University of Belgrade, Technical Faculty in Bor

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EcoTER'22

Proceedings

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Prof. Dr Snežana Šerbula

21-24 June 2022, Hotel Sunce, Sokobanja, Serbia

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STABILITY OF SOYBEAN PEROXIDASE IMMOBILIZED ONTO HYDROGEL MICRO-BEADS FROM TYRAMINE-PECTIN

Miloš Prokopijević[1*], Dragica Spasojević[1], Olivera Prodanović[1], Nevena Pantić[1], Dragana Bartolić[1], Ksenija Radotić[1], Radivoje Prodanović[2]

[1]University of Belgrade, Institute for Multidisciplinary Research, Kneza Višeslava 1, 11030 Belgrade, SERBIA

[2]University of Belgrade, Faculty of Chemistry, Studentski trg 12–16, 11158 Belgrade, SERBIA

[*]*milos.prokopijevic@imsi.rs*

Abstract

The application of enzymes for phenol removal from polluted waters is an effective and environmentally favorable method and an ongoing worldwide research topic. Oxidoreductive enzymes, like Soybean peroxidase (SBP), catalyze oxidation and polymerization of phenolic compounds in the presence of H_2O_2. The industrial application, however, requires enzyme immobilization on various carriers to overcome the disadvantages of using the soluble form. Chemically modified pectin has been chosen as a carrier for entrapment of SBP inside a three-dimensional polymeric network. Immobilization of SBP was performed in an emulsion polymerization reaction producing enzymes entrapped in covalently crosslinked tyramine-pectin in the shape of micro-beads. The specific activity of immobilized SBP was determined using pyrogallol as a substrate. In this study, the stability of the immobilized SBP onto modified pectin in three different molar ratios was tested to determine the carrier with the best performance. Immobilized peroxidase has potential for application as a biocatalyst for phenol removal from wastewater.

Keywords: immobilization, soybean peroxidase, pectin, tyramine, hydrogel

INTRODUCTION

Soybean peroxidase (SBP, E.C. 1.11.1.7) is a glycoprotein that belongs to the class III plant peroxidase family and catalyzes oxidation and polymerization of aromatic substrates using H_2O_2 as electron acceptor [1,2]. With a growing number of applications, from phenol removal from wastewater, and organic syntheses to analytical tests, SBP is an excellent candidate for wider usage due to its availability and relatively easy extraction and purification [3]. Removal of toxic aromatic pollutants from wastewaters by enzymatic method using oxidoreductive enzymes is highly specific, efficient, and causes less environmental impact compared to traditional methods [4].

The application of soluble enzymes as biocatalysts in industrial processes is limited due to some disadvantages, such as their short storage life, lack of operational stability, expensive and time-consuming recovery, and practically no reusability [5,6]. Immobilization of enzymes on solid supports can improve their catalytic efficiency, performance in organic solvents, stability and resistance to environmental changes in pH and temperature [6]. Focus on the

discovery of novel immobilization systems for wastewater treatment is a major research challenge.

Hydrogels are an insoluble, hydrophilic, polymeric network that has the ability to absorb and retain large amounts of water. Due to their properties, there is a growing interest in the application of hydrogels as carriers for enzyme encapsulation [7]. Pectin is a natural heteropolysaccharide and a major structural component of plant cell walls capable of producing hydrogels by ionotropic gelation in the presence of Ca^{2+} ions or chemical crosslinking and is frequently used due to its gelling properties and nontoxicity [8,9]. Tyramine-pectin has been synthesized by chemical modification of pectin as described by Prokopijević *et al.* [10] and used as a carrier for SBP immobilization. Entrapment is an immobilization technique defined as physical restriction where enzymes are confined inside a polymeric network that permits substrates and products to pass through [11].

The aim of this research was to test the storage stability of SBP immobilized inside tyramine-pectin micro-beads after 24 hours of storage. Three carriers with different degrees of modification were chemically crosslinked in an emulsion polymerization reaction and tested for SBP immobilization.

MATERIALS AND METHODS

Materials

Pectin, glucose oxidase (160 U/mg), tyramine hydrochloride, triton X-100 (t-octylphenoxypolyethoxyethanol), pyrogallol, span 80 (sorbitan monooleate), and mineral oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). TRIS was purchased from SERVA Electrophoresis GmbH (Heidelberg, Germany). Sodium cyanoborohydride was obtained from Fluka Chemie GmbH (Buchs, Switzerland) and glucose from Zorka Pharma (Šabac, Serbia). Soybean peroxidase was isolated from soybean hulls by coprecipitation with acetone and ammonium sulfate described in previously published research [12].

Polymer modification

Tyramine-pectin was synthesized according to the previously published procedure by Prokopijević *et al*. [10]. Pectin was oxidized using sodium metaperiodate in three molar ratios (2.5 mol%, 10 mol%, and 20 mol%), and in a reductive amination reaction tyramine was bound to newly formed vicinal hydroxyl groups in the presence of sodium cyanoborohydride.

SBP immobilization

Solution of 20 % (w/v) tyramine-pectin in 0.1 M Tris HCl buffer pH 7 was prepared, and 300 µl of that solution was mixed with 0.1 U of glucose oxidase, 0.8 U of SBP, and 10 mM glucose. The obtained mixture was instantly poured into 0.6 ml of light mineral oil with 3 % (v/v) Span 80 while gently stirring on a magnetic stirrer. After 15 min. tyramine-pectin micro-beads were formed and 1 ml of 0.5 % (v/v) Triton X-100 in 5 % (w/v) $CaCl_2$ was added to stop the reaction. Micro-beads with encapsulated enzyme were rinsed 3 times with 1 ml of 5 % (w/v) $CaCl_2$ solution containing 0.5 % (v/v) Triton X-100 and afterward 3 times with 5 % $CaCl_2$ and stored in the same solution at 4 °C. Activity of unbound soluble SBP, measured in the collected $CaCl_2$ solution after rinsing of the micro-beads, was used to calculate bound activity.

Enzyme activity

The specific activity of SBP encapsulated inside obtained micro-beads was tested with 13 mM pyrogallol in 0.1 M Tris HCl buffer pH 7. Typical enzyme assays were carried out in a 3 ml reaction mixture by adding 150 µl of 60 % hydrogel suspension into a buffered substrate with 0.1 U glucose oxidase and 0.1 M glucose. The reaction mixture was stirred and aliquots were collected after 5, 10, and 15 minutes. Absorbance was measured at 420 nm on a UV-VIS spectrophotometer (Shimadzu Corporation UV-2501PC, Japan).

RESULTS AND DISCUSSION

The specific activity of SBP immediately after immobilization onto three tyramine-pectin carriers with different degrees of periodate oxidation and after 24 h of storage was compared. Obtained results are presented in Figure 1.

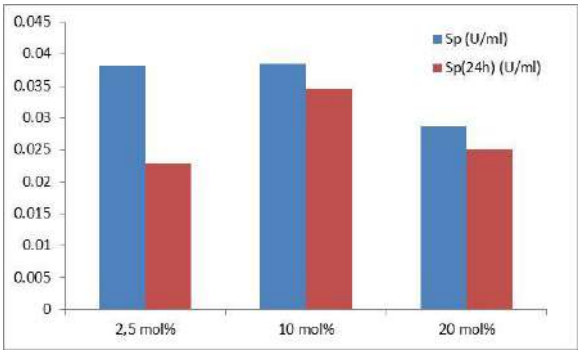

Figure 1 *Specific activity (U/ml) of SBP immobilized onto tyramine-pectin micro-beads (blue) and residual activity after 24 h of storage (red)*

Although the highest specific activity (0.038 U/ml) was obtained when 2.5 mol% and 10 mol% tyramine-pectin were used as carriers, their respective retained activity after 24 hours of storage distinctly differs: 59.95 % and 89.64 % (Table 1). Tyramine-pectin with higher degrees of modification (10 mol% and 20 mol%) possesses higher residual activity while the highest bound activity was achieved with 20 mol% modification.

Table 1 *Specific activity (U/ml), bound activity (%) and residual activity (%) of SBP immobilized onto tyramine-pectin micro-beads after 24 h of storage*

	2.5 mol%	10 mol%	20 mol%
Specific activity (U/ml)	0.038	0.038	0.029
Bound activity (%)	78.60	75.83	85.90
Residual activity after 24 h (%)	59.95	89.64	87.05

Better storage stability and higher activity after the observed period may be due to the reduced leakage of the enzyme from the carriers with a higher degree of pectin modification. Tyramine-pectin with 10 mol% periodate oxidation expressed the highest specific activity

(0.038 U/ml) and storage stability (89.64 %), therefore it is a suitable candidate for further application.

CONCLUSION

This research confirmed that SBP was successfully immobilized on all 3 tyramine coupled natural polymers with different degrees of modification. The bound activity was above 75 % for all of the used carriers. Tyramine-pectin with the molar ratio of 10 mol% periodate oxidation expressed the highest specific activity and residual activity after 24 h of storage. Immobilized SBP has the potential for application in the removal of toxic aromatic compounds from polluted waters.

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