J. Am. Chem. Soc. **2001,** *123,* 8442−8443

Supramolecular Stabilization of a Tris(imidazolyl) Zn−Aqua Complex Evidenced by X-ray Analysis: A Structural Model for Mono-Zinc Active Sites of Enzymes

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Received June 5, 2001
Revised Manuscript Received July 4, 2001

Zinc enzymes are ubiquitous in biology and constitute an essential class of metallo-proteins.[1] They are mostly specialized in hydrolytic processes but they also catalyze the transfer of hydrides, as in liver alcohol dehydrogenase, or of methyl groups as in methionine synthase. The zinc ion is usually tetrahedrally coordinated to three protein residues and a water ligand that is either displaced by the substrate or deprotonated. A tris(histidine) Zn environment is often found in mononuclear enzymes, namely, carbonic anhydrase, matrix metalloproteases, snake venom metalloproteinases to name but a few. Several chemical systems have been developed to reproduce the (His)$_3$Zn^{2+}(OH$_2$) coordination core. Surprisingly, zinc−aqua model complexes have proven extremely difficult to stabilize, and all classical models have failed to stabilize a tetrahedral dicationic Zn−aqua species.[2,3]

We have recently described a novel system based on calix[6]-arene presenting three imidazolyl arms on the narrow rim.[4] We showed that within this neutral core, the 4-coordinated aqua complex N$_3$Zn^{2+}(OH$_2$) is exceptionally stable. For example, no deprotonation occurred upon the addition of 1 mol equiv of an amine. Instead, the aqua ligand was displaced by a primary amine yielding a new 4-coordinate species N$_3$Zn^{2+}(NH$_2$R′). Such a behavior stands in contrast to the high acidity expected from a water molecule coordinated to a dicationic tetrahedral zinc center. Also surprising was the fact that the calixarene cavity appeared equally filled by a small H$_2$O ligand or an organic molecule as its cone conformation was not significantly affected by the nature of the included ligand.[5] Here, we present the X-ray structure of the aqua complex obtained with a calixarene-based tris(imidazolyl) ligand. It represents the first X-ray characterization of an aqua model complex that exhibits the same dicationic tetrahedral core as in the natural Zn systems.

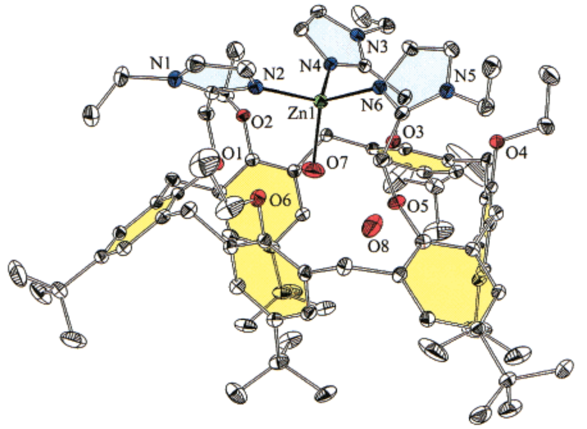
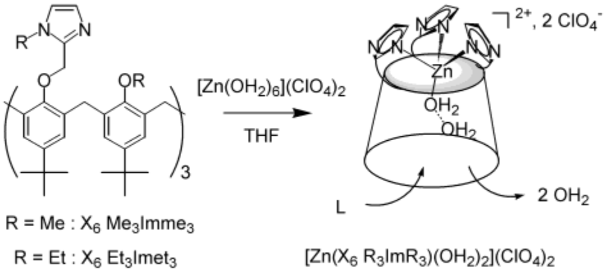


Figure 1. Crystal structure of [Zn(X$_6$Et$_3$Imet$_3$)(OH$_2$)·(OH$_2$)](ClO$_4$)$_2$ showing ellipsoids at 20% probability. Hydrogen atoms, counterions, and solvent of crystallization have been omitted for clarity. Selected bond length [Å] and angles [deg]: Zn1−N2 1.997(4), Zn1−N4 2.011(3), Zn1−N6 1.982(3), Zn1−O7 1.972(4), N2−Zn1−O7 100.1(2), N4−Zn1−O7 111.9(2), N6−Zn1−O7 112.4(2), N2−Zn1−N4 111.0 (2), N4−Zn1−N6 104.7(2), N2−Zn1−N6 117.0(2).

Scheme 1



As previously described with X$_6$Me$_3$Imme$_3$, a zinc−aqua complex was obtained upon reaction of a slightly modified calixarene-based ligand X$_6$Et$_3$Imet$_3$ [6] with zinc perchlorate (Scheme 1). Crystals suitable for X-ray diffraction were obtained by slow diffusion of Et$_2$O in a benzonitrile solution of the complex. The molecular structure is shown in Figure 1. It displays a tetrahedral zinc within the tris(imidazolyl) environment provided by the calixarene-based ligand, which is in a cone conformation. Two water molecules are buried in the calixarene cavity, with one of them coordinated to Zn. The average Zn−N bond length (1.997 Å) is quite short as was observed in the related complexes [Zn(X$_6$Me$_3$Imme$_3$)(L)](ClO$_4$)$_2$ with other guests L,[4,7] in accordance with the high Lewis acidity of the metal center.[8] The Zn−O(7) bond length [1.972(4) Å] is significantly longer than those reported for Zn−OH complexes,[9] but similar to the Zn−O distance found in the related ethanol complex [L = EtOH, *d*(Zn−

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(1) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375.

(2) (a) Parkin et al. have described the first X-ray structure of a 4-coordinate zinc−aqua complex within, however, an anionic N$_3$ coordination core [a tris-(pyrazolyl)borate]. The corresponding complex [Zn(Tp^{tBu,Me})(OH$_2$)]$^+$ was described as highly sensitive to deprotonation, being only stable with a particularly weakly basic counterion [HOB(C$_6$F$_5$)$_3$]$^-$; see: Bergquist, C.; Parkin, G. *J. Am. Chem. Soc.* **1999**, *121*, 6322. (b) Vahrenkamp et al. have reported the molecular structure of a tris(benzimidazolylmethyl)amine complex, [Zn(BIMA)(OH$_2$)](CH$_3$SO$_3$)$_2$, where Zn is 5-coordinated; see: Brandsch, T.; Schell, F.-A.; Weis, K.; Ruf, M.; Müller, B.; Vahrenkamp, H. *Chem. Ber./Recl.* **1997**, *130*, 283.

(3) Several groups have succeeded in stabilizing mononuclear N$_3$Zn(OH) species with highly sterically hindered tris(imidazolyl)phosphine [(a) Kimblin, C.; Allen, W. E.; Parkin, G. *J. Chem. Soc., Chem. Commun.* **1995**, 1813] or tris(pyrazolyl)borate ligands [(b) Alsfasser, R.; Trofimenko, S.; Looney, A.; Parkin, G.; Vahrenkamp, H. *Inorg. Chem.* **1991**, *30*, 4098. (c) Ruf, M.; Vahrenkamp, H. *Inorg. Chem.* **1996**, *35*, 6571].

(4) Sénèque, O.; Rager, M.-N.; Giorgi, M.; Reinaud, O. *J. Am. Chem. Soc.* **2000**, *122*, 6183.

(5) This stands in contrast to the [Cu(X$_6$Me$_3$Imme$_3$)(CO)]$^+$ complex, which was shown to undergo the autoinclusion of one calixarene *t*Bu group to optimize the cavity filling. See ref 6.

(6) Rondelez, Y.; Sénèque, O.; Rager, M.-N.; Duprat, A.; Reinaud, O. *Chem. Eur. J.* **2000**, *6*, 4218.

(7) Sénèque, O.; Giorgi, M.; Reinaud, O. *J. Chem. Soc., Chem. Commun.* **2001**, 984.

(8) This average Zn−N bond length is indeed shorter than that of the monocationic species [Zn(Tp^{tBu,Me})(OH$_2$)]$^+$ (2.018 Å). See ref 2a.

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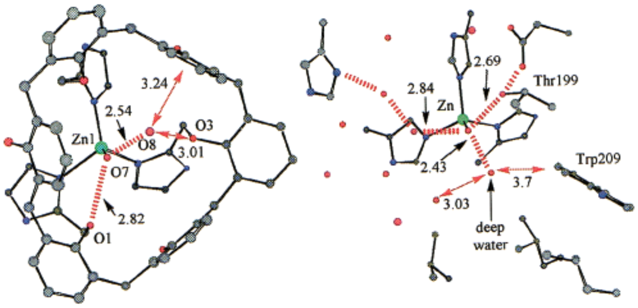

Figure 2. Comparison of the hydrogen-bond networks in the calixarene-based complex (left) and in the carbonic anhydrase (right). Alkyl substituents in the calixarene structure have been omitted for clarity. Distances are given in Å.

O) = 1.984 (5) Å],[7] thus attesting to the aqua nature of the ligand.[10−12] One of the O···O distances between the coordinated water molecule and an oxygen linked to the calixarene nitrogen arms is much shorter {*d*[O(1), O(7)] = 2.82 Å} than the two others (3.34 and 3.63 Å). This denotes the presence of a hydrogen bond linking the guest ligand to the host, as previously observed in the related heptylamine[4] and ethanol[7] adducts.[13] The second water molecule is suspended in the heart of the cavity by a very strong hydrogen bond to the aqua ligand {*d*[O(7), O(8)] = 2.539(7) Å, the Zn(1)−O(7)−O(8) and O(1)−O(7)−O(8) angle values being 129.1° and 126.2°, respectively}. It is also at a hydrogen-bond distance from an oxygen linked to another calixarene nitrogen arm {*d*[O(8), O(3)] = 3.01 Å} and stands just in front of an aromatic ring of the calixarene skeleton. The corresponding O(8)···centroid distance of 3.24 Å and O(7)−O(8)-centroid angle of 136.6° may well be indicative of an OH/π interaction stabilizing the low end of the hydrogen-bond network.[14] The proposed global connection is shown in Figure 2.

A comparison of the X-ray structure of the calixarene-based aqua complex and that of the active site of carbonic anhydrase II crystallized at pH 6[15] is displayed in Figure 2. In the acidic form of the enzyme, the aqua ligand is strongly hydrogen-bonded to a threonine residue and to two water molecules. One of them, called the deep water, is situated at a very short distance from the aqua ligand. It also stands next to another H_2O and to a tryptophane residue. Hence, the analogies between this deep water and the (O8) water molecule of the calixarene-based complex are quite remarkable. Interestingly as well, it has been noticed that, in the CAII variant structures, buried cavities larger than 50 Å^3 (the approximate size of the calix[6]arene cavity defined by the aromatic walls) are usually hydrated with water molecules.[16]

We have previously reported a quantitative study on the host−guest behavior of the aqua complex where the water ligand was displaced by diverse organic molecules.[4] The study was conducted at 300 K with constant H_2O and Zn complex concentrations. Suspecting that the second H_2O molecule is in fact involved in the exchange process, we have reinvestigated the equilibrium between $[Zn(X_6Me_3Imme_3)(OH_2)\cdot(OH_2)](ClO_4)_2$ and $[Zn(X_6Me_3Imme_3)(DMF)](ClO_4)_2$ by ^1H NMR spectroscopy.[17] For that purpose, we made large-scale variations of the concentration of each reactant and of the temperature. Indeed, we found that the equilibrium was highly sensitive to the H_2O concentration and strongly displaced in favor of the aqua adduct when the temperature was lowered. A careful quantification of the different species showed that the equilibrium constant $K_{DMF/H2O}$ (defined below) was second-order relative to H_2O concentration and first-order relative to the concentration of each DMF, zinc−aqua and zinc−DMF species. This agrees with the following equilibrium:

$$[Zn(X_6Me_3Imme_3)(OH_2)\cdot(OH_2)]^{2+} + DMF \leftrightarrows [Zn(X_6Me_3Imme_3)(DMF)]^{2+} + 2\ H_2O$$

$$K_{DMF/H2O} = [Zn(X_6Me_3Imme_3)(DMF)][H_2O]^2/[Zn(X_6Me_3Imme_3)(H_2O)\cdot(OH_2)][DMF]$$

Interestingly, whereas the water molecule coordinated to the zinc ion was in slow exchange,[12] the second water molecule in the Zn−aqua complex was found to be in fast exchange with free water on the NMR time scale at 298 K. The temperature dependence of $K_{DMF/H2O}$ in the form of a van't Hoff plot yielded the thermochemical parameters $\Delta H = 33.5(7)$ kJ·mol^{-1} and $\Delta S = 91(3)$ J·K^{-1}·mol^{-1}. This large and positive ΔS value accords well with an exchange process in which two water molecules are replaced by one molecule of DMF.[17]

In conclusion, the intriguing stability of the calixarene-based Zn−aqua complex has been clarified by X-ray structural analysis, revealing an extensive hydrogen-bond network between the host and two guest water molecules. Although only one of them is coordinated to the metal ion, both are expulsed upon exchange with an organic ligand. This indeed accounts for (i) the stabilization of the coordinated aqua molecule that appeared resistant to deprotonation compared to other model systems, (ii) the necessary filling of the cavity. All of this information is strongly reminiscent of natural systems, particularly carbonic anhydrase.

Supporting Information Available: Experimental and crystallographic information, van't Hoff plot, equilibrium constants between aqua adduct and adduct with other guests (PDF). This material is available free of charge via the Internet at http://pubs.acs.org. X-ray crystallographic files available in CIF format.

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(9) For the zinc hydroxide complexes described in ref 3, *d*(Zn−O) = 1.85−1.86 Å.

(10) This Zn−O distance is shorter than that of a 5-coordinate complex described in ref 2b (2.011 Å), in agreement with a higher Lewis acidity expected for a 4-coordinated metal ion.

(11) The perchlorate anions are separated from each other by more than 4 Å. Furthermore, the ^1H NMR spectrum was not significantly modified when the aqua complex was synthesized with PF_6 instead of ClO_4 as counteranions. This rules out the hypothesis of their protonation and indicates a global count of four hydrogen atoms associated to O(7) and O(8).

(12) Complexes based either on $X_6Me_3Imme_3$ or $X_6Et_3Imet_3$ exhibited similar structural and chemical properties: their ^1H NMR profiles could almost be superimposed and ligand exchange qualitatively occurred in the same way. As discussed in ref 4 for $X_6Me_3Imme_3$, the δ shift of the aqua ligand (3.63 ppm) strongly suggests that O(7) is a water molecule, not a hydroxide. Furthermore, this δ shift is similar to that found for the OH group in the related ethanol adduct (3.92 ppm), which has been recently X-ray characterized [see ref 7]. The δ value [3.2 (2) ppm] for the protons of the O(8) unit was estimated from the observed shifts of bulk H_2O (with the same ligand, $X_6Me_3Imme_3$). It is in good accordance with a second water molecule.

(13) Hydrogen bonds between coordinated water and counteranions were also reported for $[Zn(BIMA)(OH_2)](CH_3SO_3)_2$ and $[Zn(Tp^{tBu,Me})(OH_2)][HOB(C_6F_5)_3]$. In the latter case, one of the O−H bonds in the water ligand was shown to be considerably weakened, which may be related to the short Zn−O distance (1.937 Å). See ref 2.

(14) Similar weak interactions with the calixarene aromatic walls have been previously observed. See ref 7 and references therein.

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(17) The previously reported equilibrium constants for the aqua exchange with other guest ligands L have been reevaluated according to the new above-defined equilibrium. The corresponding values $K_{L/H20}$ are reported in the Supporting Information.