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Direct immobilization of peroxidase on DEAE cellulose from ammonium sulphate fractionated proteins of bitter gourd (*Momordica charantia*)

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Abstract

The direct immobilization of peroxidases on DEAE cellulose from ammonium sulphate fractionated proteins of bitter gourd has been investigated. The activated DEAE cellulose was quite effective in high yield immobilization of peroxidases from bitter gourd and it could bind nearly 590 enzyme units per g of the matrix. Bitter gourd peroxidase immobilized on this anion exchanger showed very high effectiveness factor 'η' as 0.95. BGP bound very strongly to the DEAE cellulose, as it did not detach even in the presence of 0.5 M NaCl. Immobilized bitter gourd peroxidase preparation was more stable to the denaturation induced by pH, heat, urea, proteolytic enzyme, detergents (Surf Excel and Rin powder), Triton X 100 and water-miscible organic solvents (dioxane, dimethyl sulphoxide and *n*-propanol). Peroxidase adsorbed on the matrix exhibited very high resistance to proteolysis mediated by the trypsin treatment. DEAE cellulose bound bitter gourd peroxidase lost 45% of its initial activity after treatment with 2.5 mg trypsin per ml of incubation mixture for 1 h at 37 °C while the soluble enzyme lost nearly 65% of the initial activity under similar incubation conditions.

Keywords: Adsorption; Direct; DEAE cellulose; Bitter gourd; Immobilization; Peroxidase; Stabilization; *n*-Propanol

1. Introduction

Recently, it has been reported that peroxidases can be used in the detoxification and biotransformation of several phenols, aromatic amines, biphenyls, bisphenols and dyes present in polluted wastewater/industrial effluents coming out from several industries [1–5]. The soluble enzyme cannot be exploited at the large-scale due to some inherent limitations to treat the huge volume of effluents. On the other hand, the immobilized enzyme has offered several advantages, such as enhanced stability, easier product recovery and purification, protection of enzymes against denaturants, proteolysis and reduced susceptibility to contamination. Several methods have been used for the immobilization of peroxidases from various sources but most of the immobilized enzyme preparations either use commercially available enzyme or expensive supports [6,7]. These factors were affecting the cost of the immobilized enzyme system [8]. These expensive immobilized systems could not meet the requirements for the treatment of huge volume of effluents. However, among the techniques used for the immobilization of enzymes, adsorption on the insoluble supports has several merits over the other known methods. It is a simple procedure and can be exploited for the direct immobilization of enzymes even from the crude cell homogenates.

Adsorption procedures are significantly useful for the immobilization of enzymes directly from the crude homogenate and thus avoiding the high cost of enzyme purification [9,10]. Ease of immobilization, lack of chemical modification and usually accompanying enhancement in stability are some of the advantages offered by the adsorption procedures. Several investigators have described the immobilization of enzymes on the bioaffinity supports for the specific

Abbreviations: BGP, bitter gourd peroxidase; DEAE, diethyl aminoethyl; DMSO, dimethyl sulphoxide

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immobilization of enzymes directly from partially purified enzyme preparation but these procedures require the use of expensive ligands, such as antibodies or lectins [6,11,12]. In order to minimize the cost of immobilization, the adsorption of proteins directly from partially purified enzyme preparation on an anion exchanger can be performed. These supports have already appeared in high yield and stable immobilization of enzymes.

Here, an effort has been made to immobilize the peroxidases on an anion exchanger, diethyl aminoethyl (DEAE) cellulose directly from the salt fractionated proteins of bitter gourd and dialyzed bitter gourd proteins. DEAE cellulose adsorbed bitter gourd peroxidase (BGP) preparation was compared with its soluble counter part for its stability against pH, heat, urea, detergents, water-miscible organic solvents and proteolytic enzyme (trypsin). DEAE cellulose adsorbed BGP preparation was significantly stable against several tested physical and chemical parameters.

2. Materials and methods

2.1. Materials

DEAE cellulose 11 was the product of SRL Chemicals, Mumbai, India. *o*-Dianisidine-HCl was purchased from the Center for Biochemical Technology, New Delhi, India. Ammonium sulphate, dioxane, dimethyl sulphoxide, *n*-propanol and Triton X 100 were obtained from the SRL Chemicals Mumbai, India. Surf Excel and Rin powder were purchased from the local market. Other chemicals and reagents employed were of analytical grade and were used without any further purification.

2.2. Ammonium sulphate fractionation of bitter gourd proteins

Bitter gourd (50 g) was homogenized in 100 ml of 50 mM sodium acetate buffer, pH 5.6. Homogenate was filtered through four layers of cheesecloth. The filtrate was then centrifuged at 10,000 × *g* on a Remi R-24 cooling centrifuge for 10 min at 4 °C. The clear supernatant was subjected to salt fractionation by adding 20–80% (w/v) $(NH_4)_2SO_4$. It was stirred overnight at 4 °C and the obtained precipitate was collected by centrifugation at 10,000 × *g* on a Remi R-24 cooling centrifuge for 10 min at 4 °C [6]. The collected precipitate was redissolved in 50 mM sodium acetate buffer, pH 5.6 and dialyzed against the assay buffer.

2.3. Activation of DEAE cellulose

DEAE cellulose (5.0 gm) was added to100 ml of distilled water and was stirred slowly, kept overnight for swelling. Swelled DEAE cellulose was filtered on a Buchner funnel and was incubated with 100 ml of 0.5N HCl for 1 h. It was collected by filtration on Buchner funnel and was washed with distilled water continuously till it attained pH 7.0. Hundred milliliters of 0.5N NaOH was added to HCl treated DEAE cellulose and it was stirred on a magnetic stirrer for 1 h at room temperature 25 °C. It was washed again with distilled water till it attained neutral pH. Further, it was suspended and stored in 100 ml of distilled water at 4 °C.

2.4. Adsorption of BGP on activated DEAE cellulose

BGP (5535 units) was added to 5.0 g of DEAE cellulose and stirred overnight at 4 °C. Unbound BGP was removed by extensive washing with the assay buffer.

2.5. Effect of ion concentrations on the DEAE cellulose adsorbed BGP

The adsorbed BGP preparation was incubated with increasing concentration of NaCl (0.1–1.0 M) in 0.1 M sodium acetate buffer, pH 5.6 for 1 h at 37 °C. In order to monitor the effect of long-time exposure of immobilized enzyme with ions, the adsorbed BGP was also incubated with 0.1 M NaCl upto 24 h.

2.6. Effect of trypsin mediated proteolysis on the activity of soluble and immobilized BGP

Soluble and immobilized BGP preparations (1.25 units) were incubated with 0.25–2.5 mg trypsin/ml of incubation mixture at 37 °C for 1 h [6]. The activities of soluble and immobilized BGP in assay buffer without any trypsin treatment were taken as control (100%), for the calculation of percent activity. Peroxidase activity was determined according to the standard procedure.

2.7. Effect of Surf Excel and Rin powder on the activity of soluble and DEAE cellulose adsorbed BGP

Soluble and immobilized BGP preparations (1.25 units) were incubated independently with varying concentration of Surf Excel and Rin powder (0.1–1.0%, w/v) in 50 mM sodium acetate buffer, pH 5.6 at 37 °C for 1 h. Peroxidase activity was assayed at all the indicated detergent concentrations and other assay conditions were same as mentioned in the text. The activity of soluble and immobilized BGP in assay buffer without any detergent were taken as control (100%), for the calculation of percent activity.

2.8. Treatment of soluble and immobilized BGP with Triton X 100

Soluble and immobilized BGP (1.25 units) preparations were incubated with increasing concentration of Triton X 100 (0.5–5%, v/v) in 50 mM sodium acetate buffer, pH 5.6 at 37 °C for 1 h. After exposure the peroxidase activity was determined according to the procedure described in the text.