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Guanidine Is a Zn^{2+}-Binding Ligand at Neutral pH in Aqueous Solution

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Arginine (Arg) residues having pK_a of 12.5 in proteins are perceived to function as guanidinium cations to bind to anions such as phosphates. Although acidic transition metals such as Zn^{2+} are common in metalloproteins and in metal-activated enzymes, their coordinate binding to Arg has not been proposed. Indeed, metal−guanidinyl complexes are barely known and unexplored.[1−3] A recent success of Pt^{2+}−guanidinyl complexes at pH 7.5 was ascribed to specific circumstances; that is the highly acidic nature of Pt^{2+} lowered pK_a values of guanidiniums by hydrophobic environment or π acceptor (guanidine)−π donor (Pt^{2+}) interaction.[2,3] Herein we report that guanidine can be a good ligand to Zn^{2+} in neutral pH aqueous solution.

A new ligand, (2-guanidinyl)ethyl-cyclen (L^1) **1** formed a stable 1:1 Zn^{2+} complex (ZnL^1) **2b**, which crystallized out as a $2ClO_4^-$ salt from pH 7.5 aqueous solution (Scheme 1).[4] X-ray crystal structure analysis revealed a distinct coordination of the pendant guanidine (through N18) to Zn^{2+} (Figure 1). Zinc(II) is thus five-coordinate with four nitrogens of the cyclen ring (the average Zn^{2+}−N bond distance is 2.16 Å) and a nitrogen of guanidine (1.95 Å). A shorter bond length (1.31 Å) between C17 and N18 with respect to those for C17−N16 (1.35 Å) and C17−N19 (1.35 Å) implies that the Zn^{2+}-bound N18 is an imine nitrogen.[1]

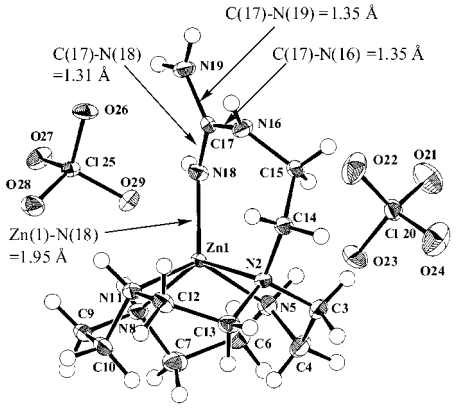

Figure 1. ORTEP drawing (50% probability ellipsoides) of ZnL^1 complex (**2b**). Selected bond distances (Å): Zn1−N2 2.188(2), Zn1−N5 2.125(2), Zn1−N8 2.180(1), Zn1−N11 2.135(2), Zn1−N18 1.953(2), C17−N16 1.352(2), C17−N18 1.310(2), C17−N19 1.347(2).

How does the guanidine in **2** prefer Zn^{2+} over protons at neutral pH? From potentiometric pH titration of the ligand L^1 (1 mM) in the absence and presence of 1 mM Zn^{2+} at 25 °C with I = 0.1 ($NaNO_3$), the pK_a values of >12, 10.13, 8.45, <2, and <2 were determined by the program "BEST".[5,6] In light of the pK_a values of 10.7, 9.7, <2, and <2 for cyclen (L^2),[7] the highest pK_a value of >12 for L^1 was assigned to the pendant guanidinium ion. In Scheme 1, the Zn^{2+} complexation constant (log K ($Zn(L^1{\cdot}H^+)$) for **1**·H^+ + Zn^{2+} ⇄ $Zn(L^1{\cdot}H^+){\cdot}(H_2O)$ (**2a**) and the deprotonation constant pK_a-($Zn(L^1{\cdot}H^+)$) for **2a** ⇄ **2b** + H_3O^+ were 12.4 ± 0.1 and 5.9 ± 0.1, respectively, at 25 °C with I = 0.1 ($NaNO_3$).[8] Zn^{2+}-cyclen complexes are good models of active centers of various zinc(II) enzymes.[9] For instance, the deprotonations from a Zn^{2+}-bound water (**4a** ⇄ **4b**)[10] and from a Zn^{2+}-bound alcohol (**5a** ⇄ **5b**)[11] are facile with the pK_a values of 7.9 and 7.7, respectively. It is thus reasonable that the deprotonation of the guanidinium for **2a** ⇄ **2b** is facile due to the close interaction between the Zn^{2+}-bound water (or OH^-) and the guanidinium ion in **2a**. An apparent complex formation constant, log K_{app}(ZnL^1) was calculated to be 10.4 in comparison to 11.0 for ZnL^2 and 10.4 for ZnL^3 at pH 7.4.[12] The speciation diagram for a mixture of 1 mM L^1 and 1 mM Zn^{2+} as a function of pH at 25 °C with I = 0.1 ($NaNO_3$) indicates that the initially formed Zn^{2+} complex $Zn(L^1{\cdot}H^+){\cdot}H_2O$ **2a** is populated most abundantly (75%) at ca. pH 5.5 (Figure 2a).

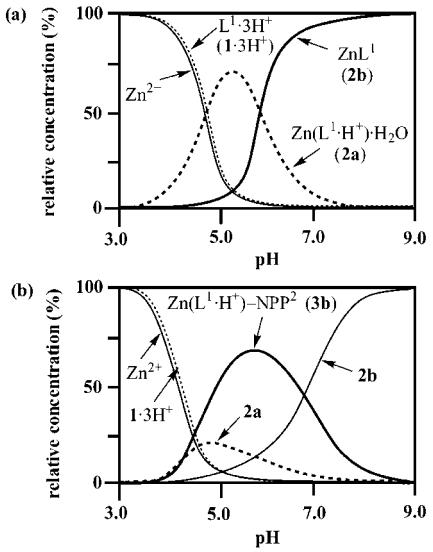

Figure 2. (a) Speciation diagrams for a mixture of 1 mM **1** + 1 mM Zn^{2+} as a function of pH at 25 °C with I = 0.1 ($NaNO_3$). (b) Speciation diagrams for a mixture of 1 mM **2** + 1 mM NPP as a function of pH at 25 °C with I = 0.1 ($NaNO_3$). Other species in less than 5% are omitted for clarity.

Another interesting question with the Zn^{2+}−guanidine bonding in **2b** was how labile is it in aqueous solution? We found that **2b** did not catalyze the hydrolysis of 4-nitrophenyl acetate at pH 5.0−9.0 (see the Supporting Information), unlike the catalytically reactive nucleophiles **4b**[10] and **5b**.[11] We then tested if an external (4-nitrophenyl)phosphate (NPP) anion could displace the pendant guanidine in **2b**. The dianionic NPP was a good ligand to Zn^{2+} in **4** to form an 1:1 complex with log K_{app} = 3.1 at pH 5.6.[13] Figure 3 compares the ^{31}P NMR (162 MHz) titration curves of NPP (5

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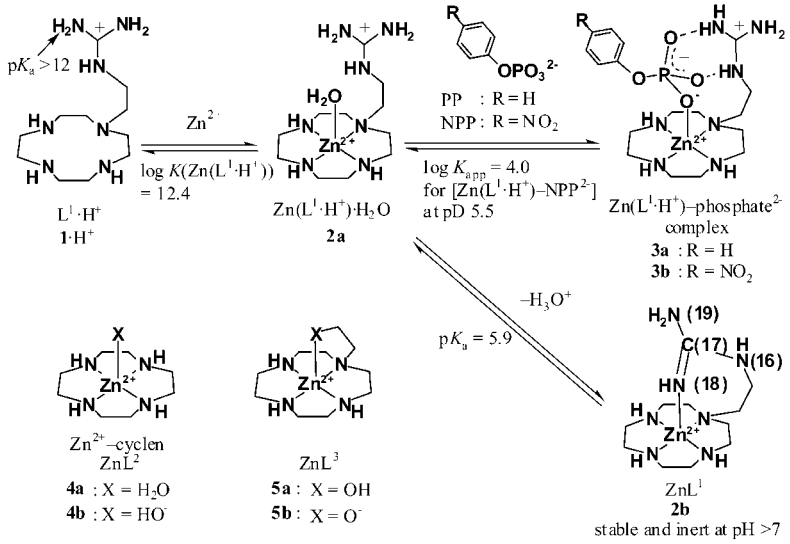
Scheme 1



mM) with **1**, **2**, and **4** at various pD, from which a 1:1 complex **3b** formation with log K_{app} (**2**−NPP) of 3.7 ± 0.1 at pD 5.5 ± 0.1 was calculated.[14] A structure of **3b** was assigned, as depicted in Scheme 1. The ligand **1** alone (mostly in the $L^1{\cdot}2H^+$ form) had little interaction with NPP at pD 5.5 ± 0.1, see Figure 3.[15] On the basis of potentiometric pH titration, a speciation diagram was obtained for a mixture of 1 mM **2** and 1 mM NPP as a function of pH at 25 °C with $I = 0.1$ ($NaNO_3$) (Figure 2b).[6] At higher pH, the pendant guanidinium became deprotonated to displace the Zn^{2+}-bound NPP^{2-} to yield **2b**. The apparent complexation constant, log K_{app}(**2**−NPP), of 4.0 ± 0.1 at pH 5.5 agreed reasonably well with 3.7 ± 0.1 obtained by the ^{31}P NMR method. A higher affinity of NPP^{2-} to Zn^{2+} in **2a** over **4a** probably arose from the phosphate−guanidinium interactions, as depicted in **3b**.

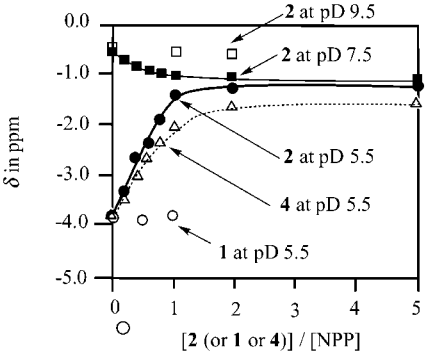


Figure 3. pH-dependent change (at pD 5.5, 7.5, and 9.5) of ^{31}P chemical shift of NPP (5 mM) upon addition of **1**, **2**, and **4** in D_2O at 35 °C.

Colorless prisms were obtained from a mixture of **2b** and phenyl phosphate (PP) in aqueous solution at pH 6.5. The X-ray crystal structure analysis proved the 1:1 **2**−PP^{2-} complex (**3a**), where the Zn^{2+}-bound PP^{2-} is stabilized by hydrogen bondings with two intermolecularly adjacent guanidinium protons (see the Supporting Information). The intermolecular interactions may come from strong crystal packing forces. The present facts may imply another function of Arg residues in the active centers of zinc(II) enzymes.[16,17]

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Supporting Information Available: Experimental section, Figures S1−S6, and Tables and CIF for **2b** and **3a** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(4) Synthesis of **1** and **2b** was described in the Supporting Information.

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(6) For the potentiometric pH titration curves, see the Supporting Information.

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(8) $Zn^{2+} + L^1{\cdot}H^+ \rightleftarrows Zn(L^1{\cdot}H^+){\cdot}H_2O$ (**2a**):

$$K(Zn(L^1{\cdot}H^+)) = [Zn(L^1{\cdot}H^+)(\mathbf{2a})]/[Zn^{2+}][L^1{\cdot}H^+]\ (M^{-1}) \quad (1)$$

2a ⇄ **2b** + H^+:

$$K_a(Zn(L^1{\cdot}H^+)) = [\mathbf{2b}]a_{H^+}/[\mathbf{2a}] \quad (2)$$

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(12) The $K_{app}(ZnL^m)$ values are defined by eqs 3 and 4. For detailed comparison, see the Supporting Information.

$$K_{app}(ZnL^m) = [\mathbf{2a} + \mathbf{2b}\ (\text{or}\ \mathbf{4a} + \mathbf{4b}\ \text{or}\ \mathbf{5a} + \mathbf{5b})]/[Zn^{2+}]_{free}[L^m]_{free}$$

(at designated pH) (M^{-1}) (3)

$$[L^m]_{free} = \Sigma[L^m{\cdot}nH_+]_{free}$$

($n = 0-5$ for $m = 1$ and 3, and $n = 0-4$ for $m = 2$) (4)

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(14) The pK_1 and pK_2 values for NPP are <2 and 5.2 ± 0.1, respectively, at 25 °C with $I = 0.1$ ($NaNO_3$) (ref 13a, b).

(15) The ^{31}P NMR titration for NPP (5 mM) and **4** was carried out at pD 5.5 ± 0.1 and 35 °C, giving log K_{app}(**4**−NPP) of 3.4 ± 0.1 (Figure 3).

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