant), Triton X-100 (TX-100; a non-ionic surfactant), and cetyltrimethylammonium bromide (CTAB; a cationic surfactant) on the stability and activity of laccase was investigated. The effect of ionic and non-ionic surfactants on the kinetic parameters K_m and k_{cat} was also explored, and the optimal temperature and pH for the enzyme activity were determined. It has been reported that the use of TX-100 as a non-ionic surfactant at concentrations above CMC could increase the laccase-catalyzed conversion of bisphenol A *via* an increase in the enzyme stability and water solubility of bisphenol A [19]. Indole is one of the most important motifs in natural bioactive molecules and marketed drugs. The indole ring system is one of the most abundant and important heterocycles in nature that is found in a wide range of biologically active natural compounds, ranging from simple derivatives such as the neurotransmitter serotonin to complex alkaloids such as vinblastine, mitomycin C, and reserpine [22]. In addition, many important synthetic drugs contain an indole nucleus, such as fluvastatin, rizatriptan, sumatriptan, and tadalafil. Therefore, it was decided to investigate the laccase-mediated bioconversion of indole in the presence of TX-100 as an effective stabilizing surfactant.

2. Experimental

2.1. Chemicals

Laccase from *Trametes versicolor* (20 U mg^{-1}), AOT (>97%), CTAB (>99%) and the laccase mediator 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, 98%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). TX-100 (European Pharmacopoeia grade) was provided from Merck (Darmstadt, Germany). The structure and properties of the three surfactants are shown in Table 1. The substrate 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, 99%) was obtained from Sigma–Aldrich (Steinheim, Germany). All other chemicals and reagents were of the highest purity available.

2.2. Laccase activity assay

The catalytic activity of laccase was determined by oxidation of ABTS as the substrate [20]. The reaction mixture consisting of 1 mL laccase (5 μg/mL) and 1 mL of 2.5 mM laccase substrate (ABTS dissolved in 0.1 M citrate buffer, pH 5) was prepared and incubated at 25 °C and 120 rpm for 10 min. A change in absorbance at 420 nm was monitored using a UV/vis spectrophotometer (ScanDrop® Spectrophotometer, Analytik Jena, Jena, Germany). The laccase activity was calculated using the molar extinction coefficient of ABTS (ε_{420} = 36,000 M^{-1} cm^{-1}) [20]. One unit of laccase activity was defined as the amount of enzyme that oxidized 1 μmol of ABTS per minute at pH 5 and 25 °C. Activity measurement was performed in triplicate.

2.3. Effect of surfactant concentration on enzyme activity

The influence of various surfactant concentrations on enzyme activity was evaluated, using the enzyme and substrate solutions containing 0.5–1.5 mM of AOT, 0.5–20 mM of CTAB, and 0.1–100 mM of TX-100. The criterion for selecting various surfactant concentrations was their water solubility. AOT, as the least water soluble surfactant, showed a maximum solubility of 1.5 mM. Therefore, a narrow concentration range of 0.5–1.5 mM was chosen. In comparison to AOT, CTAB was more soluble, and hence a concentration range up to 20 mM (maximum solubility) was evaluated. In the case of TX-100, as the most water soluble surfactant, we thought that it was rational to check a wider range of concentration. To investigate the effect of surfactants on the kinetic parameters and determine the optimal pH and temperature, enzyme assay was performed in triplicate in the presence of 1.5, 20 and 100 mM of AOT, CTAB, and TX-100, respectively.

2.4. Determination of kinetic parameters

The kinetic assay for ABTS oxidation was carried out at 25 °C. 1 mL of enzyme solution was added to 1 mL 0.1 M citrate buffer containing various concentrations of the substrate ABTS (0–5 mM). The K_m and k_{cat} values were obtained from Lineweaver–Burk plots.

2.5. pH and temperature profile of the enzyme activity

Optimum pH and temperature were determined by measuring laccase activity at various reaction temperatures (25–80 °C) over a pH range of 3.5–5.5 in 0.1 M citrate buffer. This experiment was repeated at least three times.

2.6. Enzyme stability

Ten milliliters of enzyme solutions (0.3 mg/mL) were prepared with citrate buffer (0.1 M, pH 5) containing 1.5 mM AOT, 20 mM CTAB, or 100 mM of TX-100. The solutions were then incubated in a water bath with controlled temperatures of 50, 60 and 70 °C. Periodically, 100 μL of each solution were withdrawn and the activity was then assayed at 25 °C. This experiment was repeated at least three times.

2.7. HPLC method

Chromatographic analysis was performed on Knauer HPLC system (Berlin, Germany), consisting of a pump (Smartline 1000), PDA Detector 2800, a Degasser 5000, and ChromGate software (version 3.3.1) from Knauer. A Lichrospher 100 RP & EC C8 reverse phase column (C8, 25 cm × 0.46 cm id, 5 μm particle size; Teknokroma, Barcelona, Spain) was used for the separation. When the samples were filtered through 0.45 μm PTFE filters (Schleicher & Schull, Germany), each sample (20 μL) was injected using a Smartline Autosampler 3950 with a sample loop of 100 μL. Mobile phase was a mixture of methanol:water (62:38, v/v) which was freshly prepared and degassed each day. UV detector was set at 278 nm.

2.8. Bioconversion of indole

Laccase (20 U, 4 mg) and laccase mediator TEMPO (5 mol%) were added to a magnetically stirred solution of TX-100 (100 mM) in 10 mL citrate buffer (0.1 M, pH 4) under air, and the reaction mixture was incubated at 45 °C for 10 h. The progress of the reaction was monitored with the above-mentioned HPLC method. When the reaction was completed, the reaction mixture was extracted with ethyl acetate (3 × 15 mL), and the organic layer was dried over anhydrous Na_2SO_4. The product was purified with silica gel