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Enzymology, Immobilization and Applications of Urease Enzyme

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Abstract

Urease enzyme (urea amidohydrolase, E. C. 3.5.1.5) from jack bean was the first crystallized enzyme. It is a Ni- dependent metalloenzyme that has been isolated from different sources: plants and microbial (bacteria and fungi) and all of them having similar structure and catalysis mechanism. We also discuss about the different strategies for immobilization of urease and their applications in different fields.

Keywords: Enzyme, urease, immobilization, applications.

Introduction

Urease is a nickel dependent metalloenzyme which catalyzes the hydrolysis of urea to yield ammonia and carbamate, the latter compound spontaneously hydrolyzes to form carbonic acid and another molecule of ammonia[1]. The best-studied urease is that from jack bean[2], which was identified as the first nickel metalloenzyme[3] and urease from jack bean (*Canavalia ensiformis*) was the first enzyme to be crystallized[4]. In 1926, James Sumner showed that urease is a protein. Urease is found in bacteria, yeast, and several higher plants. Urease is a cytosolic enzyme. Its major activity with some exceptions is associated with the soluble fractions of the cells[5]. The best genetic data of plant ureases are available for soybean (*Glycine max*)[6,7]. Two urease isoenzymes, a tissue-ubiquitous and embryo-specific encoded by two separate genes, as well as regulatory proteins encoded by unlinked genes was identified in soybean[8,9]. The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean[7] and *Arabidopsis*[10], while the other type of urease (called ubiquitous) is found in lower amounts in vegetative tissues of most plants[11].

Sources for occurrence of urease in living organisms include plants, some bacteria, fungi and invertebrates. In plants, urease is a hexamer - it consists of six identical chains and is located in the cytoplasm. In bacteria, it consists of either two or three different subunits. For activation, urease needs to bind two nickel ions per subunit[12]. The enzyme inhibitors can interact with enzymes and block their activity towards natural substrates[13,14]. Because of instability of enzyme, its use is limited. This problem can be overcome by recent developments in the field of biotechnology for immobilizing enzymes. Many methods exist for the immobilization of enzymes but usually used methods include entrapment; physical adsorption; co-polymerization; and covalent attachment. The immobilization of urease in nylon tubes, carboxymethyl-cellulose, polyacrylamide and gelatin has been done. Calcium alginate is commonly used for entrapment of enzymes[15-17].

A number of medical and ecological significances of microbial ureases has been described. The significance of the enzyme includes: to serve as a virulence factor in human and animal infections of the urinary and gastrointestinal tracts, play role in recycling of nitrogenous wastes in the rumens of domestic livestock, and its application in environmental transformations of nitrogenous compounds, involve urea based fertilizers[18].

Different Sources for Production of Urease Enzyme

The enzyme urease occurs in a wide variety of tissues in humans, as well as in bacteria, yeasts, molds, plants and invertebrates. In 1926, Sumner was the first chemist who showed that urease was a protein of the globulin type with an isoelectric point of five.

Microbial urease: Microbial ureases hydrolyze urea to ammonia and carbon dioxide. There are many microbial sources for this enzyme including bacteria such as *Lactobacillus ruminis*, *Lactobacillus fermentum* and *Lactobacillus reuteri* and *Klebsiella aerogenes* [19,20] and fungi such as *Rhizopus oryzae*[21]. Filamentous fungi are the sources of about 40% of all available urease enzymes[22]. Urease activity of an infectious microorganism can contribute to the development of urinary stones, pyelonephritis, gastric ulceration, and other diseases. In contrast to its pathogenic effects, microbial ureases are important enzymes in ruminant metabolism and in environmental transformations of certain nitrogenous compounds[18]. Thus urease activities serve as an indicator of pathogenic potential and of drug resistance among some groups of bacteria.

Plant urease: Urease activity is also found in plants. In plants urease is involved in urea metabolism. The primary role of urease is to allow the organisms to use external or internally generated urea as a nitrogen source[18,5]. Nitrogen present in urea is unavailable to the plant unless hydrolyzed by urease. Significant amounts of plant nitrogen flow through urea. Urease plays important role in germination and seedlings nitrogen metabolism[10]. It may function coordinately with arginase in the utilization of seed protein reserves during germination[6].

Mode of action of urease enzyme (*In vivo* urease activation): Urease catalyzes the hydrolysis of urea to yield ammonia and carbamate, carbamte spontaneously hydrolyzes to form carbonic acid and another molecule of ammonia[1] as shown in figure 1.

$$NH_2\text{-}CO\text{-}NH_2 + H_2O \rightarrow NH_3 + NH_2\text{-}CO\text{-}OH$$
$$NH_2\text{-}CO\text{-}OH + H_2O \rightarrow NH_3 + H_2CO_3$$

Figure-1
Reaction showing hydrolysis of urea to ammonia catalyzed by urease enzyme

In solution, the released carbonic acid and the two molecules of ammonia with protonated forms are in equilibrium. The net effect of these reactions is an increase in pH.

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$
$$2NH_3 + H_2O \leftrightarrow 2NH_4^+ + 2OH^-$$

A urease apoenzyme needs to be activated and this process requires participation of several accessory proteins that incorporate nickel into the urease forming catalytic site. Genetic analyses of many organisms revealed the possibility to eliminate urease activity by disrupting genes other than those encoding the urease subunits[23,24,25,26] and sequence information revealed the presence of numerous nonsubunit genes in urease gene clusters which facilitate assembly of a functional metallocenter in the protein. This result came from studies in which inactive urease was purified from a deletion mutant lacking a DNA fragment encoding part or all of the *ureE*, *ureF*, and *ureG* genes[27]. It has been found that purified protein possess insignificant levels of nickel ions. It has been demonstrated that four accessory genes (*ureD*, *ureE*, *ureF*, *ureG*) are involved in nickel metallocenter biosynthesis[28]. Hypothetical roles for the urease accessory proteins are as shown in figure 2.

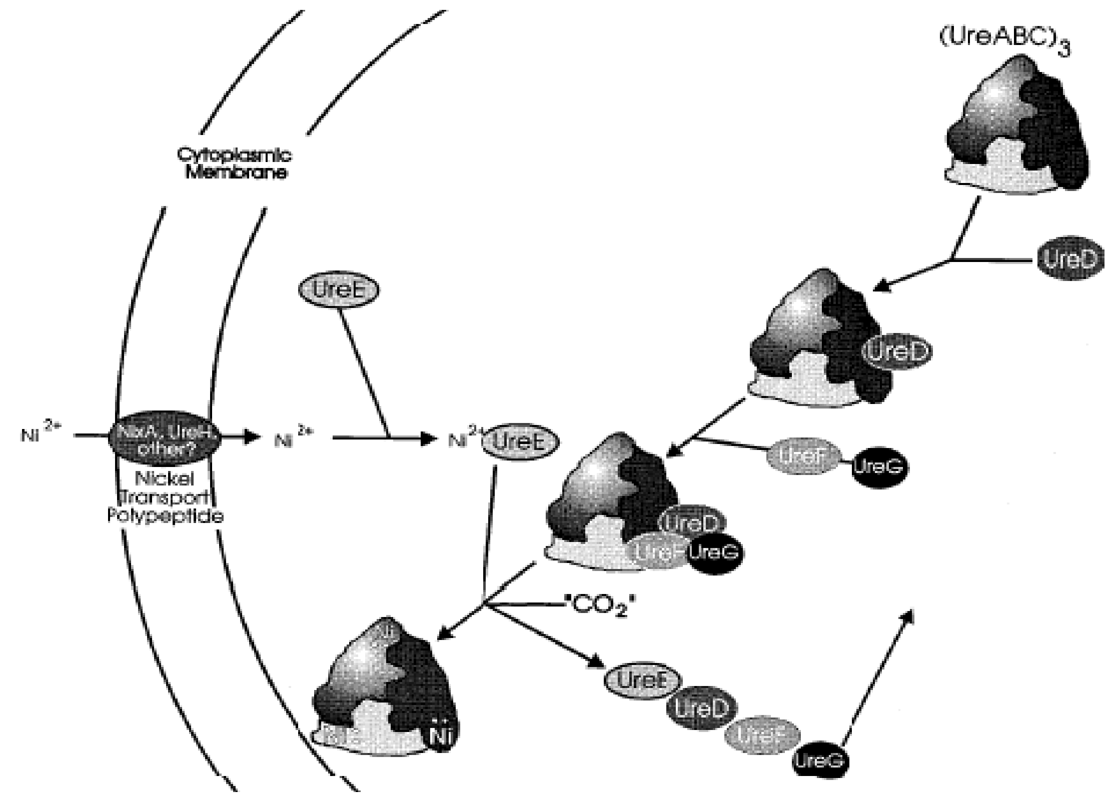

Figure-2
Role of accessory genes for urease activation. The urease apoenzyme is assembled from three copies of each distinct subunit polypeptide, UreA, UreB, and UreC, resulting in a stoichiometry of (UreABC)$_3$. *In vivo* activation of the apoprotein requires the presence of nickel ion, carbon dioxide, and numerous urease accessory gene products. UreD, serves as a urease-specific chaperone protein that facilitates proper assembly of the metallocenter[29,30]. UreF and/ or UreG join to the complex, making the apoenzyme competent to accept nickel ions (Ni^{2+}) into the active site in a productive manner. Function of these proteins include the generation or delivery of carbon dioxide to the metallocenter assembly site, facilitate productive interaction between urease apoprotein and UreE holoprotein. Upon activation, UreD, UreE, UreF, and UreG dissociate from the enzyme and are recycled for interaction with the next urease apoenzyme molecule or with other ''UreABC'' units on the same molecule. The net result is that urease binds two nickel ions per subunit[5].

Immobilization of Urease Enzyme

The use of enzymes is often limited due to their high cost, limited availability, instability and the limited possibility of economic recovery of these soluble biocatalysts from a reaction mixture. Immobilization of enzymes can overcome some of these problems. Immobilization of enzymes can be done by a number of methods but mainly it involves: entrapment; physical adsorption; co-polymerization; and covalent attachment. Immobilization changes original enzyme properties (storage stability, kinetic parameter) and customizes them for specific applications[31]. The activity and diffusion limitations in immobilization mainly depend on the properties of support material and the immobilization method. Supporting material should be of low cost and provide adequate large surface area with least diffusion limitation in transport of substrate and products for enzyme reactions[32]. Enzymes are immobilized by a variety of methods which are classified as physical and chemical methods. There are a number of publications for the immobilization of urease enzyme. The enzyme urease (jack bean meal) was immobilized by various techniques such as entrapment in calcium alginate, lac and paraffin wax[33]. The K_m and V_{max} values get altered after immobilization method. Immobilized enzyme increases stability with respect to free enzyme with increasing time and temperature[34]. An optical urea biosensor was fabricated by the stacking of the sol-gel films allowing the immobilization of urease enzyme without any chemical attachment and along with immobilization of Nile blue chromoionophore (ETH 5294)[35]. Urease from pigeon pea is partially purified using acetone fractionation and then immobilized on calcium alginate in the form of beads and this immobilized urease enzyme has application in estimation of blood urea[36]. Immobilized enzymes have applications in different fields, which are as below:

Uses of Immobilized Enzymes

Use of immobilized enzymes in medicines: Immobilized enzymes are used routinely in the medical fields, for the diagnosis and treatment of various diseases. Enzyme-based electrodes represent a major application of immobilized enzymes in medicine. Because of properties like the high specificity and reactivity of an enzyme towards its substrate, they are used in biosensor technology. Biosensors used in clinical applications possess advantages such as reliability, sensitivity, accuracy, ease of handling and low-cost compared to conventional detection methods. These characteristics in combination with the unique properties of an enzyme render an enzyme based biosensor ideal for biomedical applications[37]. Immobilized pigeon pea urease (on glutaraldehyde-activated chitosan beads) was used to analyze blood urea of some of the clinical samples from the clinical pathology laboratories. A column packed with immobilized urease beads was also prepared in a syringe for the regular and continuous monitoring of serum urea concentrations[38].

Use of immobilized enzymes as biosensors: Biosensors have wide applications including biomarker detection for medical diagnostics and pathogen and toxin detection in food and water[39]. Because of their exceptional properties such as high specificity and sensitivity, rapid response, low cost, relatively compact size and user-friendly operations of biosensors make them an important tool for detection of various chemical and biological components[40]. The development of biosensors based on immobilized enzymes came out to solve several problems such as loss of enzyme and maintenance of enzyme stability. Immobilized- urease enzyme nylon-tube reactors used for blood urea analyses. The immobilized enzyme nylon tube reactor gives reliable and reproducible results with high precision and low cost. A method involving differential colorimetry is used for determining citrulline in blood and which makes use of the immobilized urease, albeit indirectly[41].

Immobilized enzymes used for antibiotic production: Enzyme based routes are considered as an environment friendly approach as it involves working at room temperature and avoiding organic chloride solvents. There are a number of immobilized enzymes that are used for production of different antibiotics with different immobilized support. Significant advances have also been made in the resolution of racemic mixtures using B-lactam acylases by means of stereo-selective acylation or hydrolysis[42]. Immobilized Penicillin acylases find wide applications[43].

Immobilized enzymes used for bioremediation: The environment of the microorganisms is not often optimal for rapid degradation. In recent studies, it has been indicated that an enzymatic approach has attracted much interest in the removal of phenolic pollutants from aqueous solution considered as an alternative strategy to the conventional chemical as well as microbial treatments[44]. Immobilized urease is used for the treatment of industrial fertilizer effluents rich in urea by a new coupling method to immobilize crude urease onto polyester which is having high flow through property in columns[45].

Use of immobilized enzymes in food industry: Immobilized enzymes are of great value in the food industry for processing of food samples and its analysis. Immobilization of enzymes such as glucoamylase, lactase, protease and flavor modifying enzymes has received some attention recently for food processing in food industries[46]. Immobilized enzymes have advantage over free enzymes such as having higher activity as compared to free enzyme at higher temperatures and having the ability to hydrolyze raw starch such as that of potato would help in overcome problems that are related to gelatinization of starch during hydrolysis[47].

Applications of Urease Enzyme

As anticancer agent: DOS47 is an enzyme called urease derived from the jack bean. By inducing the catabolism of urea in the interstitial medium surrounding cancer cells, urease action results in the production of metabolites: ammonia and hydroxide ions. It has been estimated that these metabolic products of urease activity stress cancer cells by direct toxicity and by the induction of alkaline effects. Ammonia is toxic to cancer cells and catabolism of urea increases the local pH of the surrounding medium. Increased alkanization may counteract the adverse effect of the acidic microenvironment on weakly basic drugs and enhances the uptake of these chemotherapeutics[48].

To treat hypertension: *Cucumis melo* is the plant source of urease enzyme and *Cucumis melo* shows the diuretic effects in herbal medicines. These diuretic clinical medicines are used to lower blood pressure and work by increasing the excretion of urine from the body as well as the amount of sodium in urine[49].

Role in nitrogen metabolism: Urease plays an important role in the nitrogen metabolism of ruminants such as cattle, sheep, and other animals that contain a forestomach[50]. It has been identified that species of *Staphylococcus, Lactobacillus*, and *Klebsiella aerogenes* are ureolytic and these are responsible for the urease activity in rumen sheep[51]. Substantial amounts of animal-derived urea are recycled to the rumen, where ureolytic activity releases ammonia, the major source of nitrogen for most ruminal bacteria[52].

Urease having role in germination of nitrogen-limited *Arabidopsis thaliana* seeds: It has been shown that, urease functions to recycle nitrogen bound in urea that accumulates during early seedling development[10].

Urease-based confirmatory enzyme-linked immunosorbent assay: A new urease-based enzyme-linked immunosorbent assay[53] was evaluated for the confirmation of *Neisseria gonorrhoeae*, utilizing novel monoclonal antibodies.

Urease in vaccines: Sumner showed that jack bean urease enzyme was the first crystallized enzyme and demonstrated that this enzyme could be used as an antigen[54] having ability to stimulate a strong immunoglobulin response. It has also been studied[55] that the plant enzyme was used as a vaccine based on the inhibition of catalytic activity to explain the mechanism of protection against infection by *H. pylori* which cause gastritis ulceration disease and possibly gastric cancer.

Role of urease in wine industry: Currently, there is significant concern regarding the occurrence of ethyl carbamate (carcinogen) in wine, that is formed from ethanol and urea (if present), during storage of wine. So as a precautionary measure, these wines treated with preparation of killed cells containing an acid urease, removes urea from wine which is the potential source of ethyl carbamate and thus prevents the formation of ethyl carbamate[56,57].

Conclusion

It has been established that accessory proteins are required for Ni- activation in mechanism of action of urease enzyme. The immobilized urease enzyme has promising applications in biotechnology, clinical and industrial fields. There is significance of urease activity in both plants and microbes as discussed in this review article. In addition to this, urease is also beneficial in case of some diseases such as cancer.

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