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Review

Plant ureases: Roles and regulation✪

Agnieszka Sirko✉ and Robert Brodzik

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, A. Pawińskiego 5A, 02-106 Warszawa, Poland

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Both urea and urease were subjects of early scientific investigations. Urea was the first organic molecule to be synthesized and jack bean urease was the first enzyme ever to be crystallized. About 50 years later it was shown to be the first nickel metalloenzyme. Since then, nickel-dependent ureases have been isolated from many bacteria, fungi and higher plants. They have similar structures and mechanisms of catalysis. A urease apoenzyme needs to be activated. This process requires participation of several accessory proteins that incorporate nickel into the urease forming catalytic site. In this review, ureases from various organisms are briefly described and the similarities of their structures discussed. Moreover, the significance of urea recycling in plants is explained and recent literature data about the function and activation of plant ureases are presented.

ENZYMATIC ACTIVITY OF UREASE

Urease (EC 3.5.1.5, urea amidohydrolase), a nickel-dependent metalloenzyme, catalyzes the hydrolysis of urea to form ammonia and carbon dioxide. Hydrolysis of one molecule of urea results in the release of two molecules of ammonia and one molecule of carbon dioxide.

Several assays, mainly based on the measurement of the amounts of products released during the reaction, are available for quantifying urease activity. Ammonia can be detected by several methods including: ion-selective electrodes, reaction with phenol-hypochlorite or with Nesler's reagent. It is possible to include pH-sensitive dyes in the assays or simply observe changes with pH electrodes since release of ammonia results in an increase in pH. The amount of ammonia can also be monitored spectrophotometrically using a coupled system with NADH-dependent glutamate dehydrogenase (ammonia is a substrate for

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✉e-mail: asirko@ibb.waw.pl

this enzyme). The other product of the reaction, carbon dioxide, can be trapped and monitored by radiological methods with ^{14}C-labeled urea as a substrate. For references and a more detailed review of the methods see (Mobley & Hausinger, 1989, and Mobley *et al.*, 1995).

OCCURRENCE OF UREASE IN LIVING ORGANISMS

Many organisms, including plants, some bacteria, fungi and invertebrates, synthesize urease. Biochemically, the best-characterized plant urease is that from jack bean (*Canavalia ensiformis*) (Hirai *et al.*, 1993; Karmali & Domingos, 1993; Riddles *et al.*, 1991; Takishima *et al.*, 1988). Recently, urease from mulberry (*Morus alba*) leaves has also been purified and characterized (Hirayama *et al.*, 2000). The best genetic data concerning plant ureases are available for soybean (*Glycine max*) (Polacco & Holland, 1993; 1994). Separate genes encoding two urease isoenzymes, a tissue-ubiquitous and embryo-specific, as well as the unlinked genes encoding regulatory proteins (see below), were identified in soybean (Meyer-Bothling & Polacco, 1987; Torisky *et al.*, 1994) and mutants are available. The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean (Polacco & Holland, 1994) and *Arabidopsis* (Zonia *et al.*, 1995), while the other type of urease (called ubiquitous) is found in lower amounts in vegetative tissues of most plants (Hogan *et al.*, 1983).

Bacterial ureases play an important role in the pathogenesis of a number of bacterial species including *Proteus mirabilis*, *Staphylococcus saprophiticus*, *Yersinia enterocolitica*, *Ureaplasma urealiticum* and others (Mobley *et al.*, 1995). Due to urease activity, bacteria (e.g. *Klebsiella aerogenes*) are able to use urea as a sole nitrogen source (Mulrooney *et al.*, 1989). In the case of *Vibrio parahaemolyticus*, the ability to hydrolyze urea was proposed as a simple screening test to predict which strains are potentially pathogenic (Kaysner *et al.*, 1994). One of the most frequently mentioned examples in the recent literature is the urease from *Helicobacter pylori* because of its essential role in the pathogenesis of this microorganism and the high prevalence of this human pathogen (Eaton *et al.*, 1991). However, the best structural data are available for the urease from *K. aerogenes* (Jabri *et al.*, 1995).

Urease activity was found in several species of fungi, however, the nucleotide sequences of the genes encoding urease were reported for only a few of them, including a fungal respiratory pathogen of human *Coccidioides immitis* (Yu *et al.*, 1997) and *Schizosaccharomyces pombe* (Tange & Niwa, 1997).

In the invertebrate *Aplysia californica*, urease, together with carbonic anhydrase, is required for the formation and homeostasis of statoconia, calcium carbonate inclusions in the lumen of the gravity-sensing organ, the statocyst (Pedrozo *et al.*, 1996a; 1996b).

Urease is a cytosolic enzyme. In most of the studied cases (with exceptions that will not be discussed here) the majority of its activity is associated with the soluble fractions of the cells (Mobley *et al.*, 1995).

PROTEIN STRUCTURE OF UREASES

The plant and fungal ureases are homo-oligomeric proteins (consist of identical subunits), while the bacterial ureases are multimers formed from a complex of two or three subunits (Mobley *et al.*, 1995; Tange & Niwa, 1997). Significant amino-acid similarities were observed between all known ureases. Amino-terminal residues of the monomers of plant and fungal enzymes are similar to the small subunits of bacterial enzymes (e.g. UreA of *H. pylori*). The large subunits of bacterial ureases (e.g. UreB of *H. pylori*) resemble the carboxy-terminal portions of plant and fungal subunits (Fig. 1). The high sequence similarity indicates that all ureases

are variants of the same enzyme and are likely to possess similar tertiary structures and catalytic mechanisms (Mobley *et al.*, 1995). This conclusion is supported by the available biochemical and structural data obtained for the best-characterized ureases, e.g. from jack bean (Hirai *et al.*, 1993) and *K. aerogenes* (Jabri *et al.*, 1995). Jack bean urease exists as a homotrimer able to aggregate to a homohexamer (Hirai *et al.*, 1993). Bacterial ureases possess structures similar to the jack bean urease. They are either trimers or hexamers of subunit complexes (Fig. 1). They can also exist in aggregated forms. The activatory) proteins, which appear to act as urease-specific chaperones (Mobley *et al.*, 1995). These proteins are required for assembling an active urease in both bacteria and plants (Polacco & Holland, 1994; Mobley *et al.*, 1995).

ROLE OF UREASES IN PLANT METABOLISM

There are at least three key enzymes involved in urea metabolism in plants: arginase, urease and glutamine synthetase (Fig. 2).

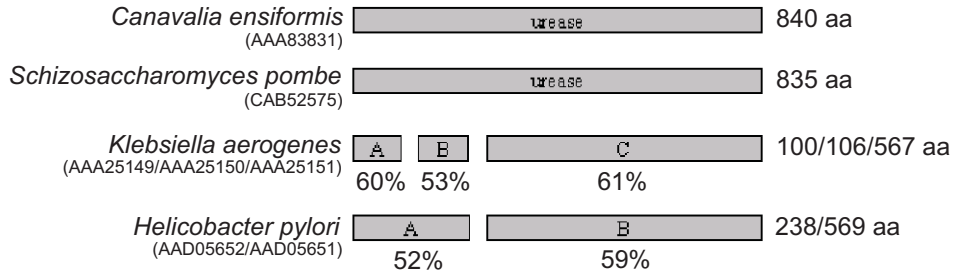

Figure 1. Schematic comparison of the structural subunits of ureases from selected organisms.

The GenBank accession numbers (in brackets) and the numbers of amino acids (on the right) are shown for each protein. The percent values below boxes indicate the degree of identity to urease from jack bean (*C. ensiformis*).

stoichiometry of subunits (1 : 1 for ureases from *Helicobacter* sp. or 1 : 1 : 1 for most bacterial ureases, including the urease from *K. aerogenes*) is always maintained. The catalytic site is located in subunit UreC of the *K. aerogenes* enzyme and in the respective regions (Fig. 1) of ureases from other organisms.

Each catalytic subunit contains the active site with two nickel ions that, in the case of crystallized *K. aerogenes* urease, were shown to be 3.5 Å apart (Jabri *et al.*, 1995). In this enzyme, one nickel atom is bound to two histidine residues (His-246 and His-272), while the second nickel atom is bound to three residues: two histidines (His-134 and His-136) and aspartic acid (Asp-360). Additionally, a carbamate ligand derived from Lys-217 bridges the two nickel ions (Mobley *et al.*, 1995). The process of nickel incorporation requires participation of many accessory (or

The primary role of ureases is to allow the organism to use external or internally generated urea as a nitrogen source (Mobley & Hausinger, 1989; Mobley *et al.*, 1995). Significant amounts of plant nitrogen flow through urea. This compound derives from arginine (Fig. 2) and possibly from degradation of purines and ureides (Polacco & Holland, 1994). The nitrogen present in urea is unavailable to the plant unless hydrolyzed by urease. The product of urease activity – ammonia – is incorporated into organic compounds mainly by glutamine synthetase (Fig. 2). It has been reported that overexpression of the pine glutamine synthetase in transgenic poplar improves the growth of the plants, probably by increasing the efficiency of nitrogen utilization (Gallardo *et al.*, 1999). On the other hand, it has been shown that increased activity of this enzyme in the roots of a legume plant, *Lotus japonicus*, leads to decreased plant biomass production,

possibly due to limited nitrate uptake (Limami *et al.*, 1999). These results, which seem contradictory at first glance, might reflect the different roles of glutamine synthetase isoenzymes in different plants and/or different plant organs. They indicate the significance of this enzyme for plant nitrogen metabolism. The activity of glutamine synthetase, the amount of its product, glutamine, and possibly the availability of its substrate, ammonia, seem to be important factors controlling nitrogen metabolism and affecting plant growth (Stitt, 1999; Wiren *et al.*, 2000).

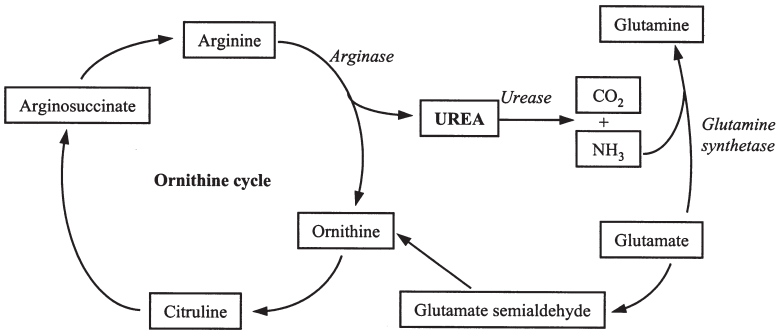

Figure 2. Urea metabolism in plants.

Modified from Gerendas *et al.* (1998). The names of the key enzymes involved in the production and conversion of urea are indicated in italics.

The ubiquitous urease (found in all plant tissues) is responsible for recycling metabolically derived urea (Polacco & Holland, 1994). In soybean, the ubiquitous urease is found at the level of 1/1000 or 1/100 that of the embryo-specific urease. The latter does not seem to have any assimilatory function. Its physiological role is unknown, however, the involvement in protection against plant pathogens (due to production of toxic ammonia) has been proposed (Polacco & Holland, 1994).

As mentioned above, urea can be assimilated exclusively by urease in higher plants. Moreover, urease is the only nickel-containing metalloenzyme yet identified in plants (Polacco & Holland, 1993; 1994). The importance of nickel for urease activity was demonstrated by the observation that urea-grown nickel-deprived rice (*Oriza sativa*) plants showed reduced growth and accumulated large amounts of urea due to reduced urease activity (Gerendas *et al.*, 1998). Urease-negative mutant plants and nickel- deprived wild type plants have the same phenotype. They accumulate urea and exhibit necrotic leaf tips, apparently due to urea "burn" (Polacco & Holland, 1993). It has been demonstrated that similar leaf-tip necrosis observed after fertilization with urea result from the accumulation of toxic amounts of urea rather than from the formation of a toxic amount of ammonia (a product of urease action) since addition of a urease inhibitor increased the leaf-tip necrosis (Krogmeier *et al.*, 1989).

Urease plays an important role in germination and in seedlings' nitrogen metabolism. It may function coordinately with arginase in the utilization of seed protein reserves during germination (Polacco & Holland, 1993). Imbibition of *Arabidopsis* seeds in water containing urease inhibitor delayed germination by 36 h and completely blocked germination of aged seeds. This inhibition could be abolished by supplying nitrogenous compounds into the imbibition medium (Zonia *et al.*, 1995).

PLANT ACCESSORY PROTEINS INVOLVED IN UREASE MATURATION

Genetic analysis of urease expression in soybean revealed several genes whose inactiva-

tion leads to the lack of urease activity (Polacco & Holland, 1993; 1994). Two of these genes, *Eu1* and *Eu4*, encode embryo-specific and tissue-ubiquitous ureases, respectively. Two other genes, *Eu2* and *Eu3*, are analogous to bacterial urease accessory genes that are involved in urease maturation i.e. the placing of essential nickel in the urease active site. Mutation in either of the two latter genes eliminates the activity of the ubiquitous and embryo ureases but has little effect on the amounts of these proteins (Meyer-Bothling & Polacco, 1987; Meyer- Bothling *et al.*, 1987). It was recently shown that *Eu3* gene encodes the 32-kDa protein (Freyermuth *et al.*, 2000). Its homologues have also been found in *Arabidopsis* (Freyermuth *et al.*, 1999) and in *Medicago truncatula* (accession AA660998). Eu3 protein interaction with the product of *Eu2* in the activation of embryo-specific urease was demonstrated (Freyermuth *et al.*, 2000) but sequence data are not yet available for *Eu2*. Freyermuth and coworkers have shown that the Eu3 protein is developmentally controlled and accumulates in developing embryos. They have proved the direct involvement of this protein in urease activation since anti-Eu3 antibodies blocked this activation *in vitro* (Freyermuth *et al.*, 2000). Lack of Eu3 protein prevented accumulation of embryo-specific urease since the enzyme is most probably unstable without nickel insertion. Although the authors could not prove the direct involvement of Eu3 in the activation of tissue-specific urease (encoded by *Eu4*) because of the low sensitivity of the method (Freyermuth *et al.*, 2000), such an involvement is rather expected, since both functional Eu3 and functional Eu2 are necessary for the activation of both types of ureases (Polacco & Holland, 1994).

Urease activation in *K. aerogenes* involves the action of four accessory proteins (UreD, UreE, UreF and UreG) (Moncrief & Hausinger, 1997). These proteins are well conserved in bacteria that possess urease activity (Mobley *et al.*, 1995). The Eu3 protein shows 55–60% identity in its C-terminal part to bacterial UreG proteins, including a nucleotide-binding P-loop that is essential for *in vivo* activation (Moncrief & Hausinger, 1997). The N-terminal part of Eu3 is rich in histidines and in this regard is similar to the C-terminus of *K. aerogenes* UreE (Freyermuth *et al.*, 2000). No plant homologues of bacterial UreD, UreE or UreF have been yet identified.

In summary, several pieces of evidence suggest that both urease enzymes and the machinery required for their activation are evolutionary conserved. First, there is a significant sequence homology not only between urease enzymes (Fig. 1) but also between activatory factors (Freyermuth *et al.*, 2000), although the latter is much less striking. Second, it was postulated that urease of soybean-commensal bacteria (*Methylobacter*) required some plant factors for activation (Polacco & Holland, 1994). Third, an increase of urease activity was observed in plants transformed with the *ureA* and *ureB* genes of *H. pylori* encoding subunits of urease (Brodzik *et al.*, 2000). This result suggests that the plant accessory factors were able to incorporate nickel into a bacterial apoenzyme. The same enzyme produced in *E. coli* was inactive unless co-expressed with the *H. pylori* genes encoding accessory proteins (Hu & Mobley, 1993).

CONCLUDING REMARKS

Significance of urea metabolism and advantages of increasing urease activity in plants

Nitrogen, after carbon, is the most limiting element in plant nutrition. Efficient recycling of reduced nitrogen present in the form of urea is important for plant growth since urea contains a significant amount of this element (Polacco & Holland, 1993). In addition to internally generated urea, externally applied urea can also be utilized by plants. Urea is a widely used fertilizer because of its low costs, ease in handling and high nitrogen content

(Mobley & Hausinger, 1989). In plants, urease is the only enzyme that is able to recapture nitrogen from urea (Polacco & Holland, 1993). Fertilization with urea through leaves could be an efficient method of plant feeding and any modifications leading to increased urease activity in leaves could result in more effective assimilation of this fertilizer. Such an increase might have a positive impact on the nitrogen metabolism in plants since more ammonia would be available for assimilation *via* glutamine into a variety of nitrogenous compounds.

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REFERENCES

Brodzik, R., Koprowski, H., Yusibov, V. & Sirko, A. (2000) Production of urease from *Helicobacter pylori* in transgenic tobacco plants. *Cell Mol. Biol. Lett.* **5**, 357–**366.**

Eaton, K.A., Brooks, C.L., Morgan, D.R. & Krakowka, S. (1991) Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. *Infect. Immun.* **59**, 2470–2475.

Freyermuth, S.K., Forde, B.G. & Polacco, J.C. (1999) Nucleotide sequence of a cDNA encoding an *Arabidopsis* urease accessory protein (accession no. AF109374) (PGR99–012). *Plant Physiol.* **119**, 364–366.

Freyermuth, S.K., Bacanamwo, M. & Polacco, J.C. (2000) The soybean *Eu3* gene encodes an Ni-binding protein necessary for urease activity. *Plant J.* **21**, 53–60.

Gallardo, F., Fu, J., Canton, F.R., Garcia-Gutierrez, A., Canovas, F.M. & Kirby, E.G. (1999) Expression of a conifer glutamine synthetase gene in transgenic poplar. *Planta* **210**, 19–26.

Gerendas, J., Zhu, Z. & Sattelmacher, B. (1998) Influence of N and Ni supply on nitrogen metabolism and urease activity in rice (*Oryza sativa* L.). *J. Exp. Bot.* **49**, 1545–1554.

Hirai, M., Kawai-Hirai, R., Hirai, T. & Ueki, T. (1993) Structural change of jack bean urease induced by addition of surfactants studied with synchrotron-radiation small-angle X-ray scattering. *Eur. J. Biochem.* **215**, 55–61.

Hirayama, C., Sugimura, M., Saito, H. & Nakamura, M. (2000) Purification and properties of urease from the leaf of mulberry, *Morus alba*. *Phytochemistry* **53**, 325–330.

Hogan, M.E., Swift, I.E. & Done, J. (1983) Urease assay and ammonia release from leaf tissues. *Phytochemistry* **22**, 663–667.

Hu, L.T. & Mobley, H.L. (1993) Expression of catalytically active recombinant *Helicobacter pylori* urease at wild-type levels in *Escherichia coli*. *Infect. Immun.* **61**, 2563–2569.

Jabri, E., Carr, M.B., Hausinger, R.P. & Karplus, P.A. (1995) The crystal structure of urease from *Klebsiella aerogenes*. *Science* **268**, 998–1004.

Karmali, A. & Domingos, A. (1993) Monoclonal antibodies against urease from *Canavalia ensiformis*. *Biochimie* **75**, 1001–1006.

Kaysner, C.A., Abeyta, C., Jr., Trost, P.A., Wetherington, J.H., Jinneman, K.C., Hill, W.E. & Wekell, M.M. (1994) Urea hydrolysis can predict the potential pathogenicity of *Vibrio parahaemolyticus* strains isolated in the Pacific Northwest. *Appl. Environ. Microbiol.* **60**, 3020–3022.

Krogmeier, M.J., McCarty, G.W. & Bremner, J.M. (1989) Phytotoxicity of foliar-applied urea. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8189–8191.

Limami, A., Phillipson, B., Ameziane, R., Pernollet, N., Jiang, Q., Roy, R., Deleens, E., Chaumont-Bonnet, M., Gresshoff, P.M. & Hirel, B. (1999) Does root glutamine synthetase control plant biomass production in *Lotus japonicus* L.? *Planta* **209**, 495–502.

Meyer-Bothling, L.E. & Polacco, J.C. (1987) Mutational analysis of the embryo-specific urease locus of soybean. *Mol. Gen. Genet.* **209**, 439–444.

Meyer-Bothling, L.E., Polacco, J.C. & Cianzio, S.R. (1987) Pleiotropic soybean mutants defective in both urease isoenzymes. *Mol. Gen. Genet.* **209**, 432–438.

Mobley, H.L. & Hausinger, R.P. (1989) Microbial ureases: Significance, regulation, and molecular characterization. *Microbiol. Rev.* **53**, 85–108.

Mobley, H.L., Island, M.D. & Hausinger, R.P. (1995) Molecular biology of microbial ureases. *Microbiol. Rev.* **59**, 451–480.

Moncrief, M.B. & Hausinger, R.P. (1997) Characterization of UreG, identification of a UreD-UreF-UreG complex, and evidence suggesting that a nucleotide-binding site in UreG is required for *in vivo* metallocenter assembly of *Klebsiella aerogenes* urease. *J. Bacteriol.* **179**, 4081–4086.

Mulrooney, S.B., Pankratz, H.S. & Hausinger, R.P. (1989) Regulation of gene expression and cellular localization of cloned *Klebsiella aerogenes* (*K. pneumoniae*) urease. *J. Gen. Microbiol.* **135**, 1769–1776.

Pedrozo, H.A., Schwartz, Z., Dean, D.D., Wiederhold, M.L. & Boyan, B.D. (1996a) Regulation of statoconia mineralization in *Aplysia californica in vitro*. *Connect. Tissue Res.* **35**, 317–323.

Pedrozo, H.A., Schwartz, Z., Luther, M., Dean, D.D., Boyan, B.D. & Wiederhold, M.L. (1996b) A mechanism of adaptation to hypergravity in the statocyst of *Aplysia californica*. *Hear Res.* **102**, 51–62.

Polacco, J.C. & Holland, M.A. (1993) Roles of urease in plant cells; in *International Review of Cytology* (Jeon, K.W. & Jarvik, J., eds.) vol. 145, pp. 65–103, Academic Press, Inc., San Diego.

Polacco, J.C. & Holland, M.A. (1994) Genetic control of plant ureases; in *Genetic Engineering* (Setlow, J.K., ed.) vol. 16, pp. 33–48, Plenum Press, New York.

Riddles, P.W., Whan, V., Blakeley, R.L. & Zerner, B. (1991) Cloning and sequencing of a jack bean urease-encoding cDNA. *Gene* **108**, 265–267.

Stitt, M. (1999) Nitrate regulation of metabolism and growth. *Curr. Opin. Plant Biol.* **2**, 178–186.

Takishima, K., Suga, T. & Mamiya, G. (1988) The structure of jack bean urease. The complete amino acid sequence, limited proteolysis and reactive cysteine residues. *Eur. J. Biochem.* **175**, 151–165.

Tange, Y. & Niwa, O. (1997) Identification of the *ure1*$^+$ gene encoding urease in fission yeast. *Curr. Genet.* **32**, 244–246.

Torisky, R.S., Griffin, J.D., Yenofsky, R.L. & Polacco, J.C. (1994) A single gene (*Eu4*) encodes the tissue-ubiquitous urease of soybean. *Mol. Gen. Genet.* **242**, 404–414.

Wiren, N., Gazzarrini, S., Gojon, A. & Frommer, W.B. (2000) The molecular physiology of ammonium uptake and retrieval. *Curr. Opin. Plant Biol.* **3**, 254–261.

Yu, J.J., Smithson, S.L., Thomas, P.W., Kirkland, T.N. & Cole, G.T. (1997) Isolation and characterization of the urease gene (URE) from the pathogenic fungus *Coccidioides immitis*. *Gene* **198**, 387–391.

Zonia, L.E., Stebbins, N.E. & Polacco, J.C. (1995) Essential role of urease in germination of nitrogen-limited *Arabidopsis thaliana* seeds. *Plant Physiol.* **107**, 1097–1103.