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Review

Ureases: Historical aspects, catalytic, and non-catalytic properties – A review

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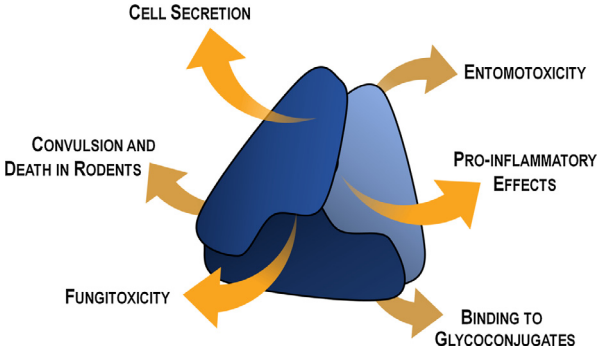
GRAPHICAL ABSTRACT



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ABSTRACT

Urease (urea amidohydrolase, EC 3.5.1.5) is a nickel-containing enzyme produced by plants, fungi, and bacteria that catalyzes the hydrolysis of urea into ammonia and carbamate. Urease is of historical importance in Biochemistry as it was the first enzyme ever to be crystallized (1926). Finding nickel in urease's active site (1975) was the first indication of a biological role for this metal. In this review, historical and structural features, kinetics aspects, activation of the metallocenter and inhibitors of the urea hydrolyzing activity of ureases are discussed. The review also deals with the non-enzymatic biological properties, whose discovery 40 years ago started a new chapter in the study of ureases. Well recognized as virulence factors due to the production of ammonia and alkalinization in diseases by urease-positive microorganisms, ureases have pro-inflammatory, endocytosis-inducing and neurotoxic activities that do not require ureolysis. Particularly relevant in plants, ureases exert insecticidal and fungitoxic effects. Data on the jack bean urease and on jaburetox, a recombinant urease-derived peptide, have indicated that interactions with cell membrane lipids may be the basis of the non-enzymatic biological properties of ureases. Altogether, with this review we wanted to invite the readers to take a second look at ureases, very versatile proteins that happen also to catalyze the breakdown of urea into ammonia and carbamate.

History and molecular features of ureases

Ureases (urea amidohydrolase, EC 3.5.1.5) are ubiquitous metalloenzymes, produced by plants, fungi and bacteria, but not by animals. The most proficient enzymes known to date, ureases

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catalyze the hydrolysis of urea into ammonia and carbamate (which then decomposes into another ammonia molecule and carbon dioxide), accelerating the rate of this reaction by a factor of at least 10^{14} when compared to the urea decomposition by elimination reaction [1–4].

Computational modeling of urease proficiency led to the proposal of a value up to 10^{32} times the theoretical rate of uncatalyzed urea hydrolysis [5]. However, one can argue that, in solution, this value is not realistic due to limits imposed by the diffusion of the substrate in water.

Urea, the natural substrate of ureases, was first isolated from human urine by Rouelle in 1773 and about a half century later, Wöhler achieved the synthesis of urea, the first organic molecule to be obtained from inorganic ones [6]. The first ureolytic microorganism, *Micrococcus ureae*, was isolated by van Tiehem in 1864, and the first enzyme with ureolytic activity was isolated from putrid urine by Musculus in 1874. The name "urease" was proposed in 1890 by Miquel [4]. Urease contributed two historical landmarks in Biochemistry. First, the crystallization of urease isolated from jack bean (*Canavalia ensiformis*) seeds by James B. Sumner, in 1926, demonstrated the proteinaceous nature of enzymes [7], a discovery laureated with the Nobel Prize in Chemistry in 1946. Second, the biological significance of nickel was recognized in 1975, after studies of Zerner's group revealing the presence of nickel ions in the active site of the jack bean urease (JBU), obligatory for its catalytic activity [8]. The identification of a plant toxin as a urease in 2001 can be considered as a third breakthrough involving ureases, as it led to the discovery of non-catalytic properties of these enzymes [9]. This finding widened our knowledge on the array of functions performed by these proteins, besides their role in nitrogen metabolism [10].

Ureases are members of the superfamily of amidohydrolases and phosphotriesterases, which display catalytically active metal (s) in their active sites. With a few exceptions reported [11,12], ureases carry two Ni^{2+} ions in their active sites [4,13]. Ureases from different sources share about 55% identity in their primary sequences suggesting divergence from a common ancestral protein. X-ray crystallography studies revealed that plant and bacterial ureases share a common basic "trimeric" structure [4,14]. The number of polypeptide chains that form the "monomer" or functional unit varies according to the source of urease. For plant and fungal ureases this functional unit is a single polypeptide chain (α). The functional unit of bacterial ureases is formed by two subunits (α and β, so far found only in the genus *Helicobacter*) or three (α, β and γ) types of polypeptide chains. The most abundant structure of plant ureases is a dimer of trimers $(\alpha_3)_2$ although a few dimeric/trimeric/tetrameric plant and also fungi ureases have been described. Bacterial ureases are trimers $([\alpha\beta\ \gamma]_3)$ while *Helicobacter pylori*'s urease has been crystalized as a tetramer of trimers of dimers $([\alpha\beta]_3)_4$ (reviewed in [10,14]). The amino acid sequences of smaller subunits of prokaryotic ureases are collinear to the corresponding region in the single chain of eukaryotic ureases [4].

Fig. 1 illustrates the structural features of ureases.

The primitive state of these proteins – single- or three-chained – is one of the unanswered questions regarding ureases. Using phylogenetic inference and two algorithms applied to three different datasets, a 3-to-1 transition in the number of urease's subunits was observed, implying a three-chained ancestral urease from which all the present enzymes derived. In that scenario, the two-chained ureases in the genus *Helicobacter* are not evolutionary intermediates of the eukaryotic single-chained ureases [15].

Table 1 presents an updated list of ureases for which molecular and kinetics characteristics are known.

Activation and catalytic properties of ureases

The active site of ureases consists, besides the two nickel atoms, of one carbamylated lysine, four histidines and one aspartate

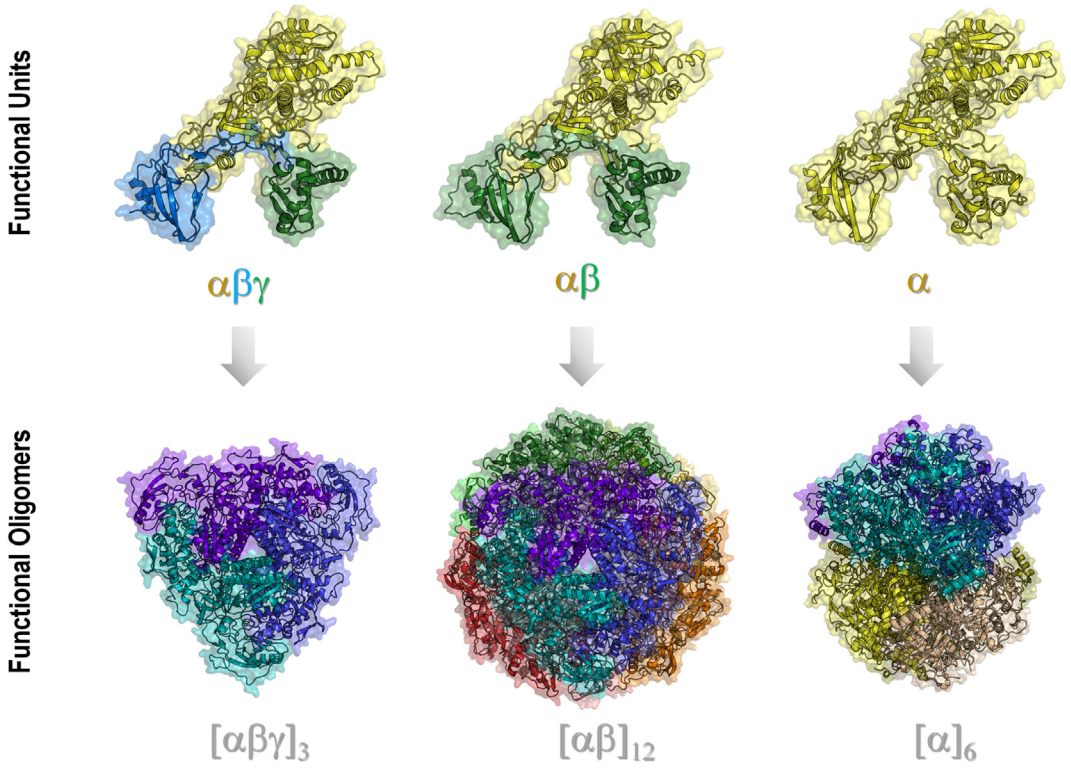

Fig. 1. Urease structural conservation. A functional unit can be formed by a heterotrimer (as in *Sporosarcina pasteurii*, PDB ID 2UBP), a heterodimer (as in *Helicobacter pylori*, PDB ID 1E9Z) or by a single unit (as in *Canavalia ensiformis*, PDB ID 3LA4). These functional units (or monomers) form larger complexes, such as trimers, hexamers or dodecamers.

Table 1
Biochemical and structural data on selected ureases of plants, bacteria and fungi.

	Source Isoform GenBank identifier	Native M_r Oligomeric state	Number of residues – M_r subunit(s)[a]	p*I*	km for urea (mM)	Optimal pH	3D structure (PDB ID)	Refs
PLANTS	*Arabidopsis thaliana* AT1G67550		838 aa					[16]
	Canavalia ensiformis JBU M65260.1	540 kDa α_6	840 aa 90.8 kDa	5.0–5.1	2.9–3.6	7.0–7.5	3LA4	[17–23]
	Canavalia ensiformis CNTX	180 kDa α_2	n.a. 95 kDa	n.a.	2–3	n.a.	n.a.	[9]
	Cajanus cajan JN107804.1	540 kDa- α_6	840 aa 90 kDa	n.a.	3.0	7.3	4G7E	[24,25]
	Glycine max Embryo-specific AY230157	α_6	840 aa 93.5 kDa	n.a.	0.2–0.6	7.0	n.a.	[26,27]
	Glycine max Ubiquitous AY276866	345 kDa α_3	837 aa	n.a.	0.8	5.25 8.75	n.a.	[26,28,29]
	Gossypium hirsutum	α_6	98.3 kDa	n.a.	0.12–0.15	8.0	n.a.	[30]
	Morus alba AB479106.1	175 kDa α_2	90.5 kDa	n.a.	0.16	9.0	n.a.	[31]
FUNGI	*Aspergillus nidulans*	540 kDa α_6	840 aa 90 kDa	n.a.	1.33	8.5	n.a.	[32]
	Aspergillus niger XM_001388748.2	540 kDa α_6	837 aa 90 kDa	n.a.	3.0	8.0	n.a.	[33]
	Cryptococcus gattii CPC735_069440	180 kDa α_2	840 aa 90 kDa	n.a.	2.0	8.0	n.a.	[34,35]
	Cryptococcus neoformans CNAG_05540	α_2	832 aa 90 kDa	n.a.	n.a.	n.a.	n.a.	[36]
	Coccidioides posadasii CPC735_069440	540 kDa- α_6	840 aa 90 kDa	n.a.	n.a.	n.a.	n.a.	[37]
	Coccidioides immitis U81509	α_4	839 aa 91.5 kDa	5.5	4.1	8.0	n.a.	[38]
	Schizosaccharomyces pombe	α_2	835 aa 91.2 kDa	n.a.	1.03	8.2	n.a.	[39]
BACTERIA	*Aerobacter aerogenes* PRL-R3			n.a.	2.8	7.5	n.a.	[40]
	Arthrobacter oxydans	242 kDa		4.3–4.7	12.5	7.6	n.a.	[41]
	Brevibacterium ammoniagenes	200 kDa $(\alpha\beta\gamma)_3$	α 67 kDa	4.1	32	7.0	n.a.	[42]
	Brucella suis Two operons	$(\alpha\beta\gamma)_3$		5	5.6	7.0	n.a.	[43,44]
	Helicobacter pylori M60398	1.06 MDa $([\alpha\beta]_3)4$	β 238 aa 30 kDa α 569 aa 62 kDa	5.9	0.2–0.8	8.0–8.2	1E9Z	[45–48]
	Klebsiella aerogenes M36068	$(\alpha\beta\gamma)_3$	γ 100 aa 11.1 kDa β 106 aa 11.7 kDa α 567 aa 60.3 kDa	n.a.	2.8	7.75	1FWJ	[49,50]
	Morganella morganii	590 kDa $(\alpha\beta\gamma)_3$	63 kDa 15 kDa 6 kDa	0.7				[51,52]
	Providencia stuartii	230 kDa $(\gamma 2\beta 2\alpha)_2$	γ 9 kDa β 10 kDa α 73	5.4	9.3	n.a.	n.a.	[53]
	Proteus mirabilis M31834	252 kDa $(\alpha\beta\gamma)_3$	γ 100 aa 11 kDa β 109 aa 12.2 kDa α 567 aa	5.2–5.9	13	7.5	n.a.	[52,54]

(*continued on next page*)

Table 1 (*continued*)

Source Isoform GenBank identifier	Native M_r Oligomeric state	Number of residues – M_r subunit(s)[a]	pI	km for urea (mM)	Optimal pH	3D structure (PDB ID)	Refs
		61 kDa					
Selenomonas ruminantium	360 kDa		n.a.	n.a.	2.2	8.0	[55]
Sporosarcina pasteurii KR133628	260 kDa $(\alpha\beta\gamma)_3$	γ 101 aa 11.1 kDa β 122 aa 14 kDa α 570 aa 61.4 kDa	4.6	17.3	8.0	4CEU	[56,57]
Staphylococcus leei	480 kDa [(γβα)5	γ 12 kDa β 21 kDa α 65 kDa	n.a.	1.66	n.a.	n.a.	[58]
Staphylococcus saprophyticus	427 kDa $(\gamma\beta\alpha)_4$	γ 13.9 kDa β 20.4 kDa α 72.4 kDa	4.7	9.5	6.0–7.0	n.a.	[59]
Staphylococcus xylosus X74600	300 kDa $(\alpha\beta\gamma)_3$	γ 16.3 kDa β 17.8 kDa α 64 kDa	4–5	n.a.	7.2	n.a.	[60]
Ureaplasma ureolyticum L40490	274 kDa $(\alpha\beta\gamma)_3$	γ 102 aa 11.2 kDa β 121 aa 13.6 kDa α 614 aa 66.6 kDa	5.0–5.2; 4.6	2.5	6.9–7.5	n.a.	[61–63]

[a] Regardless of the names given to urease's subunits in the initial or original reports, here the subunits were designated according to their homologous protein domains.
n.a. not available

residue. The crystal structures of bacterial ureases from *Klebsiella aerogenes* [50] and *Sporosarcina* (former *Bacillus*) *pasteurii* [56] first revealed the architecture of the enzymes' active site. These two ureases have nearly superimposable active sites, very similar to those of other ureases characterized afterwards, implying that this architecture is representative of all ureases. In the active site, the carbamylated lysine bridges the two nickel atoms, with Ni(1) further coordinated by two histidines and Ni(2) by the other two histidines and by an aspartate residue. Additionally, a hydroxide ion bridges the two Ni atoms, which along with other three terminal water molecules (W1, W2, W3), forms an H-bonded water tetrahedral cluster in the active site (Fig. 2) [4,14,2].

Besides the amino acid residues that compose the active site itself other residues, including a conserved cysteine, form the "mobile flap", which works as a gate for the substrate. This flap is composed by a helix-turn-helix motif and is responsible for substrate influx and product efflux in ureases, especially via motion control of a conserved histidine residue [2]. In the catalysis, amino acid residues of the mobile flap participate in the substrate binding, mainly through H bonds, thereby stabilizing the catalytic transition state and accelerating the reaction [2,4,14].

The mechanism for urea hydrolysis catalyzed by urease (Fig. 2) has been a hotly debated subject (see [64,65]). Currently, it seems to be an agreement on the mechanism, strongly supported by studies with urease inhibitors [14,66–68]. After taking the place of water molecules W1-W3 (Fig. 2A) in the urease active site, urea binds to Ni(1) ion through the carbonyl oxygen, making the urea carbon more electrophilic and, thus, more susceptible to nucleophilic attack (Fig. 2B). Then urea binds to Ni(2), through one of its amino nitrogen atoms, establishing a bidentate bond with urease (Fig. 2C). This bond is believed to facilitate the water nucleophilic attack on the carbonyl carbon resulting in a tetrahedral intermediate (Fig. 2D), from which NH_3 and carbamate are released (Fig. 2E). The main controversy point was that while Benini et al., 1999 [65] proposed that the nucleophilic attack is performed by the bridging hydroxide which provides protons to the NH_3 group, Karplus et al., 1997 [64] argued that it is a His residue from the active site mobile flap that acts as a general acid for this protonation. As an alternative, Karplus et al., 1997 [64] also considered the monodentate binding of urea to Ni(1) with Ni(2) providing the water molecule as a nucleophile for the carbonyl carbon of urea. In addition to these two hypothesis, Estiu and Merz, 2007, based on simplified computer models for the active site, proposed that hydrolysis and elimination could occur competitively in ureases, in which a "protein-assisted elimination" would be favored [69].

To achieve full ureolytic activity, the active site of ureases needs prior insertion of nickel ions and also carbamylation of its lysine residue. In bacteria, four accessory proteins (UreD, UreF, UreG, and UreE) are involved in the assembly of urease's active metallocenter. For reviews on this topic see [13,70–73]. In bacteria, the urease genes *Ure*A, *Ure*B, and *Ure*C encoding the enzyme's subunits are grouped with genes for the accessory proteins *Ure*D, *Ure*E, *Ure*F,

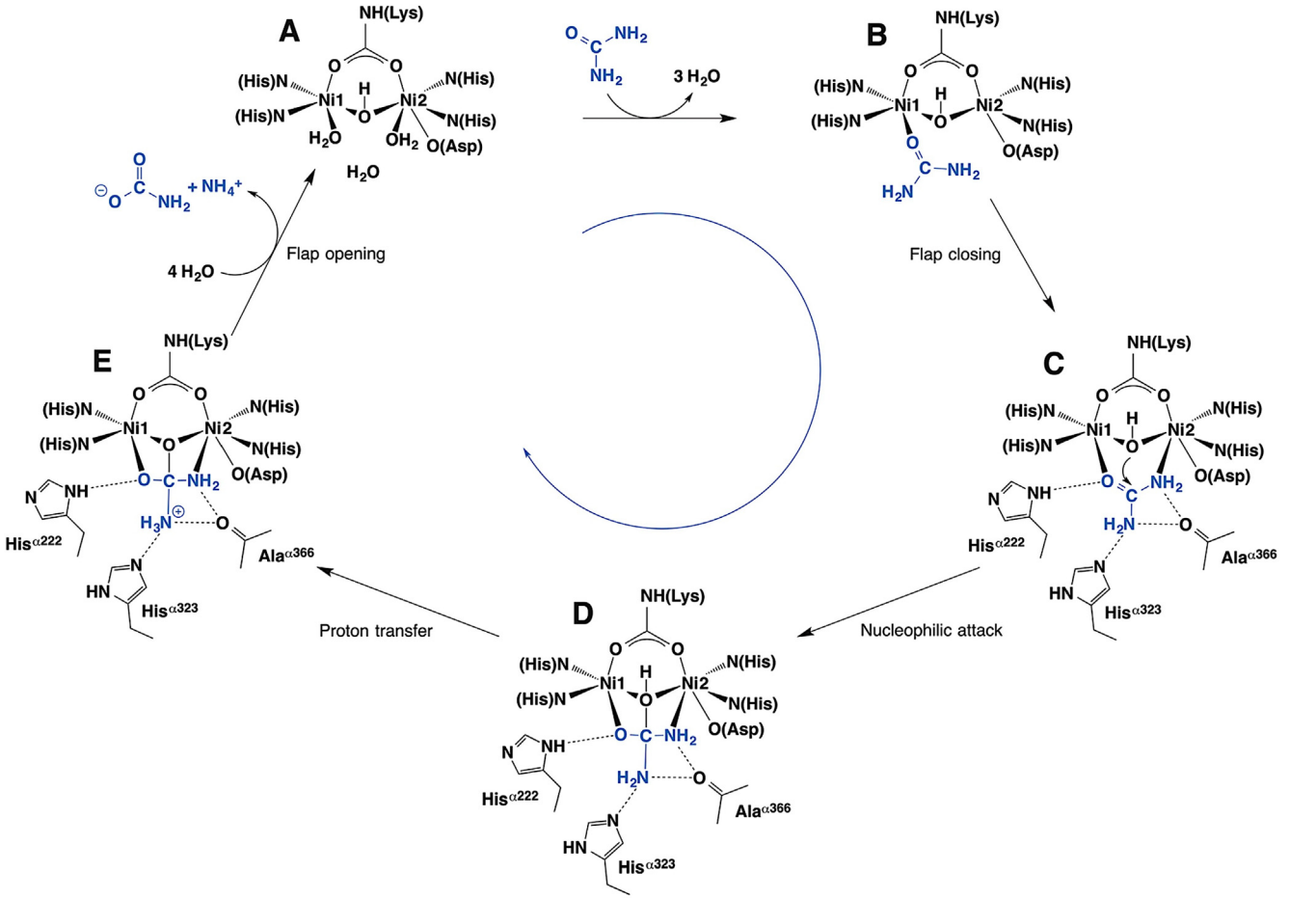

Fig. 2. Catalytic mechanism of ureases. Structure-based urease catalytic mechanism of the enzymatic hydrolysis of urea. The *Sporosarcina pasteurii* urease residue-numbering scheme is used. Please refer to the text for a stepwise description of the mechanism. Note that Ni(1) and Ni(2) are labeled Ni1 and Ni2 in this figure. Reproduced from Mazzei et al. [14] under permission from the Royal Chemical Society.

and *UreG*. In the case of *K. aerogenes*, these genes are organized in an *Ure*DABCEFG operon. Knockout and complementation studies of each accessory protein separately have shown that, *Ure*E as an exception, *Ure*D, *Ure*F and *Ure*G are crucial for the production of a fully activated "mature" urease [70,74,75].

The traditional model for urease activation starts with UreD, the first protein that binds to the apo-urease oligomer, and serves as a scaffold for the formation of the activation complex. Then UreF binds $(UreABC–UreD)_3$, and acts as a GTPase-activating protein, since its binding to $(UreABC–UreDF)_3$ correlates to the GTPase activity when further binding of UreG completes the activation complex. UreG, the first intrinsically disordered enzyme to be described [76,77], acts as a GTPase delivering energy for the urease maturation process. As GTP is hydrolyzed, the nickel-binding chaperone UreE delivers the metal ions to the $(UreABC–UreDFG)_3$ oligomer [76,77]. This model has been further refined with the increasing amount of structural information on individual urease accessory proteins [14]. In this new activation proposal, Ni^{2+}-bound UreE binds apo-UreG, facilitating GTP uptake by UreG (presence of Mg^{2+} ions is required), with Ni^{2+} ions being translocated from UreE to UreG. Then, the $(UreDF)_2$ complex competes with UreE for the Ni^{2+}-UreG to form the supercomplex apo-urease/Ni^{2+}-$(UreDFG)_2$. In the final step, $KHCO_3/NH_4HCO_3$ catalyzes GTP hydrolysis by UreG, thus completing urease activation. All urease accessory proteins are taken as metallochaperones that bind and/or transport nickel ions while driving the apo-urease into its fully active conformation. In plants and fungi, the functions of the bacterial UreG and UreE chaperones appear to be combined in a single UreG protein, which carries a histidine-rich domain with metal binding properties in its N-terminal segment [78,79]. The reason why eukaryotes lack UreE is still unknown [79].

The role of each accessory protein in the activation process has been a research hot topic in the last decade and there are some questions yet to be answered, mostly on the sequence of events and oligomerization state of each protein in the activation complex. The description at low resolution by small-angle X-ray scattering of the *K. aerogenes* $(UreABC–UreD)_3$ and $(UreABC–UreDF)_3$ oligomers started to uncover what the activation complex looks like [80]. Computational studies provided models of the activation complex [81]. The crystal structure of *H. pylori's* UreD-UreF-UreG complex revealed the presence of tunnels that span the entire length of both UreF and UreD, through which the delivery of nickel ions from UreG to the apo-urease could possibly occur [73,82].

Ureases inhibitors

Studies on urease's inhibitors have been carried out both to provide molecular insights on how the catalytic site machinery works as well as searching for effective inhibitors to counterbalance urease's catalyzed urea hydrolysis in a number of situations [83,84]. Urease inhibitors are a topic of intense investigation. The substrate urea, urea analogues and ammonium ions (products of urea hydrolysis), are weak inhibitors of urease [4]. Searching the

Web-of-Sciences database (March 6th, 2018) for articles with "urease" in the title retrieved 4509 documents, 920 were found using "urease" and "inhibit∗" of which 413 were published since 2010. Please refer to the next section, "Biological roles of ureases", for more information on the importance of ammonia release by ureases.

An extensive and detailed review on the different classes of urease inhibitors can be found in [14]. Other articles on this special issue of *Journal of Advanced Research* deal in more details with urease inhibitors.

Sulfur compounds

Thiols, particularly β-mercaptoethanol, are of historic importance as urease inhibitors that, back in 1980, provided to B. Zerner's group crucial information on the active site of JBU [85]. Thiolate anions ($R\text{-}S^-$) inhibit ureases in a competitive manner. X-ray analysis of *S. (B.) pasteurii* urease complexed with β-mercaptoethanol (PDB code 1UBP) revealed its thiolate anion bridging the two Ni^{2+} ions in the active site and the hydroxy group further chelating the metallocenter [56]. Sulfite also acts as competitive pH-dependent inhibitor of urease [86].

Hydroxamic acids

Acetohydroxamic acid, the most studied derivative of this group of metal-binding compounds, acts as a urease slow-binding competitive inhibitor. It has been found interacting with the two nickel ions in the active sites of *S.(B.) pasteurii* (PDB code 4UBP), *H. pylori* (PDB code 1E9Y) and a mutated form of *K. aerogenes* (PDB code 1FWE) ureases [86]. So far, acetohydroxamic acid is the only urease inhibitor with therapeutic application to treat hyperammonemia in cirrhosis of *H. pylori* positive-patients [87] and it has been used to reduce urinary stones and treat urinary infections due to *P. mirabilis* infections [84,88]. However, this compound induces severe side effects, including teratogenesis, psychoneurological and muscular symptoms [89], which limit its use and caused its withdraw from the general market [84].

Phosphorous compounds

Amide and esters of phosphoric/thiophosphoric acids

Studies on phosphorus-based compounds as urease inhibitors started in the 1970s after the observation that some organophosphate-based insecticides inhibit soil urease [4,90]. In 1980, Dixon et al. described that phophoramidate inhibited JBU through its binding to the two nickels in the enzyme's active site [65,85]. Derivatives of phosphoric and thiophosphoric acid are potent inhibitors of urease [4]. A great number of derivatives have been developed and patented for potential application in infections by urease-producing pathogenic microorganisms [83] and in agriculture to avoid hydrolysis of urea used as fertilizer [84]. For all the derivatives of this class of inhibitors, the initial enzymatic hydrolysis of the molecule generates diamidophosphate, which is believed to be the actual urease inhibitor [14]. The main issue involving organophosphate inhibitors of urease is related to their low stability in acidic pH. To overcome this problem non-hydrolysable aminophosphinic acids have been developed [91,92].

Phosphate

Phosphate is a pH-dependent urease competitive inhibitor in the pH range 5.0–8.0, but negligible at pH higher than 7.5–8.0 [23,93]. X-ray diffraction structural data on phosphate-inhibited *S.(B.) pasteurii* urease inhibited with phosphate elucidated that the binding mode involves the formation of four coordinated bonds with both Ni ions in the enzyme's molecule [93]. It is a weak inhibitor compared to its amides (phosphoramidates) that rank among the most active urease inhibitors.

Fluoride

The mode of inhibitory action of fluoride, explored mostly using *S.(B.) pasteurii* urease, was described as a pH-sensitive mixed inhibition, which varies from a weak competitive mode in acidic medium to a stronger uncompetitive mechanism in alkaline conditions [57]. Five crystal structures of the enzyme in its fluoride-inhibited state were analyzed to establish that one fluoride ion binds to Ni(1) of the active site, while the nickel bridging hydroxide is replaced by another fluoride ion [57]

Quinones

Ubiquitous in the nature, quinones have bactericidal and antifungal activities, and participate of biologically relevant redox mechanisms. Quinones were described as urease inhibitors in the 1970s in studies of Bremner's group, pointing to 1,4-benzoquinone as a promising inhibitor of soil urease [94]. More recently, Krajewska's group reported on the kinetics of the inhibition of JBU by quinones, demonstrating a general slow-binding concentration-dependent mechanism indicative of a covalent modification of the conserved cysteine residue in the mobile flap of the active site. In addition to the covalent modification, quinones might inhibit urease through arylation and oxidation of its thiol groups [95].

Polyphenols

Catechol, the simplest molecule with a polyphenol scaffold, was shown to inhibit soil urease by Bremner and Douglas early in 1970s [94]. Plants are rich sources of polyphenolic compounds with antioxidant and bactericidal properties, generally regarded as beneficial for human health. For instance, polyphenols present in the green tea and other herbal beverages inhibited *H. pylori* urease (HPU) *in vitro* and reduced infection by *H. pylori* in Mongolian gerbils [96,97]. The mechanism of inhibition of urease by catechol is not yet fully understood. Current hypothesis are that inhibition by catechols could be due to a time dependent oxidation to ortho-benzoquinone which acts as the actual inhibitor by modifying protein's functional groups [98,99], and/or that polyphenols can coordinate with transition metals forming catechol–metal complexes, inactivating urease's metallocenter [100].

Other urease inhibitors

Although in most cases detailed structural data are not available, other classes of urease inhibitors are known, including boron-containing acids, citrates, and heavy metals. For a review on these topics see [4,14]. Heavy metals such as Hg, Ag, and Cu are slow reacting inhibitors of ureases [101,102]. Bismuth (Bi^{3+}) was shown to inactivate HPU by interacting with the cysteine residue of the mobile flap [103]. Due to the bactericidal activity, bismuth compounds have been widely used to treat gastric ulcers associated to *H. pylori* infection [104,105].

Biological roles of ureases that require ureolytic activity

Urease activity enables microorganisms to use urea as their sole nitrogen source. Urease synthesis may be constitutive or synthesized as a stress-related response of bacteria to counteract low environmental pH [106]. Ureolytic activity of the human gut microbiota hydrolyzes up to 30% of all urea produced in our bodies

[107]. Microbial ureases are important also in dental health [108]. The production of alkali subsequent to salivary urea cleavage by oral microbiota urease was shown to inhibit dental cavities and plaque formation [109]. In ruminants, animal-derived urea is cleaved by bacterial ureases in the forestomach, releasing ammonia as nitrogen source for the rumen microbiota, which in turn serves as biomass to feed the animals [110,111].

Pathogenesis of many clinical conditions in humans and other animals are related directly to the ureolytic activity of bacterial or fungal enzymes [112,113]. Some examples are as follows. *Proteus mirabilis* is the most common organism that causes urinary stones in humans, due to urine alkalization promoted by its urease, contributing to the pathogenesis of pyelonephritis and catheter encrustation. Precipitation of urinary salts in the alkalinized urine results in struvite and carbonate apatite crystallization [114]. The bacterium *H. pylori* colonizes the stomach mucosa of half of the world's population, significantly increasing the risk of gastric ulcers and cancer [113,115,116]. HPU, which constitutes about 10% of the total cell protein, enables bacterial survival in the stomach by neutralizing the acidic medium [117]. Ureolytic organisms in the digestive or urinary tract potentially contribute to hepatic encephalopathy and coma resulting in hyperammonemia and brain intoxication [118]. Reduction of the ureolytic bacteria load and the use of acetohydroxamic acid as a urease inhibitor are considered therapeutic approaches under these conditions [119–121]. Other pathogens also produce urease to acquire acid resistance and enable colonization, among which are Shiga-toxin producing *Escherichia coli* [122], *Yersinia enterocolitica* [123], *K. pneumoniae* [124], *Brucella abortus* [125], and *Haemophilus influenza* [126]. Fungal ureases are involved in the pathogenesis of human cryptococcosis by *Cryptococcus neoformans* [127,128], and *Cryptococcus gattii* [35], and of coccidiodomycosis (San Joaquin Valley fever) by *Coccidioides immitis* and *C. posadasii* [37]. However, the role of microbial ureases as virulence factors has a still largely ignored contribution of non-enzymatic properties of these proteins, a subject that will be covered in the following section.

Urease is ubiquitous in plants and can be found in all vegetal tissues [129,130]. Nitrogen is a limiting element for plant growth, second only to carbon. Worldwide used as a soil fertilizer, urea is a relevant N source for plants, and dedicated urea transporters actively import this compound from the soil [131]. Urea hydrolysis to release ammonia and carbon dioxide is the main physiological role attributed to ureases in plants [130,132]. Urease is abundant in the soil, both in living bacteria and as extracellular urease, bound to clays and humic substances [133,134]. Ureolysis by cell-free ureases alkalinizes the soil inducing calcium carbonate precipitation and affecting the availability of minerals [135,136]. In addition to that, high levels of soil urease reduce the efficiency of urea fertilization leading to loss of ammonia into the atmosphere and ammonia-induced phytotoxicity [90,137]. The search for urease inhibitors with agricultural applicability to optimize urea fertilization is an intense field of investigation. These topics are broadly covered in other articles of this thematic issue of the *Journal of Advanced Research*.

Biological properties of ureases independent of ureolysis

Table 2 lists the biological properties of ureases found not to require ureolysis, either because urea is not available or its concentration is negligible, or the study employed ureases that were enzymatically incompetent (either with blocked active sites or in the inactive, nickel-deprived, apo-urease form).

Ureases play a role in cell-to-cell or organism-to-organism communication. Arginases with lectin properties from the lichens *Evernia prunastri* and *Xanthoria parietina* were shown to bind to a glycosylated urease in the cell wall of the homologous algae. The polygalactosylated urease is produced only in the season when the algal cells divide assuring recognition of the phycobiont by its fungal partner in the mutualistic association of these lichens [138,139].

Ureases were evaluated for a role in soybean nodulation by the diazotrophic bacterium *Bradyrhizobium japonicum* [140]. Soybean and jack bean ureases were characterized as chemotactic factors recognized by the bacterial cells *in vitro*. Independent of the urease status of the nodulating bacteria, urease-deficient mutant soybean plants had fewer but larger nodules when compared to the wild-type plant. Leghemoglobin production in wild-type plants was higher and peaked earlier than in urease-deficient plants, indicating a less efficient process of nitrogen fixation. Inhibition of urease activity in wild-type plants did not reproduce the results seen in mutated plants. These data made clear that soybean urease(s), but not the bacterial enzyme, participate(s) somehow of the plant-diazotrophic bacteria symbiosis. This role of the soybean urease does not require ureolysis and is relevant for biological nitrogen fixation by the plant [140].

Among microbial ureases that play a role as virulence factors, much attention is given to HPU because of its crucial role in the pathogenesis of gastric diseases. Production of urease proved to be essential to allow stomach colonization by *H. pylori*, however studies carried out in the early 1990s have shown that neutralization of gastric acidity is not the only function of the protein [141,142]. Following the steps of our previous observations made on ureases from jack bean (*C. ensiformis*) and from *S.(B.) pasteurii* (reviewed in [10] – see next sections), we have reported several other biological properties of the purified recombinant HPU, observed in the 10^{-6}–10^{-8} M range of protein concentration. These properties include induction of lipoxygenase-dependent activation and aggregation of rabbit [143] and human platelets [144]; induction of lipoxygenase-dependent chemotaxis and ROS production in human neutrophils [145]; delaying apoptosis in human neutrophils [145] and in gastric epithelial cells [146]; increase of the lipoxygenase content in neutrophils [145]; induction in platelets of the production of lipoxygenase-derived eicosanoids [143]; promotion of angiogenesis in human umbilical endothelial cells and in the chicken embryo chorioallantoic membrane model [146]; and induction of processing of pre-mRNA encoding pro-inflammatory cytokines in human platelets [144]. Most of these effects are also displayed by an enzyme-inhibited HPU, while some are induced by one of its isolated subunits alone [144], indicating that these biological effects do not require urea hydrolysis. Other groups also reported biological roles of HPU that are carried out by one of its subunits, implying absence of ureolysis. HPU's subunit B was shown to bind to Th17 lymphocytes [147] and to CD74 on gastric epithelial cells thereby eliciting production of IL-8 [148]. HPU's subunit A contains a nuclear localization signal (sequence $_{21}$KKRKEK$_{26}$), and it was found in the nuclei of COS-7 cells [149,150] and AGS gastric epithelial cells, inducing alterations in the cells' morphology [150].

Altogether these non-enzymatic biological effects of HPU point out to a relevant contribution (yet mostly ignored) of this protein to the inflammatory process that underlies the gastric diseases caused by *H. pylori*. Because HPU activates non-gastric cells such as platelets, neutrophils, endothelial cells, among others, it may contribute as well to the pathogenesis of extragastric illnesses, in particular cardiovascular diseases. Probably none of the future urease inhibitors that are being conceived or are presently under development will have any use to counteract HPU's pro-inflammatory effects or other unwanted contributions of this protein that are not due to its ureolytic activity. Thus, there is an urgent need to understand the structural basis of the non-enzymatic biological properties of HPU, and of other microbial

Table 2
Ureolysis-independent biological properties of selected ureases and urease-derived peptides.

Ureases and derived-peptide	Entomotoxic properties	Antifungal activity	Mammal neurotoxicity	Exocytosis in platelets	Eicosanoid signaling	Chemotactic activity
PLANTS						
CNTX	✓	✓	✓	✓	✓	✓
JBU	✓	✓	✓	✓	✓	✓
eSBU	✓	✓	n.d.	✓	n.d.	✓
uSBU**	✓	✓	n.d.	✓	n.d.	n.d.
GHU	n.d.	✓	n.d.	n.d.	n.d.	n.d.
PPU	✓	✓	n.d.	n.d.	n.d.	n.d.
BACTERIA						
SPU	✗	n.d.	✗	✓	✓	n.d.
HPU**	✗	✓	✓	✓	✓	✓
PMU**#	✓	✓	n.d.	✓	n.d.	n.d.
UREASE-DERIVED PEPTIDES						
JBTX**	✓	✓	✗	✗	✓	n.d.
SYTX**#	✓	✓	✗	n.d.	n.d.	n.d.

CNTX, canatoxin (*C. ensiformis*); JBU, jackbean urease (*C. ensiformis*); eSBU, embryo-specific soybean urease (*G.* max); uSBU, ubiquitous soybean urease; GHU, *Gossypium hirsutum* (cotton) urease; PPU, pipeon pig urease (*C. cajan*); SPU, *S. pasteurii* urease; HPU, *H. pylori* urease; PMU, *P. mirabilis* urease; BJU, *B. japonicum* urease; JBTX, jaburetox; SYTX, soyuretox.
✓ presence of biological activity; ✗ absence of biological activity; ** Recombinant protein; n.d. not determined; # unpublished result.

ureases with relevant roles as virulence factors, aiming the design of drugs that could specifically block these other activities. Such new urease inhibitors could be used alone or together with ureolysis inhibitors, to target all the noxious effect of ureases involved in pathogenesis.

Neurotoxicity of ureases

The discovery of the non-enzymatic properties of ureases is closely related to the study of their neurotoxicity, both in rodents and in insects. Canatoxin (CNTX) is an isoform of *C. ensiformis* urease, first isolated from the plant seeds as a neurotoxic protein causing convulsions and death of rats and mice, with an $LD_{50} \sim 2$ mg/kg, given by intraperitoneal route [151]. Two decades after the isolation of CNTX, it became evident that the neurotoxic protein is actually an isoform of the most abundant urease (JBU) found in the same seeds [9]. Canatoxin is a non-covalent dimer of ~95 kDa subunits with one zinc and one nickel atom per subunit [9,12] what probably explains its lower ureolytic activity. CNTX and JBU differ in one order of magnitude in their sensitivity to the irreversible inhibitor *p*-hydroxy-mercurybenzoate (*p*HMB), an oxidant of thiol groups [9] and in their metal-binding affinities [152].

Studies on CNTX have indicated that its primary mechanism of action at the cellular level is to induce exocytosis, triggering a signaling pathway that characteristically involves eicosanoids derived from the lipoxygenases pathway (reviewed in [10]). This biological property of CNTX was reported in a number of mammalian models, both *in vivo* and *in vitro*, among which are blood platelets and rat brain synaptosomes. The aggregating activity of CNTX in rabbit, rat, guinea pig or human platelets occurs in the nanomolar range [153]. CNTX-activated platelets recruit a lipoxygenase-mediated pathway that leads to influx of external Ca^{2+} through opening of voltage-gated Ca^{2+} channels and without release of intracellular [Ca^{2+}] pools. The increased cytoplasmic [Ca^{2+}] triggers exocytosis of platelet granules that contain ADP, which in turn induces the aggregation response [153,154]. Later the ability to induce platelet aggregation was reported for JBU [9], the embryo-specific [155] and the ubiquitous [156] isoforms of soybean ureases, *B.*(*S.*) *pasteurii* urease [155,157], and HPU [143], thus it is a property common to one-, two-, and three-chained ureases.

The observations that *p*HMB-treated CNTX, in which the ureolytic activity is irreversibly blocked, was still lethal to mice and still able to promote platelet aggregation set the ground for the discovery of the non-enzymatic biological properties of ureases [9]. In the following two decades, a lot more of ureolysis-unrelated effects were described for *C. ensiformis* ureases as well as for ureases from other sources (reviewed in [10]).

The exocytosis inducing effect of CNTX was later characterized in rat brain synaptosomes, which responded dose-dependently to the neurotoxin by releasing neurotransmitter vesicles previously loaded with radiolabeled serotonin or dopamine. At 500 nM CNTX, the amount of neurotransmitter released from the synaptosomes was similar to that obtained by depolarization with 50 mM KCl [158]. The ability of CNTX to promote secretion in synaptosomes correlates with the neurotoxicity it induces *in vivo* in mice and rats. The medullar origin of CNTX-induced seizures and other CNS-related effects were described in rodents [159].

More recent data have shown that JBU (10–100 nM) induces Ca^{2+} events in cultured rat hippocampal neurons, an effect also observed for HPU (Piovesan, A.R., unpublished results). In patch clamp experiments, it was observed that JBU increases the frequency of spontaneous firing action potentials in cultured rat hippocampus neurons, rising the amplitude of sodium currents, and apparently not affecting potassium currents. A higher frequency of spontaneous excitatory post synaptic currents was also seen, consistent with a seizure-like activity (Dal Belo, C. A., unpublished data). Studies using microPET (Positron Emission Tomography) indicated an increase of ~30% in the uptake of ^{18}Fluor-desoxy-glucose in the brain of CNTX-treated anaesthetized rats, particularly affecting the hippocampus, a typical finding for seizure-inducing drugs (De Almeida, C.G.M., unpublished results).

Similar to our observations, JBU had been previously reported to be lethal and to produce seizures in mice and rabbits after intravenous administration [160]. Likewise, purified HPU was shown to kill mice upon intraperitoneal injection, producing hypothermia, convulsions and death [161]. In both studies, the neurotoxicity of the ureases was attributed to the high levels of ammonia found in the animal's blood. Although hyperammonemia probably contributes to the neurotoxic effects induced by CNTX in mice and rats, surely it does not tell the whole story, considering that *p*HMB-treated CNTX still caused neurotoxic symptoms and seizures leading to death of the animals [9].

Contributions of ureases to plant defense against predators and pathogens

The first description of the insecticidal effect of a urease was published in 1997 showing that ingestion of CNTX killed insects

[162]. The susceptibility of the insects to CNTX's lethal effect depended on the type of their digestive enzymes. Insects with acidic midguts and cathepsin-like proteinases, like the cowpea weaver *Callosobruchus maculatus* (Bruchidae) and the kissing bug *Rhodnius prolixus* (Hemiptera), were susceptible to CNTX while insects with alkaline midguts and trypsin-like enzymes were not. These data were interpreted as evidence for the need of proteolytic activation of CNTX that, once ingested, is hydrolyzed by insect cathepsin-like enzyme(s) releasing an internal peptide(s) with insecticidal activity. In fact preventing CNTX hydrolysis by adding a cathepsin B inhibitor simultaneously with the toxin in the insects' diet protected them against the lethal effect [162]. In the following years we described that JBU/CNTX and the embryo specific soybean urease were insecticidal against the hemipterans *Nezara viridula* [163], *Dysdercus peruvianus* [155,164], *Oncopeltus fasciatus* [165], and K. Ponnuraj's group in India reported the insecticidal effect of the pigeon pea urease (*Cajanus cajan*) against *Callosobruchus chinensis* [25].

The proteolytic activation of CNTX by insect cathepsin-like enzymes was further investigated. Insecticidal peptides were isolated from CNTX's fragments after digestion with *C. maculatus* enzymes [166]. The most active peptide, pepcanatox, with a molecular mass of ~10 kDa had its N-terminal sequence determined and, based on this information, a recombinant peptide named jaburetox was obtained by heterologous expression in *E. coli* [167]. Cathepsin D-like enzymes from *D. peruvianus* midgut that were able to perform hydrolysis of CNTX/JBU and release the insecticidal peptide were characterized [164,168,169]. A similar study was performed with JBU and the milkweed bug *Oncopeltus fasciatus,* identifying a cathepsin L that hydrolyzed the urease to release a ~10 kDa entomotoxic peptide [165].

The recombinant peptide jaburetox was cloned using as template the cDNA of JBURE-II, a third isoform of urease found in *C. ensiformis* [170,171]. Based on jaburetox's sequence, a recombinant insecticidal peptide called soyuretox was produced [172] having as template the cDNA of the ubiquitous soybean urease which, like the embryo-specific urease, also kills *R. prolixus* [156]. Interestingly, the region that encompasses the jaburetox/soyuretox sequence, comprising about 90 amino acid residues, displays a lower similarity when compared to that of the complete sequence of different ureases, suggesting less evolutionary pressure to conserve this entomotoxic "domain" of plant ureases [15,167].

But the proteolytic release of entomotoxic peptides does not tell the whole story of urease's entomotoxicity. Evidences showing that the entire urease molecule is entomotoxic *per se* started to add up with studies on the anti-diuretic effect of *C. ensiformis* ureases. In Carlini et al., [162], we showed that CNTX produced an important anti-diuretic effect in *R. prolixus* that peaked about 4 h after the insects received the "meal" containing the toxin, disappearing after 24 h. However, the hydrolysis of CNTX in the insect midgut was not detected before 18 h, suggesting that the anti-diuretic effect was produced by the entire protein. Later, JBU and the jaburetox peptide were shown to cause anti-diuresis in *R. prolixus*' isolated Malpighian tubules in the concentration range of 10^{-10} and 10^{-15} M, respectively [173]. Surprisingly, although both molecules induced antidiuretic effects, JBU and jaburetox triggered different signaling pathways leading to antidiuresis [173]. In the following years other papers were published by our group describing a list of entomotoxic effects of JBU, some of which are not shared with jaburetox, such as alteration in water transport and of the contractility in the crop of *R. prolixus* [174]. Similar to the data indicating recruitment by ureases of eicosanoid-mediated pathways in mammalian systems (reviewed in [10]), JBU effects in insects required a phospholipase A_2 type XII [175] and prostaglandins [176]. JBU and jaburetox targeted the immune system of *R. prolixus*, inducing an eicosanoid-dependent aggregation of hemocytes and alterations in cell morphology [176,177] that render the insect more susceptible to entomopathogenic bacteria [177].

Both JBU and jaburetox are neurotoxic to insects from different orders. Jaburetox was immunolocalized in the brain of *Triatoma infestans* (Hemiptera) and neurotoxic symptoms preceded death of the insects injected with the peptide [178]. JBU-induced effects were studied in the cockroach *Nauphoeta cinerea* (Blatodea) revealing that both, the central and the peripheral nervous systems are targeted by the urease, with alterations of the cholinergic, octopaminergic and GABAergic pathways as part of its entomotoxic mode of action [179]. The effects of JBU were also investigated on neuromuscular junctions of *Locusta migratoria* (Orthoptera) and of *Drosophila melanogaster* (Diptera), and the resulting data pointed to interference of JBU on neurotransmitter release, probably by disruption of the calcium machinery in the pre-synaptic region of insect neurons [180].

Previous studies with *B.*(*S.*) *pasteurii* urease suggested lack of insecticidal properties for microbial ureases, which was attributed to the absence of part of jaburetox's sequence in those proteins [155]. However, later reports on insecticidal activity of ureases of bacteria from *Photorhabdus* and *Xenorhabdus* genera [181], *Yersinia pseudotuberculosis* [182] and *P. mirabilis* (Broll, V. et al., unpublished results) indicated that bacterial ureases are indeed entomotoxic and insecticidal, in agreement to the fact that ureases contain other entomotoxic domains besides the sequence corresponding to jaburetox.

Ureases are toxic against filamentous fungi and yeasts [183]. The fungitoxic activity of CNTX was the first reported showing that the protein at 2% concentration caused growth inhibition of the phytopathogenic filamentous fungi *Macrophomina phaseolina, Colletotrichum gloesporioides* and *Sclerotium rolfsii* [184]. Becker-Ritt et al., 2007, reported that JBU and the soybean embryo-specific ureases inhibited growth and/or spore germination of seven other species of filamentous fungi at submicromolar concentrations and caused damage to cell wall, even after blockage of their ureolytic active sites. In this same study, the two-chained HPU also inhibited fungal growth although with less efficiency [185]. The native ureases of cotton seeds (*G. hirsutum*) [30] and of pigeon pea [25], and the recombinant non-ureolytic apoureases, JBURE-IIb [171] and a ubiquitous soybean urease fused to glutathione transferase [156], were also shown to be detrimental to filamentous fungi.

In Postal et al., 2012, JBU was tested in the 10^{-6}–10^{-7} M range against different yeast species and caused inhibition of proliferation and of glucose metabolism, morphological alterations with pseudohyphae formation, and cell membrane permeabilization, eventually leading to cell death [186]. Jaburetox induced similar effects against the yeasts but at one to two orders of magnitude higher doses. Studies with peptides from a papain-hydrolyzed JBU indicated the presence of other fungitoxic domains in the protein, besides jaburetox [186]. Soyuretox, a peptide derived from the soybean ubiquitous urease, is also fungitoxic in the same concentration range as observed for jaburetox [172]. Detached leaves of "urease-null" soybean transgenic plants, due to co-suppression of ureases genes, and infected with uredospores of the Asian rust fungus *Phakopsora pachyrhizi* developed more lesions and pustules when compared to leaves of wild plants with normal levels of ureases, suggesting a protective role of ureases against fungal diseases in the wild plants [187].

Interestingly, a non-catalytical urease was identified in the soybean genome. This urease lacks critical features of the enzyme's active site, but it is expressed in various plant tissues [188], reinforcing the multifunctional characteristics of the protein, especially when related to plant defense. It is tempting to predict that more of these non-catalytical ureases will be found as more plant

genomes are decoded. Altogether these data suggests that urease-overexpressing plants or transgenic plants jaburetox/soyuretox may represent alternatives to achieve resistance to insect herbivory and/or fungal disease in agriculture. In this context it is important to mention that ureases can be generally regarded as biosafe proteins, which are present in relatively large quantities in most edible plants and are particularly abundant in seeds of legumes and in fruits such as tomatoes, melon, and watermelon, that are eaten in raw state [129,132]. Although more studies are needed to ascertain the biosafety of urease-derived peptides, no acute toxicity was detected for jaburetox given in high doses either injected or by oral route to mice and neonate rats [167]. Preliminary data obtained for soyuretox in the zebrafish (*Danio rerio*) model indicated toxicity only in the highest tested doses (Kappaun, K. et al., unpublished results).

Structural aspects of jaburetox

Models of the tridimensional structure of jaburetox [167,189] indicated the existence in the C-terminal half of the peptide of a prominent β-hairpin motif, a feature that could be related to a pore-forming activity eventually leading its neurotoxicity. A β-hairpin in the region of JBU corresponding to jaburetox was found in its crystallographic structure [22]. Aiming to carry out structure versus activity studies on jaburetox, three mutants corresponding to truncated versions of the peptide were obtained: Jbtx Δ−β, which lacked the β-hairpin motif (residues 61–74 deleted); Jbtx N-ter (residues 1–44), corresponding to the N-terminal half; and Jbtx C-ter (residues 45–93), corresponding to the C-terminal half of jaburetox [190]. In insect bioassays, the Jbtx Δ−β peptide kept the entomotoxic properties of the whole peptide, clearly indicating that the β-hairpin motif is not required for the insecticidal effect. On the other hand, while Jbtx N-ter remained entomotoxic, the Jbtx C-ter peptide, which contains the β-hairpin motif, was less active or inactive when tested on two different insect models. The data support the conclusion that the N-terminal half of jaburetox carries its most important entomotoxic domain [190].

Molecular dynamics studies employing long simulations of jaburetox in aqueous medium suggested that the peptide becomes largely unstructured after 500 ns, more accentuated in its N-terminal domain, while the initial structure observed for its moiety in JBU's crystals is completely lost [190]. Subsequently light scattering, circular dichroism and nuclear magnetic resonance spectroscopy studies of jaburetox in solution determined that it is an intrinsically disordered polypeptide [191]. Regions of jaburetox which exhibited tendency to form one small alpha-helix close to the N terminus, and two turn-like motifs, in the central portion and close to the C terminus, respectively, were predicted as sites of potential interaction with other proteins or lipids, suggesting that upon such interactions structural changes could be triggered to drive the peptide into a biologically active conformation [191]. The solution structure of soyuretox was determined using the same methodologies and revealed its intrinsically disordered nature, although with more secondary structure elements when compared to jaburetox (Kappaun, K. et al., unpublished results).

Interaction of ureases and urease-derived peptides with lipids and membranes

The interaction of jaburetox with lipid membranes was first reported by Barros et al., 2009 [189]. In this study, jaburetox was shown to cause leakage of carboxyfluorescein entrapped inside large unilamellar vesicles, without lysis of the liposomes. The leakage was greater in vesicles composed by acidic lipids and depended on the state of aggregation of jaburetox. Molecular dynamics applied to jaburetox suggested that its β-hairpin motif could anchor at polar/non-polar interfaces [189]. However, as mentioned earlier, even if the β-hairpin does interact with insect membranes, it is not essential for the entomotoxic properties of jaburetox. Moreover all three truncated versions of jaburetox developed by Martinelli et al., 2014, disrupted liposomes, revealing the presence of more than one lipid interacting domain in the peptide [190].

In another study, JBU, jaburetox and its mutated peptides were tested for an ion channel forming activity in planar lipid bilayers [192]. All proteins formed well resolved, highly cation-selective channels exhibiting two conducting states (7–18 pS and 32–79 pS, respectively). Urease (20 nM) and Jbtx N-ter (1 μM) were more active at negative potentials, while the channels formed by the other peptides were not voltage-dependent. This study was the first direct demonstration of the capacity of *C. ensiformis* urease and jaburetox to permeabilize membranes through an ion channel-based mechanism, which may be the basis of their diverse biological activities. Molecular models of JBU showed that the moiety corresponding to jaburetox is well exposed at the protein's surface, from where it can probably "enforce" the interaction of the entire urease with lipid bilayers, a hypothesis formulated to explain why the polypeptides share many, although not identical, biological properties [192].

To elucidate whether an interaction with lipids could induce conformational changes in the intrinsically disordered molecule of jaburetox, the structural behavior of the peptide was probed using nuclear magnetic resonance and circular dichroism spectroscopies when in contact with membranes models [193]. The interaction of jaburetox with SDS micelles increased its content of secondary and tertiary structure elements. When exposed to large unilamellar vesicles and bicelles prepared with phospholipids, conformational changes were observed mostly in N-terminal regions, but without significant acquisition of secondary structure motifs. Fluorescence microscopy was used to demonstrate that the lipid vesicles could displace the interaction of jaburetox with lipid-rich membranes of the cockroach nervous chord. These data suggested that contacts of the N-terminal moiety of jaburetox with membrane phospholipids lead to its anchorage to cell membranes and promote conformation changes of jaburetox into a more ordered structure that could facilitate its interaction with membrane-bound target proteins [193].

Further studies aiming to elucidate the mechanism of interaction of JBU and jaburetox with lipid membranes were carried out using multilamellar liposomes with a lipid composition simulating that of human platelets, subjected to dynamic light scattering and small angle X-rays scattering (SAXS) analyses [194]. Results were obtained indicating that both JBU and jaburetox are able to insert themselves into the lipid bilayers, reducing the hydrodynamic radius of the vesicles, altering the lamellar repeat distance, the number of lamellae, and decreasing the membrane's fluidity. The interaction of jaburetox affected the vesicle's internal bilayers and caused more drastic effect on the multilamellar organization of the liposomes than did JBU. In the same study, the interaction of JBU with giant unilamellar vesicles (GUVs) made of fluorescent phospholipids showed that JBU caused membrane perturbation with formation of tethers. The data reinforced the idea that JBU can interact with multilamellar liposomes, probably by inserting its jaburetox "domain" into the vesicle's external membrane [194].

Conclusions and future perspectives

While the history of research on urease as an enzyme is almost 150 years old, dating back to the 1870s, the knowledge that ureases perform other biological roles unrelated to ureolysis is

considerably younger, not 50 years yet. An unbiased view of these molecules as more than enzymes is needed to allow discovery of yet unsuspected biological properties of ureases. Finding ureases in sources little explored so far, deciphering the structural characterization of a broad range of ureases including non-ureolytic proteins, and to investigate a potential synergy between catalytic and non-catalytic properties of ureases, are only a few of the open fields in the study of these enzymes.

There are many proposed technological application of ureases [195–200] all of which explore exclusively the enzyme's catalytic activity. There are though certainly much more to be explored with these proteins, starting with the biotechnological use of ureases (and of urease-derived peptides) as transgenes to protect crops against insect herbivory and disease-causing fungi, or as eco-friendly insecticides to control insect borne diseases. The comprehension of ureases as virulence factors not only as an ammonia-producing and alkalinizing agent but acting in a much more complex way, endowed of exocytosis-inducing and pro-inflammatory activities, and recruiting the participation of eicosanoids, may show the way to finding new pharmacological approaches to many pathologies.

Finally, with this review, apart from historical and structural aspects of ureases, we wanted to encourage the readers to take a second look at ureases, very versatile proteins that happen also to catalyze the breakdown of urea into ammonia and carbamate.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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