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Urease Purification from the Seeds of *Cajanus Cajan* and Its Application in a Biosensor Construction

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ABSTRACT

Urease has been purified from the seeds of *Cajanus Cajan*. The purification process involves three solvent extraction steps followed by DEAE-cellulose column chromatography. The specific activity of the purified enzyme is found to be 1920 U/mg with the recovery of 8%. The application of the purified enzyme in a biosensor construction is discussed.

Index Entries: Enzyme purification; enzyme electrode; biosensor.

INTRODUCTION

Urease has gained considerable attentions owing to its widespread interest in the medical and technical fields. A number of publications are available on purity (*1,2*), purification (*3,4*), and physical and chemical properties (*5*) of Jack Beans urease.

Activity of the enzyme is expressed in term of units, where one unit is the amount of enzyme that catalyzes the transformation of 1 μmol of the substrate per minute under defined conditions. Specific activity (SA) is defined as the ratio [enzyme]/[protein] that is expressed in terms of [U]/[mg]. Sumner has reported purified urease enzyme with maximum SA of 133 U/mg from Jack Beans. Later on, Zerner et al. (*1*) have reported the purified urease preparation with SA of 182 U/mg from the same

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source. There is a still need of purified urease preparation of high SA for the construction of a urease-based biosensor from the following angles: (i) to increase the selectivity of the measurements; (ii) to reduce the quantity of the enzyme to be used in a biosensor construction; and (iii) to avoid the interferences. In this paper, we report a new method of enzyme purification from a new source seeds of *Cajanus Cajan* with a high purity. The applications of this urease preparation in the construction of an enzyme electrode has been discussed.

EXPERIMENTAL

Extraction of Crude Enzyme

Seeds of *Cajanus Cajan* (300 g) were soaked in 600 mL of Tris-acetate buffer (0.025*M*, pH 6.5) for 3 h. The soaked seeds were crushed in a blender for 1 min followed by the addition of another 200 mL of buffer and the mixture was filtered using cotton. The filterate was centrifuged at 0–4°C (20 min, 6000 rpm). Extract (700 mL) {9.0×10^4 U, SA 5.8 U/mg} was obtained.

Acetone Fractionation, Step 1

Into the extract (700 mL) obtained from above, precooled acetone (−20°C, 234 mL) was added dropwise and after 15 min, the mixture was centrifuged at 0°C (15 min, 10,000 rpm). Into the supernatant obtained, another 234 mL of precooled acetone was added dropwise followed by immediate centrifugation at 0°C (15 min, 15,000 rpm). The precipitate was suspended overnight in 46 mL of Tris-acetate buffer (0.05*M*, pH 6.5) and centrifuged at 4°C (15 min, 10,000 rpm) the next day. Extract (46 mL) {4.7×10^4 U, SA 189 U/mg} was obtained.

Acid Fractionation Step

Into the extract (46 mL) obtained from Step 1, acetic acid (0.1*M*, 23 mL) was added dropwise at 4°C. Care was taken to control the local pH variations in the enzyme solution by ensuring that the solution was completely mixed during acid addition. The mixture was centrifuged immediately at 0–4°C (15 min, 15,000 rpm) followed by immediate adjustment of the pH of enzyme solution to pH 6.5 using 0.1*M* Tris solution. Acid extract (92 mL) {4.5×10^4 U, SA 445 U/mg} was obtained.

Acetone Fractionation Step 2

Into the 92 mL solution obtained after acid fractionation, 62 mL of precooled acetone (−20°C) was added dropwise followed by immediate centrifugation at 0°C (15 min, 15,000 rpm). The precipitate was suspended

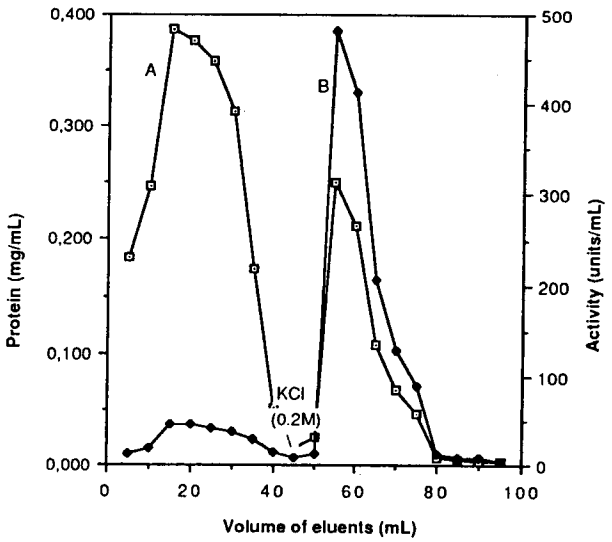

Fig. 1. Typical DEAE-cellulose chromatographic elution profile of urease. [A = protein (mg/mL) and B = activity (U/mL)].

in 20 mL of Tris-acetate buffer (0.1*M*, pH 6.5) and centrifuged next day at 0–4°C (15 min, 15,000 rpm). Extract (20 mL) {1.25×10^4 U, SA 1080 U/mg} was obtained.

DEAE-Cellulose Column Chromatography

DEAE-cellulose, washed and suspended in Tris-acetate buffer (0.1*M*, pH 6.5), was packed into a glass column (id = 1 cm, packing height, 15 cm). Before loading the enzyme the column was thoroughly washed with Tris-acetate buffer. After loading the enzyme, fractions of 5 mL were collected using fraction collector (Pharmacia Fine Chemical, FRAC-100) at the flow rate of 0.81 mL/min. After passing the enzyme solution, the column was thoroughly washed with Tris-acetate buffer (0.1*M*, pH 6.5) until the fractions were free from protein. Finally, the enzyme was eluted with 0.2*M* KCl solution in Tris-acetate buffer. The enzyme activity and protein content were analyzed for each fractions and recorded in Fig. 1. The fractions containing the enzyme were mixed and precipitated using precooled acetone (at −20°C). The precipitate was immediately collected by centrifugation at 0°C (10 min, 15,000 rpm). The precipitate was suspended in 2 mL of Tris-acetate buffer (0.1*M*, pH 6.5) and centrifuged again. A clear yellow solution (2 mL) was obtained (6.9×10^3 U, SA 1920 U/mg).

Measurement of Enzyme Activity

The enzyme activity was measured by incubating the appropriate amount of enzyme (1 mL in Tris-acetate buffer, pH 7.4) with a solution of urea (0.2*M*, 0.5 mL) for 10 min at 25°C. The enzymatic reaction was

Table 1
Parameters of Importance Recorded
During the Purification of Urease from 300-g Seeds of *Cajanus cajan*

Extract	Volume mL	Activity U	Protein mg/mL	Specific Activity U/mg	Fold purification	Recovery %
Original extract	700	9.0×10^4	22	5.8	-	-
Acetone fractionation step 1	46	4.7×10^4	5.4	189	32	52
Acid fractionation	92	4.5×10^4	1.1	445	77	50
Acetone fractionation step 2	20	1.25×10^4	0.58	1080	186	13.8
DEAE-Cellulose column chromatography	2	6.9×10^3	1.79	1920	331	7.7

arrested after 10 min by adding 0.2 mL of 10% trichloroacetic acid. After sufficient dilution of the reaction mixture with water, 1 mL of Nessler's reagent was added and its optical density was recorded at 405 nm. The activity of the enzyme was calculated from a standard curve obtained by using standard ammonium chloride solution.

Measurement of Protein

Protein concentration was measured by placing 0.1 mL of appropriately diluted samples in a clean and dry test tube followed by the addition of 5 mL of 5× diluted and filtered solution of Bio-Rad dye. The mixture was stirred gently. After 30 min the optical density of the solution was reorded at 535 nm. The concentration of protein was calculated from a standard curve prepared by using standard bovine serum albumin solution.

RESULTS AND DISCUSSION

Table 1 shows evolution of specific activity and recovery at various stages of purification of urease from the seeds of *Cajanus Cajan*. The specific activity of the purified enzyme after DEAE-cellulose column chromatography is 1920 U/mg. The enzyme is about 330-fold purified with the

total recovery of 8%. Figure 1 shows the DEAE-cellulose chromatographic elution profile of the enzyme showing two maxima, of which the second one corresponds to urease as identified by the activity measurements for each fraction.

In the construction of an enzyme electrode, it is important that highly purified enzyme be used so that only a small amount of enzyme need to be used in the construction of the electrode. This will ensure a fast response time, approaching that of base probe. We have used this enzyme for the construction of an enzyme electrode using prepolymer over the surface of a ammonium ion-selective electrode (*6*), with the slope of 40–50 mV/decade, which may be as a result of the use of modified commercially available single-junction reference electrode (Radiometer calomel electrode, K 401) replacing saturated KCl by Tris-HCl buffer (0.1*M*, pH 7.4) as internal filling solution. Other parameters (e.g., response time, storage, and operational stability) have been improved over the earlier design (*7*). We have recently observed that clear solution of highly soluble enzyme may undergo chemisorption onto the modified glass surfaces, leaving a thickness of enzyme layer to the order of 10–20 μm. This showed a very fast improvement in response time (<10 s). The commercially available urease from Jack Bean is unsuitable for this purpose.

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