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Ureases display biological effects independent of enzymatic activity. Is there a connection to diseases caused by urease-producing bacteria?

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Abstract

Ureases are enzymes from plants, fungi and bacteria that catalyze the hydrolysis of urea to form ammonia and carbon dioxide. While fungal and plant ureases are homo-oligomers of 90-kDa subunits, bacterial ureases are multimers of two or three subunit complexes. We showed that some isoforms of jack bean urease, canatoxin and the classical urease, bind to glycoconjugates and induce platelet aggregation. Canatoxin also promotes release of histamine from mast cells, insulin from pancreatic cells and neurotransmitters from brain synaptosomes. *In vivo* it induces rat paw edema and neutrophil chemotaxis. These effects are independent of ureolytic activity and require activation of eicosanoid metabolism and calcium channels. *Helicobacter pylori*, a Gram-negative bacterium that colonizes the human stomach mucosa, causes gastric ulcers and cancer by a mechanism that is not understood. *H. pylori* produces factors that damage gastric epithelial cells, such as the vacuolating cytotoxin VacA, the cytotoxin-associated protein CagA, and a urease (up to 10% of bacterial protein) that neutralizes the acidic medium permitting its survival in the stomach. *H. pylori* whole cells or extracts of its water-soluble proteins promote inflammation, activate neutrophils and induce the release of cytokines. In this paper we review data from the literature suggesting that *H. pylori* urease displays many of the biological activities observed for jack bean ureases and show that bacterial ureases have a secretagogue effect modulated by eicosanoid metabolites through lipoxygenase pathways. These findings could be relevant to the elucidation of the role of urease in the pathogenesis of the gastrointestinal disease caused by *H. pylori*.

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- Eicosanoids

Introduction

Ureases (urea amidohydrolase; EC 3.5.1.5) are nickel-dependent enzymes (1) that catalyze the hydrolysis of urea to form ammonia and carbon dioxide. They have been isolated from a wide variety of organisms including plants, fungi and bacteria. While fungal and plant (e.g., jack bean and soybean) ureases are homo-oligomeric proteins of ca. 90 kDa subunits, bacterial ureases are multimers of two or three subunit complexes (Figure 1) (2,3). Amino-terminal residues of the monomers of plant and fungal enzymes

are similar in sequence to the small subunits of bacterial enzymes (e.g., UreA and UreB of *Klebsiella aerogenes*). The large subunits of bacterial ureases (e.g., UreC of *K. aerogenes*) resemble the carboxy-terminal portions of plant and fungal subunits. So far only bacterial ureases have had their 3-D crystallographic structure successfully resolved, e.g., *K. aerogenes* (1FWJ), *Bacillus pasteurii* (4UBP), and *Helicobacter pylori* (1E9Z) (2, 3). However, the high sequence similarity of all ureases indicates they are variants of the same ancestral protein and are likely to possess similar tertiary structures and catalytic mechanisms (2,3). Despite their highly conserved structures and enzymatic action, little is known about the physiological role of ureases in the source organisms. Urease activity enables bacteria to use urea as a sole nitrogen source. Some bacterial ureases play an important role in the pathogenesis of human and animal diseases such as those from *Proteus mirabili*s and *H. pylori* (2).

The wide distribution of ureases in leguminous seeds as well as the accumulation pattern of the protein during seed maturation suggest an important physiological role. Soybean mutants lacking the embryo-specific highly active isoform of urease do not exhibit any of the abnormalities associated with loss of the less active ubiquitous isoform, suggesting that this enzyme probably does not have an essential physiological role (4). *In vitro* cultures of developing soybean cotyledons have indicated that ureases do not play an important role in embryo nutrition since urea is an extremely poor nitrogen source (4). The obvious question from this observation is why would the developing soybean embryo invest in producing a very active ureolytic protein when it usually does not "encounter" urea. Polacco and Holland (4) have proposed that plant ureases may have a role in plant defense against predators due to the high toxicity of the ammonia released.

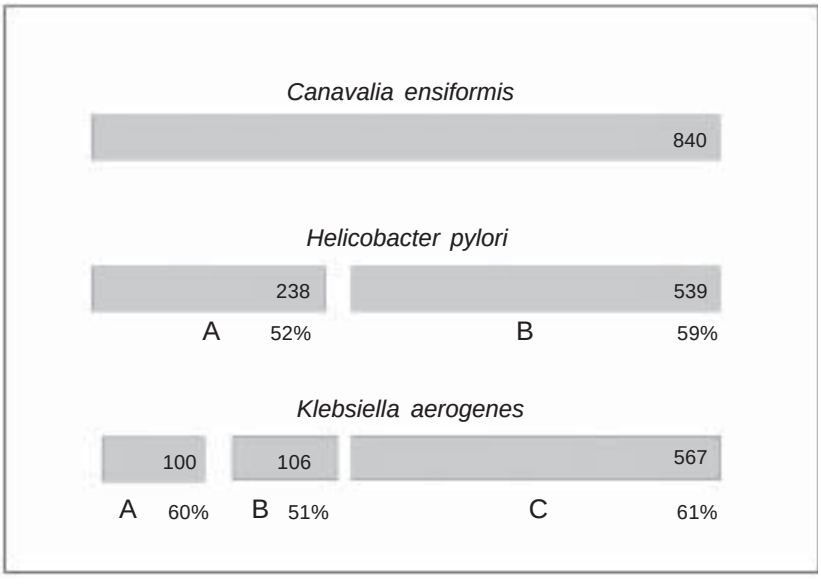

Figure 1. Schematic structure of ureases. Plant ureases such as the jack bean (*Canavalia ensiformis*) enzyme have a single subunit while bacterial ureases have two (*Helicobacter pylori*) or three (*Klebsiella aerogenes*) subunits. The number of amino acid residues of each subunit is indicated. Note that the length of each box is not drawn to scale of total amino acids. The percentage of identity to the corresponding fragment of the jack bean urease is shown under the bacterial proteins.

Plant-derived ureases: canatoxin and jack bean urease

The jack bean *Canavalia ensiformis* is the source of interesting proteins which have contributed significantly to modern Biochemistry. One of these is no doubt urease, the first protein ever crystallized (5), and the first nickel-containing enzyme described (1). In 1981, we isolated a toxic protein, named canatoxin, which accounts for 0.5% of seed dry weight of jack beans (6). Canatoxin is lethal to rats and mice by intraperitoneal injection, but it is inactive when given orally (7). Canatoxin, which consists of a noncovalently linked dimer of 95-kDa acidic polypeptide chains, was characterized recently as a variant form of the classical more abundant jack bean urease (8). RT-PCR applied to mRNA isolated from *C. ensiformis* tissues and Southern blots confirmed the presence of a family of urease-related genes with at least two members sharing 86% similarity (9). Jack bean ureases presented differential behavior in immobilized metal affinity chro-

matography enabling separation of the isoenzymes (8,10). A particle-induced X-ray emission technique applied to determine the metal content of the isoforms showed that canatoxin displays ca. 1 atom of nickel and 1 of zinc per monomer, contrasting with 2 atoms of nickel and absence of zinc in the monomer of the major form of urease in *C. ensiformis* (8,11).

Insecticidal properties of plant ureases

The insecticidal properties of plant ureases were first described for canatoxin (12) and later for *C. ensiformis* major urease and soybean embryo-specific urease (13). The kissing bug *Rhodnius prolixus*, and three economically important crop pests, the cowpea weevil *Callosobruchus maculatus*, the green stinkbug *Nezara viridula* and the cotton stainer bug *Dysdercus peruvianus*, are susceptible to the lethal effect of these proteins when they are added to their diets at 0.02 to 0.1% (w/w) levels (14,15). Susceptible insects have cathepsins of type B and D as their main digestive enzymes. Canatoxin and urease are hydrolyzed by these enzymes to release an internal entomotoxic peptide of 10 kDa (16). No effects of intact canatoxin/urease were seen in insects relying on trypsin-like digestive enzymes, which apparently degrade the proteins more extensively (12). A recombinant peptide, equivalent to that produced by hydrolysis of canatoxin with insect cathepsins, was obtained by heterologous expression in *Escherichia coli*. This peptide presented potent insecticidal effects (17) and did not affect mice or neonate rats upon oral or intraperitoneal administration. In contrast, the urease from the soil bacterium *B. pasteurii* is devoid of insecticidal properties, as expected from its three-chain structure, since part of the sequence of the entomotoxic peptide is absent in microbial ureases. In plant ureases this corresponds to a fragment located between the UreB and UreC chains of *B. pasteurii* urease (13).

Taken together, our results indicate that plant ureases are probably involved in defense mechanisms of plants against insect predation and that their insecticidal properties are independent of their enzymatic activity, being associated with an internal peptide of these proteins.

Biological properties of canatoxin and similarities with other ureases

Canatoxin administered intraperitoneally to rats or mice (LD_{50} 0.4-0.6 and 2-3 mg/kg, respectively) induces respiratory distress, convulsion, and death (6,18). At subconvulsant doses canatoxin promotes increased gonadotropin (19) and plasma insulin levels (20) and pro-inflammatory effects in rats (21). *In vitro*, canatoxin displays potent secretagogue activity at nanomolar doses in several isolated cellular systems, inducing platelet secretion and aggregation (22,23), secretion of labeled dopamine and serotonin from rat brain synaptosomes (23), histamine release from mast cells (24), and secretion of insulin from isolated pancreatic islets (23,25). Thus, canatoxin induces dose-dependent aggregation of platelets from different species at concentrations as low as 20 nM (22). Rat isolated pancreatic islets secrete insulin when exposed to canatoxin (1 µM), making this protein about 20,000-fold more potent than glucose in provoking insulin release (25). Most of these effects, either *in vivo* or *in vitro*, apparently involve activation of arachidonic acid metabolism mainly through the lipoxygenase pathway, since they are blocked by lipoxygenase inhibitors such as nordihydroguaiaretic acid and esculetin, but not by cyclooxygenase inhibitors (Table 1) (21-23,25,26). Pretreatment of animals with lipoxygenase inhibitors protected them also against the lethal effect of canatoxin (26).

Canatoxin was shown to disrupt Ca^{2+} transport by the Ca^{2+},Mg^{2+}-ATPase of sarcoplasmic reticulum membrane vesicles (27)

and to alter Ca^{2+} flux across the plasma membrane of platelets through a verapamil-inhibitable Ca^{2+} channel (28). Canatoxin does not activate phospholipase C, and the intracellular calcium mobilization mediated by inositol 1,4,5-triphosphate does not play a role in platelet activation by this toxin. Preincubation of platelets with 8-bromo-guanosine 3',5'-cyclic monophosphate inhibited the canatoxin-evoked calcium influx, arachidonate release, ATP secretion, and cell aggregation, showing that the calcium influx is an early step in the mechanism of platelet activation by canatoxin, being modulated by cGMP (28).

Canatoxin displays pro-inflammatory activity (21). Thus, intraplantar injection of 50-300 µg canatoxin induced a dose-dependent rat hind-paw edema characterized after 3 h by an intense cellular infiltration at the site of administration. Pharmacological studies suggested that canatoxin-induced edema is a phenomenon mediated by several components. Initially histamine, serotonin, platelet aggregating factor, and prostaglandins play a role as agonists while lipoxygenase metabolites, probably leukotrienes, may account for the development of an intense cellular infiltration at the inflammatory site. Canatoxin also induced neutrophil migration into rat peritoneal and pleural cavities and into air pouches (29). This effect was dependent on the resident macrophage population and was inhibited by glucocorticoids but not by non-steroidal anti-inflammatory drugs. It has also been shown that rat macrophage monolayers treated with canatoxin release a neutrophil chemotactic factor (29). Mouse peri-

Table 1. Modulation of canatoxin-induced effects by inhibitors of the lipoxygenase pathway.

Model/Effect	Canatoxin EC_{50}	Inhibitor	Dose	% inhibition	Ref.
Rabbit platelets					
Aggregation	300 nM	NDGA	0.52 mM	50	22
		ETYA	0.02 mM	50	22
		BW755C	0.05 mM	50	22
5-HT secretion	300 nM	NDGA	0.5 mM	75	23
		Esculetin	0.1 mM	85	23
Rat brain synaptosomes					
5-HT secretion	500 nM	NDGA	0.2 mM	90	23
		Esculetin	0.1 mM	90	23
Dopamine secretion	2 µM	NDGA	0.5 mM	42	23
Rat pancreatic islets					
Insulin secretion	200 nM	NDGA	0.2 mM	76	25
		Esculetin	0.1 mM	36	25
Rat mast cells					
Histamine secretion	0.5 mM	Not tested	–	–	24
Mouse macrophages					
Release of lysosomal enzymes	0.3 mM	NDGA	0.15 mM	No inhibition	#
Rat - *in vivo*					
Hypoglycemia	0.4 mg/kg	NDGA	125 mg/kg	100	26
		Esculetin	125 mg/kg	100	26
Hyperinsulinemia	0.4 mg/kg	NDGA	125 mg/kg	100	20
Hypoxia	0.4 mg/kg	NDGA	125 mg/kg	72	26
		Esculetin	125 mg/kg	50	26
Paw edema	0.3 mg/paw	NDGA	100 mg/kg	66	21
		Esculetin	50 mg/kg	No inhibition	21
Convulsions	0.4 mg/kg	NDGA	125 mg/kg	75	26

5-HT = 5-hydroxytryptamine; NDGA = nordihydroguaiaretic acid; ETYA = eicosatetraynoic acid; BW755C = 3-amino-l-[m-(trifluoromethyl)-phenyl]-2-pyrazoline. #Ghazaleh FA, unpublished results.

toneal macrophages release lysosomal enzymes when exposed to canatoxin through a pathway involving nitric oxide (NO) signaling and guanyl cyclase activation (Ghazaleh FA, unpublished data).

In addition to lethality to insects, jack bean urease unexpectedly also displays other relevant biological properties observed for canatoxin, such as activation of blood platelets and monovalent lectin activity, but no toxicity when administered intraperitoneally to mice, probably due to its larger size (540 kDa as opposed to 185 kDa) (8). Canatoxin as well as urease interact with polysialogangliosides (GD1b and GT1b) and sialoproteins (mucin, tireoglobulin, fetuin) on the surface of erythrocytes and in ELISA microplates (7,8). This property of binding carbohydrates probably "directs" the proteins to cell surfaces enriched with this type of glycoconjugates and may provide an explanation for their selective tissue specificity. Pretreatment of the proteins with the thiol oxidant *p*-hydroxymercuribenzoate (pHMB) irreversibly abolished the ureolytic activity of urease (IC_{50} 0.5 mM) and of canatoxin (IC_{50} 5 mM) (8). In contrast, pHMB-treated canatoxin or urease was still fully active to promote platelet aggregation and binding to glycoconjugates. Moreover, the intraperitoneal toxicity of canatoxin was also not affected by pHMB treatment, indicating that these biological effects are not related to the enzymatic activity (8,10,13).

In order to determine if ureases from other sources share with jack bean ureases the property of inducing biological effects independent of their ureolytic activity, soybean embryo-specific urease and *B. pasteurii* urease (30) were tested in rabbit platelets. Both ureases induced platelet aggregation even after being treated with pHMB (13). Furthermore, purified recombinant *H. pylori* urease also displays platelet aggregating activity (Figure 2) (Wassermann GE, unpublished data).

The pattern of platelet response to all the ureases tested so far was very similar, with a collagen-type shape-change reaction. As is the case for canatoxin (22,23), platelet aggregation induced by *B. pasteurii* or *H. pylori* ureases also depends on lipoxygenase-derived metabolites. Thus, treatment of platelets with indomethacin, a cyclooxygenase inhibitor, potentiates urease-induced aggregation while the lipoxygenase inhibitor esculetin blocks platelet responses to the microbial enzymes (Figure 3).

Taken together, our data indicate that plant and microbial ureases form a group of multifunctional proteins with at least two distinct domains: 1) a thiol-dependent domain containing the ureolytic active site, and 2) thiol-independent domain(s) involved in toxic effects on insects (and mice, only for canatoxin), binding to glycoconjugates and in the activation of blood platelets.

In addition to the platelet aggregating activity found for *B. pasteurii* and *H. pylori*, these and other bacterial ureases may share other biological activities and pro-inflammatory properties with canatoxin, including the ability to activate lipoxygenases and the metabolism of eicosanoids. If true, these considerations could change our present understanding of the pathogenesis of some diseases caused by urease-producing bacteria, such as urolithiasis due to *Proteus mirabilis* or gastric ulcers consequent to *H. pylori* infection.

Helicobacter pylori, its urease and its implications in gastric diseases

H. pylori is a micro-aerophilic spiral-shaped Gram-negative bacterium with polar flagella that colonizes the human stomach mucosa (31,32). The presence of this bacterium has now been established as the main risk factor in the development of diseases such as duodenal and stomach ulcers, gastric carcinomas and lymphomas (33,34). Gastric cancer is the first or second most common cancer in many developing countries, affect-

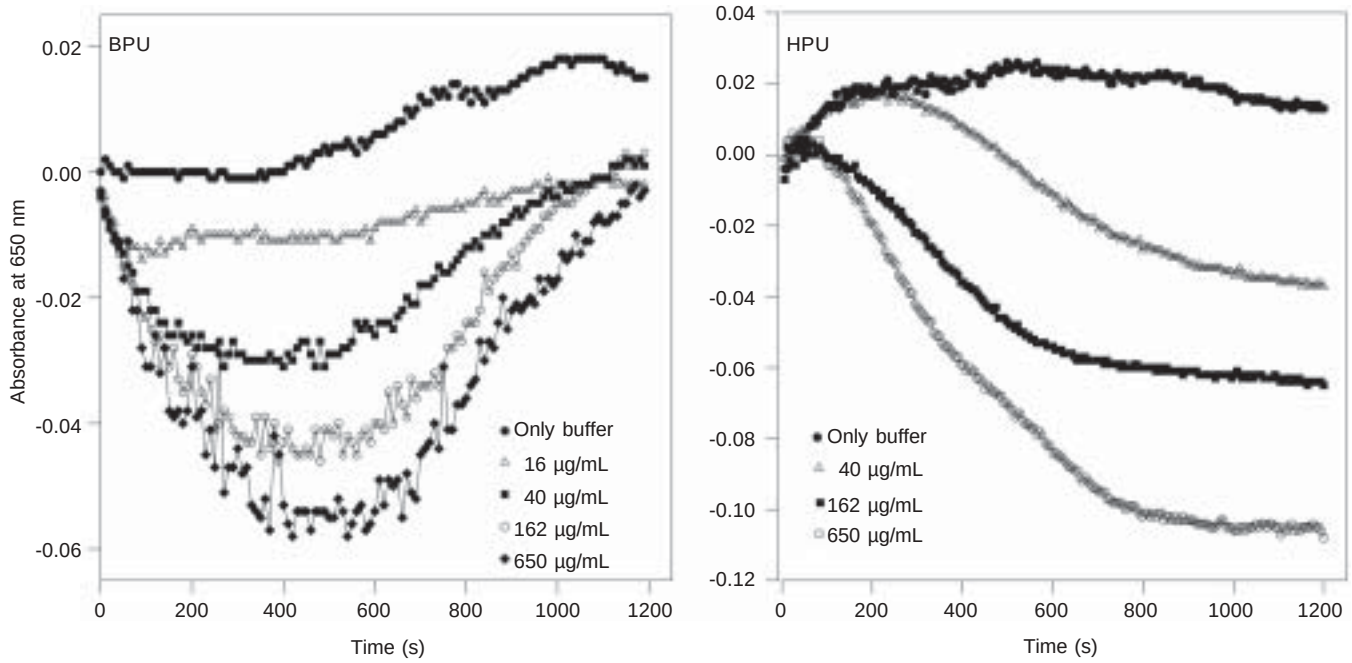

Figure 2. Dose-response curve of platelet aggregation induced by purified *Bacillus pasteurii* (BPU, left panel) and *Helicobacter pylori* ureases (HPU, right panel). Rabbit platelet-rich plasma suspensions were challenged with different concentrations of purified *B. pasteurii* (Sigma, St. Louis, MO, USA) or recombinant *H. pylori* (Ref. 44) ureases. The decrease in absorbance at 650 nm indicating platelet aggregation was monitored every 11 s for 20 min with a plate reader (13). Typical experiments are shown.

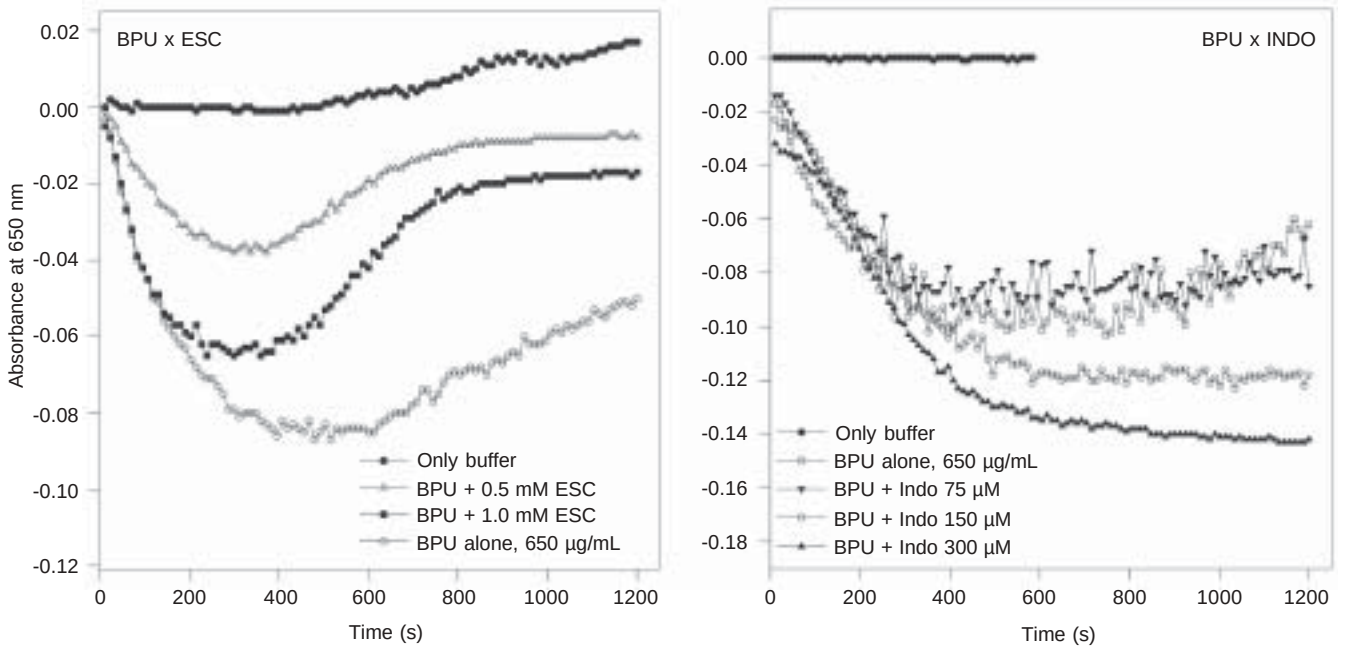

Figure 3. Effect of inhibitors of arachidonate metabolism on platelet aggregation induced by *Bacillus pasteurii* urease. Rabbit platelet-rich plasma suspensions were pretreated for 2 min with the indicated final concentrations of esculetin (ESC, left panel) and indomethacin (INDO, right panel) and then exposed to 0.7 mg/mL *B. pasteurii* urease (BPU; Sigma). The decrease in absorbance readings at 650 nm indicating platelet aggregation was monitored every 11 s for 20 min with a plate reader (13). Typical experiments are shown.

ing 40 to 60 persons per 100,000 yearly. Population-based intervention studies have shown the beneficial effect of early treatment of *H. pylori* in the prevention of gastric cancer. Notably, the most common lymphoma of the stomach, mucosa-associated lymphoid tissue (MALT) lymphoma, is strongly associated with *H. pylori* infection and *H. pylori* eradication therapy is today the widely accepted initial treatment of stage I gastric MALT lymphoma, leading to remission rates of as much as 70% (34).

H. pylori is estimated to infect half of the world's population, with peaks of 90% of the population infected in countries with poor sanitary conditions and of low socioeconomic level (33,34). The prevalence of the organism in the Brazilian population ranges from 34% among urban children in the richer Southeast region to over 82% among adults in the poorer Northeast region (35,36). For the discovery of *H. pylori* and demonstration of its association with gastric disease, the Australian gastroenterologists Warren and Marshall (31) received the 2005 Nobel Prize in Medicine.

H. pylori-induced gastroduodenal disease depends on the inflammatory response of the host and on the production of main virulence factors, such as the vacuolating cytotoxin VacA and the cytotoxin-associated protein CagA, and of urease, all of which cause damage to gastric epithelial cells (37-39). *H. pylori* resides within the mucus and on the apical surface of epithelial cells, where it attaches firmly via adhesin molecules. All *H. pylori* isolates, as well as each of the gastric *Helicobacter* species identified to date, produce large quantities of the enzyme urease, which may account for 10-15% of the bacterial protein. Urease and urea influx through UreI, a pH-gated urea channel, have been shown to be essential for gastric colonization and for acid survival *in vivo*. Intrabacterial urease generation of ammonia and a membrane-anchored periplasmic carbonic anhydrase regulate inner membrane potential and periplasmic pH to approximately 6.1 under acidic conditions, allowing adequate bioenergetics for survival and growth (40). Upon bacterial autolysis urease is released and adsorbed onto the extracellular surface of viable bacteria where it represents about 30% of the total cell urease content (38-39).

The native urease of *H. pylori* has a molecular mass of approximately 540 kDa and is a nickel-containing hexameric molecule consisting of two subunits, UreA (26.5 kDa) and UreB (60.3 kDa), in a 1:1 molar ratio (41,42). The residues involved in the coordination of the two active site Ni^{2+} ions are completely conserved between *H. pylori* and *K. aerogenes* ureases (42). The circular genome of *H. pylori* encodes about 1500 genes, depending on the strain (43). The biosynthesis of urease is coordinated by a gene cluster composed of two structural genes encoding the UreA and UreB subunits and five accessory proteins which are responsible for Ni^{2+} uptake and insertion into the active site of the apoenzyme (44). Active recombinant *H. pylori* urease was produced in *E. coli* transformed with pHP8080, a plasmid encoding the whole operon with the two subunit structural genes and the NixA nickel transporter (44).

Pathogenesis of gastroduodenal diseases and *Helicobacter pylori*

The exact mechanisms by which *H. pylori* contributes to the development of gastroduodenal injury is unclear. Studies have shown that *H. pylori* infection has no direct effect on basal or peak acid secretion, thus raising doubts as to the importance of hypergastrinemia in mucosal injury pathogenesis (38,39). The main role of urease is thought to be the neutralization of the acidic microenvironment by producing ammonia. A stable urease-negative ($ureB^-$) mutant strain was reported to be unable to colonize the stomach of nude mice (45). The co-inoculation of a urease-negative (ure^-) strain with a urease-positive strain in the

stomach of gnotobiotic piglets resulted in preferential colonization of the urease-positive bacteria (46), suggesting that neutralization of the gastric acidity is not the sole role of urease for colonization.

Several reports have demonstrated *H. pylori*-induced apoptosis in gastric epithelial cells *in vivo* and *in vitro* (47). Neutrophil apoptosis and subsequent clearance by phagocytes are critical to the resolution of acute inflammation (48), a process modulated by the NF-kappaB pathway and inflammatory regulators such as interleukin-8 (IL-8), lipopolysaccharide, or leukotriene B4 (48,49).

Although this organism is known to be noninvasive, *H. pylori* infection elicits gastric mucosal infiltration of inflammatory cells, especially neutrophils (38,50). Histological observation in humans has indicated that the degree of *H. pylori* infection and the severity of mucosal injury are directly correlated with the extent of neutrophil infiltration into the mucosa. Superfusion of the exposed mesentery with aqueous extracts of *H. pylori*, which are rich in urease and devoid of significant contamination by lipopolysaccharide from the cell wall, resulted in a three-fold increase in adherent leukocytes within the venules and a four-fold increase in those that had emigrated into the interstitium resulting in gastric mucosal injury (50). Kim and co-workers (51) reported that supernatants of *H. pylori* cultures enhance neutrophil degranulation and adhesion capacity and up-regulate IL-8 expression by activated neutrophils. Purified *H. pylori* urease was shown to directly activate primary human blood monocytes and to stimulate dose-dependent production of inflammatory cytokines (IL-1b, IL-6, IL-8, and tumor necrosis factor-alpha) (52). Recently, Enarsson et al. (53) reported that *H. pylori* induced significant T-cell migration in a model system using human umbilical vein endothelial cells. $CD4^+$ and $CD8^+$ T cells migrated to the same extent in response to *H. pylori*. Although the presence of a functional *cag* pathogenicity island contributed to the transendothelial migration, purified *H. pylori* urease alone induced a migration effect similar to that of whole live bacteria. On the other hand, mutant *H. pylori* negative for urease A subunit still promoted significant cell migration (53), suggesting that the ability of the bacterial urease to induce this effect may rely only on its B chain.

Inducible NO-synthesizing enzyme (iNOS) is expressed after activation by pro-inflammatory cytokines in macrophages, endothelial cells and other cells. NO acts as a messenger in various inflammatory pathways and contributes to defense mechanisms against microorganisms but can also have cytotoxic effects. iNOS levels are up-regulated in a subgroup of patients with chronic active gastritis (54). Recombinant *H. pylori* urease was shown to stimulate directly macrophage iNOS expression (55).

Ischemic lesions due to vascular insufficiency may lead to the development of ulcers within the gastric mucosa. Studies using fluorescent *in vivo* microscopy have shown that *H. pylori* infection alters blood flow, the endothelial lining of the vessels, and leukocyte activity and often induces the formation of circulating or adherent platelet aggregates, consistent with epidemiological studies that suggest a possible association between *H. pylori* infection and the incidence of cardiovascular diseases, as reviewed by Kalia and Bardhan in 2003 (56). So far, there are no published studies on the effects of purified *H. pylori* urease on platelets. On the other hand, it is well known that platelets participate in the inflammatory response by modulating the activity of other inflammatory cells and as a storage site of vasoactive substances and inflammatory mediators such as histamine, serotonin, platelet aggregating factor, thromboxane A2 and other eicosanoids, as well as by generating cytotoxic superoxide and hydroxyl radicals which may induce microcirculatory disturbances (56).

Histamine not only contributes to gastric

secretion but is also a major vasoactive mediator in microcirculatory physiology. A major source of histamine within the gastrointestinal tract is the mast cell and it was demonstrated that *H. pylori*, and in particular its cell wall materials, could potentiate secretogogue-induced histamine release from isolated mast cells (57).

H. pylori adhesion to the gastric mucosa represents the initial contact between the bacterium and its host. Numerous adhesive properties of *H. pylori* have been described, including hemagglutination, attachment to epithelial cells, and binding to oligosaccharides or proteins of the extracellular matrix. Several research groups have reported that *H. pylori* cells contain proteins that bind Neu5Ac (39,58).

Adhesins are bacterial proteins, glycoconjugates, or lipids involved in the initial stages of colonization mediating the interaction between the bacterium and the host cell surface. It is predicted by genome sequencing that *H. pylori* possesses a supergene family of 32 genes encoding putative outer membrane proteins. Among these, the bacterial adhesin BabA2 has been identified to bind human blood group antigen Lewis b in the gastrointestinal mucosa. Two other members of this family, Alp A and B, are necessary for *H. pylori* to attach to human gastric tissue. A sialic acid-binding adhesin, SabA, was identified using a sialyl-Lex saccharide as a probe. The adhesion of *H. pylori* to fibronectin and lactoferrin is not dependent on BabA or SabA activities because the *babA/sabA* double mutant still binds to these proteins. Thus, the presence of an additional binding activity of *H. pylori* has been suggested (58,59).

In general, adhesin receptors are carbohydrate moieties on glycoproteins or glycosphingolipids. Extracellular matrix proteins such as laminin and collagen type IV have been proposed as receptors for *H. pylori*. For cellular receptors, phosphatidylethanolamine, laminin, and sialic acid-containing molecules are regarded as potential receptors other than Lewis b. *H. pylori*-binding gangliosides and sialylated glycoproteins are present in relatively high amounts in human neutrophils (59). Bacterial binding to normal gastric cells may be through nonsialylated receptors, like Lewis b antigenic structures, or lactotetraose. However, the level of sialylated structures increases accompanying inflammation. Sialyllactose has been reported to inhibit binding of *H. pylori* to cultured gastrointestinal epithelial cells and chronic atrophic gastritis in mice has been shown to be associated with increased synthesis of Neu5Aca3Gal structures (58,59).

It has become increasingly clear that urease has other functions in the physiology of *H. pylori* besides alkalization of the medium. Icatlo's group (60) has shown that purified *H. pylori* urease binds to gastric mucin and sulfated cell membrane glycolipids in an acidic setting. This property is expressed independently of its ureolytic activity which requires pH above 5.0. The interaction of urease with sulfated glycoproteins, heparin and heparinoids at pH 4.0 was shown to be dose- and time-dependent, and affected by the pH and salt concentration of the medium.

Reports of antibiotic-resistant *H. pylori* clinical isolates are increasing. Therefore, specific drugs targeting factors important for bacterial colonization, such as urease and chemotaxis, may be useful to minimize generation of drug-resistant bacteria. Carbohydrates and their chemical analogs are relevant candidates for anti-adhesion therapy (58-60).

Perspectives

In view of our data on the biological activities of jack bean ureases, particularly the secretagogue, platelet-activating and pro-inflammatory effects described for the canatoxin isoform, our present research is based on the hypothesis that bacterial ureases display the same properties. There are several

lines of evidence that point in this direction for *H. pylori* urease. These include a) pro-inflammatory activity accompanied by mononuclear phagocyte activation and neutrophil chemotaxis; b) platelet aggregating activity; c) histamine release from mast cells; d) lectin-like activity towards sialic-acid-containing glycoconjugates. Most studies reported so far were carried out with whole *H. pylori* cells or non-fractionated aqueous extracts and therefore the conclusion of involvement of urease in these phenomena is merely circumstantial.

As reviewed here, similar findings have been reported for the plant urease canatoxin. Thus, it is possible that *H. pylori* urease may also have other biological activities presented by canatoxin, particularly the secretagogue effect modulated by eicosanoid metabolites through lipoxygenase pathways, dependent on verapamil inhibitable calcium channels, and involving cGMP and NO signaling. Another important aspect to be investigated is whether or not the biological activities displayed by *H. pylori* urease depend on its ureolytic activity. If proven to be true, these findings could be extremely relevant to the elucidation of mechanisms leading to gastrointestinal disease caused by this bacterium and should be taken into consideration in the development of more efficient therapeutic approaches.

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