

Molecular Recognition of an Amino Acid by a Novel Macrocyclic Monoxo-tetraamine and Its Zn^{II} Complex

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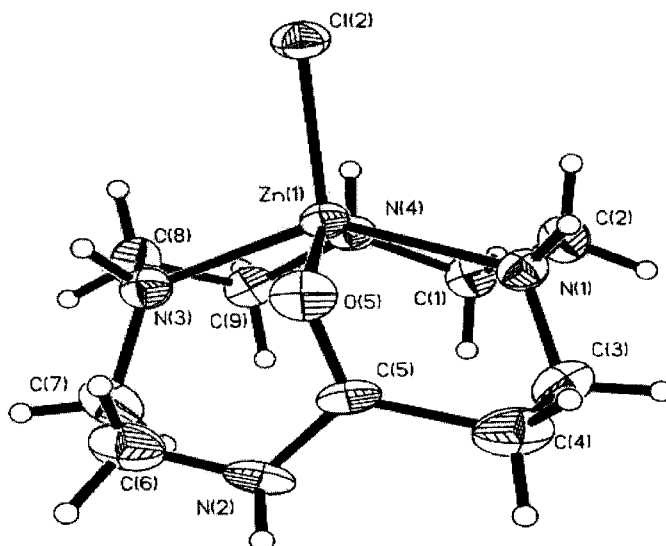
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The macrocycle monoxo-tetraamine, 1,4,8,11-tetraazacyclotridecane-5-one (mota), was found to be extremely efficient in the recognition of the amino acid, glycine (G1), in aqueous solution. The single-crystal structure of a five-coordinated mota-Zn^{II} complex is reported. This Zn^{II} complex acts as host for G1 to form a stable ternary complex. The recognition constants and the recognition mechanism for the binary and ternary complexes are based on potentiometric data.

Introduction. – Molecular recognition of amino acids and their derivatives is important in studying both biological and artificial recognition processes, especially in understanding the mechanism of aminoacyl-transfer RNA synthases [1–5]. Synthetic receptors for amino acids can be of great utility in model studies of biological molecular recognition and also in separation of complex mixtures. A variety of receptors have been designed to recognize amino acids, but most of them bind the ammonium or carboxylate parts of protected amino acid derivatives [6–10]. Since amino acids exist as zwitterions in aqueous solution, their desolvation is a costly energetic process. Thus, effective receptors should have multiple binding sites complementary to zwitterions. A few host molecules have been reported in which two different kinds of binding sites were functionally arranged [11–15]. In this work, we first utilize the asymmetric monoxo-tetraamine, mota, as an acceptor for glycine. The crystal structure, stability, and recognition ability of mota and its Zn^{II} complex toward glycine are investigated.

Results and Discussion. – *Crystal Structure of mota-Zn^{II} Complex.* The ligand mota was synthesized according to the literature method [16]. Recrystallization of mota-Zn^{II} complex from MeOH yielded colorless crystals from which the crystal structure was determined. *Fig. 1* shows a perspective view of the cationic [Zn(mota)Cl]⁺ complex. The asymmetric unit of the crystal consists of a complex cation and one chloride counterion, giving the overall formulation [Zn(mota)Cl](ClO₄). The Zn^{II}-atom has an irregular five-coordinate geometry, the donors being three N-, and one O-atom from the macrocyclic ligand and one coordinated chloride. The bond lengths are unexceptional (see *Table 1*). The Cl(2)–Zn(1)–N(3), Cl(2)–Zn(1)–N(1), Cl(2)–Zn(1)–N(4), and Cl(2)–Zn(1)–O(5) angles are 109.97°, 111.87°, 105.42°, and 102.34°, respectively. Thus, the geometry around Zn^{II} is a distorted square pyramid with N(3), N(1), N(4), and O(5) at the nearly basal plane, and Cl[–] at the axial site.

Protonation Constants of mota and G1. The protonation constants of mota have been reported previously [17]. They were redetermined under the experimental conditions employed in this work. The results and comparisons are located in *Table 2*.

Fig. 1. Crystal structure of $[Zn(mota)Cl](ClO_4)$ Table 1. Selected Bond Lengths [\AA] and Bond Angles [deg] for mota- Zn^{II} Complex

Bond lengths					
Zn(1)–N(3)	2.108(4)	Cl(1)–O(2)	1.414(4)	N(1)–C(3)	1.486(5)
Zn(1)–N(1)	2.109(4)	Cl(1)–O(1)	1.428(4)	N(1)–C(2)	1.485(5)
Zn(1)–N(4)	2.132(3)	Cl(1)–O(3)	1.434(3)	N(2)–C(5)	1.365(12)
Zn(1)–O(5)	2.209(3)	Cl(1)–O(4)	1.433(3)	N(2)–C(6)	1.361(10)
Zn(1)–Cl(1)	2.232(11)	O(5)–C(5)	1.268(10)	N(3)–C(8)	1.483(5)
Bond angles					
N(3)–Zn(1)–N(1)	138.12(13)	O(2)–Cl(1)–O(1)	110.0(4)		
N(3)–Zn(1)–N(4)	83.71(12)	O(2)–Cl(1)–O(3)	108.1(3)		
N(1)–Zn(1)–N(4)	83.74(12)	C(3)–N(1)–C(2)	111.6(3)		
N(3)–Zn(1)–O(5)	86.86(12)	C(5)–N(2)–C(6)	117.6(9)		
N(1)–Zn(1)–O(5)	86.05(12)	C(3)–N(1)–Zn(1)	121.6(3)		
N(4)–Zn(1)–O(5)	152.24(12)	C(2)–N(1)–Zn(1)	103.5(2)		
N(3)–Zn(1)–Cl(1)	109.97(10)	C(8)–N(3)–Zn(1)	103.8(2)		
N(1)–Zn(1)–Cl(1)	111.87(10)	C(7)–N(3)–Zn(1)	123.6(3)		
N(4)–Zn(1)–Cl(1)	105.42(9)	C(1)–N(4)–Zn(1)	107.9(2)		
O(5)–Zn(1)–Cl(1)	102.34(8)	C(9)–N(4)–Zn(1)	107.7(2)		

Table 2. Protonation Constants of mota and Gl ($\mu = 0.1\text{M}$ KCl, 25° , $[\text{mota}] = [\text{Gl}] = 0.001\text{M}$)

Substrate	$\log K_1$	$\log K_2$	$\log K_3$
mota ^{a)}	9.82 (9.82)	7.33 (7.33)	2.09 (2.08)
Gl ^{b)}	9.55 (9.50)	2.22 (2.31)	–

^{a)} Values in parentheses from [27]. ^{b)} Values in parentheses from [28].

Also listed are protonation constants of glycine that were re-evaluated. The differences between the values determined in this work and determined by others [18] are minor.

Glycine Recognition by mota. The titration curve for the mota-Gl system (Fig. 2) indicate a buffered region between a values 0, 1, and 2 by a break with the remainder of the curve buffered at high pH. The first buffer region corresponds to the deprotonation of motaH₃ to the bis-protonated state motaH₂. The following buffer region is the formation of successive deprotonated states of the ligand. The equilibrium constants calculated for the binding of glycine to protonated forms of mota are listed in Table 3.

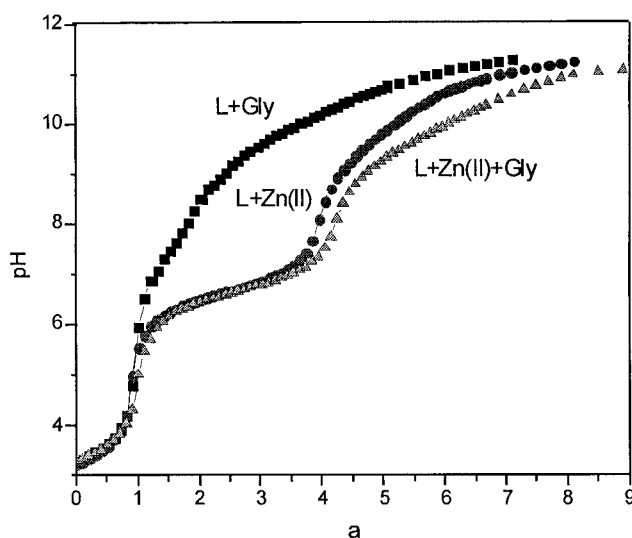


Fig. 2. Potentiometric equilibrium curves for mota-Gl, mota-Zn^{II}, and mota-Zn^{II}-Gl systems (L = mota)

Table 3. Stability Constants for the mota-Gl System ($\mu = 0.10\text{M KCl}$, 25° , $[\text{mota}] = [\text{Gl}] = 0.001\text{M}$)

Stoichiometry			Quotient K	$\log K$
mota	Gl ($\text{H}_2\text{NCH}_2\text{COOH}$)	H		
1	1	0	$[\text{motaGl}]/[\text{mota}][\text{Gl}]$	5.30
1	1	1	$[\text{motaGlH}]/[\text{motaGlH}][\text{Gl}]$	12.32
1	1	2	$[\text{motaGlH}_2]/[\text{motaGlH}_2][\text{Gl}]$	12.87
1	1	3	$[\text{motaGlH}_3]/[\text{motaGlH}_3][\text{Gl}]$	13.93

The species distribution curves are shown in Fig. 3. It was found that three-protonated ligand-glycine species are formed between pH 2.0 and 11.0. The stability of the species decreases as the degree of protonation decreases. This is in accordance with the coulombic effects in that increased positive charge of the ligand N-atoms will more strongly coordinate the anionic portion of the glycine zwitterions. A proposed binding mode of motaGlH₃ is illustrated in Scheme 1. A glycine unit in its neutral zwitterion

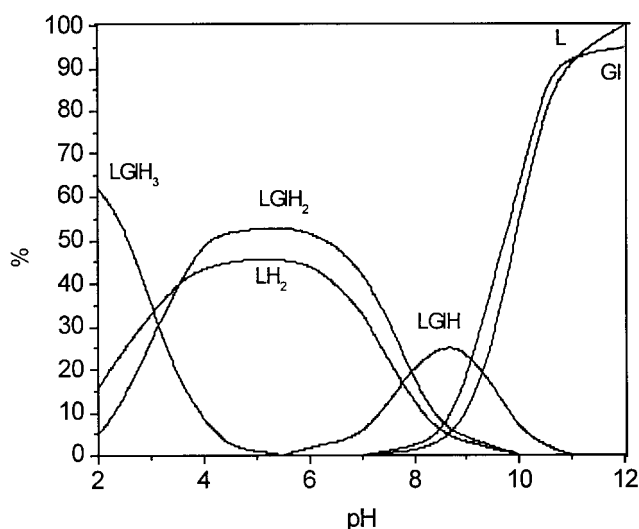
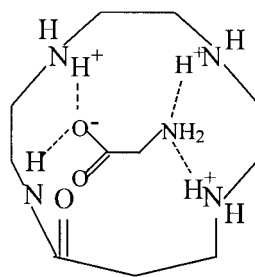


Fig. 3. Species-distribution diagram for mota-Gl system in 1:1 ratio ($\mu=0.100\text{M}$ KCl, $t=25^\circ$, $T_{\text{MOTA}}=T_{\text{GI}}=0.001\text{M}$). Only mota- and Zn^{II} -related species are shown.

Scheme 1

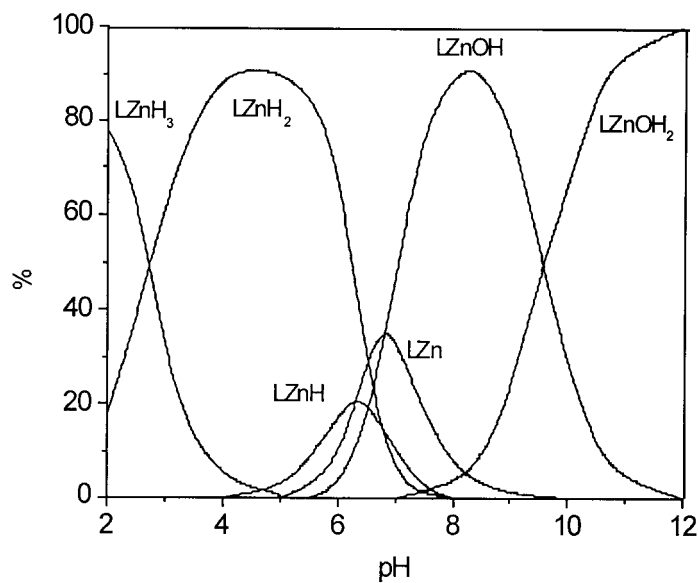
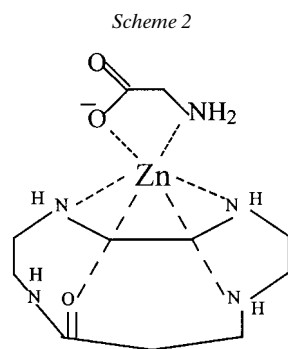


form is shown to bind the tris-protonated macrocycle through H-bonding. Further protonation of the tris-protonated binary species may not be possible because the positively charged glycine would not be attracted to the positively charged macrocyclic ligand.

Binding of Glycine by the Zn^{II} Complex of mota. The equilibrium constants determined for the mota- Zn^{II} and mota- Zn^{II} -glycine systems are listed in Table 4. The species distribution curves are shown in Figs. 4 and 5, respectively. In the presence of Zn^{II} , the recognition for the amino acid is increased considerably in the neutral to basic region (Fig. 5). Glycine approaches 33.3% maximum formation as the mota- Zn^{II} -GI⁻ complex. The monoprotonated complex mota- Zn^{II} -GIH exists only as a minor species at the lower-pH region of 2.0–6.0 (22.2%). The predominant factor in the complex binding is the glycine amine- Zn^{II} coordination bond. The carboxylate group is capable of forming a coordination bond with Zn^{II} in the basic region. Meanwhile, the acidic H-atom of the amide on mota may also favor recognition. As shown in Scheme 2, the

Table 4. Stability Constants for the mota-Zn^{II}-Gl System ($\mu = 0.10\text{M KCl}$, 25° , $[\text{mota}] = [\text{Zn}] = [\text{Gl}] = 0.001\text{M}$)

Stoichiometry				Quotient K	log K
mota (L)	Zn	Gl	H		
1	1	0	0	$[\text{LZn}]/[\text{L}][\text{Zn}]$	9.33
1	1	0	1	$[\text{LZnH}]/[\text{LZn}][\text{H}]$	6.44
1	1	0	2	$[\text{LZnH}_2]/[\text{LZn}][\text{H}]^2$	6.65
1	1	1	0	$[\text{LZnGl}^-]/[\text{LZn}][\text{Gl}^-]$	12.25
1	1	1	1	$[\text{LZnGlH}]/[\text{LZnH}][\text{Gl}]$	2.58
1	1	1	-1	$[\text{LZnGlOH}]/[\text{LZnOH}][\text{Gl}]$	1.00
1	1	1	-2	$[\text{LZnGlOH}_2]/[\text{LZnOH}_2][\text{Gl}]$	0.88

Fig. 4. Species-distribution diagram for mota-Zn^{II} system in 1:1 ratio ($\mu = 0.100\text{M KCl}$, $t = 25^\circ$, $T_{\text{MOTA}} = T_{\text{Zn}} = 0.001\text{M}$). Only mota- and Zn^{II}-related species are shown.

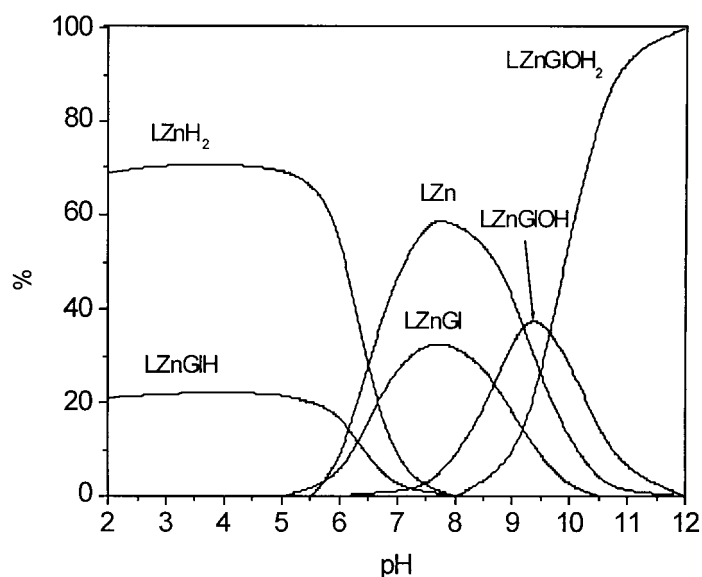


Fig. 5. Species-distribution diagram for mota- Zn^{II} -GI system in 1:1:1 ratio ($\mu = 0.100M$ KCl, $t = 25^\circ$, $T_{MOTA} = T_{Zn} = T_{GI} = 0.001M$). Only mota- and GI-related species are shown.

formation of a stable ternary complex, $motaZn^{II}GI^-$ leads to high binding constant ($\log K = 12.25$) in recognition of glycine.

Kimura *et al.* have studied Zn-cyclen and its analogues for interaction with RNA and DNA [19][20]. The results showed that these kinds of complexes could serve as a new type of HIV-1 RNA-targeting molecule. The especially strong recognition of amino acids (binding constants, $\log K > 12$) for mota and its Zn^{II} complex reported in this work provides valuable information for the research in this direction. Further investigation in selective recognition of various amino acids and peptides is in progress in our laboratory.

Experimental Part

Materials. All of the metal stock solns. for potentiometric studies are reagent-grade chloride salts prepared with doubly distilled H_2O and standardized against EDTA. CO_2 -free *Dilute-it* ampules of KOH were obtained from *J. T. Baker Inc.* KOH Solns. (ca. 0.1M) were prepared with doubly distilled H_2O and standardized. The extent of carbonate accumulation (<1.8%) was checked periodically by titration with a standard HCl soln.

Potentiometric Equipment. A *Corning 250* digital pH-meter, fitted with *Fisher* full-range blue-glass and *Fisher* calomel reference electrodes, was used for potentiometric titrations. A *Metrohm* 10-ml-capacity piston buret was used for precise delivery of standard KOH. The soln. to be studied was contained in a 75-ml jacketed glass cell thermostated at $25.00 \pm 0.05^\circ$ by a circulating constant-temp. water bath.

Potentiometric Determinations. All pH calibrations were performed with standardized aq. HCl solns. to measure H^+ ion concentrations directly ($p[H] = -\log [H^+]$). The ionic strength was adjusted to 0.100M with KCl. Titrations of the ligand in the presence of metal ions in aq. soln. were conducted in the manner described by *Martell and Motekaitis* [21]. Cell solns. (in general 50.00 ml) were purged with a purified Ar stream. Standard base was introduced into the sample solns. with a *Metrohm* piston buret. Exper. runs were carried out by adding increments of standard base to a soln. containing mota $\cdot 3$ HCl plus other components such as KCl soln. and metal soln. The concentrations of the sample solns. were 1×10^{-3} M for mota $\cdot 6$ HCl. The titrations of mota- Zn^{II} -

GI in a 1:1:1 ratio were carried out to investigate the recognition process. The pH range for accurate measurements was considered to be 2–12. The pK_w for the aq. system, defined as $-\log([H][OH])$ at the ionic strength employed, was found to be 13.78.

Computations. Protonation constants and stability constants from the direct titrations were calculated from the potentiometric data with the program BEST. For the ternary system, the stability constants of the mononuclear species were fixed. Only the recognition set of constants were refined within the 1:1:1 titration data, until the differences between the calculated and observed p[H] values were minimized. Species distribution diagrams were computed from the measured equilibrium constants with SPE and plotted with SPEPLOT.

Preparation of [Zn(mota)Cl](ClO₄). To a soln. containing 0.05 mmol of mota · 3 HCl in 15 ml of EtOH were added 0.15 mmol of Et₃N and 0.05 mmol of Zn(ClO₄)₂ · 6 H₂O. After stirring for 2 h, white microcrystals formed. Chemical analyses for the complex confirmed the composition given.

Crystallography. The structure was solved by direct methods and subsequent Fourier difference techniques, and refined anisotropically by full-matrix least-squares on F^2 . Crystal data and structure refinements are summarized in Table 5. Crystallographic data for the structure reported in this paper are available through the internet at martell@mail.chem.tamu.edu.

Table 5. Crystal Data and Structure Refinement Details for mota-Zn^{II} Complex

Molecular formula	C ₉ H ₂₀ Cl ₂ N ₄ O ₅ Zn
Formula weight	400.56
Crystal color; habit	white
Crystal system	monoclinic
Lattice type	primitive
<i>a</i> /Å	9.5127(19)
<i>b</i> /Å	12.181(2)
<i>c</i> /Å	13.743(3)
<i>a</i> /deg	90
<i>β</i> /deg	90.462
<i>γ</i> /deg	90
<i>V</i> /Å ³	3517.6(5)
Space group	P2(1)/n
<i>Z</i>	4
ρ (calc.)/c/gcm ⁻³	1.671
<i>F</i> (000)	824
θ Range/deg	2.59–28.23
Abs. coeff/mm ⁻¹	None
<i>T</i> /K	110(2)
λ /Å	0.71073
Reflections collected	9732
Independent reflections	3680; ($R_{int} = 0.0393$)
Final <i>R</i> indices ($I > 2.0\sigma(I)$)	$R1 = 0.0602$, $wR2 = 0.1517$
<i>R</i> ^a)	0.0786
<i>Rw</i> ^b)	0.1724
GOF on F^2	1.045
Peak, hole in final difference map/eÅ ⁻³	2.410; –0.706

^a) $R = \sum ||F_o| - |F_c|| / \sum F_o$. ^b) $Rw = \{ \sum (F_o^2 - F_c^2)^2 / [\sum w(F_o^2)^2] \}^{1/2}$.

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