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Bioaffinity-based an inexpensive and high yield procedure for the immobilization of turnip (*Brassica rapa*) peroxidase

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Abstract

This study demonstrates the immobilization of carbohydrate containing turnip peroxidase on an inexpensive bioaffinity adsorbent, Concanavalin A-cellulose support. The bioaffinity support was prepared simply by incubating cellulose powder with jack bean extract at 4 °C. Cellulose powder adsorbed 30 mg concanavalin A/g of the matrix. Concanavalin A adsorbed cellulose has been employed for the simultaneous purification and immobilization of glycoenzymes directly from ammonium sulphate fractionated proteins of turnip. The obtained bioaffinity support was quite effective in high yield immobilization of peroxidase from turnip and it retained 672 U/g. Turnip peroxidase immobilized on concanavalin A-cellulose support retained 80% of the initial activity. Immobilized turnip peroxidase preparation was quite resistant against the denaturation mediated by pH, heat, urea, guanidinium-HCl, Surf Excel, cetyltrimethylammonium bromide and water-miscible organic solvents; dimethyl formamide, dioxane and *n*-propanol. Low concentration of detergents like Surf Excel and cetyltrimethylammonium bromide enhanced the activity of soluble and immobilized turnip peroxidase.

Keywords: Bioaffinity support; Concanavalin A; Cellulose; Immobilization; Peroxidase; Purification; Stabilization; Turnip roots

1. Introduction

Peroxidase (E.C. 1.11.1.7) are ubiquitous heme-proteins, which utilize hydrogen peroxide to catalyze the oxidation of a wide spectrum of organic and inorganic substrates (Duran et al., 2002; Duarte-Vazquez et al., 2003). Plant peroxidases are receiving increasing attention due to their extensive potential applications in clinical, biochemical, biotechnological, industrial and in the synthesis of useful compounds (Ryu et al., 1993; Lobarzewsky and Ginalska, 1995; Kim and Moon, 2005; Duran and Esposito, 2000). These enzymes could also be exploited for the detoxification and remediation of various aromatic pollutants such as phenols, aromatic amines, 2,4,6-trinitrotoluene and dyes, etc. present in wastewater/industrial effluents coming out from several industries such as textile, dyes, printing, paper and pulp (Husain and Jan, 2000; Akhtar et al., 2005a,b; Lee et al., 2003; McEldon and Dordick, 1996). The use of soluble enzymes has some inherent limitations whereas their immobilized form has several advantages over the soluble enzymes such as enhanced stability, easier product recovery and purification, protection of enzymes against denaturants, proteolysis and reduced susceptibility to contamination.

Numerous efforts have been made to develop the procedures for the immobilization of peroxidase from various sources but most of the immobilized enzyme preparations either use commercially available enzyme or expensive supports which increased the cost of the processes (Husain and Jan, 2000; Akhtar et al., 2005a; Tischer and Kasche, 1999). However, such immobilized enzyme preparations could not be exploited for the treatment of large volume of effluents coming out of the industrial sites. Among the techniques used for the immobilization of enzymes, bioaffinity supports have attracted the attention of the enzymologists due to several merits over the other known classical methods. Researchers have shown remarkable interest in the immobilization of enzymes on bioaffinity supports due to ease of immobilization, lack of chemical modification and usually accompanying an enhancement in stability (Saleemuddin and

Abbreviations: CTAB, cetyltrimethylammonium bromide; TP, turnip peroxidase; S-TP, soluble turnip peroxidase; I-TP, immobilized turnip peroxidase; Con A, concanavalin A; DMF, dimethyl formamide; SDS, sodium dodecyl sulphate

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Husain, 1991; Akhtar et al., 2005c; Saleemuddin, 1999). Besides the mentioned advantages offered by the bioaffinity-based procedures, there is some additional benefit, such as proper orientation of enzyme on the support (Turkova, 1999; Mislovicova et al., 2000; Khan et al., 2005). These supports have been used for high yield and stable immobilization of glycoenzymes/enzymes. A large number of bioaffinity-based procedures have already been developed for the immobilization of enzymes directly from the crude homogenate or partially purified enzyme preparation (Saleemuddin and Husain, 1991; Akhtar et al., 2005c; Saleemuddin, 1999; Khan et al., 2005).

In this article an effort has been made to select an inexpensive and easily available source of peroxidase, turnip. The purpose of this study was to find a cheaper and easily available alternative for the commercially available enzymes and its immobilization and utilization at large scale. Con A-cellulose immobilized turnip peroxidase (TP) preparation was compared with its soluble counter-part for its stability against various physical and chemical parameters.

2. Materials and methods

2.1. Materials

Methyl α-D-mannopyranoside was the products of Sigma Chem. Co. (St. Louis, MO), USA. Jack bean meal was procured from the Loba Chem. Co., India. *o*-Dianisidine-HCl was obtained from the Center for Biochemical Technology, New Delhi, India. Cetyltrimethylammonium bromide, dioxane, dimethyl formamide and *n*-propanol were obtained from the SRL Chemicals, Mumbai, India. Cellulose powder (0.02–0.15 mm) was obtained from Centron Research Labs, Mumbai, India. Surf Excel was the product of Hindustan Lever Ltd., Mumbai, India. Turnip roots were purchased from the local vegetable market. Other chemicals and reagents employed were of analytical grade and were used without any further purification.

2.2. Ammonium sulphate fractionation of turnip proteins

Turnip root (200 g) was homogenized in 200 ml of 0.1 M sodium acetate buffer, pH 5.5. Homogenate was filtered through four layers of cheesecloth. The filtrate was then centrifuged at 10,000 × *g* on a Remi Cooling Centrifuge C-24. The clear solution thus obtained was subjected to salt fractionation by adding 20–80% (w/v) $(NH_4)_2SO_4$. The mixture was stirred overnight at 4 °C to obtain the maximum precipitate. The precipitate was collected by centrifugation at 10,000 × *g* on a Remi Cooling Centrifuge C-24. The obtained precipitate was re-dissolved in 0.1 M sodium acetate buffer, pH 5.5 and dialyzed against the assay buffer (Matto and Husain, 2006).

2.3. Preparation of bioaffinity support

Cellulose (5.0 g) was incubated and stirred with 100 ml of clear solution of jack bean extract prepared in 0.1 M sodium phosphate buffer, pH 6.2 overnight at 4 °C. The unbound proteins were removed by extensive washing with assay buffer (Akhtar et al., 2005c). The specific binding of Con A with cellulose was confirmed by eluting the bound lectin using 1.0 M methyl α-D-mannopyranoside.

2.4. Measurement of peroxidase activity

Peroxidase activity was determined from a change in the optical density ($A_{460\ nm}$) by measuring the initial rate of oxidation of 6.0 mM *o*-dianisidine-HCl in the presence of 18 mM hydrogen peroxide in 0.1 M sodium acetate buffer, pH 5.5 for 15 min at 37 °C.

The immobilized preparation was continuously agitated for the entire duration of assay. The assay was highly reproducible with immobilized preparations (Musthapa et al., 2004).

One unit of peroxidase activity (U) was defined as the amount of enzyme protein that catalyzes the oxidation of 1 (mol of *o*-dianisidine-HCl per min at 37 °C into colored product (ε_m = 30,000 M^{-1} L^{-1}).

2.5. Immobilization of TP on Con A-cellulose support

TP (7240 U) were added to 5.0 g of Con A-cellulose support and stirred in sodium phosphate buffer, pH 6.2 at 4 °C overnight. The unbound TP was removed by extensive washing with the assay buffer (Akhtar et al., 2005c).

2.6. Effect of pH on soluble and immobilized TP

The activities of soluble and immobilized TP preparations (1.15 U) were measured in buffers of various pH values (3.0–10.0). The molarity of each buffer was 0.1 mol L^{-1}.

2.7. Effect of temperature on soluble and immobilized TP

The activities of soluble and immobilized TP preparations (1.15 U) were measured at various temperatures (20–80 °C) under standard assay conditions. The activity obtained at 30 °C was taken as 100% for the calculation of percent activity.

Soluble and immobilized TP preparations (1.15 U) were incubated at 60 °C in 0.1 M sodium acetate buffer, pH 5.5. Aliquots of each preparation were removed at each indicated time interval and activity was measured. The activity obtained without incubation at 60 °C was taken as control (100%) for the calculation of percent activity.

2.8. Effect of detergents on soluble and immobilized TP

Surf Excel (0.1–1.0%, w/v) and CTAB (0.2–2.0%, w/v) were used as final assay concentration to observe the effect of detergents on the activity of TP. Soluble and immobilized TP preparations (1.15 U) were incubated with the detergents in 0.1 M sodium acetate buffer, pH 5.5 at 37 °C for 1 h. Peroxidase activity was determined at all the indicated detergent concentrations. The activity obtained without exposure to detergent was taken as 100% for the calculation of percent activity.

2.9. Effect of water-soluble organic solvents on soluble and immobilized TP

Soluble and immobilized TP preparations (1.15 U) were incubated with 10–60% (v/v) of water-miscible organic solvents; DMF/dioxane/*n*-propanol prepared in 0.1 M sodium acetate buffer, pH 5.5 at 37 °C for 1 h. Peroxidase activity was determined at all the indicated organic solvent concentrations after the incubation period (Jan et al., 2001). Other assay conditions were the same as described in the text.

2.10. Determination of protein concentration

The protein concentration was determined according to the procedure described by Lowry et al. (1951). Bovine serum albumin was used as standard.

3. Results

3.1. Preparation of bioaffinity support and immobilization of TP

Cellulose adsorbed nearly 30 mg protein/g cellulose powder from jack bean extract. Con A-cellulose matrix was selected as a bioaffinity media for the direct immobilization of