At Last—the Crystal Structure of Urease

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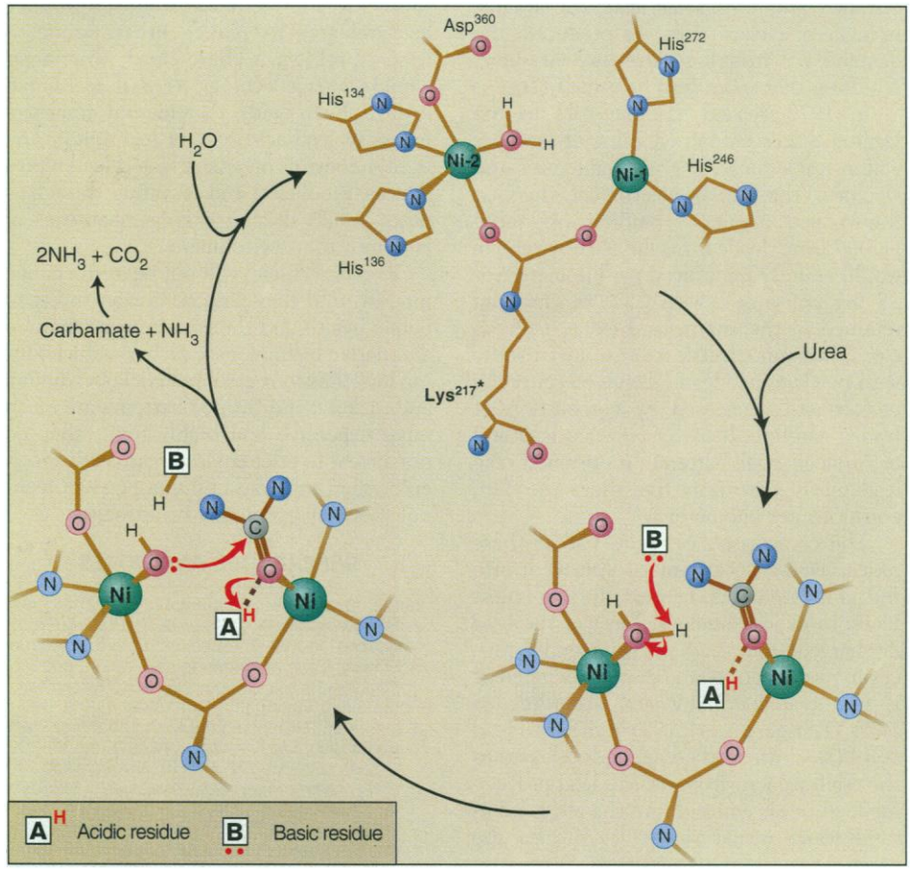
Urease has a long and distinguished history in the development of enzymology. This enzyme catalyzes the hydrolysis of urea, an abundant end product of metabolism (humans release 10 kilograms per year), to form ammonia and carbamate. Accordingly, urease is harbored by bacteria, fungi, various plants, and some invertebrates where it degrades urea at about 10^{14} times the uncatalyzed rate in order to supply these organisms with a source of nitrogen for growth (*1*).

Three significant events mark urease for special attention. It was the first enzyme ever to be crystallized, a feat accomplished in 1926 by James B. Sumner who used material isolated from jack beans (*2*). Although met with considerable skepticism by segments of the biological chemistry community, this accomplishment afforded incontrovertible proof that enzymes were well-defined chemical compounds (*3*). Sumner achieved the ultimate vindication of his claim by being awarded the 1946 Nobel Prize in Chemistry. The second development in the history of urease was the discovery that it contains nickel at the active site. This work, by Blakeley, Zerner, and co-workers, was prefaced by the remark that it was "with some sadness" that they had proved wrong Sumner's "proposition that enzymes could be proteins devoid of organic coenzymes *and metal ions*" (*4*) (my italics). The third achievement in the study of urease is the announcement by Jabri and co-workers in this issue of *Science* (p. 998) of the 2.0 Å resolution determination of the x-ray crystal structure of the enzyme from *Klebsiella aerogenes*. This fascinating structure, obtained nearly 70 years after urease was first crystallized, reveals the intimate details of the molecular geometry at the active site.

As indicated in the figure, the active site of urease consists of two nickel atoms 3.5 Å apart bridged by the carboxylate side chain of a rather unusual protein-derived ligand. The ε-amino residue of Lys^{217} has been modified by reaction with carbon dioxide to form a carbamate group. It was recently determined that carbon dioxide is required for nickel binding to metal-free urease (*5*), and the present structure determination clearly delineates the reason for that requirement. Urease is not the first enzyme in which a lysine residue has been converted to a carbamate, since similar chemistry occurs in ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) (*6*). Nevertheless, it is an unusual and clever way in which nature can expand its repertoire of metal-binding ligands from a rather limited set. The lysine–carbon dioxide metalation reaction is synergistic in the sense that the metal ion—nickel in the case of urease and magnesium in rubisco—stabilizes the carbamate, which in turn binds to and holds the metal in the active site.

The advantage of converting a lysine to a carbamate in order to bridge two metal ions, rather than encoding aspartate or glutamate in that position, is addressed by Jabri *et al.* Although urease has no obvious sequence similarity to any other protein, the $(\alpha\beta)_8$ barrel of its α subunit, which contains the metal-binding site, is nearly identical to that of the zinc-containing enzyme adenosine deaminase. But adenosine deaminase has only a single metal ion, whereas urease has two. The difference appears to be the modified Lys^{217} residue, designated $Lys^{\alpha 217*}$, which is equivalent to Asp^{181} in the sequence and structure of adenosine deaminase. The side chain of Asp^{181} is too short to coordinate to the zinc in adenosine deaminase, but the longer $Lys^{\alpha 217*}$ polymethylene side chain together with the carbamoyl moiety allow this modified lysine residue to reach the dinickel center in urease where it serves as a bridging carboxylate ligand.

Carboxylate-bridged dimetallic centers in proteins, such as that found in urease, and the related phosphodiester-bridged dimetallic units postulated to occur in RNA enzymes are becoming one of the most ubiquitous and functionally diverse units in bioinorganic chemistry. Examples include nonheme diiron proteins like (i) hemerythrin, a reversible dioxygen carrier; (ii) *Escherichia coli* ribonucleotide reductase, in which the diiron center reacts with dioxygen to generate a tyrosyl radical; and (iii) soluble methane monooxygenase, which uses such a center to hydroxylate methane



Possible mechanism for the chemistry at the catalytic site of urease.

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selectively to methanol. Similar carboxylate-bridged dimanganese centers occur in enzymes such as pseudocatalase. In these and related cases, two redox-active metal ions are held in close proximity, and the resulting dimetallic centers undergo two-electron redox reactions. The strategic importance of this chemistry is presumably to facilitate similar two-electron transfer steps connecting the dioxygen-derived species

$$O_2 \xrightarrow{2e^-} O_2^{2-} \xrightarrow{2e^-} 2O^{2-}$$

The carboxylate side chains of aspartate or glutamate provide convenient ligands which, unlike one possible alternative, cysteine, are redox-inactive and thus excellent spectators rather than participants in the reaction chemistry.

In contrast to these redox-active carboxylate-bridged dimetallic proteins, there is a growing class of carboxylate- and phosphodiester-bridged hydrolase enzymes that use zinc(II), magnesium(II), manganese(II), and calcium(II) ions, which do not undergo electron transfer reactions during catalysis (*7*). Included are phosphate ester–processing enzymes such as alkaline phosphatase, ribonuclease H of human immunodeficiency virus–1 (HIV-1) reverse transcriptase, the Klenow fragment of DNA polymerase β from *Escherichia coli*, and ribozymes, for which a two-metal-ion mechanism has been proposed (*8*). Although detailed mechanisms are not known for most of these systems, it has been suggested that one metal ion serves as a Lewis acid to polarize and stabilize the leaving group, whereas the other deprotonates water and provides a local source of hydroxide ion for nucleophilic attack on the substrate—for example, the phosphorus atom of the phosphate ester moiety. It is to this latter category that urease was postulated to belong (*9*), and the crystal structure now offers strong circumstantial support for such an idea.

The figure reveals how such a mechanism might operate. As can be seen from the crystal structure, one of the nickel atoms, designated Ni-1, has two histidine nitrogen ligands ($His^{\alpha 246}$ and $His^{\alpha 272}$) in addition to the oxygen atom of the carbamate group of $Lys^{\alpha 217*}$ in its coordination sphere. The geometry at Ni-1 is pseudo-tetrahedral, if one includes the site that in the figure is depicted as being empty. Because in the crystal this site is only partially occupied, presumably by a water molecule, it seems a likely candidate for binding of urea during catalysis. This possibility is reinforced by a recently synthesized inorganic model complex in which urea is coordinated through its oxygen atom to one nickel atom of a carboxylate-bridged dinickel(II) center (*10*). The coordination sphere of the second, pentacoordinate nickel atom of urease is approximately trigonal bipyramidal. Its ligands comprise the oxygen atom of the bridging $Lys^{\alpha 217*}$ carbamate, two histidine nitrogen atoms ($His^{\alpha 134}$ and $His^{\alpha 136}$), a monodentate carboxylate of aspartate ($Asp^{\alpha 360}$), and a water molecule. Such an arrangement in itself may not be sufficient to promote the hydrolysis of urea, however. The active site of the enzyme contains, in addition to the dinuclear metal center, a potential urea-binding pocket adjacent to the coordinated water molecule and lined by several acidic and basic side chains that could participate in the catalysis. When in a model study urea was placed on Ni-1, its oxygen atom was close to the ε nitrogen atom of one such residue, $His^{\alpha 219}$, which might aid the nickel(II) center in polarizing the carbonyl group for nucleophilic attack (A^H in figure). Similarly, either an oxygen atom of $Asp^{\alpha 360}$ or, if it were to move upon substrate binding, a nitrogen atom of $His^{\alpha 320}$ (not shown) could act as a base (B in figure) to remove a proton from the coordinated water molecule to generate hydroxide ion, the requisite nucleophile. Other models are possible, however, and an important goal for the near future will be to determine the structure of urease containing a bound inhibitor or of a catalytically incompetent mutant containing urea in the active site.

With the crystal structure of urease in hand, an important chapter is ended in the history of enzymology. For the field of bioinorganic chemistry, however, the structure helps to open new frontiers, one of which is to understand the principles by which carboxylate-bridged dimetallic centers function. The presence of two nickel(II) ions at the active site serves to focus attention on the special ability of such two-metal centers to catalyze hydrolysis reactions, a topic already of considerable interest in the RNA enzymes community (*8*). Another point of interest is to discern the various roles of nickel in biology. Although nickel is less frequently encountered in metalloenzymes than are other first-row transition metals such as manganese, iron, copper, and zinc, it is nevertheless an important cofactor in methyl-coenzyme M reductase, carbon monoxide dehydrogenase, and hydrogenase. Each of these latter enzymes catalyzes redox reactions. Since nickel(II) is not usually redox-active under physiological conditions, its role in these systems is uncertain. Urease, on the other hand, is the only hydrolase enzyme known to use nickel, and it seems likely that a mechanism similar to that shown in the figure will apply. The crystal structure has clearly revealed the players on the catalytic stage. It remains now for the bioinorganic chemist to delineate the roles of the other, nonligated amino acid residues in the active site, in the reaction mechanism that allows the remarkable rate enhancement of the hydrolysis of urea.

References

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