Molecular Recognition of a Peptide by the Nickel(II) Complex of 1,4,7,10-Tetraazacyclododecane-2,9-dione

by **Jian Gao**, **Joseph H. Reibenspies**, **Yizhen Sun**, and **Arthur E. Martell***

Department of Chemistry, Texas A & M University, College Station, TX 77842-3012, USA
(e-mail: martell@mail.chem.tamu.edu)

The nickel(II) complex of the macrocycle 1,4,7,10-tetraazacyclododecane-2,9-dione (dota) was found to be efficient in the recognition of the dipeptide, glycyl-glycine (Gly-Gly) in aqueous solution. This (dota)Ni^{II} complex serves as a targeting molecule to form a stable ternary complex with the dipeptide at pH 8.3 in aqueous solution. The recognition constant ($\log K = 19.20$) and the recognition mechanism were investigated based on the potentiometric method. The single-crystal of a six-coordinated $(dota)_2Ni^{II}$ complex is also reported.

Introduction. – Molecular recognition and selective cleavage of peptides by metal complexes have become increasingly important in amino acid sequencing [1] and in the study of protein function and solution structure [2–5]. Although the stability of peptide amide bonds has placed limits on the number of available cleavage reagents, there is evidence that metal ions and their corresponding complexes of Cu^{II}, Ni^{II}, Pd^{II}, Pt^{II}, and Zn^{II} effectively hydrolyze unactived amide bonds in small peptides [6–10]. The initial stage of cleavage is believed to be the effective recognition and binding of the target molecules (peptide) by a metal complex. Thus, it becomes critical to study the coordination structure of the metal complex and peptide molecules in the process of recognition. The binding constants obtained may provide useful information in the study of hydrolysis of peptides.

As a derivative of cyclen, mota [11] and dota bear the dual features of macrocyclic polyamines and oligopeptides. Other than tetraamine and monooxo-tetraamine ligands, dota has the potential to be a bi-, tri-, and tetra-dentate chelating ligand [12]. This property provides an opportunity to investigate the coordination structure of a dipeptide molecule in binding with a metal complex of dota. Our former investigation showed that the Zn^{II} complex of mota is efficient in the recognition of amino acids [11]. The increased recognition ability was ascribed to the introduction of the amido group into the macrocyclic ligand. In this work, the binary Ni^{II} complex of dota was used as a small targeting molecule in the recognition of a dipeptide. The ternary dota Ni^{II}-Gly-Gly system was investigated structurally and potentiometrically.

Results and Discussion. – *Structure of [Ni^{II}(dota)] Complex.* The tridentate coordination model of dota with Ni^{II} was confirmed by crystallographic analysis of its Ni^{II} complex. Recrystallization of $[Ni^{II}(dota)_2]$ complex from aqueous solution at pH 7.0 yielded purple crystals from which the crystal structure was determined. *Fig. 1* shows a perspective view of a $[Ni^{II}(dota)_2)](ClO_4)_2$ molecule. The symmetric structure of the crystal consists of a complex cation and two perchloride counterions. The Ni^{II}-

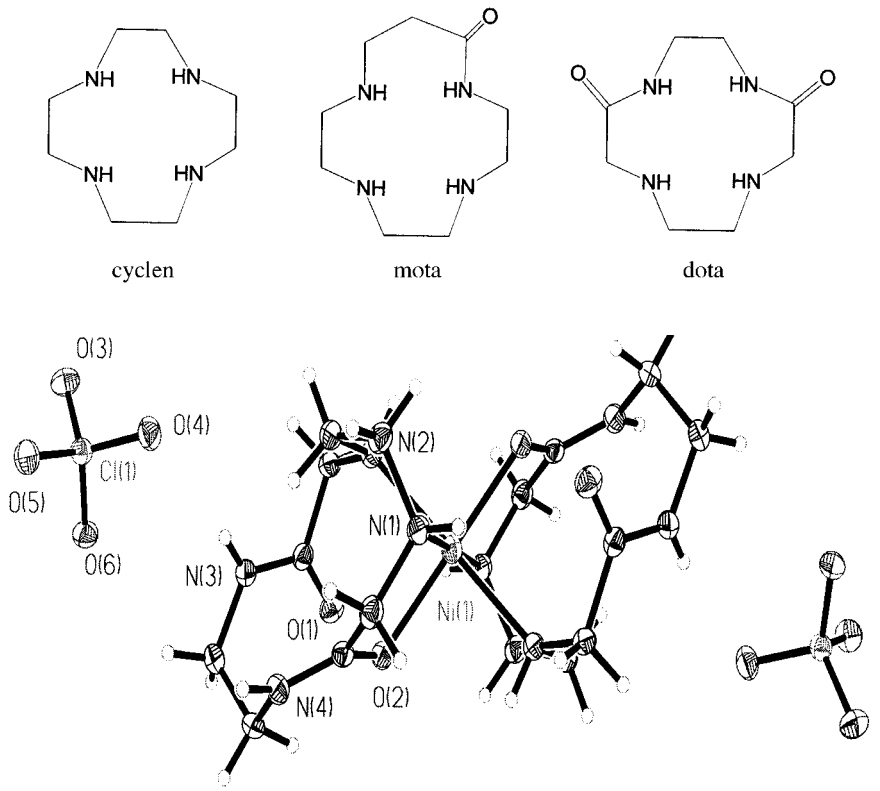


Fig. 1. *Crystal structure of* $[Ni^{II}(dota)_2](ClO_4)_2$

atom has a regular six-coordinate geometry, the donors being four N- and two O-atoms from two macrocyclic ligands. The bond lengths are unexceptional (see *Table 1*). The N(1)–Ni–N(1A), N(2)–Ni–N(2A), and O(2)–Ni–O(2A) angles are all 180.00°. Thus, the geometry around the Ni^{II}-atom is an octahedron with N(1), N(1A), N(2), and N(2A) at the basal plane, and O(2) and O(2A) at the axial sites.

Stability Constants and Species Distribution of 1:1 (dota) Ni^{II} *Complex System.* The titration curves for dota · 2 HBr and dota-Ni^{II} in 1:1 ratio were shown in *Fig. 2*. The protonation constants of dota and Gly-Gly have been reported previously [12][13]. They were redetermined at the experimental conditions employed in this work. The difference in the constants between the values determined in this work and those determined by others are minor (*Table 2*). The stability constants calculated for the 1:1 complex system are listed in *Table 3*.

The species-distribution curves for the 1:1 dota-Ni^{II} system are shown in *Fig. 3*. It was found that the mono- and diprotonated Ni^{II} complexes, (dota)H–Ni^{II} and (dota)H_2–Ni^{II} exist only at lower pH region of *ca.* 2.0–6.0. The system dota-Ni^{II} prevails between pH 4.0–10.0. At physiological pH 7.4, it approached 98.0%. In the higher pH range (>10.0), dota-Ni^{II}OH and dota-$Ni^{II}(OH)_2$ become the dominant species. Since the displacement of hydrogen ions from the amido groups is possible,

Table 1. *Selected Bond Lengths* [Å] *and Bond Angles* [deg] *for [Ni^{II}(dota)$_2$]*

Bond lengths					
Ni(1)–O(2)	2.087(2)	Cl(1)–O(3)	1.435(2)	N(1)–C(1)	1.478(4)
Ni(1)–O(2A)	2.087(2)	Cl(1)–O(5)	1.437(2)	N(1)–C(8)	1.482(4)
Ni(1)–N(1)	2.135(3)	Cl(1)–O(6)	1.449(2)	N(2)–C(2)	1.484(4)
Ni(1)–N(1A)	2.135(3)	Cl(1)–O(4)	1.453(2)	N(2)–C(3)	1.494(4)
Ni(1)–N(2)	2.176(2)	O(1)–C(4)	1.233(4)	N(3)–C(4)	1.338(4)
Ni(1)–N(2A)	2.176(2)	O(2)–C(7)	1.259(4)	N(3)–C(5)	1.456(4)
Bond angles					
O(2)–Ni(1)–O(2A)	180.00(4)	O(2)–Ni(1)–N(2A)	88.28(9)		
O(2)–Ni(1)–N(1)	80.60(9)	O(2A)–Ni(1)–N(2A)	91.72(9)		
O(2A)–Ni(1)–N(1)	99.40(9)	N(1)–Ni(1)–N(2A)	97.83(9)		
O(2)–Ni(1)–N(1A)	199.40(9)	N(1A)–Ni(1)–N(2A)	82.17(9)		
O(2A)–Ni(1)–N(1A)	180.60(9)	N(2)–Ni(1)–N(2A)	180.0(2)		
N(1)–Ni(1)–(N1A)	180.00(12)	O(3)–Cl(1)–O(5)	110.07(14)		
O(2)–Ni(1)–N(2)	91.72(9)	O(3)–Cl(1)–O(6)	109.65(14)		
O(2A)–Ni(1)–N(2)	88.28(9)	O(5)–Cl(1)–O(6)	109.41(15)		
N(1)–Ni(1)–N(2)	82.17(9)	O(3)–Cl(1)–O(4)	109.36(14)		
N(1A)–Ni(1)–N(2)	97.83(9)	O(5)–Cl(1)–O(4)	109.21(15)		

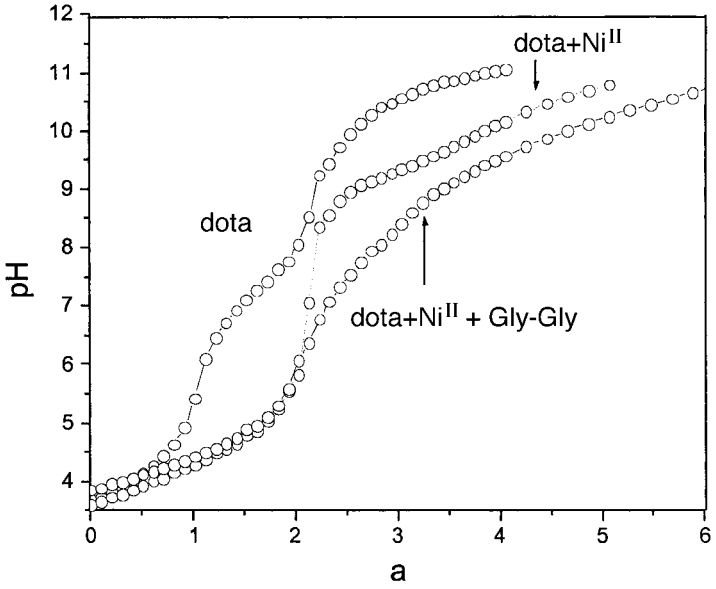


Fig. 2. *Potentiometric equilibrium curves for dota, dota + Ni^{II}, and dota + Ni^{II}-Gly-Gly systems*

Table 2. *Protonation Constants of dota and Gly* (μ = 0.1M KCl, 25°, [dota] = [Gly-Gly] = 0.001M)

Substrate	log K_1	log K_2
dota	6.98(6.98)[a])	3.74(3.75)
Gly-Gly	8.08(8.09)[b])	3.13(3.11)

[a]) Values in parentheses from [12] [b]) Values in paretheses from [13].

Table 3. *Stability Constants for the dota-Ni^{II} System* ($\mu = 0.10$M KCl, 25°, [dota] = [Ni^{2+}] = 0.001M; L denotes dota)

	Stoichiometry		Quotient K	log K
dota (L)	Ni^{II}	H		
1	1	0	[LNi]/[L][Ni]	15.51
1	1	1	[LHNi]/[LNi][H]	4.50
1	1	2	[LH_2Ni]/[LHNi][H]	4.11
1	1	−1	[LNiOH]/[LNi][OH]	6.31
1	1	−2	[$LNiOH_2$]/[LNiOH][OH]	−9.64

equilibria between the hydrolyzed species and the deprotonated species dota$H_{-1}Ni^{II}$ and dota$H_{-2}Ni^{II}$ exist in solution (the notations dotaH_{-1} and dotaH_{-2} represent one and two H-atoms, resp., released into solution during the formation of the Ni^{II} complex). A proposed structural variation upon stepwise deprotonation is illustrated in the *Scheme*.

Binding of Gly-Gly by Ni^{II} Complex of dota. The equilibrium constants determined for the dota + Ni^{II} + Gly-Gly systems are listed in *Table 4*. The species distribution curves are shown in *Fig. 4*. In the presence of Ni^{II}, the recognition for the peptide molecules is significant in the neutral to basic region. At pH 8.3 there is 50.9% maximum formation of the mota-Zn^{II}-Gly-Gly^{-} complex. At physiological pH (7.4), 33.6% Gly-Gly molecules are bound by dota-Ni^{II} complex. No neutral peptide complex, mota-Zn^{II}-Gly-Gly, could be found at the lower pH region (2.0–6.0). This

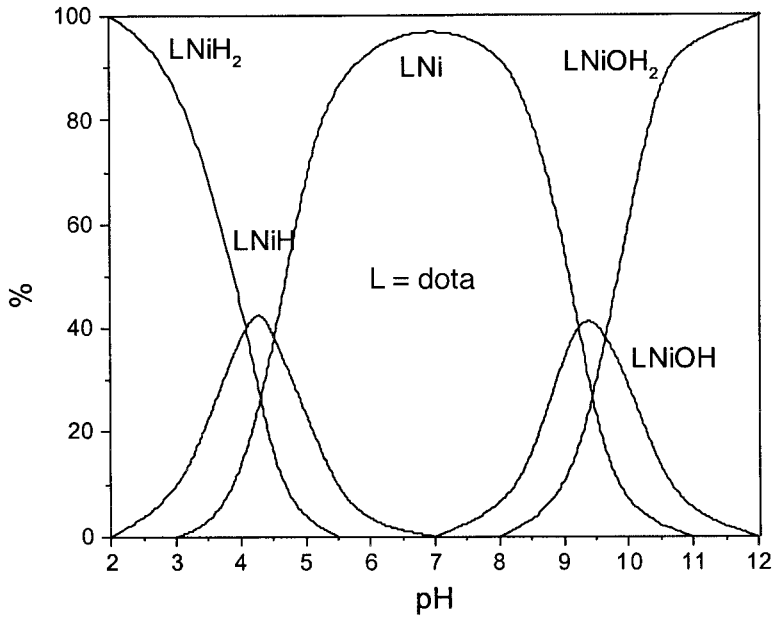
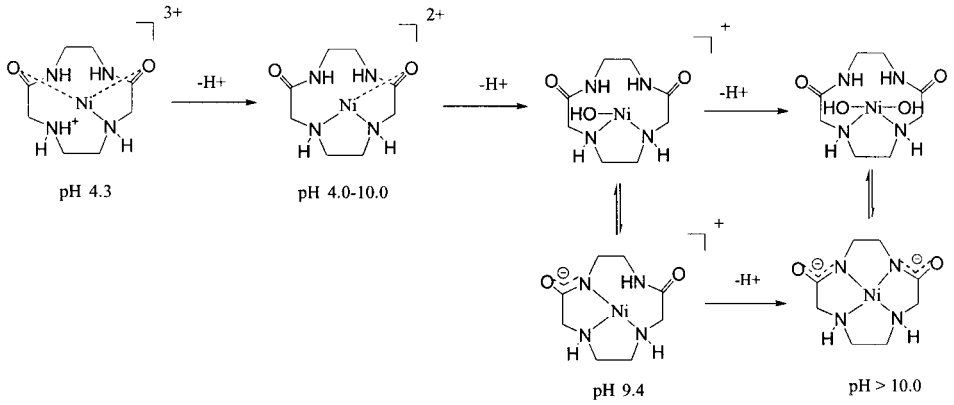


Fig. 3. *Species-distribution diagram for dota-Ni^{II} in 1:1 ratio.* (μ = 0.100M KCl, 25°, [dota] = [Ni] = 0.001M)

Scheme



indicated that the Gly-Gly amine-Ni^{II} coordinate bond is not strong enough to form a stable species. The coordinated carboxylate group is necessary in the formation of a ternary complex.

As shown below, Gly-Gly is coordinated with Ni^{II} as a tridentate side chain. The formation of a stable ternary complex, dota-Ni^{II}-Gly-Gly$^-$, leads to a very high binding constant of $\log K = 19.20$. The strong recognition may also be partially attributed to the formation of a H-bond between dota and the dipeptide. Further investigations of hydrolysis of peptide bond will be published in due course.

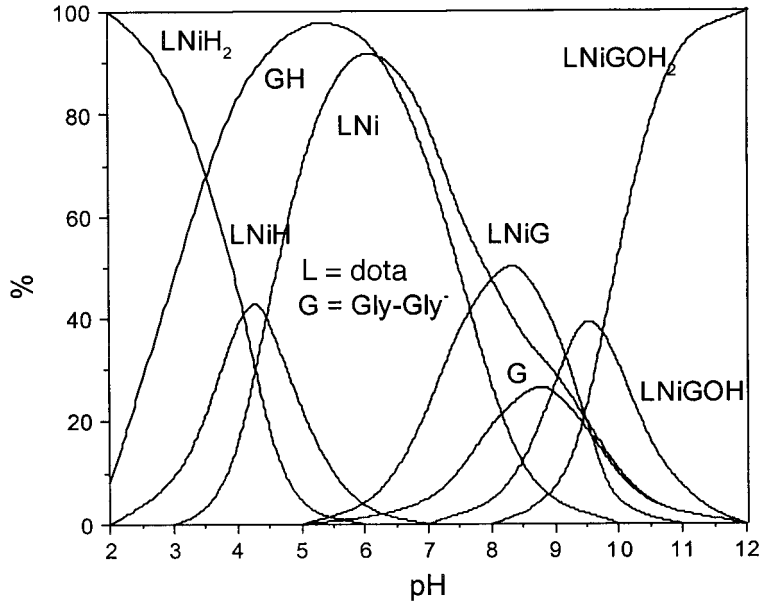
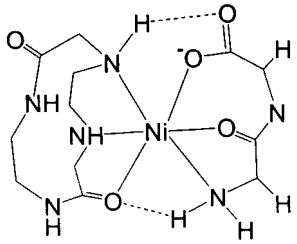


Fig. 4. *Species-distribution diagram for dota + Ni^{II} + Gly-Gly system in a 1:1:1 ratio.* ($\mu = 0.100$M KCl, 25°, [dota] = [Ni] = [Gly-Gly] = 0.001M)

Table 4. *Stability Constants for the dota-Ni^{II}-Gly-Gly System* ($\mu = 0.10$M KCl, 25°, [dota] = [Ni] = [Gly-Gly] = 0.001M, L = dota, G = Gly-Gly

	Stoichiometry			Quotient K	log *K*
dota (L)	Ni	G	H		
1	1	1	0	[LNiG]/[LNi][G]	19.20
1	1	1	−1	[LNiGOH]/[LNiG][OH]	10.07
1	1	1	−2	$[LNiGOH_2]/[LNiGOH][OH_2]$	0.29



Experimental Part

Materials. All of the metal stock solns. for potentiometric studies are reagent-grade chloride salts prepared with doubly distilled water and standardized by 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 water 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 $25.00 \pm 0.05°$ 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. 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* [14]. 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. Experimenta1 runs were carried out by adding increments of standard base to a soln. containing dota · 2 HBr plus other components such as KCl soln. and metal soln. The concentrations of the sample solns. were 1×10^{-3} M for dota · 2 HBr. The titrations of dota + Ni^{II} + Gly-Gly 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 and stability constants from the direct titrations were calculated from the potentiometric data with the program BEST [14]. 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 pH values were minimized. Species distribution diagrams were computed from the measured equilibrium constants with SPE and plotted with SPEPLOT.

Preparation of [Ni^{II}[(dota)$_2$](ClO$_4$)$_2$. To a soln. containing 0.05 mmol of dota · 2 HBr in 15 ml of EtOH were added 0.10 mmol of EtOH and 0.05 mmol of $Ni(ClO_4)_2 \cdot 6\ H_2O$. After stirring for 2 h, purple micro-crystals formed. Chemical analyses for the complex confirmed the composition given. Recrystallization of the dota-Ni^{II} complex from aq. soln. at pH 7.0 yielded purple crystals, for which the crystal structure was determined.

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 (excluding structure factors) for the structure reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*) as deposition No. CCDC-196017. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB21EZ UK (fax: + 44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk).

Table 5. *Crystal Data and Structure Refinement Details for dota-Ni^{II} Complex*

Empirical formula	$C_{16}H_{32}Cl_2N_8NiO_{12}$	
Formula weight	658.11	
Temp.	273(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2(1)/c$	
Unit cell dimensions	$a = 9.7522(8)$ Å	$\alpha = 90^\circ$
	$b = 12.2442(10)$ Å	$\beta = 105.1610(10)^\circ$
	$c = 10.7119(9)$ Å	$\gamma = 90^\circ$
V	1234.57(18) Å^3	
Z	2	
Density (calc.)	1.770 mg/m^3	
Absorption coefficient	1.082 mm^{-1}	
F(000)	684	
θ Range for data collection	2.16 to 28.27°	
Index ranges	$-12 < h < 12$, $-14 < k < 16$, $-13 < l < 13$	
Reflections collected	6778	
Independent reflections	2670 [R(int) = 0.0253]	
Completeness to $\theta = 28.27^\circ$	87.3%	
Absorption correction	None	
Refinement method	Full-matrix least-squares on F^2	
Data/restraints/parameters	2670/0/178	
Goodness-of-fit on F^2	1.043	
Final R indices [$I > 2\sigma(I)$] [a] [b]	$R1 = 0.0574$, $wR2 = 0.1688$	
R indices (all data)	$R1 = 0.0661$, $wR2 = 0.1934$	
Largest diff. peak and hole	2.200 and −0.968 e.Å^{-3}	

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

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