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POTENTIAL CLINICAL SIGNIFICANCE OF UREASE ENZYME

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Abstract

Urease belongs to the super family of amidohydrolases and phosphotriestreases. Urease involves the nitrogen metabolism; urea degradation; generating CO_2 and NH_3 from urea. Urease has wide clinical applications. Urease enzyme serves as a virulence factor and is responsible for pathogenesis in humans. Urease activity of microbial sources has contributed to the development of many diseases and urease from plant sources is used as vaccine against microbial infection on the basis of its inhibitory activity.

Keywords: Urease, Microbe, Plant, Pathogenic, Clinical

Introduction

Urease (EC 3.5.1.5), 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 (Andrews, Blakeley, & Zerner, 1984). The reaction occurs as follows:

$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$

Functionally, ureases belong to the super family of amidohydrolases and phosphotriestreases (Holm & Sander, 1997). The best-studied urease is that from jack bean (Andrews, Blakeley, & Zerner, 1984; Blakeley, & Zerner, 1984) which was identified as the first nickel metalloenzyme (Dixon, Gazzola, Blakeley, & Zerner, 1975) and urease from jack bean (*Canavalia ensiformis*) was the first enzyme to be crystallized (Sumner, 1926). In 1926, James Sumner showed that urease is a protein.

Urease activity tends to increase the pH of the environment by producing ammonia as a product and ammonia is a basic molecule. Ureases are found in numerous bacteria, fungi, plants and some invertebrates, as well as in soils, as a soil enzyme (Krajewska, Van-Eldik, & Brindell, 2012; Banerjee & Aggarwal, 2012). Urease of *Helicobacter* species plays a

significant function for neutralizing gastric acid by allowing urea to enter into periplasm via a proton-gated urea channel. The presence of urease is used in the diagnosis of *Helicobacter* species (Strugatsky et al., 2012).

A number of medical and ecological significances of microbial ureases have 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 (Mobley & Hausinger, 1989; Banerjee & Aggarwal, 2013). A new urease-based enzyme-linked immunosorbent assay was evaluated for the confirmation of *Neisseria gonorrhoeae*, utilizing novel monoclonal antibodies (Carballo et al., 1992). A clinical evaluation of an endoscopic tube type urease sensor system for the detection of *H. pylori* on the gastric mucosa has been carried out. It is now accepted that the curing of *H. pylori* infection will result in healing of chronic active gastritis and will change the natural history of gastroduodenal ulcer disease (Sato et al., 1999).

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. The microbial and plant ureases have profound clinical significance.

Microbial urease

Microbial ureases hydrolyze urea to ammonia and carbon dioxide. Urease activity of an infectious microorganism can contribute to the development of infection stones, pyelonephritis, peptic ulceration and hepatic encephalopathy (Mobley & Hausinger, 1989) 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 (Mobley & Hausinger, 1989). Thus urease activities serve as an indicator of pathogenic potential and of drug resistance among some groups of bacteria.

There are different microbial sources for the urease enzyme, as discussed below. Here, these microbial sources are described with their botanical names, sources and their functional descriptions.

Staphylococcus leei

Staphylococcus leei isolated from patients with gastritis, colonize the skin and upper respiratory tracts of humans. *Staphylococcus leei* having high urease catalytic activity that causes degradation of urea and urease subunit beta protein is responsible for this urease catalytic activity. Its mechanism pathway involves the nitrogen metabolism; urea degradation; generating CO_2 and NH_3 from urea (Jin, Rosario, Watle, & Calhouna, 2004).

Actinomyces naeslundii

It is a mesophilic microorganism that grows in temperature range from 15°C - 40°C, optimum growing temperature is 37°C (the normal human body temperature). *Actinomyces naeslundii* is an important ureolytic organism in the oral cavity and a major component of dental plaque (Morou-Bermudez & Burne, 1999). Ammonia production from urea by these ureolytic oral bacteria has significant impact on oral health and ecological balance of oral microbial populations. This ureolysis activity has role in bacterial aciduricity and is capable to modulate pH homeostasis (Yaling, Tao, Jingyi, & Xuedong, 2006).

Proteus mirabilis

Proteus mirabilis is the most common organism implicated in stone formation (Rosenstein, 1986). When the pH increases from 6.5 to 9.0, the soluble polyvalent ions become supersaturated, and this occurs when ammonia is released by microbial urease-catalyzed urea hydrolysis. This alkalinization results in the stone crystallization. *Proteus mirabilis* is the primary urease-producing pathogen in humans (Rubin, Tolkoff-Rubin, & Cotran, 1986). The role of *Proteus* urease in pyelonephritis by using both a rat model and a tissue culture system (Braude & Siemienski, 1960) has been studied. *Proteus mirabilis* (urease source) plays role in the etiopathogenesis of Rheumatoid arthritis **(**RA). It has been determined that the presence of HLA antigens and elevated *Proteus* antibodies in RA helps in the identification of patients with these conditions during early stages of disease (Rashid & Ebringer, 2007).

Bacillus pasteurii

Bacillus pasteurii urease (BPU) also induces aggregation of rabbit platelets, similar to the canatoxin-induced effect (ED_{50} 0.4 and 0.015 mg/mL, respectively). Platelet aggregation induced by BPU is mediated by lipoxygenase-derived eicosanoids and secretion of ADP from the platelets through a calcium-dependent mechanism. Canatoxin, is an isoform of *Canavalia ensiformis* urease, has several biological properties unrelated to its ureolytic activity, like platelet-aggregating and pro-inflammatory effects. Potential relevance of these findings for bacterium-plant interactions and pathogenesis of bacterial infections are known (Olivera-Severo, Wassermann, & Carlini, 2006).

Helicobacter pylori

The bacterium *H. pylori* causes peptic ulcers and gastric cancer in human beings. *H. pylori* produces urease which neutralizes the acidic medium permitting its survival in the stomach **(**Wassermann, Olivera-Severo, Uberti, & Carlini, 2010). *H. pylori* urease (HPU) induces blood platelet aggregation independently of their enzyme activity by a pathway requiring platelet secretion, activation of calcium channels and lipoxygenase-

derived eicosanoids. There is link between *H. pylori* (urease source) infection and Alzheimer's disease. *H. pylori* that is the infectious agent has been proposed as potential cause of Alzheimer's disease (AD). Serum anti-Hp-specific IgG level was analysed by enzyme-linked immunosorbent assay. *H. pylori* positive AD patients were treated with application of urease inhibitors e.g. omeprazole, that eradicates *H. pylori* **(**Kountouras et al., 2006).

Brucella suis

The *Brucella* genomes contain two urease operons designated as *ure*1 and *ure*2 (Contreras-Rodriguez et al., 2008; Bandara et al., 2007). The role of the two *Brucella suis* urease operons on the infection, intracellular persistence, growth, and resistance to low-pH killing has been examined but these findings suggest that only one operon *ure*1 operon is responsible for encoding an active urease and necessary for optimal growth in culture, urease activity, resistance against low-pH killing and *in vivo* persistence of *B. suis* when inoculated by gavage (Bandara et al., 2007). The urease reactivity of sera from patients diagnosed with brucellosis has also been examined (Contreras-Rodriguez et al., 2008).

Helicobacter hepaticus

Helicobacter hepaticus has a strong urease activity that catalyzes the hyrdolysis of urea into bicarbonate and ammonia, both of which neutralize gastric acid and therefore allows the bacteria to colonize the acidic environment of the gastrointestinal tract. *Helicobacter hepaticus* tests positive in both oxidase and catalase tests (Benoit, Zbell, & Maier, 2007).

Bacillus sphaericus

It is naturally-occurring urease positive bacteria found in soil, survives in water that is rich in organics, and lives in the human digestive tract. A whole cell electrochemical lead biosensor has been developed using urease producing *B. sphaericus* isolate (MTCC 5100) used for m**onitoring Lead ions in Milk** (Verma, Kaur, & Kumar, 2011).

Streptococcus salivarius

Streptococcus salivarius is a highly ureolytic organism which is present in large numbers on the soft tissues of the oral cavity. The hydrolysis of urea by urease enzymes of these oral bacteria has major impact on oral microbial ecology and is involved in oral health and diseases. The ability to genetically engineer plaque bacteria that can modulate environmental pH through ureolysis will open the way to using recombinant *Streptococcus salivarius* to test hypotheses regarding the role of oral ureolysis in dental caries, calculus formation, and periodontal diseases. Such recombinant organisms may eventually prove useful for controlling dental caries by replacement therapy (Chen, Clancy, & Burne, 1996).

Cryptococcus neoformans

Fungal *Cryptococcus neoformans* is a urease positive organism and catalyzes the hydrolysis of urea to ammonia and carbamate and has been found to be an important pathogenic virulence factor for certain bacteria. This fungus *Cryptococcus neoformans* is significant in human pathogenicity, produces large amounts of urease and the role of urease in the pathogenesis of *cryptococcosis* has been investigated **(Cox et al.,** 2000).

Plant urease

Urease activity is also found in plants. There are different plant sources for the urease enzyme, as described below. Besides the presence of ureases in plants, their physiological roles have also been known. Urease catalyzes the conversion of urea into ammonia, which is subsequently assimilated by the plant via glutamine synthesis. The main function of plant ureases is thought to be related to nitrogen recycling from urea either formed endogenously or derived from external sources (Sirko & Brodzik, 2000; Follmer, 2008). Urease also has a fundamental role in recycling exogenous urea used as fertilizer (Witte et al., 2002).

Canavalia ensiformis (Jack bean)

Makham Sem is the local name of *Canavalia ensiformis*. Jack bean urease enzyme was the first crystallized enzyme and demonstrated that this enzyme could be used as an antigen (Kirk & Sumner, 1934) having ability to stimulate a strong immunoglobulin response. Sera collected from rabbits immunized with the purified enzyme were shown to inhibit urease activity. Thus, it has been described (Leveen et al., 1973; Thomson & Visek, 1963) 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. The synergistic inhibition of *H. pylori* urease activity has also been described by two monoclonal antibodies raised against the purified enzyme (Nagata et al., 1992).

Cucumis melo (Muskmelon)

Herbs and botanicals offer a natural safeguard against the development of certain conditions and be a putative treatment for some diseases like the lowering of blood pressure in those where it is elevated (i.e. hypertension). Therefore, there is increasing interest in the health and wellness benefits of herbs and botanicals. Studies have been made to identify extracts that promote diuresis and species from the genuses *Cucumis (Cucumis melo* and *Cucumis trigonus*) are found to be very effective (Wright, Van-Buren, Kroner, & Koning, 2007). Kharbooza and Banggi are common names of *Cucumis melo***.** *Cucumis melo* is a plant source of urease enzyme. Urease was extracted and purified from the seeds of *Cucumis melo* (Prakash, Talat, Hasan, & Pandey, 2008). *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 (Wright, Van-Buren, Kroner, & Koning, 2007).

Gossypium hirsutum (Cotton)

Rui, kapas and Narma are the local names of *Gossypium hirsutum*. Cotton seed urease has low ureolytic activity but exhibits potent antifungal properties at sub-micromolar concentrations against different phytopathogenic fungi (Menegassi et al., 2008). The fungitoxic effect of urease is independent of ureolysis, since enzymatically inactivated ureases (obtained after pretreatment with p-hydroxy-mercurybenzoate, an irreversible and covalent inhibitor) are still fully active (Becker-Ritt et al., 2007). Thus, it has been suggested that this urease plays an important role in plant defense.

Conclusion

Thus, urease enzyme from microbial sources has a significant role towards human pathogenecity and plant urease enzyme is used as a vaccine on the basis of its catalytic inhibition activity for protection against microbial infection. Plant ureases can thus be applied for the treatment of many health disorders like gastrointestinal infection and hypertension as discussed in the above article.

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