REVIEW ARTICLE

An overview of urease and its relation to the ureolytic bacteria and the search for new urease inhibitors

Ureasa a její vztah k ureolytickým bakteriím a hledání nových inhibitorů ureasy

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Summary

In this review, an overview of the available literature on urease is presented. Urease is an enzyme which catalyzes the hydrolysis of urea. The occurrence of ureases and their functions are discussed thoroughly. The relationship of urease to ureolytic bacteria is examined, and the currently available urease inhibitors, both inorganic and natural, are presented. Finally, the importance of urease and current and future applications of new inhibitors and explored.
Keywords: bacteria • inhibitor • urease

Souhrn

V následujícím souhrnu je uveden přehled dostupné literatury na aktuální téma týkající se enzymu ureasy. Ureasa je enzym katalyzující hydrolýzu močoviny. V přehledu je diskutován výskyt ureasy, její funkce a význam a rovněž role ureasy a ureolytických bakterií v patogenezi různých onemocnění. Jsou prezentovány známé anorganické i přírodní inhibitory ureasy. Práce se zabývá důležitostí hledání nových inhibitorů ureasy a potenciálem přírodních látek v této oblasti.
Klíčová slova: bakterie • inhibitor • ureasa

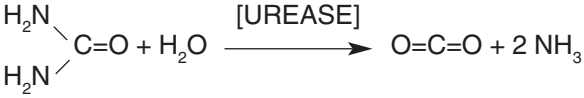
Overview of ureases

Overview

Urease (urea amidohydrolase; EC 3.5.1.5) catalyzes the hydrolysis of urea to produce carbon dioxide and ammonia[1]. Ureases occur in plants, bacteria, fungi, algae and invertebrates[2]. The presence of urease is a known virulence factor for a number of bacteria. *Helicobacter pylori* is a ureolytic bacteria that infects the intestinal tract and causes gastric and duodenal ulcers. The inhibition of urease would prevent these bacteria from alkalizing their environment which is a factor that causes most complications. The well-known inorganic urease inhibitors, hydroxamic acid and its derivatives, have been shown to be reversible, slow-binding inhibitors of both plant and bacterial ureases[1, 3]. Unfortunately, acetohydroxamic acid is associated with severe adverse effects[4]. A number of plant extracts have been investigated for their antiurease activity[5]. In this paper we explore the new advancements in this field and the practical applications, such as treatment of peptic ulcers and urinary stones.

Introduction to ureases

Urease (urea amidohydrolase; EC 3.5.1.5) is an enzyme which catalyzes the hydrolysis of urea. Urea is cleaved by urease to produce one molecule of ammonia and one of carbamate. Carbamate spontaneously decomposes to ammonia and carbonic acid. The carbonic acid equilibrates in water, as do the two molecules of ammonia, which become protonated to yield ammonium and hydroxide ions. The reaction results in a rise in the pH of the reaction environment[6]. This reaction can be summarized as following:



Urea is stable in aqueous solutions. The uncatalyzed reaction is very slow and leads to an elimination reaction that results in isocyanate and ammonia[2].

The general protein structure of ureases

The best studied plant urease is from the jack bean

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(*Canavalia ensiformis*). Although jack bean urease was the first enzyme to be crystallized, its structure is yet to be determined[7]. The protein structure of urease from *Klebsiella aerogenes* was first solved in 1995, and since then several other structures have been determined, including structures from *Bacillus pasteurii* and *Helicobacter pylori*[8].

Plant and fungal ureases are homo-oligomeric proteins of 90 kDa identical subunits, while bacterial ureases are multimers of two- or three-subunit complexes. The bacterial and plant ureases have high sequence similarity. The active-site architecture of jack bean urease is similar to that of bacterial ureases containing a binickel center. Although the amino acid sequences of plant and bacterial ureases are closely related, some biological activities differ significantly[7].

Plant ureases are made up of a single-chain polypeptide in contrast to bacterial ureases, which consist of two or three polypeptides designated as α, β, and γ[7]. Active sites of all known ureases are located in the α subunit[2]. The amino acid sequences of the active site are principally conserved in all known ureases, and the catalytic mechanism of their action is believed to be the same[9].

The occurrence of ureases and their function

Ureases occur in plants, bacteria, fungi, algae and invertebrates[2]. They are produced by bacteria, fungi, yeast, and plants where they catalyze the urea degradation to supply these organisms with a source of nitrogen for growth[10]. In plants (*Canavalia ensiformis, Glycine max*), urease catalyzes urea assimilation after uptake into plant cells and participates in the metabolism of N-containing compounds[2].

The role of ureases in microbial virulence

Urea represents an assimilable nitrogen source for bacteria that can colonize the human body. A significant proportion of urea produced in the liver ends up in the intestines, where it can be hydrolyzed and assimilated by several different species of ureolytic bacteria. Similarly, an abundance of oral bacteria *Streptococcus salivarius* can use urea as a primary nitrogen source of growth. Ammonia release appears to have the greatest impact in terms of pathogenesis. Ureolysis can lead to the formation of ammonium hydroxide, toxic to mammalian cells. The formation of ammonia in the presence of the oxidative burst created by immune cells can lead to the formation of monochloramine, which has been shown to be able to induce DNA damage[6]. The resulting increase in the pH of the reaction environment can reach up to 9.2[11].

H. pylori is a ureolytic bacteria infecting the intestinal tract that causes gastric and duodenal ulcers. The ammonia production increases pH which allows the bacteria to grow in the strongly acidic environment[1]. Ureolytic bacteria, such as *Proteus vulgaris* and *P. mirabilis,* are the most common causes of urinary tract infections. An increase in the pH leads to the formation of urinary stones through the precipitation of normally soluble polyvalent ions present in urine[4, 6]. Additionally, ammonia has a direct cytotoxic effect on epithelial cells[2, 6].

The inhibition of urease prevents these bacteria from alkalizing their environment. The well-known inorganic urease inhibitor hydroxamic acids and its derivatives have been shown to be reversible, slow binding inhibitors of both plant and bacterial ureases[1, 3, 12]. Unfortunately, the use of acetohydroxamic acid is associated with severe side effects[4]. Plant extracts or natural compounds would be a better choice, because they are relatively free of side effects and well tolerated.

Bacteria and its relation to urease inhibition

Helicobacter pylori

H. pylori is a helix-shaped Gram-negative bacterium. It requires oxygen for its growth, but at a lower concentration than is found in the atmosphere. Besides urease it also produces oxidase and catalase[2, 3, 13]. At least half of the world's population is infected by the bacterium, making it the most widespread infection in the world[14]. *H. pylori* persists in the stomach for decades in most people without showing any clinical symptoms. Approximately 10–20% of those colonized by *H. pylori* will develop gastric or duodenal ulcers. *H. pylori* has been proved to be the main cause of peptic ulcers, urease deficient *H. pylori* strain was not able to colonize gastric mucosa[3]. The bacterium also induces chronic gastritis, a long- lasting inflammation of the stomach. 1 to 2% of people with chronic *H. pylori* infection will develop stomach cancer[15].

The survival of *H. pylori* in the acidic stomach is dependent on urease. The ammonia is converted to ammonium by taking a proton from water, which leaves only a hydroxyl ion. Hydroxyl ions then react with carbon dioxide, producing bicarbonate which neutralizes gastric acid[16].

Once *H. pylori* is detected in patients with a peptic ulcer, the normal procedure is to eradicate it and allow the ulcer to heal. To prove the presence of *H. pylori*, various diagnostic tests are available, and most of them have high sensitivity and specificity. Invasive tests include endoscopy and histology, which is followed by a rapid urease test, *H. pylori* culture and antibiotic susceptibility testing or molecular methods. Endoscopy can be used as a functional imaging technique to detect mucosal barrier defects in patients with *H. pylori* infection. The most promising high-resolution imaging technologies include high-resolution microendoscopy, optical coherence tomography, endocytoscopy, and confocal laser endoscopy. None of these techniques are widely available nor are they specific enough at present to obtain a real-time diagnosis of *H. pylori* infection[17]. Histological evaluation of antral biopsy specimens can discover acute and chronic inflammation[18]. Currently, the conventional Giemsa staining (the differential stain that can be used to study the adherence of pathogenic bacteria to human cells) is the most widely used technique[17]. Rapid Urease Test can be performed when a biopsy of mucosa is placed into a medium containing urea and phenol red as an indicator. The urease produced by *H. pylori* hydrolyzes urea to ammonia, which raises the pH of the medium, and changes the colour of the specimen from yellow (negative) to red (positive)[17]. The urea breath test using ^{13}C-labelled urea remains the best non-invasive test to diagnose *H. pylori* infection. A patient swallows urea labelled with either radioactive carbon-14 or non-

radioactive carbon-13. The detection of isotope-labelled carbon dioxide in exhaled breath indicates that the urea was split, which indicates that urease is present in the stomach, and hence that *H. pylori* bacteria are present[17]. The monoclonal stool antigen test is a suitable and widely available test for the primary as well as for post-treatment diagnosis of *H. pylori* infection[17]. Serological evaluation for immunoglobulin G and IgA antibodies to *H. pylori* is also a frequently used non-invasive method with high specificity[18].

The standard first-line therapy is a one week triple-therapy consisting of a proton pump inhibitors such as omeprazole, lansoprazole and the antibiotics clarithromycin and amoxicillin or metronidazole. Treatment success with this therapy has fallen below 80%, largely due to clarithromycin or metronidazole resistance[15, 19].

An increasing number of infected individuals are found to harbour antibiotic-resistant bacteria. This results in initial treatment failure and requires additional rounds of antibiotic therapy or alternative strategies. Additionally, many antibiotics possess adverse effects that decrease patient compliance. Rising microbial resistance and adverse effects of antibiotics increase the need for new antibacterial agents that can be found among natural products.

Other urease producing bacteria and their relation to urease inhibition

The most clinically significant bacteria producing urease are *Proteus, Klebsiella, Pseudomonas* and *Staphylococcus*. The main cause of urinary tract infection is *Escherichia coli* and *Proteus vulgaris*. Urinary stones are a consequence of urinary tract infection in 10–15% of patients with stone disease and their formation is one of the most troublesome complications of urinary tract infection[4, 20].

Compounds present in urine that are soluble at a normal pH (5.8–6.0) precipitate and form urinary stones when urine becomes alkaline (7.2) due to the urease activity. Stones caused by urinary tract infection are mainly composed of magnesium ammonium phosphate (struvite), carbonate apatite and mono-ammonium urate[4].

Klebsiella pneumoniae and *K. oxytoca* is a Gram-negative rod-shaped bacteria and its infection can lead to a wide range of disease states: urinary tract infections, respiratory tract infections, pneumonia[21]. *Corynebacterium urealyticum*, a Gram-positive rod was also found to be a cause of struvite stones formation[22].

The ammonia produced by urease activity is directly toxic to the epithelial cells of the urinary tract. Crystals can easily adhere and become a source of stone formation. If the infection is not treated, a continual source of the component ions is provided, allowing the stones to grow very rapidly and also serve as a reservoir for bacteria. They are present inside the stones and deteriorate the condition even more[4].

Eradication of bacterial infection is necessary and stones have to be removed. Surgery, such as percutaneous nephrolithotomy or extracorporeal shock wave lithotripsy (stones are fragmented by shock waves) is required[4]. Urease inhibitors could play an important role in the prevention of early stage of urinary tract infection treatment and, in combination with antibiotics, in the eradication process. Therefore, it is necessary to continue looking for safe and effective inhibitors of urease.

Urease inhibitors

Ureases are inhibited by a number of compounds. They were studied or designed for following purposes:

1. The therapy of bacterial infections causing peptic ulcers (*H. pylori*) or urinary stones (*Klebsiella, Proteus*).
2. The protection of soil from pH elevation and the loss of nitrogen after the use of urea as a fertilizer.
3. Manure odour control in livestock production facilities. Urease inhibitors can be used to reduce urea hydrolysis in cattle feedlot pens, conserve nitrogen, and inhibit ammonia emission that contributes to odour[23].

Inorganic compounds

Urease inhibitors can be classified into two categories: substrate structural analogues and inhibitors that affect the mechanism of reaction[3]. Substrate analogues, such as hydroxyurea and N-substituted hydroxyurea derivatives are weak inhibitors of urease[2, 24]. Inhibitors affecting the mechanism of reaction can be further divided by chemical structure into the following groups: thiols, hydroxamic acid and its derivatives, phosporamide compounds and nickel chelators[3].

Thiols inhibit urease in their thiolate anion form. They react directly with the metallocenter of urease – the S-atom bridges the Ni ions and reduces the distance between two nickel atoms. A similar mechanism was found for acetohydroxamic acid and its derivatives. The bridging of Ni atoms is provided by the acidic hydroxamate oxygen and the carbonyl oxygen ligating the Ni atom[2] (Krajewska, 2009). Hydroxamic acids are reversible, slow binding inhibitors of both plant and bacterial ureases[3, 12]. The use of acetohydroxamic acid is associated with severe adverse effects, such as dermatological, neurological and hematological[4]. Amides and esters of phosphoric acid are classified as the strongest inhibitors. The inhibition is always caused by a product of their hydrolysis – diamidophosphate[2]. *N,N*-dimethylaminomethane-P-methylphosphinic acid was the most active compound of all tested derivatives[25]. Heavy metal ions inhibit urease with different efficiency. The fluoride ion was found to be active against both plant and bacterial urease and classified as a competitive inhibitor[2].

Natural compounds or plant extracts

A number of plant extracts and natural compounds have been investigated for their antiurease activity. A lot of plant extracts, fractions and pure compounds from various sources have been proven to possess an inhibitory potential. Current efforts are focused on seeking new urease inhibitors with good availability and low toxicity. The number of research papers increases every week and some findings and results are controversial. The following chapter gives a brief preview on what has been done so far among natural products.

The most frequent groups of secondary metabolites that have been found responsible for the antiurease activity are

flavonoids and tannins. It only makes sense that plants or plant extracts with high flavonoid and tannin content show antiurease activity. Some examples include flavonoid and tannin rich extracts from *Geranium robertianum*, *Helleborus purpurascens* and *Hyssopus officinale*[26], flavonoids from *Allium cepa* and *Psidium guajava*[27] or catechins from green tea[28].

Various types of constituents were found to be active against urease: oxindole alkaloids from *Isatis tinctoria*[29], stilbene resveratrol[30], xanthones from *Hypericum oblongifolium*[31] or quinones such as juglone[32]. Fresh garlic extract inhibited jack bean urease due to the presence of alk(en)yl thiosulfinates. Alk(en)yl thiosulfinates are the primary components of a fresh garlic extract and are produced from alk(en)ylcysteine sulfoxides in the enzymatic reaction after crushing the garlic[33]. Phytochemical investigations on the roots of *Ranunculus repens* led to the isolation of methyl gallate, R(+)-4-methoxydalbergione and R(+)-dalbergiophenol. These compounds showed a potent inhibitory activity against *Bacillus pasteurii* urease and jack bean urease. The authors suggested that the inhibitory activity depends on the number of hydroxyl groups (the more hydroxyl groups, the better the inhibition)[34]. Even a diterpene ester that was isolated from *Euphorbia decipiens* showed an inhibitory activity against jack bean urease[35]. Benzoquinone and naphtoquinone derivatives have also been reported to have urease inhibitory activity. Inactivation of urease by quinones is concentration dependent. They act through arylation of thiols in the urease and this results in conformational changes in the enzyme molecule[36]. Twenty synthetic polyphenols were evaluated for their inhibitory activity against *H. pylori* urease. The structure-activity relationship was investigated. Compounds with catechol skeleton exhibited potent inhibitory activities and the authors came to a conclusion that the two ortho hydroxyl groups presented on the aromatic ring may be responsible for inhibitory activity and coordinate nickel ions in the active site of urease[37]. Replacing one of the hydroxyl groups on the catechol skeleton by a methoxy group lead to partial or complete loss of activity. Any compounds with a resorcinol skeleton showed no or extremely weak inhibitory activity. The long distance between hydroxyl groups probably prevented an interaction with the active site. Inhibitors based on isoflavones showed a significant activity and it was revealed that the carbonyl group was critical to the inhibitory effect[37].

Potential other promising role of urease

Urease in the therapy of cancer

A potential new targeted drug product for the treatment of adenocarcinoma of the lung, the most common form of cancer in the world today, has been designed. An immunoconjugate L-DOS47 consists of a lung adenocarcinoma specific single domain antibody and urease enzyme. In order to specifically target the tumour, each L-DOS47 molecule has, in addition to its urease core, a number of linked antibody fragments that specifically recognize lung adenocarcinoma cells. After an intravenous injection of L-DOS47, molecules concentrate on the extracellular surface of lung adenocarcinoma cells. Once attached to the cancer cells, L-DOS47 rapidly produces ammonia through urea hydrolysis. By doing so at the site of cancerous tissues in the body, L-DOS47 is believed to modify the micro environmental conditions of the lung cancer cells in a manner that leads to their death. The ammonia molecules are believed to react with naturally occurring water to release hydroxyl ions and cause an increase in the pH of the tumour environment reversing the acidic extracellular conditions that are known to be necessary for cancer cell survival. In addition, ammonia is believed to diffuse into the cancer cells and exert its direct cytotoxic effect[38].

Conclusion

This work presents a conclusive thorough review of the available literature on the enzyme urease and its inhibitors. There are many prospective uses of the inhibitors of ureases including the treatment of peptic ulcers and urinary stones. Potential applications of this enzyme can be used in the treatment of certain types of cancer. For these reasons and others, there is an urgent need to screen more plants to isolate and identify new natural urease inhibitors and work to develop and improve the existing ones.

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Conflicts of interest: none.

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