JOURNAL OF BACTERIOLOGY, May 2004, p. 2520–2522 Vol. 186, No. 9
0021-9193/04/$08.00+0 DOI: 10.1128/JB.186.9.2520–2522.2004
Copyright © 2004, American Society for Microbiology. All Rights Reserved.

Metabolic Versatility of Prokaryotes for Urea Decomposition

Robert P. Hausinger*

Department of Microbiology and Molecular Genetics and Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824

In this issue of the *Journal of Bacteriology*, Kanamori et al. (6) present evidence that *Oleomonas sagaranensis*, an α-proteobacterium, metabolizes urea by two distinct pathways. On the one hand, this microorganism directly hydrolyzes urea by action of urease, an enzyme that is widely distributed in prokaryotes, fungi, and plants. In addition, *O. sagaranensis* decomposes urea by use of urea amidolyase, an activity heretofore observed only in selected yeast and green algae. Of more general interest, sequence database searches suggest that the latter pathway is present in numerous other prokaryotes; thus, this alternative pathway for urea decomposition may be more prevalent than generally appreciated. These results inspire questions about why a microbe would possess two routes for urea degradation, how the two pathways are regulated and utilized in the cell, and whether one pathway might function in a role other than urea degradation.

UREASE

Urease catalyzes the deceptively simple reaction illustrated in Fig. 1, i.e., the hydrolysis of urea to yield one molecule each of ammonia and carbamate, with the latter decomposing spontaneously to yield a second molecule of ammonia and carbonic acid (3). The substrate is highly resonance stabilized (30 to 40 kcal/mol), thus decreasing the reactivity of its carbonyl carbon so that spontaneous hydrolysis of urea has never been observed. Rather, urea decomposes in solution (with an estimated half-life of 3.6 years at 38°C) by the slow elimination of ammonia to form cyanic acid (17). Remarkably, urease exhibits a catalytic rate that is at least 10^{14} times that of this elimination reaction. Of historical interest, this enzyme was the first to be crystallized (16) and the first shown to require nickel ions (2). The metal ions are present in a binuclear active site and are bridged by a carbamylated lysine residue plus a catalytic hydroxide ion, as depicted in Fig. 2 (4, 10). Acetohydroxamic acid binds tightly to this metallocenter (10), and the ability of this compound to inhibit ATP-independent urea decomposition was used to confirm the identity of urease in *O. sagaranensis* (6). Recent interest in urease has centered on its action as a virulence factor, such as its participation in gastroduodenal infections by *Helicobacter pylori* (15) and stimulation of urinary stone formation by *Proteus mirabilis* (7).

* Mailing address: Department of Microbiology and Molecular Genetics, 6193 Biomedical Physical Sciences Building, Michigan State University, East Lansing, MI 48824. Phone: (517) 355-6463, ext. 1610. Fax: (517) 353-8957. E-mail: hausinge@msu.edu.

UREA AMIDOLYASE

Urea amidolyase represents a second pathway for urea metabolism and is comprised of three activities, biotin carboxylase, urea carboxylase, and allophanate hydrolase (Fig. 3). A biotin cofactor, covalently attached to a carboxyl carrier domain of the protein (at a Lys residue found in a Met-Lys-Met sequence), is carboxylated in an ATP- and bicarbonate-dependent step (5). This reaction is presumed to involve a transient carboxyphosphate intermediate, but such a species has never been observed and is predicted to have a half-life of only 70 ms (14). Making use of the long side arm of the bound cofactor, the carboxybiotin translocates to a second active site where it serves as the carboxyl group donor to urea. The product, allophanate, is hydrolyzed by the third active site. Hydrolysis of this compound is far easier than for urea because of the greatly reduced resonance stabilization involving the carbonyl carbon. This elaborate mechanism for urea decomposition was first identified over 30 years ago (11–13). The biotin-dependent reactions are avidin sensitive, a property utilized by Kanamori et al. (6) to verify these activities, and are always present in the same protein. Allophanate hydrolase sometimes is associated with a separate domain fused to urea carboxylase, as in *Saccharomyces cerevisiae* (1), or it can be present as a distinct protein. Until the studies described in this issue (6), urea carboxylase activity had not been observed in any prokaryote. In contrast, allophanate hydrolase is predicted from sequence analyses to be present in cyanuric acid-degrading bacteria (8) and other bacterial species (6) and its activity was observed in an atrazine-degrading bacterium (8), but in no case had it previously been associated with urea decomposition in a prokaryote.

REMAINING QUESTIONS

The finding that a single bacterium possesses two pathways for decomposing urea raises the question: why? Both of these urea-degrading systems require a large investment on the part

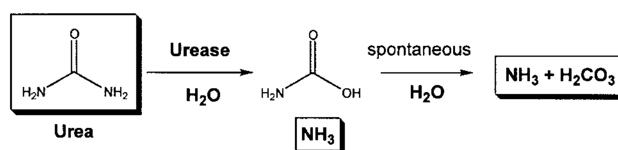

FIG. 1. Urease catalyzes the hydrolysis of urea, yielding one molecule each of ammonia and carbamate.

Downloaded from http://jb.asm.org/ on April 14, 2021 by guest

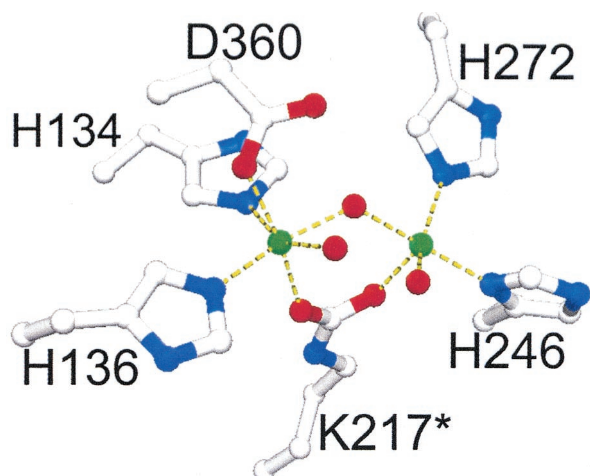

FIG. 2. Dinuclear nickel active site of urease. The structure depicted is that of urease from *Klebsiella aerogenes* (PDB accession code 1FWJ). A carbamylated lysine residue (indicated by K217*) bridges the two metal ions (shown in green), and metal-bound waters are illustrated in red.

of the microorganism. In the case of urease, enzyme synthesis requires the structural subunits, a collection of metallocenter biosynthesis accessory proteins, CO_2/bicarbonate (for lysine carbamylation), GTP hydrolysis, and a mechanism to take up

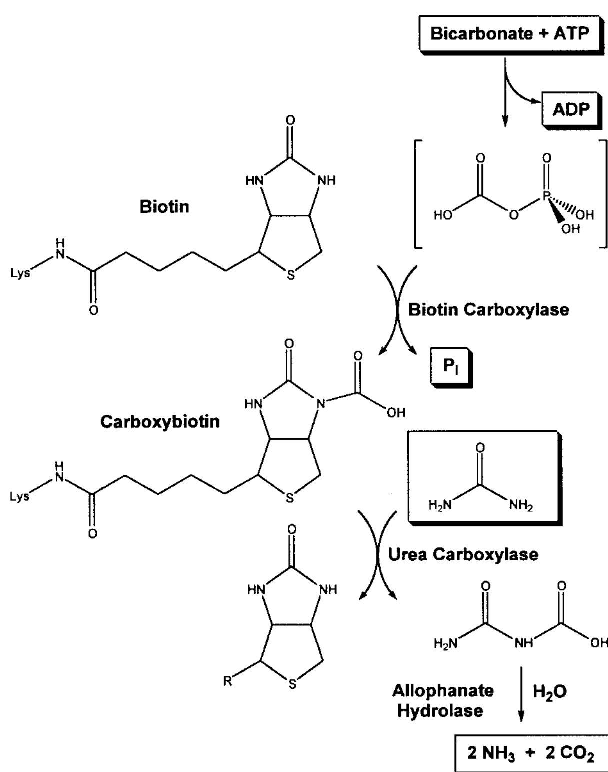

FIG. 3. The three activities of urea amidolyase, biotin carboxylase, urea carboxylase, and allophanate hydrolase, are shown.

nickel ions from the environment (9). In particular, urease activity was found in *O. sagaranensis* even when the cells were grown without supplemental nickel ions, consistent with the presence of an efficient uptake system for this metal ion that concentrates trace levels found in the medium (6). Cells similarly require a host of components in order to generate an active urea amidolyase: the structural subunits of this complex enzyme system, biotin synthase or another source of biotin, biotin ligase to connect the cofactor to the protein, and bicarbonate plus ATP as substrates for the reaction. Further efforts are needed to examine the regulation and kinetic properties of the two systems. It is possible that the concentrations of nickel ions or bicarbonate available in the cellular environment differentially affect transcription of the genes for the two systems. Alternatively, one pathway may be utilized at high substrate concentrations and the other at low concentrations. Another possibility is that one of these enzyme systems preferentially functions in a role other than urea hydrolysis. Ureases are generally highly specific for urea, with significantly reduced (a factor of less than 10^{-3}) activity when using semicarbazide, formamide, acetamide, *N*-hydroxyurea, *N*,*N'*-dihydroxyurea, and various phosphoroamides (3). On the other hand, it is possible that the urease found in *O. sagaranensis* exhibits broader substrate specificity, and the properties of this enzyme deserve further characterization. Of great potential significance is that the prokaryotic urea carboxylase utilizes acetamide and formamide (larger acyl amides were not examined) with a catalytic efficiency of approximately 5% of that of urea (6). A role in acyl amide decomposition is conceivable and needs to be further explored. As one example, the substrate range of allophanate hydrolase should be examined in order to test whether its physiological role involves the decomposition of carboxylated acyl amides.

I thank Scott Mulrooney for preparation of Fig. 2.Urease studies in the Hausinger laboratory are supported by the National Institutes of Health (DK 45686).

REFERENCES

1. **Cooper, T. G., C. Lam, and V. Turoscy.** 1980. Structural analysis of the *dur* loci in *S. cerevisiae*: two domains of a single multifunctional gene. Genetics **94:**555–580.
2. **Dixon, N. E., C. Gazzola, R. L. Blakeley, and B. Zerner.** 1975. Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel? J. Am. Chem. Soc. **97:**4131–4133.
3. **Hausinger, R. P., and P. A. Karplus.** 2001. Urease, p. 867–879. *In* K. Wieghardt, R. Huber, T. L. Poulos, and A. Messerschmidt (ed.), Handbook of metalloproteins. John Wiley & Sons, Ltd., West Sussex, United Kingdom.
4. **Jabri, E., M. B. Carr, R. P. Hausinger, and P. A. Karplus.** 1995. The crystal structure of urease from *Klebsiella aerogenes.* Science **268:**998–1004.
5. **Jitrapakdee, S., and J. C. Wallace.** 2003. The biotin enzyme family: conserved structural motifs and domain rearrangements. Curr. Protein Pept. Sci. **4:**217–229.
6. **Kanamori, T., N. Kanou, H. Atomi, and T. Imanaka.** 2004. Enzymatic characterization of a prokaryotic urea carboxylase. J. Bacteriol. **186:●●●.**
7. **Li, X., and H. L. Mobley.** 2002. Vaccines for *Proteus mirabilis* in urinary tract infection. Int. J. Antimicrob. Agents **19:**461–465.
8. **Martinez, B., J. Tomkins, L. P. Wackett, R. Wing, and M. J. Sadowsky.** 2001. Complete nucleotide sequence and organization of the atrazine catabolic plasmid pADP-1 from *Pseudomonas* sp. strain ADP. J. Bacteriol. **183:**5684–5697.
9. **Mulrooney, S. B., and R. P. Hausinger.** 2003. Nickel uptake and utilization by microorganisms. FEMS Microbiol. Rev. **27:**239–261.
10. **Pearson, M. A., L. O. Michel, R. P. Hausinger, and P. A. Karplus.** 1997. Structure of Cys319 variants and acetohydroxamate-inhibited *Klebsiella aerogenes* urease. Biochemistry **36:**8164–8172.
11. **Roon, R. J., and B. Levenberg.** 1968. An adenosine triphosphate-dependent

Downloaded from http://jb.asm.org/ on April 14, 2021 by guest

avidin-sensitive enzymatic cleavage of urea in yeast and green algae. J. Biol. Chem. **243:**5213–5215.
12. **Roon, R. J., and B. Levenberg.** 1970. CO_2 fixation and the involvement of allophanate in the biotin-enzyme-catalyzed cleavage of urea. J. Biol. Chem. **245:**4593–4595.
13. **Roon, R. J., and B. Levenberg.** 1972. Urea amidolyase. 1. Properties of the enzyme from *Candida utilis.* J. Biol. Chem. **247:**4107–4113.
14. **Sauers, C. L., W. P. Jencks, and S. Groh.** 1975. The alcohol-bicarbonate-water system. Structure-reactivity studies on the equilibria for formation of alkyl monocarbonates and on the rates of their decomposition in aqueous alkali. J. Am. Chem. Soc. **97:**5546–5553.
15. **Stingl, K., K. Altendorf, and E. P. Bakker.** 2002. Acid survival of *Helicobacter pylori*: how does urease activity trigger cytoplasmic pH homeostasis? Trends Microbiol. **10:**70–74.
16. **Sumner, J. B.** 1926. The isolation and crystallization of the enzyme urease. J. Biol. Chem. **69:**435–441.
17. **Zerner, B.** 1991. Recent advances in the chemistry of an old enzyme, urease. Bioorg. Chem. **19:**116–131.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.

Downloaded from http://jb.asm.org/ on April 14, 2021 by guest