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Activity and stability of urease enzyme immobilized on Amberlite resin

Jawad Kadhim Jawad AL-SHAMS, Mustafa Abdul Kadhim HUSSEIN*, and Hussein Kadhem AL-HAKEIM

Department of Chemistry, College of Science, University of Kufa, Iraq

Abstract. Immobilization of enzymes is a good field of study to extend the life of enzyme and reduce the cost of the chemical processes, such as separation processes. Urease is an important enzyme with medical and industrial applications. The aim of the present study is to prepare an immobilized urease on a strong cation exchange resin (Amberlite IR120 Na) and study its activity and stability. We monitored the release of Na ions in the collected fractions and searching for enzyme in the fractions as indicators of immobilization by ion exchange phenomenon. Sodium is determined by using atomic absorption spectroscopy technique, while the enzyme concentration was tested by Bradford's method. Immobilized urease activity was evaluated by salicylate-hypochlorite method. The results indicated a complete immobilization of urease enzyme on the resin surface with reserving 92% of the activity of free enzyme. The immobilized urease enzyme on resin showed good stability and it has a 62% of its activity after 154 days of storage at room temperature. It is concluded that a new immobilized urease enzyme system is prepared with good enzyme activity and stability.

Keywords: Amberlite IR120 Na resin, urease, enzyme immobilization.

1. Introduction

Immobilization of enzymes on different surfaces is an important method to increase the life and stability of the enzymes and to decrease the cost of the enzyme reactions [1]. Urease (EC 3.5.1.5) is an enzyme that convert urea to CO_2 and ammonia [2]. Immobilized urease on various materials have many classic applications [3-6]. Furthermore, some new applications of urease immobilization are introduced including hemodialysis membranes [7], enzymatic bio-electrochemical devices [8], biosensor for the quantitative determination of urea [9, 10], and study of protein-surface interaction [11]. The enzyme may be immobilized in several ways such as adsorption on a surface where the enzyme is bound to an inactive substance [7] or by ion exchanging [8], but some immobilization processes can reduce the enzyme activity [12] or change the urease structure (e.g. when immobilization occurred on the nanoceria surface) [13]. Specific protein (prolactin) was found to be adsorbed on the active surfaces with various findings and interactions including changes in the activity [14], secondary or tertiary structure [15], or stability [16].

Previously, we had succeeded in the immobilization of urease enzyme on anionic exchange resin with good stability and activity [17].

This work aims to identify the ability of urease enzyme to be active when it immobilized on a strong cation exchange resin (Amberlite IR120 Na) and study the stability of enzyme-resin system.

2. Experimental

2.1. Materials

Amberlite IR120 Na resin was supplied from Rohm and Haas Inc. USA. Coomassie Brilliant Blue Fast Staining Solution was supplied by Cusabio Biotech®, China. Type III powder (15,000—50,000 units/gram solid) of urease (EC3.5.1.5, from *Canavalia ensiformis*) with a molecular weight of 480,000 Da was obtained from Sigma-Aldrich®. Urea solution (20 mM urea in 2 mM $CaCl_2$), Reagent A (62 mM of sodium salicylate, 3.4 mM sodium nitroprusside, and phosphate buffer 20 mM – pH = 6.9) and Reagent B (sodium hypochlorite 7 mM and sodium hydroxide 150 mM) were supplied by Spinreact®, Spain. Hydrochloric acid 37% was supplied by Mana Scientific Products, India. Phosphate buffer solution (1.0 M), pH = 7.4, was supplied by Reagene Biosciences Private Ltd, India.

2.2. Preparation of immobilized enzyme on cation exchange resin

2 g of Amberlite IR120 Na resin were suspended in 25 ml of distilled water, put in a glass chromatography column after swelling in 75 ml of distilled water and regenerated by 1 M HCl. After 2 hours, it was carefully washed with distilled water until neutralization. The urease enzyme (0.8 mg enzyme in 0.2 ml of phosphate buffer, pH = 7.4) was added to the top of the column. The mixture was kept for 45 min at room temperature in order to obtain equilibrium and complete the interaction between the enzyme molecules and the resin.

The fractions (containing urease in phosphate buffer) were collected from the column in 2 ml for each fraction at a flow rate of 1 ml/min. The concentration of Na^+, H^+ and urease was measured in each fraction. These ions are the exchangeable cations from the surface of the

* Corresponding author. *E-mail address*: mustafa.rabeea@uokufa.edu.iq (Mustafa Abdul Kadhim Hussein)

resin. If they appeared in the eluent, it means replacement of these cations with the urease molecules.

2.3. Methods

Measuring of urease concentration in fractions. The Bradford method for proteins [18] was used for estimation of amount of urease in solution and the difference between quantities before and after immobilization is equal to the quantity of immobilized enzyme on the cationic resin. For calibration curve we used the albumin as a standard at a concentration ranged from 5 to 100 µg in 100 µl. The protein solutions were added onto 5 ml of Coomassie Brilliant Blue dye solution and the mixtures were kept for 5 min at room temperature. The absorbance was measured at 595 nm [18]. Absence of protein in the collected fractions indicated the immobilization of urease enzyme.

Assay of urease activity. In order to see if the enzyme is still active after immobilization, we measure the urease activity after adding urea as substrate and measuring the ammonia released from the hydrolysis of urea by the action of urease. Briefly, 2 ml of urea solution (20 mM urea in 2 mM $CaCl_2$) as an enzyme substrate was added on the top of the exchanger and kept for 30 min at room temperature. The immobilized urease will hydrolyze urea into ammonia and CO_2. Then we collect these fractions that contain ammonia (1 ml) and added 1 ml of "Reagent A". The resulted solutions were kept for 10 minutes. Then, the solutions were mixed thoroughly with 1 ml of "Reagent B" and maintained for 10 min at room temperature [19]. Ammonium ions will react with salicylate in the presence of sodium hypochlorite to produce indophenol, which absorb light at 600 nm. The absorbance of the produced green color measured spectrophotometrically is proportional to the concentration of ammonium ions released from the hydrolysis of urea by urease [19]. The urease activity assay was also repeated on free urease enzyme to compare the activity between free and immobilized enzyme.

Determination of sodium by atomic absorption spectroscopy. Sodium concentration in solution was determined by atomic absorption spectrometry [20] after constructing a calibration curve from six known sodium solutions from 0.01 to 1.0 mg/L. The blank was double deionized water to set the digital display to zero.

Stability of the immobilized urease. The activity of immobilized urease was measured weekly to estimate the stability of the complex as cited in the previous paragraph.

3. Results and discussion

The results of the immobilization process, as estimated by Bradford's test method, indicated lack of urease in the collected fraction after addition of urease to the resin column. These results revealed a complete immobilization of urease on the resin (negative test). Furthermore, the decrease of pH (measured by pH-meter) on the collected fractions indicated occurrence of ion exchange between urease molecules and the hydrogen ions from the surface of resin. The addition of distilled water to the resin-urease mixture leads to evacuation of acidic solution until it became neutral. These results refer to a strong interaction between the resin and protein. Furthermore, sodium ions were also released from the exchange process. Figure 1 showed the release of sodium ion after adding the urease solution on the resin as a result of another ion exchange with the urease molecules.

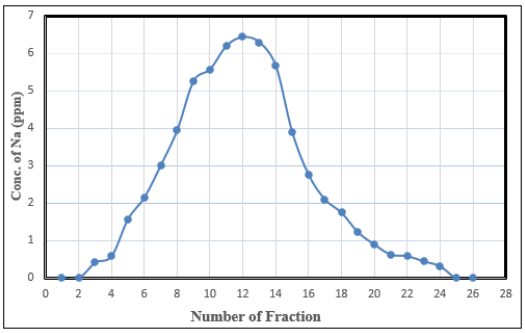

Figure 1. The concentration of sodium released in the collected fractions after exchange with the urease enzyme on the surface of Amberlite.

The urease action on the urea was determined by salicylate-hypochlorite method [19] and showed the ability of immobilized urease to hydrolyze urea. Figure 2 showed the concentration of ammonia produced by the action of immobilized urease on urea substrate.

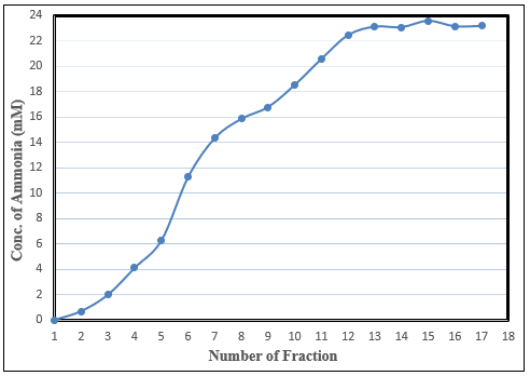

Figure 2. Ammonia concentrations in the fractions collected after adding urea solution to the immobilized urease enzyme on the Amberlite resin.

This result indicated an interaction between the resin and urease by sites far away from the active site and the immobilization reserves the enzyme active site intact. The ability of urease to be immobilized with reserving its activity was seen previously with different stationary phases [2, 4, 5, 17]. Furthermore, in a separate experiment on free urease, the activity of immobilized enzyme is about 92% of the activity of the free enzyme. This reduction in activity can be explained by the generation of hydrogen ions leading to slight increase in acidity that may affecting the activity of urease. Previous work showed 87% activity of the free enzyme after immobilization of urease on the Dowex resin (strong anion exchanger) [17]. These results indicated a different behavior of the urease on different surfaces according to the charges on the surface of resin.

Even though the immobilized enzyme has lower catalytic activity comparing with free enzyme, it has

more stability, reusability, and low cost effective [21, 22]. This result indicated an interaction between the resin and the sites beyond the active site of the enzyme. Hence, the activity is not affected dramatically due lack of involvement of the active sites in the exchange process. The slight change in activity is due to the change in spatial configuration and the orientation of groups beyond the active site. In another study, for comparison, urease covalent immobilization on the template of polysiloxane was reported to be less efficient due to the lack of the enzyme activity [23]. It is found that sloping immobilized urease presents higher temperature resistance, higher similarity to the substrate, and higher constancy of operation [24].

In some previous works [25], the immobilization of urease enzyme needed a chemical cross-linking using formaldehyde, glutaraldehyde and chromium (III) acetate while in the present work, there is no need for this treatment and the urease still binds to the resin tightly. Our study is safer as chromium ion is toxic and some important application of immobilized urease is for reducing urea levels in uremia patients [26].

Many factors affect the activity of protein adsorbed on any surface. It is found previously that high ionic strength produces huge interaction between protein and the adsorbing surface [27]. The activity of immobilized urease on strong cation exchange resin in hydrogen form showed good stability but release of H^+ during exchange increased the acidity which may cause a decrease in the stability of adsorbed urease.

The results of immobilized enzyme activity expressed as ammonia concentrations with time are presented in Figure 3.

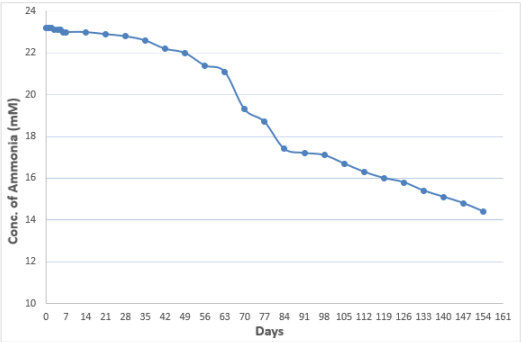

Figure 3. Variation of ammonia concentration, as a measure of immobilized urease activity, with time of storage.

The immobilization of urease on the Amberlite IR120 Na resin is easy, and the enzyme still active with good stability even after 154 days of keeping in distilled water. The enzyme has 62.07% of its activity after 154 days of preservation in the sterile condition to keep it free from contamination with foreign organisms. In our previous study, the immobilized urease on Dowex® resin showed good stability and slight change noticed in its activity after 15 days of storage at room temperature. However, this study did not monitor the activity of immobilized enzyme for a longer time as done in our study. The thermal stability of the immobilized urease [28] was higher than free enzymes in terms of the pH and the stop action of some metal ions or organic substances. The stability over time of the immobilized urease is high, its enzymatic activity was stable at 85% of the first value three months after synthesis [28].

4. Conclusions

A new system of immobilized urease enzyme on the strong cation exchange resin in sodium form (Amberlite IR120 Na) is prepared with good enzyme activity (92% of free enzyme activity) and stability up to 154 days. This new system can be used efficiently to hydrolysis urea in various medical and industrial applications.

Conflict of interest

The authors declared no conflict of interest.

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