Amide Hydrolysis Effected by a Hydroxo-Bridged Dinickel(II) Complex: Insights into the Mechanism of Urease

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Received July 12, 1999

Abstract: A series of dinuclear nickel complexes, [Ni$_2$(H$_2$O)$_4$(bdptz)](OTs)$_4$, [Ni$_2$(OH)(H$_2$O)$_3$(bdptz)](OTs)$_3$, and [Ni$_2$(H$_2$O)(OH)$_2$(bdptz)]$_2$(OTs)$_4$, where bdptz is the dinucleating ligand 1,4-bis(2,2′-dipyridylmethyl)-phthalazine and OTs is *p*-toluenesulfonate, were prepared as models for urease. Potentiometric titration of [Ni$_2$(H$_2$O)$_4$(bdptz)](OTs)$_4$ revealed two deprotonation constants with pK_a values of 4.38 ± 0.02 and 8.51 ± 0.02. The product of the first deprotonation, [Ni$_2$(OH)(H$_2$O)$_3$(bdptz)](OTs)$_3$, effected the stoichiometric hydrolysis of picolinamide in ethanol, a heretofore unprecedented transformation in a dinuclear metallohydrolase model complex. Picolinamide hydrolysis by [Ni$_2$(H$_2$O)$_4$(bdptz)](OTs)$_4$ or [Ni(terpy)(H$_2$O)$_3$](OTs)$_2$ (terpy = 2,2′:6′,2″-terpyridine) under the same conditions was not observed. This amide hydrolysis mimics the hydrolysis of urea by the metalloenzyme urease in that a hydroxide nucleophile is produced by the dinuclear metal center and the amide is activated for nucleophilic attack by coordination to the dinuclear nickel center.

Introduction

The hydrolysis of biomolecules by dinuclear metallohydrolases has been a subject of considerable interest in recent years.[1−3] Perhaps one of the most remarkable metallohydrolases is urease, the only known nickel-dependent enzyme in this class.[4] Urease catalyzes the hydrolysis of urea and small amide substrates at a dinuclear nickel active site.[5] It is of fundamental interest to understand the mechanisms by which these transformations occur and to discover the essential elements required for amide hydrolysis by a metalloenzyme or a suitable model complex.[6,7] Insight into the principles governing amide hydrolysis by dinuclear metal centers could lead to the design of artificial peptidases and the development of urease inhibitors.[2,8]

Urease catalyzes the hydrolysis of urea into ammonia and carbamic acid[9] and hydrolyzes small amides such as formamide and acetamide to their corresponding carboxylic acids.[5] The activation and hydrolysis of an amide bond is no trivial task; the half-life of an amide bond in a small polypeptide chain at neutral pH and room temperature is approximately 7 years.[10] Urea is even more resistant to hydrolysis; its spontaneous hydrolysis has never been observed.[11] The stability of urea and amides is attributed to their resonance energy; the resonance energy of urea has been estimated at 30−40 kcal/mol.[12] The

zwitterionic resonance forms decrease the electrophilicity of the carbonyl carbon atom, making it less susceptible to nucleophilic attack.

Crystal structures of urease from two different microorganisms have recently been solved, providing a detailed picture of the active site.[13−16] As shown in Figure 1, the enzyme contains two nickel(II) ions separated by 3.5 Å. The nickel ions are each ligated by two histidine residues and bridged by a carbamylated lysine residue.[13,17] One of the nickel ions is further ligated by an aspartate residue. In addition to these protein-based ligands, a solvent-derived water molecule or hydroxide ion bridges the two metal ions and additional water molecules bind terminally to each metal ion. With this solvent coordination, Ni(1) is pentacoordinate with a distorted square pyramidal geometry and Ni(2) has a pseudo-octahedral six-coordinate geometry.[16]

It has been proposed that the amide substrate binds to Ni(1), replacing the terminally bound water molecule.[13,16] The urea molecule can be further activated for nucleophilic attack by linking the two metal ions through a μ-1,3 N,O bridging mode[13,16] or through the carbonyl oxygen atom in a single-atom bridging mode.[13] Hydrogen-bonding interactions with protein side chains can also serve to polarize the molecule.[14] A possible mechanism for amide hydrolysis at the dinuclear nickel active site of urease is shown in Scheme 1. The bridging hydroxide ion serves as the nucleophile, attacking the polarized carbonyl moiety either from a bridging position or from a transient terminally bound form. Protonation of the amine group by an
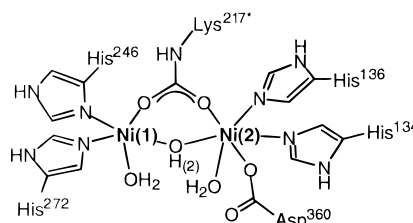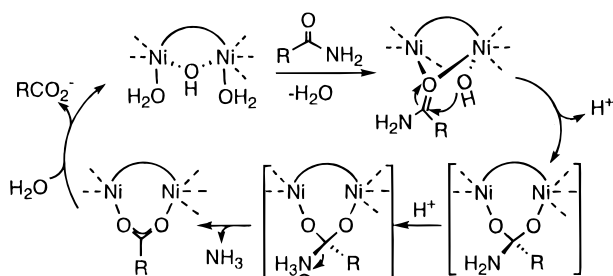
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Figure 1. Representation of the active site of urease. See refs 13 and 14.

Scheme 1

acidic residue in the active site results in loss of ammonia and collapse of the tetrahedral intermediate. To prepare a functional urease model, at least some of the requirements proposed above must be incorporated.

Numerous complexes have been reported as models for urease. Early work showed that the nickel(II) ion effected the hydrolysis and ethanolysis of 2-pyridylmethylurea.[11] Mononuclear palladium(II) complexes that catalyze the hydrolysis and ethanolysis of urea have also been reported.[18,19] This hydrolysis occurs via a nitrogen-bound urea molecule and proceeds through a nitrogen-bound carbamate intermediate rather than the oxygen-bound species that have been proposed for urease. Included among the dinuclear nickel urease models are complexes with urea bound to the dinuclear nickel center,[20-24] showing that urea binds preferentially via the carbonyl oxygen atom. A dinuclear nickel complex bridged by a deprotonated O−C−N bridging urea molecule was reported recently, demonstrating another possible mode of urea binding.[25] This latter mode of urea binding is not likely to be relevant to a species formed in the catalytic cycle of urease, since deprotonation of the urea would render it even more resistant to hydrolysis. Indeed, when this complex was heated under vacuum, ammonia was released and a cyanate complex was formed.[26] In addition to these urea complexes, several other dinuclear nickel complexes have been reported.[27,28] A few of these complexes support the formation

of a coordinated hydroxide ion at near-neutral pH,[29-31] and others have coordination sites available for substrate binding,[23,29] but all of the complexes reported thus far are conspicuous for their lack of activity in the hydrolysis of urea or amides. A report of the ethanolysis of urea at a dinuclear nickel center is the closest approximation yet.[24]

For the present study, we wished to design a dinuclear nickel complex that would be capable of producing a hydroxide ion at physiological pH and also have coordination sites available for substrate binding. Phthalazine- and pyridazine-based ligands, although not strictly biomimetic, incorporate many properties that are desirable in a ligand that will be used to model metalloenzymes. They form stable dinuclear complexes,[32-35] and the dinuclear metal centers retain additional coordination sites for exogenous ligand binding.[36,37] In addition, the ligand system is flexible enough to allow a range of metal−metal distances and a variety of exogenous ligands.[38] Hydroxo-bridged dinuclear copper(II) complexes of phthalazine- and pyridazine-based ligands have been reported,[33-35,39] but the reactivity of these complexes remains largely unexplored.

In this study we synthesized dinuclear nickel complexes with coordinated water and hydroxide molecules and investigated their ability to effect the hydrolysis of amide substrates in order to gain insight into the essential elements of amide hydrolysis by dinuclear metal centers. Our results indicate that a metal-bound hydroxide ion serves as the nucleophile for amide hydrolysis. Complexes that did not contain a hydroxide ion were unable to effect hydrolysis, whereas complexes with a hydroxide ion showed hydrolytic activity. In addition, the coordination of the substrate to the dinuclear metal center is crucial to hydrolysis.

Experimental Section

General Considerations. 1,4-Dichlorophthalazine[40] and 2,2′-dipyridylmethane[41] were prepared by following published procedures and characterized by ^{1}H NMR spectroscopy. All other starting materials were obtained from commercial sources and used as received. THF was distilled from sodium benzophenone ketyl under nitrogen.

1,4-Bis(2,2′-dipyridylmethyl)phthalazine (bdptz, 1). The synthesis of bdptz was adapted from the preparation of an analogous ligand, 3,6-bis(2,2′-dipyridylmethyl)pyridazine, reported in the literature.[36] A 4.75-g, 27.9-mmol sample of 2,2′-dipyridylmethane was dissolved in 50 mL of freshly distilled THF under argon and cooled to −78 °C in a dry

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ice/acetone bath. An 11.4-mL, 28.5-mmol aliquot of a 2.5 M solution of *n*-butyllithium in hexane was added slowly to the cold 2,2′-dipyridylmethane solution. The resulting orange solution was allowed to stir for 15 min. A solution of 2.74 g, 13.8 mmol of 1,4-dichlorophthalazine in 100 mL of freshly distilled THF was added by cannula to the lithiated dipyridylmethane solution over a period of 15 min. The resulting brown solution was then allowed to warm to room temperature. After careful addition of ∼5 mL of H_2O to destroy any unreacted *n*-butyllithium, the solvents were removed under reduced pressure, and the resulting yellow powder was recrystallized from ethanol/Et_2O to yield 5.19 g (81%) of 1,4-bis(2,2′-dipyridylmethyl)-phthalazine. ^1H NMR ($CDCl_3$): δ 8.553 (d, $J = 8$ Hz, 4H), 8.250 (m, 2H), 7.765 (m, 2H), 7.630 (t, $J = 7.6$ Hz, 4H), 7.388 (d, $J = 9$ Hz, 4H), 7.177 (t, $J = 6$ Hz, 4H), 6.927 (s, 2H). IR (KBr, cm^{-1}): 2972, 2868, 1589, 1568, 1543, 1496, 1468, 1432, 1357, 1152, 1082, 1048, 985, 880, 787, 760, 659, 614, 584, 554. Anal. Calcd for $C_{30}H_{22}N_6$: C, 77.23; H, 4.75; N, 18.01. Found: C, 77.63; H, 4.80; N, 18.04.

[Ni$_2$(H$_2$O)$_4$bdptz](OTs)$_4$ (2, OTs = *p*-Toluenesulfonate). To a 170-mg, 334-μmol solution of [Ni(H$_2$O)$_6$](OTs)$_2$ in 2 mL of acetonitrile was added a solution of 73 mg, 155 μmol of bdptz in 2 mL of 1:5 (v/v) H_2O/CH_3CN in a dropwise manner. Slow evaporation of the resulting violet solution yielded purple crystals of **2**. Recrystallization from CH_3OH/Et_2O provided **2** as blue/purple dichroic blocks, which were characterized by X-ray crystallography (170 mg, 82%). IR (KBr, cm^{-1}): 3500−3000, 1607, 1566, 1495, 1476, 1448, 1376, 1227, 1178, 1122, 1033, 1009, 877, 815, 769, 683, 566. UV−vis (EtOH) (λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 599 (11), 779 (sh), 899 (26). Anal. Calcd for $C_{58}H_{60}N_6O_{17}Ni_2S_4$ (**2**·H_2O): C, 51.27; H, 4.45; N, 6.18. Found: C, 51.21; H, 4.47; N, 6.10.

[Ni$_2$(OH)(H$_2$O)$_3$bdptz](OTs)$_3$ (3). This compound was prepared by the method described for **2**, but 1 equiv of NaOH was added to the violet solution before slow evaporation was allowed. The resulting purple-brown crystals (146 mg, 80%) were characterized by X-ray crystallography. IR (KBr, cm^{-1}): 3500−3000, 1657, 1604, 1571, 1495, 1474, 1445, 1373, 1221, 1178, 1120, 1034, 1011, 815, 779, 681, 559. UV−vis (EtOH) (λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 579 (10), 776 (sh), 898 (21). Anal. Calcd for $C_{51}H_{52}N_6O_{14}Ni_2S_3$ (**3**·H_2O): C, 51.62; H, 4.42; N, 7.08. Found: C, 51.58; H, 4.55; N, 7.01.

[Ni$_2$(OH)$_2$(H$_2$O)bdptz]$_2$(OTs)$_4$ (4). Addition of 2 equiv of NaOH to a violet solution of **2** prepared as described above produced a green solid that could be recrystallized by slow evaporation of a CH_3CN solution. The resulting green blocks were suitable for X-ray crystallographic structure determination. IR (KBr, cm^{-1}): 3444, 3079, 3019, 1669, 1603, 1572, 1495, 1470, 1446, 1362, 1221, 1109, 1007, 812, 769, 688, 577. UV−vis (EtOH:H_2O, 5:1 v/v) (λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 568 (14), 768 (sh), 898 (53). Anal. Calcd for $C_{88}H_{90}N_{12}O_{23}Ni_4S_4$ (**4**·5H_2O): C, 51.64; H, 4.43; N, 8.21. Found: C, 51.50; H, 4.42; N, 8.00.

[Ni$_2$(pic)$_2$(bdptz)](OTs)$_2$ (5, pic = picolinate). A 9.6-mg, 78.6-μmol portion of picolinamide was dissolved in 1.7 mL of ethanol along with 92 mg (78.7 μmol) of **3**. The resulting solution was heated to reflux for 24 h. The solution was then allowed to cool to −20 °C, and 36 mg (80% yield based on picolinamide) of a pale blue precipitate of **5** was isolated by filtration. The product was recrystallized by slow diffusion of diethyl ether into a methanolic solution of **5**. The resulting blue plates were suitable for X-ray crystallographic structure determination. Complex **5** could also be synthesized independently by adding 2 equiv of sodium picolinate to **2** in CH_3CN. IR (KBr, cm^{-1}): 3441, 3075, 3027, 2932, 2868, 1657, 1603, 1570, 1477, 1445, 1371, 1333, 1291, 1219, 1186, 1155, 1119, 1032, 1010, 825, 764, 700, 680, 649, 566. UV−vis (EtOH) (λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 527 (sh), 601 (32), 803 (sh), 894 (28). Anal. Calcd for $C_{56}H_{46}N_8O_{11}Ni_2S_2$ (**5**·H_2O): C, 56.59; H, 3.90; N, 9.43. Found: C, 56.42; H, 3.87; N, 9.20.

Physical Measurements. NMR spectra were obtained by using a Varian VXR-300 NMR spectrometer. UV−vis spectra were recorded on a Cary 1E spectrophotometer. FTIR spectra of solid samples pressed into KBr pellets were recorded on an FTS-135 FTIR spectrometer.

Potentiometric Titrations. Titrations were performed with a Brinkmann Metrohm 716 DMS Titrino autotitrator. A nominally 0.01 M NaOH solution was freshly prepared and standardized by using oven-dried potassium hydrogen phthalate. A 10-mL, 1 mM aqueous solution of **2** was titrated with the standardized NaOH at 23 °C. The ionic

strength was maintained at 0.10 M with KNO$_3$. Protonation constants were calculated by using PKAS, a computer program for the calculation of protonation constants from potentiometric data.[42]

Hydrolysis of Picolinamide by Dinuclear Nickel(II) Complexes. In a typical experiment, 20 mM solutions of **3** and picolinamide in ethanol were prepared separately and combined in a 0.2−1:1 v/v ratio. The total volume of the resulting solution was brought to 1 mL by addition of an appropriate volume of ethanol. The mixture was incubated at 59 °C for 5 h. At the end of this period, the reaction mixtures were allowed to cool to room temperature, and 300-μL aliquots of each were removed and assayed to determine the ammonia content. Substrate hydrolysis by **2**, a dinuclear nickel complex without a coordinated hydroxide ion, was also investigated. A 500-μL aliquot of the 20 mM picolinamide solution was combined with 500 μL of a 20 mM ethanolic solution of **2**. The resulting solution was incubated at 59 °C for 24 h, allowed to cool to room temperature, and assayed for ammonia production.

Hydrolysis of Picolinamide by a Mononuclear Nickel(II) Complex. A stock solution (40 mM) of [Ni(H$_2$O)$_6$](OTs)$_2$ and 1 equiv of 2,2′:6′,2″-terpyridine (terpy) in ethanol was prepared. Equal volumes of a 20 mM picolinamide solution and the stock solution were combined and incubated at 59 °C for 24 h. At the end of this period, the reaction mixture was assayed to determine the ammonia content.

Ammonia Assay. Ammonia was assayed by using an indophenol assay procedure involving two reagent solutions.[43] The first reagent solution consists of 5 g of phenol and 25 mg of Na$_2$[Fe(CN)$_5$(NO)] dissolved in enough water to make 500 mL, and the second reagent solution is a mixture of 2.5 g of NaOH and 5 mL of a commercially available NaClO solution (4% available chlorine) diluted to 500 mL with water. These reagents can be stored in a refrigerator for about 1 month without decomposition. A calibration curve was obtained by adding 2−10 μL of a 5.9 mM aqueous ammonium sulfate solution to 2 mL of the phenol−nitroprusside reagent, followed by addition of 2 mL of the alkaline hypochlorite reagent. The assay was mixed thoroughly and allowed to stand at room temperature for 1 h. After this time, the blue color had developed fully and the absorbance at 625 nm was measured. This procedure was used to analyze the ammonia produced in amide hydrolysis trials. When the complexes and substrates themselves were assayed, no absorbance at 625 nm was observed.

Collection and Reduction of X-ray Data. The general procedures for collection and reduction of X-ray data were reported previously.[44] All crystals were mounted on the tips of glass fibers with Paratone-N (Exxon) and rapidly cooled in the −85 °C cold stream of a Bruker (formerly Siemens) CCD X-ray diffraction system controlled by a Pentium-based PC running the SMART software package.[45] The direct methods programs SIR-92[46] or XS, part of the TEXSAN[47] and SHELXTL[48] program packages, respectively, were used to solve all structures. Structure refinements were carried out with XL, part of the SHELXTL program package.[48] All non-hydrogen atoms were refined by a series of least-squares cycles. Hydrogen atoms were assigned idealized positions and given a thermal parameter 1.2 times that of the atom to which each was attached, or 1.5 times that of the atom in the case of methyl and hydroxyl groups. Empirical absorption corrections were calculated and applied for each structure with the SADABS program.[48]

The structure of **2** has a disordered methanol molecule in the lattice. The carbon atom position was fixed, and the oxygen atom was distributed over two sites and refined with occupancy factors of 67% and 33%, respectively. In the structure of **3** there is rotational disorder

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Table 1.   Summary of X-ray Crystallographic Data

	2·2CH$_3$OH·H$_2$O	3·8H$_2$O	4·6CH$_3$CN·4H$_2$O	5·3CH$_3$OH·1H$_2$O
formula	C$_{60}$H$_{68}$N$_6$O$_{19}$Ni$_2$S$_4$	C$_{51}$H$_{66}$N$_6$O$_{21}$Ni$_2$S$_3$	C$_{94}$H$_{93}$N$_{15}$O$_{20}$Ni$_4$S$_4$	C$_{59}$H$_{58}$N$_8$O$_{12}$Ni$_2$S$_2$
fw	1422.86	1312.70	2115.91	1252.67
space group	*Pc*	*P*$\bar{1}$	*P*$\bar{1}$	*Pbcn*
a, Å	14.6594(1)	13.054(5)	13.781(6)	30.3178(19)
b, Å	12.5026(2)	14.113(5)	14.368(7)	13.5543(8)
c, Å	18.1204(2)	17.717(5)	15.012(4)	14.4248(9)
α, deg		85.151(5)	94.09(2)	
β, deg	105.137(1)	69.662(5)	115.29(3)	
γ, deg		75.172(5)	94.19(3)	
V, Å^3	3205.89(6)	2958.6(18)	2663.0(18)	5927.7(6)
Z	2	2	1	4
ρ_{calcd}, g/cm^3	1.474	1.474	1.319	1.404
T, °C	−85	−85	−85	−85
μ(Mo Kα), mm^{-1}	0.795	0.822	0.845	0.774
2θ limits, deg	3−57	3−56	3−57	3−57
total no. of data	19 628	18 038	16 861	35 674
no. of unique data	11 022	12 652	11 797	7085
observed data^a	10 348	10 521	5218	4776
no. of parameters	1027	1040	811	486
R(%)^b	2.76	4.03	6.09	5.50
w*R*^{2}(%)^c	6.90	10.13	12.97	15.04
max, min peaks, e/Å^3	0.401−0.448	0.577, −0.535	1.509, −0.990	0.941, −0.525

^{a} Observation criterion: $I > 2\sigma(I)$. ^{b} $R = \sum||F_o| - |F_c||/\sum|F_o|$. ^{c} w$R^2 = \{\sum[w(F_o{}^2 - F_c{}^2)^2]/\sum[w(F_o{}^2)^2]\}^{1/2}$.

in the −SO$_3$ group of one of the tosylate anions. The three oxygen atoms were distributed over six positions and refined, with one orientation 80% occupied and the other 20% occupied.

Pertinent crystallographic information for each complex is provided in Table 1. All bond distances and angles, as well as atomic coordinates and equivalent isotropic displacement parameters, are provided in Tables S1−S20 in the Supporting Information.

Results and Discussion

Synthesis of bdptz (1). An appropriate ligand system is crucial in the design and synthesis of dinuclear metal complexes for use in metalloenzyme modeling. A number of dinuclear nickel(II) and copper(II) complexes with phthalazine- and pyridazine-based ligand systems have been reported in the literature,[32−35,37,38] including some hydroxo-bridged dicopper-(II) complexes.[33−35,39] Although some of these complexes have a potentially nucleophilic hydroxide ion and coordination sites available for substrate binding, none have been investigated as metallohydrolase mimics.

To enhance complex solubility[39] as well as improve the yield of ligand synthesis, we designed a new ligand analogous to a previously reported system.[36,39] This new ligand, bdptz, is shown in Figure 2. The ligand bdptz was formed from the reaction of lithiated 2,2′-dipyridylmethane with 1,4-dichlorophthalazine in over 80% yield. Although this ligand is not strictly biomimetic, it contains structural features that are analogous to those found in the active site of urease. The pyridine donor arms of the ligand provide two nitrogen donor ligands to each metal ion, similar to the two histidine residues ligated to each metal ion in the active site. In addition, the phthalazine moiety, while electronically distinct from the bridging carbamate moiety in the enzyme, plays a similar structural role in bringing the two nickel ions into close proximity. With two terminal and two bridging coordination sites available for nucleophile and substrate
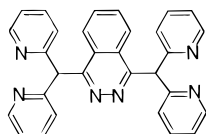

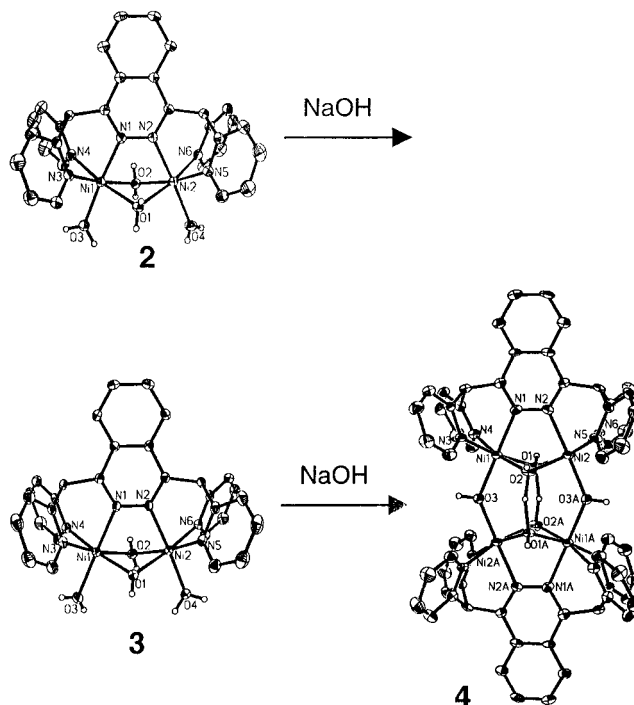**Figure 2.** Ligand 1,4-bis(2,2′-dipyridylmethyl)phthalazine, bdptz.

Figure 3. Sequential deprotonation of **2** to form **3** and **4**. The ORTEP diagrams of the cations of **2**, **3**, and **4** display 50% probability ellipsoids for all non-hydrogen atoms. Most of the hydrogen atoms have been omitted for clarity.

binding, the bdptz ligand shows promise for use in metallohydrolase modeling.

Preparation and Characterization of [Ni$_2$(H$_2$O)$_4$bdptz]-(OTs)$_4$ (2), [Ni$_2$(OH)(H$_2$O)$_3$bdptz](OTs)$_3$ (3), and [Ni$_2$(OH)$_2$-(H$_2$O)bdptz]$_2$(OTs)$_4$ (4). The treatment of bdptz with 2 equiv of [Ni(H$_2$O)$_6$](OTs)$_2$ in aqueous acetonitrile solution resulted in the formation of the dinuclear nickel complex **2** in good yield. Complex **2** is soluble in common polar organic solvents, including methanol, ethanol, acetonitrile, and DMF, and crystallizes out of most of these solvents upon slow solvent evaporation. In addition, this complex is water soluble.

The crystal structure of **2** is shown in Figure 3, and selected bond lengths and angles are listed in Table 2. The phthalazine

Table 2. Selected Interatomic Distances (Å) and Angles (deg) for **2**, **3**, and **4**[a]

	2	**3**	**4**
Ni(1)−Ni(2)	3.0598(9)	3.1398(4)	3.064(1)
Ni(1)−N(1)	2.025(2)	2.046(2)	2.082(4)
Ni(1)−N(3)	2.064(2)	2.063(2)	2.094(4)
Ni(1)−N(4)	2.041(2)	2.068(2)	2.070(4)
Ni(1)−O(1)	2.159(2)	2.042(2)	2.061(3)
Ni(1)−O(2)	2.135(2)	2.208(2)	2.121(4)
Ni(1)−O(3)	2.002(2)	2.033(2)	1.991(3)
Ni(2)−N(2)	2.049(2)	2.014(2)	2.079(4)
Ni(2)−N(5)	2.035(2)	2.071(2)	2.080(4)
Ni(2)−N(6)	2.063(2)	2.075(2)	2.084(4)
Ni(2)−O(1)	2.170(2)	2.054(2)	2.075(3)
Ni(2)−O(2)	2.128(2)	2.219(2)	2.110(4)
Ni(2)−O(4)	2.026(2)	2.039(2)	1.985(3) (O3a)
Ni(1)−N(1)−N(2)	115.8(2)	115.6(1)	114.5(3)
Ni(2)−N(2)−N(1)	116.2(2)	113.9(1)	114.1(3)
Ni(1)−O(1)−Ni(2)	92.97(7)	96.67(7)	95.49(14)
Ni(1)−O(2)−Ni(2)	94.88(7)	87.45(7)	92.68(15)
N(1)−Ni(1)−O(3)	175.23(8)	178.17(8)	177.46(15)
N(2)−Ni(2)−O(4)	176.7(1)	173.15(7)	176.22(15) (O3a)

[a] Numbers in parentheses are estimated standard deviations of the last significant figure. Atoms are labeled as indicated in Figure 3.

moiety bridges the two nickel(II) ions, and the pyridine donor arms provide two nitrogen donors to each metal ion. Each nickel ion is in a pseudo-octahedral coordination environment with three nitrogen and three oxygen donors. In addition to the coordination afforded by the ligand bdptz, two water molecules bridge the two nickel ions and each nickel is coordinated to a terminally bound water molecule. The coordinated water molecules are hydrogen bonded to the four tosylate counterions and solvent molecules in the lattice, forming an extended hydrogen-bonding network.

The tetraaqua complex **2** can be singly and doubly deprotonated to yield **3** and **4**, respectively, as outlined in Figure 3. Raising the pH of a solution of **2** to approximately 7 results in the formation of complex **3** in good yield. The crystal structure of **3**, shown in Figure 3, displays a core structure that is very similar to that of **2**. The two pseudo-octahedral nickel ions are each coordinated by three nitrogen donors from bdptz and three oxygen donor ligands derived from water. Selected bond lengths and angles are listed in Table 2. One of the bridging water molecules has been deprotonated, as evidenced by a shortening of the Ni−O bond lengths from an average of 2.148 to 2.048 Å. The formation of a bridging hydroxide ion at neutral pH and the presence of coordinated solvent molecules that could readily be replaced by substrate make complex **3** a good candidate for amide hydrolysis studies.

Raising the pH of a solution of **2** to approximately 11 followed by slow evaporation of the solvent resulted in the formation of **4**. The crystal structure of **4**, shown in Figure 3, reveals an unexpected dimerization. In this complex, there are two phthalazine-bridged dinuclear units connected by two bridging hydroxide ions. Each dinuclear unit is bridged by the diazine moiety of bdptz, a water molecule, and a hydroxide ion as in complex **3**. Two pyridine donor arms of bdptz bind to each metal ion, and the two remaining coordination sites are filled by hydroxide ions, which link one dimer unit to the other. Each nickel ion is pseudo-octahedral with a coordination environment similar to that in **2** and **3**. Selected bond lengths and angles are listed in Table 2.

Potentiometric Titration. To determine the pK_a values of the coordinated water molecules in **2**, a potentiometric titration was performed. A typical curve obtained from the titration of **2** with 0.01 M NaOH is shown in Figure 4, along with the
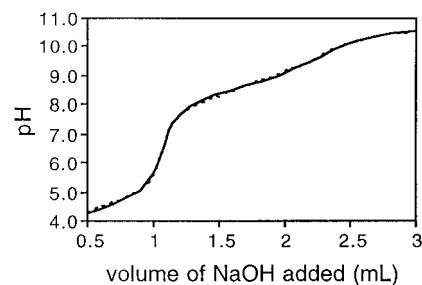

Figure 4. Representative titration curve obtained by titrating **2** with 0.01 M NaOH. The experimental curve (solid line) is in good agreement with a theoretical curve (dashed line) from which the pK_a values were obtained.

theoretical fit. The first equivalence point is at pH 6.49, and the second occurs at pH 9.71. The pK_a values of the coordinated water molecules were calculated to be 4.38 ± 0.02 and 8.51 ± 0.02 for the conversions of **2** to **3** and **3** to **4**, respectively. These numbers are quite low for metal-bound water; for comparison, the pK_a of [Ni(H$_2$O)$_6$]$^{2+}$ is approximately 9.9.[49]

Hydrolysis of Substrates. Although numerous dinuclear nickel complexes have been reported as urease models, few have been investigated for urea or amide hydrolysis activity.[26,50,51] Of those that have been tested, none has shown activity, with the exception of the ethanolysis of urea, reported recently.[24] Some authors have reported using activated substrate analogues such as *p*-nitrophenylacetate,[52] but since the requirements for hydrolysis of this activated ester are likely to be different than those for amide hydrolysis,[2] we chose to study the hydrolysis of urea and amide substrates directly. Amide hydrolysis by complex **3**, a dinuclear nickel complex with a potential hydroxide nucleophile and several coordinated water molecules that could be replaced by substrate, was investigated.

The initial rate of picolinamide hydrolysis by **3** was studied at a series of substrate and complex concentrations (2.0−10.0 mM in ethanol). The reaction is first order with respect to both the concentrations of picolinamide and **3**. When the hydrolysis of picolinamide by complex **2**, [Ni(terpy)(H$_2$O)$_3$](OTs)$_2$, or NaOH was investigated under the same conditions, no ammonia formation was detected. By fitting the experimental data to the "improved" steady-state equation[53] with a prior equilibrium approximation,[54] numerical values for the binding constant of picolinamide to **3** (K_1) and the first-order rate constant for conversion of the picolinamide complex to products (k_2) were obtained. The binding constant of picolinamide to **3** was calculated to be 70 ± 20 M^{-1}, which is in good agreement with previously published data.[55] The rate constant of the hydrolysis is (3.2 ± 0.8) × 10^{-4} min^{-1}, which represents at least a 100-fold rate enhancement over the control reactions. The data and calculated fits are shown in Figure 5.

A representation of the reaction between **3** and picolinamide is shown in Scheme 2. It is proposed that, in order for a dinuclear metal complex to effect amide hydrolysis, the amide must bind

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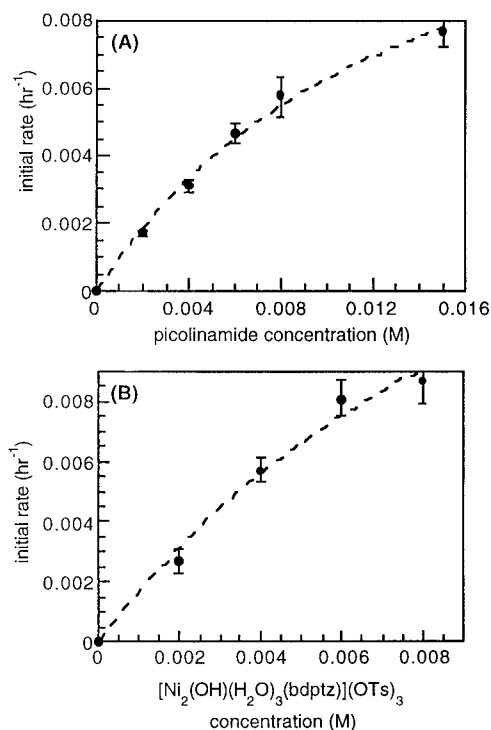
Figure 6. Infrared vibrational spectra of (A) 85 mM **3** in ethanol-d_6, (B) 50 mM picolinamide in ethanol-d_6, and (C) a deuterated ethanolic solution 50 mM in picolinamide and 75 mM in **3**. The band at 1605 cm^{-1} is assigned to **3**, and the bands at 1673 and 1591 cm^{-1} are assigned to the C=O stretching and N–H bending frequencies of picolinamide, respectively. In spectrum C it is apparent that the carbonyl stretching frequency has decreased from 1673 to 1642 cm^{-1} upon coordination to **3**.

Figure 5. Plot of the initial rate of picolinamide hydrolysis vs the concentration of (A) picolinamide and (B) **3**. The concentrations of **3** in (A) and picolinamide in (B) were fixed at 0.010 M. The curve represents the fit of the data to the "improved" prior equilibrium equation as found in ref 50.

Scheme 2
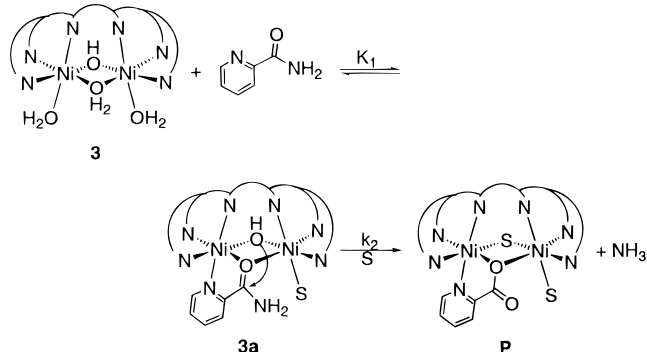
to the metal center. In this system, amides such as acetamide are not significantly hydrolyzed, whereas picolinamide, with an exogenous pyridine ligand that can serve to bring the amide functionality close to the metal center, is hydrolyzed. Further evidence that the amide binds to the metal center through the carbonyl oxygen atom, as depicted by complex **3a** in Scheme 2, was obtained by monitoring the reaction of **3** with picolinamide by FTIR spectroscopy. When **3** is added to a solution of picolinamide in ethanol-d_6, the carbonyl stretching frequency decreases from 1673 to 1642 cm^{-1}, as shown in Figure 6, indicating that the carbonyl moiety is bound to the metal center.[56–58] Based on the experimentally determined binding constant of 70 M^{-1} and the concentrations of picolinamide and **3** (50 and 75 mM, respectively), there should be no observable remnant of the carbonyl stretching band of picolinamide. This

expectation agrees well with the data shown in Figure 6c. Coordination to the metal center through the amide oxygen atom will make the carbonyl carbon atom more electrophilic, activating it for nucleophilic attack. Attack of a nucleophile on the activated amide and protonation of the leaving amine group results in the release of ammonia and the formation of products. Because the bridging hydroxide ion in **3** is the only nucleophile available under the reaction conditions, we propose that it is, indeed, the active nucleophile in this transformation, attacking the substrate either from the bridging position or from a transient, terminally bound site. The failure of **2** to hydrolyze picolinamide indicates that neither a terminally bound water molecule nor a water molecule from the solution acts as a nucleophile under these conditions.

The product of picolinamide hydrolysis by **3** was isolated and characterized to ensure that hydrolysis, not ethanolysis, was taking place. A 1:1 mixture of picolinamide and **3** in ethanol was heated to 80 °C for 24 h. Recrystallization of the resulting powder-blue product provided blue crystals of [Ni$_2$(pic)$_2$(bdptz)]-(OTs)$_2$ (**5**), corresponding to an 80% yield of picolinate based on picolinamide. The structure of **5** was solved by X-ray crystallography and is shown in Figure 7. In this complex, the diazine moiety of bdptz bridges the two metal ions and the pyridine arms of the ligand supply two additional nitrogen donors to each nickel(II) ion. Completing the coordination spheres of the two metal ions are two picolinate anions. Each picolinate ion supplies a nitrogen atom donor to the terminal position of one of the metal ions and a carboxylate oxygen atom to bridge the two metal ions. Selected bond lengths and angles are shown in Table 3. An axis of C_2 symmetry runs down through the middle of the molecule.

Although the ethanolysis of picolinamide to yield a minor amount of ethyl picolinate cannot be ruled out, the isolation of **5** in good yield from the reaction of **3** with picolinamide indicates that complex **3** almost exclusively effects the hydrolysis of picolinamide, in contrast to the ethanolysis of urea reported recently.[24] The stoichiometry of the reaction, in which one molecule of picolinamide is hydrolyzed by each dinuclear nickel complex, agrees well with the observation that only the

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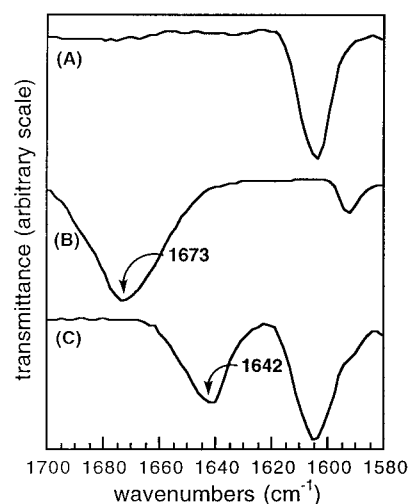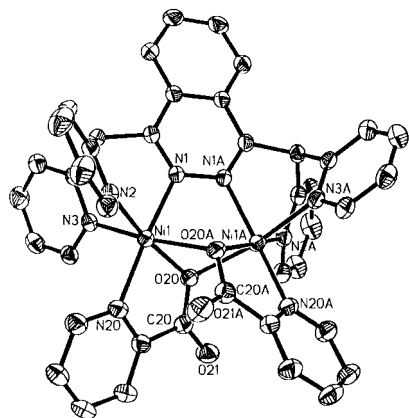(58) Barnes, D. J.; Chapman, R. L.; Stephens, F. S.; Vagg, R. S. *Inorg. Chim. Acta* **1981**, *51*, 155–162.

Figure 7. ORTEP diagram of the cation of **5** showing 50% probability ellipsoids for all non-hydrogen atoms.

Table 3. Selected Interatomic Distances (Å) and Angles (deg) for **5**[a]

Ni(1)···Ni(1a)	3.079(3)	Ni(1)−O(20)	2.088(2)
Ni(1)−N(1)	2.035(3)	Ni(1)−O(20a)	2.192(2)
Ni(1)−N(2)	2.054(3)	C(20)−O(20)	1.292(4)
Ni(1)−N(3)	2.075(3)	C(20)−O(21)	1.227(4)
Ni(1)−N(20)	2.027(3)		
Ni(1)−N(1)−N(1a)	114.90(8)	N(1)−Ni(1)−N(20)	170.08(11)
Ni(1)−O(20)−Ni(1a)	91.93(9)	O(20)−C(20)−O(21)	125.5(3)

[a] Numbers in parentheses are estimated standard deviations of the last significant figure. Atoms are labeled as indicated in Figure 7.

coordinated hydroxide ion, and not a coordinated water molecule, can effect hydrolysis. Isolation of **5** from the product mixture occurs not because of double hydrolysis of picolinamide at a single dinickel(II) site, but rather because **5** is a stable complex that crystallizes out under the conditions applied.

Comparison to Urease. The hydrolysis of picolinamide by **3** provides insight into the principles of amide hydrolysis by dinuclear metal centers. The two nickel ions act in concert to lower the pK_a of a coordinated water molecule enough that it will be deprotonated at physiological pH. The amide substrate coordinates to the dinuclear nickel complex, possibly bridging the two metal ions through the carbonyl carbon atom. Direct coordination to the dinickel complex activates the carbonyl bond for nucleophilic attack. Activation of both the water molecule and the amide substrate at the dinuclear nickel center is advantageous because, once activated, the two reagents are in close proximity to one another, facilitating hydrolysis. In this study, the cooperation of the two metal ions in producing a hydroxide ion, activating the amide substrate, and facilitating hydrolysis resulted in a 100-fold rate enhancement over hydrolysis by a mononuclear complex.

These principles can be extended to amide hydrolysis by the metalloenzyme urease. In the active site of urease, there are several water molecules coordinated to the dinuclear nickel center. It has been proposed that a bridging water molecule, which will most likely be deprotonated at physiological pH based on this work, is the nucleophile in the hydrolysis of urea.[13] Urease-catalyzed urea hydrolysis proceeds through the replacement of a coordinated water molecule by urea, which is believed to bind via the carbonyl oxygen atom. Based on the proposed mode of picolinamide coordination to complex **3**, and the recent synthesis of an analogous dinuclear nickel complex with a urea molecule bridging the two metal ions through the carbonyl oxygen atom,[59] we suggest that urea may bind to the active site of urease in a similar bridging coordination mode. Coordination of the urea molecule to both nickel ions in the active site of urease would polarize the carbonyl bond, activating it for nucleophilic attack.

Summary and Conclusion

Urease is the only known metallohydrolase that requires nickel in its active site. At the dinuclear active site, urea is hydrolyzed to carbamic acid and ammonia. Although numerous dinuclear nickel complexes have been reported as urease models, none has shown the ability to hydrolyze urea or amide substrates. To prepare an active urease model it is necessary to incorporate the fundamental requirements for amide hydrolysis at a dinuclear metal center. These requirements include the ability to produce a water-derived nucleophile and the availability of coordination site(s) for substrate binding. In this study, a series of water-stable dinuclear nickel complexes was prepared by using the dinucleating ligand bdptz. These complexes are capable of producing a hydroxide ion at neutral pH. In addition to activating water at neutral pH, complex **3** effects picolinamide hydrolysis in ethanol, providing a well-characterized example of amide hydrolysis by a dinuclear nickel complex.

Supporting Information Available: Figures S1−S4, showing fully labeled ORTEP diagrams for complexes **2**−**5**, respectively. X-ray crystallographic files, in CIF format, are also available. This material is available free of charge via the Internet at http://pubs.acs.org.

JA992447H

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