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Short Communication

Enzymatic detection of mercuric ions in ground-water from vegetable wastes by immobilizing pumpkin (*Cucumis melo*) urease in calcium alginate beads

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Abstract

Present report describes a quick and simple test based on enzyme inhibition for the detection of mercury in aqueous medium by urease immobilized in alginate beads. Urease was extracted from the discarded seeds of pumpkin (*Cucumis melo*) and was purified to apparent homogeneity (5.2-fold) by heat treatment at 48 ± 0.1 °C and gel filtration through Sephadex G-200. The homogeneous enzyme preparation (Sp activity 353 U/mg protein, $A_{280}/A_{260} = 1.12$) was immobilized in 3.5% alginate leading to 86% immobilization. Effect of mercuric ion on the activity of soluble as well as immobilized enzyme was investigated. Hg^{2+} exhibited a concentration-dependent inhibition both in the presence and absence of the substrate. The alginate immobilized enzyme showed less inhibition. There was no leaching of the enzyme over a period of 15 days at 4 °C. The inhibition was non-competitive and the K_i was found to be 1.26×10^{-1} μM. Time-dependent interaction of urease with Hg^{2+} exhibited a biphasic inhibition behavior in which approximately half of the initial activity was lost rapidly (within 10 min) and reminder in a slow phase. Binding of Hg^{2+} with the enzyme was largely irreversible, as the activity could not be restored by dialysis. The significance of the observations is discussed.

Keywords: Urease; Pumpkin; *Cucumis melo*; Hg^{2+} ions; Calcium alginate beads

1. Introduction

Many substances alter the activity of an enzyme by combining it in a way that influences the binding of the substrate. These substances are known as inhibitors (Voet and Voet, 1993). Thus, enzymatic reactions have proved to be very promising tool to identify major pollutants, such as heavy metals, enabling a very accurate toxicity identification evaluation (TIE) based on their inhibition (Brack et al., 2000).

Assay based on the inhibition of urease show a high selectivity for the sensitive and effect based screening of heavy metals (Brack et al., 2000; Jung et al., 1995; Wittekindt et al., 1996). Heavy metals are well known to inhibit the activity of enzymes and enzymes are often specific to inhibitor and in many cases the inhibition effect of investigated pollutant is related to its biological toxicity (Krawczyk et al., 2000). Interestingly, different metals exhibit quite different behavior in their ability to act as urease inhibitor (Prakash and Vishwakarma, 2001). For example, the silver ion is an extremely efficient inhibitor while the manganous ion is relatively very weak.

Owing to its pronounced sensitivity, urease (urea amidohydrolase, EC 3.5.1.5) has been considered as a model enzyme for application as a probe for heavy metal ions in industrial effluents, drinking water, surface water, ground water, wastewater, soil extracts, etc. Urease, a Ni-containing metallo-protein present in many plants, bacteria and in soil, catalyses hydrolysis of urea to ammonia and carbon dioxide at a rate approximately 10^{14} times the rate of

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uncatalysed reaction. Most of the studies have utilized urease obtained from jack bean (Krajewska et al., 2004). The information regarding interaction of heavy metal ions with this enzyme from other sources is only sketchy (Fahmy et al., 1998; Prakash and Vishwakarma, 2001). Therefore, there still exits a need to have economical urease preparation for versatile immobilization applications. During the search of an alternate and inexpensive urease preparation, an attempt has been made to purify it to apparent homogeneity from the dehusked seeds of pumpkin (*Cucumis melo*) employing very simple steps. The present communication describes the interaction of mercury ion with thus purified urease, obtained from a rather new source, i.e., dehusked seeds of pumpkin.

2. Methods

2.1. Materials

Pumpkin seeds were procured from the local market and dehusked just before soaking. Tris was obtained from Boehringer Mannheim Gmbh, Germany. Bovine serum albumin was obtained from Sigma Chemical Co., USA. Sephadex G-200 was from Pharmacia Fine Chemicals, Uppsala, Sweden. Urea (enzyme grade), Mercuric acetate, Nessler's and Folin-Ciocalteau reagents were from Qualigens Fine Chemicals, Mumbai. All other reagents were analytical grade chemicals either from BDH or E. Merck, India.

2.2. Isolation of urease

Dehusked pumpkin seeds (6 g) were soaked in 50 mM sodium phosphate buffer (pH 7.5) for 8 h at 4–6 °C. The soaked seeds along with the buffer was homogenized in a kitchen blender giving high strokes for 2 min, filtered through muslin cloth and centrifuged for 15 min at 0–4 °C at 21,500*g*. The supernatant was filtered through a thick layer of prewashed glass wool to remove fat layer.

2.3. Purification of urease

The following steps were taken to purify the crude enzyme preparation.

2.3.1. Heat treatment

Crude enzyme preparation (3.0 ml) was heated at 48 ± 0.1 °C in a water bath for 10 min and was immediately chilled in crushed ice. This was centrifuged at 21,000*g* for 15 min at 0–4 °C. The supernatant was collected.

2.3.2. Gel filtration

Heat treated enzyme (1.5 ml, 2.4–2.6 mg protein) was loaded on a Sephadex G-200 column (1.5×40 cm). The elution was carried out at 4–6 °C at a flow rate of 20 ± 2 ml/h with degassed extraction buffer. Various 2.0 ml fractions containing urease activity were pooled and concentrated against solid sucrose. The enzyme preparation was kept frozen (at −20 °C) in small aliquots until further use.

The enzyme preparation (Sp activity 353 ± 12 U/mg protein) showing a single enzyme and protein band on native 7.5% PAGE (at pH 8.3) was employed for the study.

2.4. Calcium alginate beads preparation

A 3.5% solution of sodium alginate was prepared in 25 mm Tris–acetate buffer (pH 7.5) by stirring for 2 h at room temperature and was stored at 4 °C. Suitably diluted enzyme solution (0.7 mg protein/ml) was mixed in chilled alginate solution and dropped in 100 ml of chilled and continuous stirring 400 mM calcium chloride solution with the help of a micropipette. The beads were allowed to stir for 90 min at 4 °C for complete calcium alginate formation. Beads were thoroughly washed with the buffer and stored at 4 °C.

2.5. Urease activity assay

Enzyme activity was assayed in 50 mM Tris–acetate buffer (pH 8.0) as described elsewhere (Prakash and Upadhyay, 2006).

A unit of enzyme activity was defined as the amount of enzyme required to liberate 1 μmol of ammonia in 1 min (30 °C, 50 mM Tris–acetate buffer, pH 8.0, 250 mM urea). Protein was estimated by the method of Lowry et al. (1951) with Folin-Ciocalteau reagent calibrated with crystalline bovine serum albumin.

2.6. Effect of mercuric ions on the activity

A stock solution of mercuric acetate was made in 50 mM Tris–acetate buffer (pH 8.0) and diluted with the same buffer as required. The activity of suitably diluted enzyme was determined in the presence of varying concentrations of Hg^{2+} added in the standard assay mixture (comprising of 250 mM urea, 50 mM Tris–acetate buffer, pH 8.0, at 30 °C). For the direct effect of Hg^{2+}, enzyme alone was incubated with the desired concentration of inhibitor for 10 min at 30 °C and the treated enzyme was assayed for the activity. The inhibition pattern and inhibition constant (K_i) was obtained from a Lineweaver–Burk plot.

The results reported are the mean of 5–8 replicate experiments carried out with a fresh batch of purified enzyme.

3. Results and discussion

3.1. Extraction of enzyme

The procedure for extraction of urease from dehusked seeds of pumpkin was optimized by varying pH and molarity of the extraction buffer. The soaked seeds were homog-