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UREA METABOLISM AND REGULATION BY RUMEN BACTERIAL UREASE IN RUMINANTS – A REVIEW*

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Abstract

Urea is used as non-protein nitrogen in the rations of ruminants as an economical replacement for feed proteins. Urea transferred from the blood to the rumen is also an important source of nitrogen for rumen microbial growth. It is rapidly hydrolyzed by rumen bacterial urease to ammonia (NH_3) and the NH_3 is utilized for the synthesis of microbial proteins required to satisfy the protein requirements of ruminants. Urea has commonly become an accepted ingredient in the diets of ruminants. In recent decades, urea utilization in ruminants has been investigated by using traditional research methods. Recently, molecular biotechnologies have also been applied to analyze urea-degrading bacteria or urea nitrogen metabolism in ruminants. Combining traditional and molecular approaches, we can retrieve better information and understanding related to the mechanisms of urea metabolism in ruminants. This review focuses on urea utilization in ruminants and its regulation by rumen bacterial urease in the host. The accumulated research provides foundations for proposing further new strategies to improve the efficiency of urea utilization in ruminants.

Key words: rumen, urea utilization, ureolytic bacteria, urease, regulation

Urea has been used as non-protein nitrogen (NPN) in ruminant rations for some time. Kertz (2010) wrote that more than one hundred years ago, German workers suggested that urea could be used to replace a portion of dietary protein in ruminants. Thereafter, some studies were conducted on the use of NPN in ruminant diets. During the 1970s and 1980s, multiple studies were conducted on the utilization of urea

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as a replacement for protein in ruminant diets, especially its effect on dry matter intake (Wilson et al., 1975; Polan et al., 1976), rumen fermentation (Pisulewski et al., 1981; Kertz et al., 1983), milk yield and reproduction-related parameters (Ryder et al., 1972; Erb et al., 1976). Since then, research attempting to understand the mechanisms of urea utilization in ruminants has been conducted (Balcells et al., 1993; Huntington and Archibeque, 2000; Stewart and Smith, 2005).

Studies for improving urea utilization in ruminants are ongoing. It is known that the performance and metabolism of dairy cows depend upon the amount of urea they are fed (Sinclair et al., 2012; Giallongo et al., 2015). For example, ruminal nitrogen metabolism and urea kinetics of Holstein steers fed diets containing either rapidly degrading or slowly degrading urea at various levels of degradable intake protein (DIP) were estimated by Holder et al. (2015). They found that the rapidly degrading urea group had higher dry matter digestibility than the slow-release urea group, and gastrointestinal entry of urea nitrogen (urea-N), urea-N lost to feces and urea-N apparently used for anabolism were not different between treatments, while plasma urea concentrations were greater in higher DIP diets and higher for the rapidly degrading urea group than the slow release urea group. When 2% of urea was fed to lactating dairy cows as a replacement for soybean meal, both the milk protein content and milk yield decreased, while plasma urea-N increased (Imaizumi et al., 2015). Urea supplementation could also increase nitrogen availability for ruminal microorganisms. A study by Wanapat et al. (2016) showed that when swamp buffaloes were fed rice straw supplemented with urea, the feed intake, nutrient digestibility, and microbial protein synthesis increased. More importantly, the authors also tried to determine the effect of urea supplementation on rumen microbes and they found that fungal zoospores, total bacteria and the three predominant cellulolytic bacteria (*Ruminococcus albus*, *Fibrobacter succinogenes*, and *Ruminococcus flavefaciens*) were increased by urea supplementation.

Following extensive research on urea utilization in rumens, interests began to focus on urea-degrading microbes and urea utilization mechanisms in dairy cows. Research studying the regulation of bacterial urease for improving urea utilization has also been conducted. Advanced molecular biotechnologies provide new strategies to reveal the mechanisms of urea hydrolysis, transportation, and utilization in ruminants, and provide more knowledge for the improvement of nitrogen utilization efficiency in practical ruminant production systems. This review focuses on urea recycling in ruminants, urea hydrolysis, utilization and its regulation by rumen bacterial urease in recent research.

Urea nitrogen recycling in ruminants

In ruminants, ammonia arises in the rumen from the diet and recycled urea. Urea in the rumen is rapidly hydrolyzed to ammonia and CO_2 by the bacterial enzyme urease. Ammonia from urea or from degraded dietary protein is used by the ruminal microbiota for the synthesis of microbial proteins, which are subsequently digested in the intestine. The excess ammonia is transported to the liver for endogenous urea synthesis, and urea recycling via the ruminal wall, and salivary secretion. Urea recycling to the rumen is an evolutionary advantage for ruminants because it provides

part of the N required for microbial protein synthesis and enhances survival (Reynolds and Kristensen, 2008).

Reutilization of endogenous urea

Ruminants fed on diets with high NPN had higher portal blood flow, greater hepatic uptake of excess NH_3 and increased rates of urea synthesis (Symonds et al., 1981; De Visser et al., 1997; Holder et al., 2015). Redundant NH_3 transported to the liver is likely to enter the ornithine cycle (Zhou et al., 2015). Therefore, ammonia detoxification in the liver is likely to be one of the reasons for increased plasma urea concentration (Law et al., 2009). Blood urea-N concentrations are influenced by many parameters, especially dietary nitrogen intake (Puppel and Kuczynska, 2016), and it also has been used to predict nitrogen excretion and efficient nitrogen utilization in cattle and several different species of farm animals (Kohn et al., 2005).

Ruminants recycle substantial amounts of nitrogen as urea by the transfer of urea across the ruminal wall, and salivary secretion (Huntington and Archibeque, 2000). In ruminants, urea that is recycled to the rumen is an important source of nitrogen for microbial growth and reported data indicate that 40 to 80% of endogenously produced urea-N is returned to the gastrointestinal tract (Harmeyer and Martens, 1980; Lapierre and Lobley, 2001). There is a reciprocal change between urea recycling and excretion in urine depending on the crude protein intake (Reynolds and Kristensen, 2008). When growing cattle were fed prairie hay with very low protein concentrations, virtually almost all urea entering the blood pool was returned to the gut, and little was excreted in urine. In addition, ruminal fermentation products such as short-chain fatty acids and CO_2 acutely stimulate urea transport across the ruminal epithelium, and the effects are pH-dependent (Abdoun et al., 2010). The presence of ammonia has a negative impact on urea transport rates and is concentration dependent, with saturation at 5 mmol/l (Lu et al., 2014). At physiological pH, uptake of $NH4^+$ into the cytosol may be a key signaling event regulating ruminal urea transport. Therefore, in ruminants, urea-N recycling is affected by a number of factors including plasma urea-N concentration and fermentable carbohydrates in the gastrointestinal tract.

Urea kinetics has been obtained by the infusion of labeled urea to provide an estimate of urea entry rate. Wickersham et al. (2008) evaluated the effect of increasing the amount of rumen- DIP on urea kinetics in steers consuming prairie hay with jugular infusions of $^{15}N^{15}N$-urea. They found that the transfer of urea from the blood to the rumen contributes between one-fourth and one-third of the N utilized by ruminal microbes for the synthesis of microbial protein. Provision of supplemental DIP increased forage utilization and N retention in cattle consuming low-quality forage. Zhou et al. (2015) also used $^{15}N^{15}N$-urea to detect urea kinetics and nitrogen balance in Tibetan sheep when fed oat hay. Urea-N entry rate, gastrointestinal tract entry rate, return to ornithine cycle and fecal urea-N excretion all increased linearly with an increase in dry matter intake. The estimated N requirements for maintenance were 0.50 g/kg bodyweight$^{0.75}$ per day, that is, only 66% of the amount recommended by NRC for growing sheep of its size. The Tibetan sheep demonstrated low N requirements for maintenance compared with other ruminants. Therefore, for different ru-

minants, there are a number of differences in N metabolism and recycling except for in some common responses.

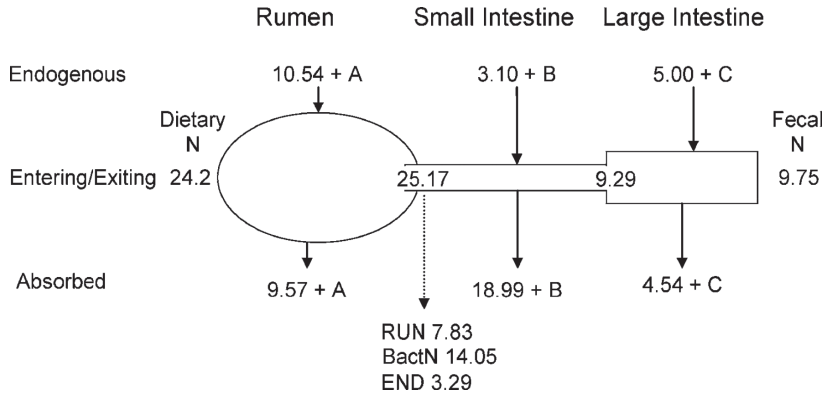

Figure 1. Nitrogen transactions along the gastrointestinal tract of cattle in an example diet (32% neutral detergent fiber [NDF], carbohydrates of medium degradation rate, consumed at 2% of bodyweight daily, on an OM basis) containing 24.2 g of N/kg of OM. All fluxes expressed in g of N/kg of OM intake. Nitrogen entering the small intestine is composed of ruminal undegraded feed N (RUN), bacterial N (BactN), and free endogenous N reaching the duodenum (END). 24.2 is N from the diet entering the rumen; 25.17 is the total duodenal N; 9.29 is the total ileal N; 9.75 is the total fecal N; 10.54, 3.10 and 5.00 are the endogenous N entering the rumen, the small intestine and the large intestine, respectively; 9.57, 18.99 and 4.54 are N absorbed by the foregut, in the small intestine and in the large intestine, respectively; xyz is absorbed and xyz is secreted etc. for the other parts of gastrointestinal tract. The estimates for the endogenous N entering each compartment are minimal estimates, which is indicated by the letters A, B, and C (Marini et al., 2008)

Currently, meta-analytical approaches have been used to evaluate the efficiency of urea utilization in ruminants. In the study of Marini et al. (2008), by utilizing a statistical approach and data obtained from studies reporting duodenal, ileal, and fecal N flows in cattle, the endogenous N losses and true digestibility of N were estimated for different segments of the gastrointestinal tract. The N transactions for the reference diet (24.2 g of N/kg of organic matter [OM], 32% neutral detergent fiber [NDF] and carbohydrates of medium fermentation rate) were estimated as shown in Figure 1. The results showed that the minimal contribution of endogenous N to the N available in the rumen was 39%. In addition, Batista et al. (2017) also estimated urea kinetics and microbial usage of recycled urea-N in ruminants by combining data from studies with ruminants (beef cattle, dairy cows, and sheep), which were published from 2001 to 2016 and analyzed according to meta-analysis techniques using linear or non-linear mixed models. They concluded that urea-N synthesized in the liver and urea-N recycled to the gut linearly increased as N intake (g/body weight$^{0.75}$) increased, with increases corresponding to 71.5% and 35.2% of N intake, respectively. However, increasing dietary crude protein intake led to decreases in the fractions of urea-N recycled to the gastrointestinal tract and of recycled urea-N incorporated into microbial N. Therefore, a better understanding of the factors involved in endogenous urea losses will allow for a more accurate estimation of both N supply and N

requirements. Since urea-N recycling to the gut is influenced by many dietary and ruminal factors, some modulation could be made in the rations of ruminants in order to improve the efficiency of utilizing endogenous urea.

Urea transport across the rumen epithelium

Urea produced in the liver is transferred across the rumen wall from the blood and then it is hydrolyzed to ammonia by resident bacteria (Lapierre and Lobley, 2001). As is already known, urea transport across the ruminant wall is mediated via urea transporters in the epithelium membrane (Abdoun et al., 2006). These transporters allow the passage of urea across cell membranes, down a concentration gradient (Smith and Rousselet, 2001). Facilitative urea transporters are derived from the UT-A and UT-B genes (Bankir et al., 2004). UT-B mRNA or protein expressions have been characterized in the rumen epithelium (Stewart et al., 2005; Simmons et al., 2009; Lu et al., 2015). In the study of Coyle et al. (2016), UT-B transporters were identified to be specifically localized to certain regions of tissue in the bovine gastrointestinal tract. UT-B2 was the predominant UT-B mRNA transcript expressed in dorsal, ventral and cranial ruminal sacs, while alternative UT-B transcripts were present in other gastrointestinal tissues (Figure 2).

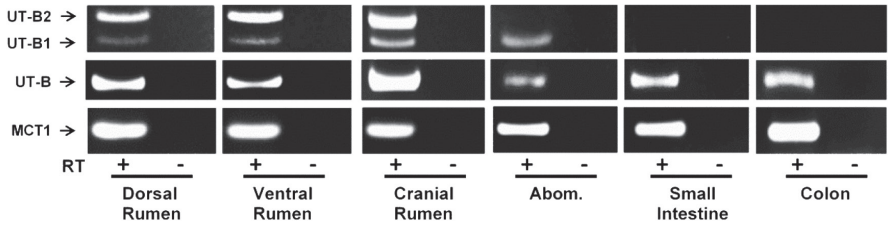

Figure 2. RT-PCR experiments investigating cDNA derived from total RNA samples from bovine gastrointestinal tissues. Analysis with BSF/BODR UT-B primers confirmed strong UT-B2 (900 bp) and weak UT-B1 (750 bp) expression in the dorsal, cranial and ventral rumen. Using these isoform-specific BSF/BODR primers, only UT-B1 was detected in the abomasum and no signals at all in either the small intestine or colon. In contrast, using BODF/BODR primers, general UT-B transcripts were detected in all six tissues tested. In addition, strong MCT1 signals were also detected in ruminal sac, abomasum, small intestine, and colon samples. MCT 1, monocarboxylate transporter 1; RT, reverse transcriptase; Abom., Abomasum (Coyle et al., 2016)

In addition to the UT-B transporters, some alternative transport mechanisms are also involved in urea transport across the epithelium. The aquaporins (AQP) are a family of membrane-spanning proteins predominantly involved in water movement, and some of them also play a role in urea movement. AQP-3, -7, -9 and -10 have been proven to be involved in urea uptake or transport, while AQP-8 is permeable to ammonia (Rojek et al., 2008; Litman et al., 2009). Rojen et al. (2011) showed that messenger RNA expression of AQP3, AQP7, and AQP10 and the abundance of AQP8 increased with increasing nitrogen intake, but their findings do not point to

these proteins as the cause of increased rumen epithelial urea permeability in dairy cows fed a low N diet. Walpole et al. (2015) investigated the roles of UT-B and AQP in the serosal-to-mucosal urea flux across rumen epithelium using Ussing chambers. The urea flux markedly decreased when Phloretin and $NiCl_2$ were added to inhibit UT-B- or AQP-mediated urea transport, respectively, which proved that both AQP and UT-B play significant functional roles in urea transport. Gene transcript abundance for UT-B and AQP was observed to be significantly correlated with the ruminal serosal to mucosal urea fluxes. However, the mechanism by which the increased gene expression occurred is unclear. Transcriptome analysis has been used to analyze the rumen epithelium metabolic pathway changes under various conditions (Baldwin et al., 2012; Dionissopoulos et al., 2014; Naeem et al., 2014), and this approach may provide a better means to understand the regulation of these urea transport mechanisms across the rumen wall.

Rumen ureolytic bacteria

Rumen ureolytic bacteria play an important role in dietary urea hydrolysis, for they produce ureases that catalyze the breakdown of urea to ammonia (NH_3) and carbon dioxide (Owens et al., 1980). In the rumen, the ammonia can be assimilated by many rumen bacteria for the synthesis of microbial proteins (Owens et al., 1980; Milton et al., 1997). However, the efficiency of urea N utilization in ruminants is low and this is attributed to the rapid hydrolysis of urea to NH_3, which occurs at a higher rate than NH_3 utilization by rumen bacteria (Patra, 2015). Due to the difficulty in cultivating rumen bacteria, only a small number of bacteria have been isolated (Kim et al., 2011). The lack of sufficient understanding of the ruminal microbiome is one of the major knowledge gaps that hinder effective enhancement of rumen functions (Firkins and Yu, 2006). In addition, limited information about rumen urea-degrading bacteria makes regulation of the urea hydrolysis rate by targeting predominant ureolytic bacteria difficult.

Ureolytic bacteria isolated using culture-dependent methods

Early studies have isolated some ureolytic bacteria from the rumen (Cook, 1976; On et al., 1998). Wozny et al. (1977) described a rapid qualitative procedure to detect urease in strains isolated from the bovine rumen, and found that many species including *Succinivibrio dextrinosolvens*, *Treponema* sp., *Ruminococcus bromii*, *Butyrivibrio* sp., *Bifidobacterium* sp., *Bacteroides ruminicola*, and *Peptostreptococcus productus* had urease activity and most *P. productus* strains contain urease. Kakimoto et al. (1989) assayed about 16,000 isolates from animal feces and intestines for the production of acid urease and found that most of the selected strains belonged to the genera *Streptococcus* and *Lactobacillus*. In a similar study by Lauková and Koniarová (1994), 909 strains from the rumen of 104 domestic and wild ruminants were tested for urease activity, and their results showed that some *Selenomonas ruminantium* strains and *Lactobacilli* manifested medium urease activity and most of the *Enterococcus faecium* and all of the *E. faecalis* isolates expressed urease activity. In addition, *Howardella ureilytica*, a Gram-positive bacterium that has been isolated from the rumen fluid of sheep, was found to be strongly ureolytic and generated

ATP through the hydrolysis of urea (Cook et al., 2007). All these above studies were conducted using culture-based methods; however, due to the difficulty in cultivating rumen bacteria, those that have been isolated represent only 6.5% of the community (Kim et al., 2011), and, therefore, only very limited information is known about rumen ureolytic bacteria. These previous studies exploring ureolytic bacteria only identified the urease activity of isolated bacteria and did not consider the information about the urease genes that express the urease. With the help of modern molecular technologies, we can acquire more information of the ureolytic bacteria at the DNA level.

Culture-independent methods of studying ureolytic bacteria

In order to get further information about the function of rumen microbes, sequencing and phylogenetic analysis of 16S rRNA and functional genes have been extensively carried out in studies focused on members of uncultivable bacteria (Chaucheyras-Durand and Ossa, 2014). For ureolytic bacteria, the *ureC* gene encodes the largest urease functional subunit and contains several highly conserved regions that are suitable as PCR priming sites (Mobley et al., 1995). Previously, Reed (2001) successfully designed urease PCR primers that can amplify a 340 bp fragment of the *ureC* gene from a variety of urease producing bacteria. Primers for *ureC* genes have been developed and applied to the analysis of urea-degrading microorganisms in various environments (Collier et al., 2009; Singh et al., 2009; Su et al., 2013).

Because rumen ureolytic bacteria are the key organisms that produce urease for the breakdown of urea, further insights into the abundant ureolytic bacteria or urease functional genes could provide the basis for designing strategies to efficiently manipulate the rumen bacteria and improve urea utilization in ruminants. Zhao et al. (2015) attempted to examine rumen ureolytic bacterial diversity by cloning and sequencing *ureC* genes and found that among the total 317 *ureC* sequences from the rumen digesta, some were about 84% identical (based on amino acid sequence) to the *ureC* gene of *Helicobacter pylori*. They also developed a vaccine based on *ureC* of *H. pylor*i, vaccinated cows had significantly reduced urease activity in the rumen compared to control cows that were mock immunized. Therefore, a vaccine based on *ureC* of *H. pylori* could be a useful approach to decrease bacterial ureolysis in the rumen. A vaccine prepared from a combination of representatives of different rumen *ureC* clusters may be more effective than *ureC* of *H. pylori* or a single rumen bacterial *ureC*.

In order to get more accurate information about the rumen ureolytic bacteria, Jin et al. (2016) investigated abundant ureolytic bacterial communities by high-throughput sequencing when treated with an activator (urea) or inhibitor (acetohydroxamic acid, AHA) of ureolytic bacteria *in vitro*. Results from 16S rRNA gene sequencing showed that rumen ureolytic bacteria were abundant in the genera of *Pseudomonas*, *Haemophilus*, *Neisseria*, *Streptococcus*, *Actinomyces*, *Bacillus*, and unclassified Succinivibrionaceae. Recently, Jin et al. (2017) studied the differences in ureolytic bacterial composition between the rumen digesta and rumen wall based on *ureC* gene classification, and found that more than 55% of the *ureC* sequences did not affiliate with any known taxonomically assigned urease genes, and the most abundant

ureC genes were affiliated with the families of Methylococcaceae, Clostridiaceae, Paenibacillaceae, Helicobacteraceae, and Methylophilaceae (Figure 3).

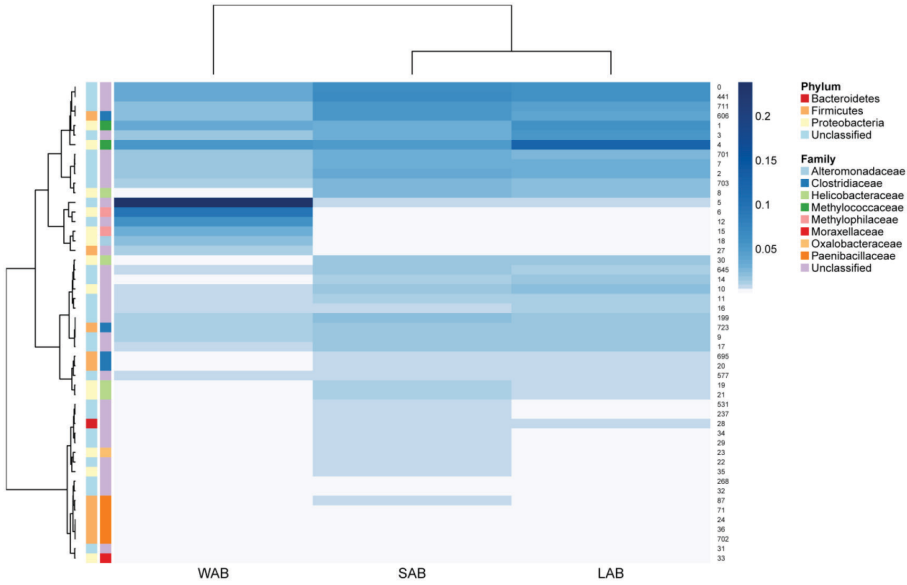

Figure 3. Rumen *ureC* gene community heatmaps and clustering of the most abundant 50 operational taxonomic units (OTUs) from different rumen fractions. Ward's minimum variance method was used for hierarchical clustering of the computed distance matrix for samples based on the Jaccard dissimilarity indices of the OTU data in the vegan package. LAB, liquid-associated bacteria; SAB, solid-adherent bacteria; WAB, wall-adherent bacteria (Jin et al., 2017)

Studies which target the *ureC* genes provide a basis for obtaining the full-length urease functional gene information (Yuan et al., 2012). This survey has expanded our knowledge of information relating to the predominant *ureC* gene in the rumen ureolytic microbial community, and provides a basis for obtaining vaccine targets of urease for regulating rumen bacterial urease activities, and moderating urea hydrolysis and utilization in the rumen.

Bacterial urease

Characterization and activation of bacterial ureases

Ureases (urea amidohydrolases, EC 3.5.1.5) are nickel-dependent enzymes, found in plants, fungi, and bacteria, which are commonly composed of two or three subunits (encoded by genes *ureA*, *ureB*, and *ureC*), and require up to several accessory proteins for activation (Mobley et al., 1995). For example, the urease of *Klebsiella aerogenes* has three subunits $(UreABC)_3$ (Jabri et al., 1995). The urease of *H. pylori* consists of two subunits $((ureAB)_3)_4$, and *ureB* in the *Helicobacter* species is equivalent to *ureC* in the organisms possessing a three-subunit enzyme (Hu and Mobley, 1990). Urease accessory genes (such as *ureD*, *ureE*, *ureF*, *ureG*, *ureH*, and

ureI) are required for synthesis of catalytically active urease when the gene clusters are expressed in a recombinant bacterial host. Some of the accessory genes have been shown to play a role in the activation of the apoenzyme, and these genes are known to be required for assembly of the nickel metallocenter within the active site of the enzyme (Mehta et al., 2003; Witte et al., 2005; Boer and Hausinger, 2012). Taking the urease activation of *K. aerogenes* as an example, the UreD, UreF, UreG, and UreE are sequentially complexed to UreABC as required for its activation (Farrugia et al., 2013).

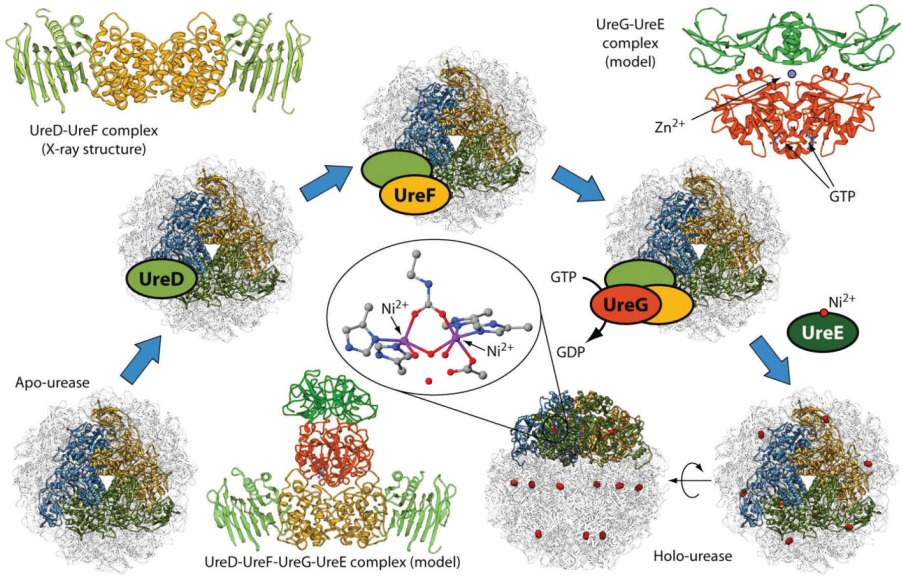

Figure 4. The *Helicobacter pylori* urease activation process starting from the apoenzyme and leading to holo-urease. The ribbon diagrams show the structure of *H. pylori* urease in its $[(ab)_3]_4$ quaternary structure; each blue chain, gold chain, and green chain represents one (ab) heterodimer, and together they reveal the similarity of the $(ab)_3$ moiety in this urease with the $(abc)_3$ quaternary structure of other bacterial ureases, such as those of *Sporosarcina pasteurii* and *Klebsiella aerogenes*. The details of the coordination environment of the Ni^{2+} ions in the active site are shown in the central inset. The crystal structures or models of the various protein complexes involved in the process are also shown as ribbon diagrams: *H. pylori* UreD (HpUreD) in light green, *H. pylori* UreF (HpUreF) in orange, *H. pylori* UreG (HpUreG) in red, and *H. pylori* UreE in dark green. GDP guanosine 5′-diphosphate, GTP guanosine 5′-triphosphate (Zambelli et al., 2014)

Urease inhibitors are targeted to the functional area of active urease. Therefore, investigation of the bacterial urease structure and activation of urease are important for finding the binding sites between urease inhibitor and urease, and for the regulation of the activation process of urease. Some studies have been done to explore the structures for the activation complex of urease (Biagi et al., 2013; Fong et al., 2013). Ligabue-Braun et al. (2013) provided an atomic-level model for the $(UreABC–UreDFG)_3$ complex from *K. aerogenes* by employing comparative modeling

associated to sequential macromolecular dockings, validated through small-angle X-ray scattering profiles. The resulting model included a putative orientation for UreG at the $(UreABC–UreDFG)_3$ oligomer. Fong et al. (2013) proposed a mechanism on how urease accessory proteins facilitate the maturation of urease. They reported the crystal structure of the UreG/UreF/UreH complex in *H. pylori*, which illustrates how UreF and UreH facilitate dimerization of UreG and assembles its metal binding site by juxtaposing two invariant Cys66-Pro67-His68 metal binding motifs at the interface to form the $(UreG/UreF/UreH)_2$ complex. Furthermore, Zambelli et al. (2014) identified the nickel binding properties of *H. pylori* UreF in the nickel-based activation of urease (Figure 4). UreF binds two Ni^{2+} ions per dimer, with a micromolar dissociation constant. Two nearly identical and symmetric tunnels were found, going from the central cavity in the UreG/UreF/UreH complex, and UreF was involved in the metal ion transport through these tunnels during urease activation. Currently, many aspects of the urease metallocenter assembly still remain obscure. The activation mechanism and roles of each accessory protein in urease maturation still need to be answered.

Regulation of bacterial urease synthesis

The regulation of urease synthesis in ureolytic bacteria is complex. In some organisms such as *Bacillus pasteurii,* and *Morganella morganii* isolated from soil, urease synthesis is constitutive (Mörsdorf and Kaltwasser, 1989; Burbank et al., 2012). However, urease synthesis in some bacteria is regulated by environmental conditions, such as the concentration of urea and nitrogen or pH (Weeks and Sachs, 2001; Dyhrman and Anderson, 2003; Belzer et al., 2005; Liu et al., 2008). Urease activity of *Providencia stuartii*, for example, is induced by the presence of urea (Armbruster et al., 2014), while *Klebsiella pneumoniae*, a facultative anaerobic organism, can use urea as the sole source of nitrogen, and the urease expression is regulated by the supply of nitrogen in the growth medium (Liu and Bender, 2007). The regulation of urease gene expression of *Actinomyces naeslundii* under different environmental conditions has been investigated by Liu et al. (2008). *A. naeslundii* is considered anaerobic or microaerophilic, the conditions of neutral pH, fast dilution rate, increased carbohydrate supply or low nitrogen supply in the medium all resulted in the enhancement of urease activity in *A. naeslundii*.

Helicobacter are Gram-negative, microaerophilic bacteria. In research comparing the regulation of urease activity in *Helicobacter hepaticus* and *H. pylori*, the urease activity of *H. hepaticus* was found to be acid-independent, which contrasts with the acid-induced urease system of *H. pylori* (Belzer et al., 2005).

When the model rumen Firmicutes organism *Ruminococcus albus* 8 were supplied with different nitrogen sources (urea, ammonia, and peptides), the urease activity was higher in the presence of urea than in the presence of ammonia and peptides (Kim et al., 2014). However, urease transcript abundance in *R. albus* 8 is not predicated by the presence of urea in the medium. This urease activity may demonstrate that *R. albus* 8 expresses urease to acquire urea as an alternative nitrogen source when the ammonia concentration in the medium is limited. Because the regulation of urease activity is complex and the rumen harbors a large diversity of ureolytic

bacteria, the mechanisms controlling urease synthesis in the complicated rumen environment need further research.

Improved urea utilization in ruminants with urease inhibitors

In ruminants, reducing the rate of urea hydrolysis in the rumen is of great importance for improving urea utilization and minimizing ammonia wastage. Urease inhibitors are one available option found to be effective in control urea hydrolysis. Several urease inhibitors, including AHA (Brent et al., 1971; Jones and Milligan, 1975), phenylphosphorodiamidate (Voigt et al., 1980 a; Voigt et al., 1980 b; Whitelaw et al., 1991), and N-(n-butyl) thiophosphoric triamide (NBPT) have been investigated (Ludden et al., 2000). Zhang et al. (2001) also studied the effect of hydroquinone on ruminal urease activity and found that concentrations of 0.01 ppm to 10 ppm inhibited urease activity of intact rumen microbes *in vitro* by 25% to 64%. Urease inhibitors also provide an insight into understanding the mechanism of enzyme catalytic activity present at the active site of the enzyme and the importance of nickel to urease, the metalloenzyme (Upadhyay, 2012).

The mechanism of *B. pasteurii* urease inhibition with acetohydroxamic acid has been resolved. The inhibitor anion symmetrically bridges the two Ni ions in the active site through the hydroxamate oxygen and chelating one Ni ion through the carbonyl oxygen (Benini et al., 2000). Although recent studies have already evaluated the function of different urease inhibitors in improving urea utilization efficiency (Ludden et al., 2000; Giallongo et al., 2015), further research is needed to investigate the response of the rumen bacteria community, especially ureolytic bacteria, to these inhibitors.

Summary

Urea is one of the major non-protein nitrogen feeds for ruminants and the optimal utilization of urea in feed can alleviate to some extent the cost of dietary protein. Urea is hydrolyzed quickly by ureolytic bacteria in the rumen. Because about 90% of rumen microbes have not been pure-cultured to date, only limited information about active ureolytic bacteria communities is known, which limits the regulation and efficient application of urea in ruminant production. Increased knowledge about ureolytic microbiomes will permit the development of mitigation strategies, such as urease inhibitors and vaccines, to target the dominant ureolytic bacteria species or urease successfully. There are breakthroughs in molecular strategies, the rapid advancement of "~omics" technologies, including metagenomics, metatranscriptomics, metabolomics, and bioinformatics could give a better understanding of the microbial and molecular mechanisms of ruminal urea hydrolyzation and utilization, and will provide knowledge for manipulating urea utilization efficiency in ruminants.

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