Stabilities of the alkaline earth and divalent transition metal complexes of the tetraazamacrocyclic tetraacetic acid ligands

Eric T. Clarke and Arthur E. Martell*

Department of Chemistry, Texas A&M University, College Station, TX 77843-3255 (USA)

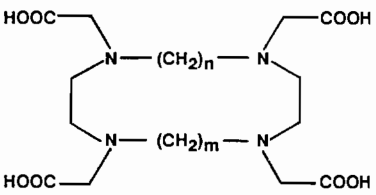
(Received May 21, 1991)

Abstract

The protonation constants of the tetraazamacrocyclic tetraacetates (L^{4-}), 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA), 1,4,7,10-tetraazacyclotridecane-*N,N',N'',N'''*-tetraacetic acid (TRITA) and 1,4,8,11-tetraazacyclotetradecane-*N,N',N''',N''''*-tetraacetic acid (TETA), have been determined by potentiometric titration in KCl electrolyte (0.100 M, 25.0 °C). A pattern of two high protonation constants (log $K^{\mathrm{H}}_1 = 10.82$ to 11.79; $K^{\mathrm{H}}_2 = 9.20$ to 9.69) and two low protonation constants ($K^{\mathrm{H}}_3 = 4.00$ to 4.85; $K^{\mathrm{H}}_4 = 2.57$ to 3.95) was observed for these ligands. The stability constant of the potassium complex formed with DOTA in KCl electrolyte was calculated from the protonation constants of DOTA in 'inert' tetramethylammonium chloride (0.100 M, 25.0 °C) with tetramethylammonium hydroxide. The metal binding constants of the alkaline earth metal complexes with DOTA, TRITA and TETA were determined by direct potentiometric titration in KCl electrolyte (0.100 M, 25.0 °C), while the stabilities of the complexes of the bivalent transition metals Co(II), Ni(II) and Zn(II) with the tetraazamacrocyclic tetraacetates were determined potentiometrically by a batch method which allowed equilibrium measurements over long periods of time. Potentiometric measurement of competition with EDTA was used to find the binding constants of the Cu(II) complexes. The results of the present work are interpreted in terms of variation in structure of the ligands and are compared to stability constant data previously reported.

Introduction

The stabilities of metal complexes of a series of macrocyclic tetraazatetracarboxylate ligands, 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid ([12]AneN$_4$-ac$_4$, DOTA), 1,4,7,10-tetraazacyclotridecane-*N,N',N'',N'''*-tetraacetic acid ([13]-AneN$_4$-ac$_4$, TRITA) and 1,4,8,11-tetraazacyclotetradecane-*N,N',N''',N''''*-tetraacetic acid ([14]AneN$_4$-ac$_4$, TETA), should provide an opportunity to show the influence of variation of the macrocyclic ring size on the values of the complex stability constants.



1 n=2, m=2 1,4,7,10-tetraazacydododecane-tetraacetic acid (DOTA)
2 n=2, m=3 1,4,7,10-tetraazacyclotridecane-tetraacetic acid (TRITA)
3 n=3, m=3 1,4,8,11-tetraazacyclotetradecane-tetraacetic acid (TETA)

However, the literature stands in disarray, in view of the wide variation in constants and the metal binding constants reported by three research groups [1–3] in three different supporting electrolytes (i.e. KNO$_3$, KCl and tetramethylammonium nitrate). The results are not comparable, even when adjustments are made for the effect of the supporting electrolyte [3] (0.100 M, 25.0 °C). Reversals of metal binding constants for the alkaline earth complexes with DOTA, TRITA and TETA reported by Stetter and Frank [2] were seemingly corrected by the work of Delgado and Frausto Da Silva [3]. The report by the former group did not contain details of the experimental methods employed, and the lists of complex species detected in solution seem to be incomplete (i.e. protonated complex species were not reported). The list of metal ions studied was expanded by the latter research group to include the bivalent transition metals Co(II), Ni(II), Zn(II) and Cu(II). Yet even in this work there are reversals of relative magnitudes of metal binding constants

---

*Author to whom correspondence should be addressed.

0020-1693/91/$3.50

(K_{ML}) for the bivalent transition metal ions for the series DOTA, TRITA and TETA. It seemed possible that the reported reversals are not real and that they may involve errors in attaining equilibrium because of slow formation of the metal complexes being measured.

It was the purpose of this research to re-examine the metal complex equilibria in these systems in a single supporting electrolyte with careful attention to the attainment of equilibrium. The new self-consistent data obtained are interpreted in terms of the variation in structures and basicities of the ligands.

Experimental

Synthesis of ligands

1,4,7,10-Tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid ([12]AneN$_4$-ac$_4$, DOTA), 1,4,7,10-tetraazacyclotridecane-*N,N′,N″,N‴*-tetraacetic acid ([13]AneN$_4$-ac$_4$, TRITA) and 1,4,8,11-tetraazacyclotetradecane-*N,N′,N‴,N‴*-tetraacetic acid ([14]-AneN$_4$-ac$_4$, TETA) were synthesized by alkylation of the parent cyclic tetraamine ligands with bromoacetic acid at a pH controlled at 11.2 to 11.3. The alkylation procedure was a modification of that described by Desreux [4]. The parent tetraazamacrocycles were synthesized by the method described by Richman and Atkins [5] and Fabbrizzi *at al.* [6], with the exception of [14]AneN$_4$, which was obtained from Aldrich. All reagents were purified and dried according to the methods of Perrin *et al.* [7].

The macrocyclic compounds were purified by ion-exchange with Dowex-50W, followed by recrystallization from water. The ligands were recovered from the ion-exchange column as ammonium salts. The ammonium cation was subsequently displaced by potassium cation by adjusting the pH of the solution to pH 11.5 with KOH, and then concentrating the solution under vacuum. The ligands were reprotonated by addition of concentrated HCl and were removed by recrystallization from hot water.

Characterization of the ligands

Proton NMR were measured with a Varian XL-200 spectrometer operating at 200 MHz. The solvent in all cases was deuterium oxide and the pD was adjusted with sodium deuteroxide. *para*-Dioxane was used as the internal standard. Shifts are reported in ppm. Elemental analyses of C, N, H, K and Cl were performed by Galbraith Laboratories of Knoxville, TN.

[12]AneN$_4$-ac$_4$ (DOTA)

DOTA crystallized from hot water containing residual KCl. The molecular weight was 530 by potentiometric titration, and the ligand was determined to contain 1.1 mole of KCl and 2.5 mole of water. ^{1}H NMR: 2.962 (s, 8H, acetate protons); 2.5 and 2.3 (m, 16H, α-ring protons). *Anal.* Calc. for $C_{16}N_4O_8H_{28} \cdot 1.1(KCl) \cdot 2.5(H_2O)$: C, 36.22; N, 10.57; H, 6.22; K, 8.09; Cl, 7.26. Found: C, 36.31; N, 10.56; H, 5.22; K, 7.89; Cl, 7.61%.

[13]AneN$_4$-ac$_4$ (TRITA)

TRITA could not be recrystallized from water, but was recovered by lyophilization and was determined to have a molecular weight of 886 by titration and to contain 2.5 mole of HCl, 4.0 mole of KCl and 0.75 mole of ammonium that was not removed quantitatively. ^{1}H NMR: 3.094, 3.024, 2.87 (s, s, m, 8H, acetate protons); 2.724, 2.528, 2.3 (s, s, m, 16H, α-ring protons); 1.6 (q, 2H, β-ring protons). *Anal.* Calc. for $C_{17}N_4O_8H_{30} \cdot 2.5(HCl) \cdot 4.0(KCl) \cdot 4.0(H_2O)$-$0.75(NH_4{}^+)$: C, 23.23; N, 7.51; H, 4.85; K, 17.60; Cl, 25.68. Found: C, 23.29; N, 7.45; H, 4.38; K. 17.34; Cl, 26.66%.

[14]AneN$_4$-ac$_4$ (TETA)

TETA crystallized from water free of salts. The molecular weight was 421 by potentiometric titration (432, theoretical). ^{1}H NMR: 3.463 (s, 8H, acetate protons); 3.1 (m, 16H, α-ring protons); 1.851 (s, 4H, β-ring protons). *Anal.* Calc. for $C_{18}N_4O_8H_{32}$: C, 50.00; N, 12.96; H, 7.41. Found: C, 49.93; N, 12.83; H, 7.43%.

Potentiometric measurements

The methodology employed for equilibrium potentiometric determinations of metal complex stability is described in a handbook by Martell and Motekaitis [8]. The specifications of the potentiometric apparatus require the use of a constant temperature bath (Haake, 25.0 ± 0.2 °C), a 100 ml glass-jacketed titration cell that is fitted with a Schott Blue Stem glass electrode, a reference calomel electrode, a pair of N_2 gas-line fittings and the tip of a 10 ml capacity Metrohm piston buret.

Prior to each potentiometric equilibrium determination, the Corning model 130 pH meter was calibrated in a cell thermostated at 25.0 °C, with standard acid and standard base, at an ionic strength adjusted to 0.100 M with KCl. All pH values were directly expressed as $-\log [H^+]$ or p[H]. The calibration was performed over the p[H] range of 2.2 to 11.3, for which the electrodes gave a reliable Nernstian response. The calibration of the electrodes was checked in each instance by setting the acid response curve in the shallow-sloped region so that the resulting response to the whole p[H] was linear.

Standard KOH solution was prepared with the exclusion of atmospheric CO_2 gas by dilution of an ampoule of carbonate-free 'DILUT-IT' analytical concentrate with boiled doubly-distilled water under a stream of purified N_2 purge gas. Titrant solutions were changed when the extent of the carbonate accumulation in the standard base solution was determined by Gran's method [9] to exceed two percent. Atmospheric CO_2 was further excluded from the titration cell during the course of the titration by passing purified N_2 across the top of the experimental solution in the cell and otherwise sealing the cell contents from the atmosphere. The carbonate content of the N_2 purge gas was minimized by bubbling the gas through a scrubbing container filled with 0.1 M KOH, and the humidity of the gas was controlled by passing the scrubbed N_2 through a container of 0.1 M KNO_3 solution.

Standardization of metal ion solutions

Metal ion solutions were prepared at about 0.02 M from the nitrate and chloride salts of the metals and were standardized by titration against Na_2H_2EDTA in triplicate, as described by Schwarzenbach and Flaschka [10]. The EDTA solution was standardized against zinc(II) acetate as described [10].

Calculation of equilibrium constants

Ligand protonation constants

Protonation constants ($K^{H}_{n} = [H_nL]/[LH_{(n-1)}][H]^n$) were calculated by fitting the potentiometric p[H] data (0.100 M, 25.0 °C) to the BEST [11] program which employs a Newton–Raphson type iterative technique to fit the titration curves and calculate thermodynamic equilibrium constants [8]. The protonation constants of DOTA, TRITA and TETA were determined in KCl and tetramethylammonium chloride (TMACl) supporting electrolytes at concentrations adjusted to provide 0.100 M ionic strength.

Metal binding constants

Stoichiometric experimental solutions of the 1:1 metal to ligand complexes were prepared directly in the titration cell and sufficient KCl was added to bring the solution to 0.100 M ionic strength. All solution systems were initially acidic to assure the complete elimination of CO_2, and the p[H] of the solution was then slowly raised by adding standard KOH.

The solutions containing equimolar amounts of alkaline earth metal ions were titrated directly to determine metal binding constants ($K_{ML} = [ML^{2-}]/[L^{4-}][M^{2+}]$). Relatively rapid equilibria periods were observed for these so that complete potentiometric equilibrium titration curves were obtained within 5 to 6 h. Equilibrium with the Co(II), Ni(II) and Zn(II) metal ions was found to be very slow and required the use of the batch potentiometric equilibrium method described below.

Because the Cu(II) complexes of DOTA, TRITA and TETA are nearly completely formed at the beginning of the titrations, the Cu(II) constants were determined by competition with EDTA (L') according to the following equilibrium:

$$CuL' + H_nL \rightleftharpoons CuL + H_mL' + (n-m)H^+$$

The p[H] of the ternary solution containing equimolar amounts of Cu(II), EDTA and macrocyclic ligand was monitored in the titration cell from acid to alkaline p[H] over periods of 24 to 48 h. Equilibrium at each p[H] point was approached from two directions by titrating the ternary solution in both the forward direction (from low p[H]) and the reverse direction (from high p[H]). The stability constants for the Cu(II) macrocyclic complexes were calculated by computer as averages of the determinations for both directions. The stability constants and protonation constants for the Cu(II)–EDTA complexes were taken from ref. 12.

Batch equilibrium method

The complexes of Co(II), Ni(II) and Zn(II) with the macrocyclic ligands were measured by a batch technique involving separate containers with equivalent amounts of metal ion and ligand but differing in the amount of titrant. Neutral and alkaline p[H] points were excluded from the range of p[H] employed because of the possibility of contamination from atmospheric CO_2 when the containers were opened for measurement. In all cases sufficient standard base was titrated in increments into each solution to place it at various positions of the p[H] profile.

For each batch determination, a stock solution containing 0.1 mmol of metal complex and 5 mmol of KCl was diluted with water in a 50 ml volumetric flask to provide 10^{-3} M metal complex and 0.10 M ionic strength. The p[H] of each aliquot of metal complex was adjusted with standard acid and base to set the desired proton stoichiometry or *a* value of the solution, and the p[H] of each series of batch solutions was monitored.

A reverse titration series was subsequently prepared by adding acid to the series of batch solutions after equilibrium had been attained in the forward series. The time to reach equilibrium varied from 6 to 10 days. Stability constants (K_{ML}) for the bivalent transition metal complexes were determined by a BEST fit of the potentiometric p[H] data for both directions and are expressed as an average.

Results

Protonation constants of tetraazamacrocyclic ligands

Potentiometric p[H] measurements of DOTA, TRITA and TETA were carried out in KCl and tetramethylammonium chloride (TMACl) electrolytes (0.100 M, 25.0 °C) (Figs. 1–3) and the extent of potassium(I) complex formation with these macrocyclic ligands (Table 1) was determined from the differences in the titration curves. The protonation constants of the parent macrocyclic ligands are shown in Table 2 for comparison. The protonation constants calculated as described in 'Experimental' reveal that only DOTA forms a complex with the K^+ cation as indicated by the value of its first protonation constant (11.14 ± 0.01), measured in 0.100 M KCl/KOH, which is lowered relative to the 'true' value of 11.22 ± 0.01 measured in 0.100 M TMACl/TMAOH. On the basis of the differences in $K^H{}_1$, and the supporting electrolyte concentration, the potassium ion binding constant K_{ML} for DOTA was calculated as 1.5 ± 0.1. The remaining three protonation constants of DOTA were not appreciably affected by the choice of electrolyte. For DOTA, the second, third and fourth protonation constants are 9.69 ± 0.02, 4.85 ± 0.02 and 3.95 ± 0.01 in KCl electrolyte, while they are 9.64 ± 0.02, 4.86 ± 0.02 and 3.69 ± 0.01 in TMACl electrolyte.

The pattern of protonation constants suggests that the potassium complex with DOTA forms only when four protons have been dissociated from the ligand (e.g. with the L^{4-} species) as shown by the species distribution curves in Fig. 4. The close internal agreement of the protonation constants for TRITA and TETA measured in KCl and tetramethylammonium chloride (Table 1) rules out any significant formation of a potassium complex with these ligands. The species distribution curves for the protonation of TRITA and TETA are similar to those of DOTA (except for K^+ binding) and are not shown.

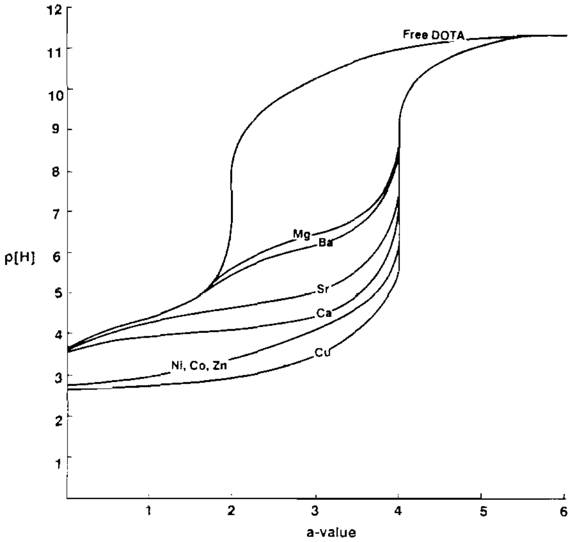


Fig. 1. Equilibrium p[H] profiles of solutions of DOTA and of 1:1 molar ratios of DOTA and the metal ions indicated. $[DOTA]_T = 2.00 \times 10^{-3}$ M ($\mu = 0.100$ M (KCl), 25.0 °C). a = mmol base/mmol ligand.

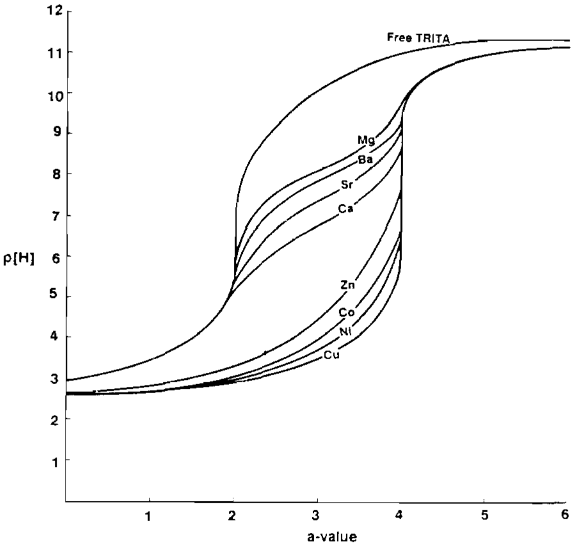


Fig. 2. Equilibrium p[H] profiles of solutions of TRITA and of 1:1 molar ratios of TRITA and the metal ions indicated. $[TRITA]_T = 2.00 \times 10^{-3}$ M ($\mu = 0.100$ M (KCl), 25.0 °C). a = mmol base/mmol ligand.

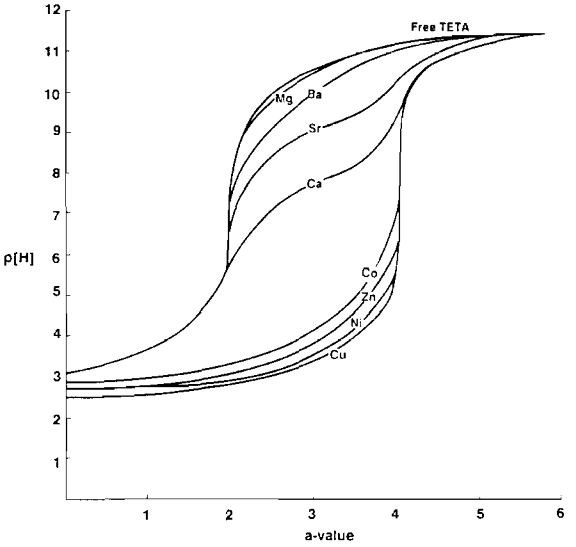


Fig. 3. Equilibrium p[H] profiles of solutions of TETA and of 1:1 molar ratios of TETA and the metal ions indicated. $[TETA]_T = 2.00 \times 10^{-3}$ M ($\mu = 0.100$ M (KCl), 25.0 °C). a = mmol base/mmol ligand.

TABLE 1. Protonation constants of the tetraazatetracarboxylate macrocyclic ligands, DOTA, TRITA and TETA. Determinations were made in KCl and tetramethylammonium chloride (TMACl) or tetramethylammonium nitrate (TMAN) at 0.100 M, 25.0 °C

Equilibrium quotient	Present work 0.100 M KCl 25.0 °C	Present work 0.100 M TMACl 25.0 °C	Ref. 1 1.00 M KNO_3 25.0 °C	Ref. 2 0.100 M KCl 25.0 °C	Ref. 3 0.100 M KNO_3[a] 25.0 °C
DOTA					
$[HL]/[L][H]$	11.14±0.01	11.22±0.01	11.08	11.36	11.22±0.03
$[H_2L]/[HL][H]$	9.69±0.02	9.64±0.02	9.23	9.73	9.68±0.01
$[H_3L]/[H_2L][H]$	4.85±0.02	4.86±0.02	4.24	4.54	4.37±0.01
$[H_4L]/[H_3L][H]$	3.95±0.01	3.68±0.01	4.18	4.41	4.36±0.01
TRITA					
$[HL]/[L][H]$	11.79±0.01	11.81±0.01		11.22	11.35±0.05
$[H_2L]/[HL][H]$	9.20±0.01	9.21±0.01		9.18	9.73±0.01
$[H_3L]/[H_2L][H]$	4.00±0.02	4.03±0.02		4.59	4.16±0.01
$[H_4L]/[H_3L][H]$	2.57±0.02	2.62±0.02		3.28	3.32±0.01
TETA					
$[HL]/[L][H]$	10.82±0.01	10.92±0.01	11.56±0.04	11.07	10.68±0.01
$[H_2L]/[HL][H]$	10.10±0.02	10.09±0.01	10.18±0.02	9.75	10.14±0.01
$[H_3L]/[H_2L][H]$	4.15±0.01	4.08±0.01	4.05±0.02	4.31	4.09±0.01
$[H_4L]/[H_3L][H]$	3.21±0.01	3.19±0.01	3.38±0.04	3.46	3.35±0.01

[a] Log protonation constants for DOTA determined by Delgado and Frausto Da Silva [3] in 0.100 M TMAN, 25.0 °C, have the values of 12.09±0.04, 9.68±0.01, 4.55±0.01 and 4.13±0.01.

TABLE 2. Protonation constants of the parent tetraazamacrocyclic ligands

Equilibrium quotient	Refs. 13, 14, 15 0.2 M $NaClO_4$ 25.0 °C	Ref. 16 0.5 M KNO_3 25.0 °C	Ref. 17 0.5 M KNO_3 25.0 °C
$[12]AneN_4$			
$[HL]/[L][H]$	10.7	10.97	
$[H_2L]/[HL][H]$	9.7	9.87	
$[H_3L]/[H_2L][H]$	1.7	2	
$[H_4L]/[H_3L][H]$	0.9	2	
$[13]AneN_4$			
$[HL]/[L][H]$	11.1	11.19	
$[H_2L]/HL][H]$	10.1	10.12	
$[H_3L]/[H_2L][H]$	1.7	2	
$[H_4L]/[H_3L][H]$	1	2	
$[14]AneN_4$			
$[HL]/[L][H]$	11.50	11.83	11.58±0.01
$[H_2L]/[HL][H]$	10.30	10.76	10.62±0.01
$[H_3L]/[H_2L][H]$	1.6	2	1.61±0.01
$[H_4L]/[H_3L][H]$	0.9	2	2.42±0.01

Stability constants of the alkaline earth metal complexes

The potentiometric equilibrium p[H] profiles of the macrocyclic complexes with the alkaline earth metal ions (Figs. 1–3) are coincident with those of the free ligands in the acid region up to $a=2$. Two protons must be dissociated from the ligands before complexes with the alkaline earth metals can be formed. Stability constants (K_{ML}) for the alkaline earth complexes calculated from these potentiometric p[H] data (see 'Experimental') appear in Table 3. Log K_{ML} for Ca(II)–DOTA is 16.37±0.01 and is about 0.9 log units less than that reported by Delgado and Frausto Da Silva [3] (17.23). This stability constant is higher than that of any other Ca(II) complex previously reported. The trend of the stabilities of alkaline earth complexes of all three macrocyclic ligands is Ca(II) > Sr(II) > Ba(II) > Mg(II). The stability constants vary with ligand in the order

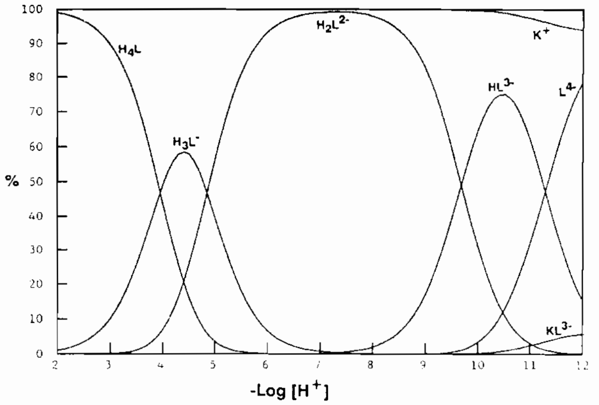


Fig. 4. Species distribution curves for the protonated forms of DOTA and its 1:1 metal complex with K(I). L = DOTA. $[L]_T=[M]_T=2.00\times10^{-3}$ M, $\mu=0.100$ M (KCl), 25.0 °C. Protonation constants and the K(I) binding constant appear in Table 1.

DOTA > TRITA > TETA, for all alkaline earth metal ions.

Stability constants of the Co(II), Ni(II) and Zn(II) complexes

The stability constants determined by the batch potentiometric methods for the metal complexes of Co(II), Ni(II) and Zn(II) with DOTA, TRITA and TETA appear in Table 4. Each stability constant is reported as an average of the batch determinations for two directions, as described in 'Experimental'. The errors associated with each determination reflect the variation of equilibrium p[H] values at given *a* values (not shown).

The protonation constants for the metal complexes were also determined by the batch method and appear in Table 4. The stability constants for the hydroxo–metal complexes (K_{MLOH}) were determined by the direct (continuous) potentiometric method. The magnitudes of the protonation constants range from 2.54 to 5.35, while most of the stability constants for the hydroxy–metal complexes are greater than 10.0. As has been noted for many other types of complexes, the constants are seen to decrease as the stabilities of the metal complexes K_{ML} increase.

Stability constants of the Cu(II) complexes

The stability constants for the Cu(II) complexes with DOTA, TRITA and TETA (Table 4) were determined by competition with EDTA in the titration cell. The good match of the potentiometric p[H] data points (Fig. 5) indicated that all of the protonated and hydroxo metal complex species had

TABLE 3. Stability constants for the 1:1 metal complexes of Mg(II), Ba(II), Sr(II) and Ca(II) with the tetraazatetracarboxylate macrocyclic ligands, DOTA, TRITA and TETA

Cation	Quotient	DOTA $[12]AneN_4\text{-}ac_4$	TRITA $[13]AneN_4\text{-}ac_4$	TETA $[14]AneN_4\text{-}ac_4$
K(I)	[ML]/[L][M]	1.5 ± 0.1		
		(1.64 ± 0.05)[a]		
Mg(II)	[ML]/[L][M]	11.15 ± 0.01	8.18 ± 0.01	3.01 ± 0.01
		(11.92 ± 0.01)	(7.62 ± 0.01)	(1.97 ± 0.01)
		[11.03][b]	[6.36]	[3.02]
	[MHL]/[ML][H]		8.12 ± 0.03	
		(4.09 ± 0.04)	(6.51 ± 0.05)	
Ba(II)	[ML]/[L][M]	11.75 ± 0.01	8.56 ± 0.01	4.37 ± 0.01
		(12.87 ± 0.01)	(8.34 ± 0.01)	(3.85 ± 0.01)
		[11.30]	[7.24]	[4.32]
	[MHL]/[ML][H]		8.13 ± 0.03	
		(5.63 ± 0.04)	(6.65 ± 0.05)	
Sr(II)	[ML]/[L][M]	14.38 ± 0.01	9.64 ± 0.01	5.91 ± 0.01
		(15.22 ± 0.02)	(10.00 ± 0.01)	(5.73 ± 0.01)
		[12.80]	[11.70]	[6.15]
	[MHL]/[ML][H]	4.52 ± 0.04	7.68 ± 0.03	
		(4.67 ± 0.03)	(5.04 ± 0.05)	
Ca(II)	[ML]/[L][M]	16.37 ± 0.01	11.99 ± 0.01	8.53 ± 0.01
		(17.23 ± 0.01)	(12.09 ± 0.01)	(8.32 ± 0.01)
		[15.88]	[8.06]	[9.48]
	[MHL]/[ML][H]	3.60 ± 0.04	8.22 ± 0.02	6.99 ± 0.03
		(3.54 ± 0.03)	(4.71 ± 0.05)	(7.45 ± 0.01)

[a] Parenthetic values were determined by Delgado and Frausto Da Silva [3]: 0.100 M tetramethylammonium nitrate (TMAN), 25.0 °C, for complexes of DOTA; and 0.100 M KNO_3, 25.0 °C, for complexes of TRITA and TETA. [b] Bracketed values were determined by Stetter and Frank [2]: 0.100 M KCl, 20.0 °C, for complexes of DOTA, TRITA and TETA.

TABLE 4. Stability constants for the 1:1 metal complexes of Co(II), Ni(II), Zn(II) and Cu(II) with DOTA, TRITA and TETA

Cation	Quotient	DOTA	TRITA	TETA
Co(II)	[ML]/[L][M]	19.3±0.1	17.50±0.08	16.70±0.01[a]
		(20.17±0.02)[b]	(20.10±0.01)	(16.56±0.01)
		[18.42][c]	[14.98]	[15.00]
	[MHL]/[ML][H]	5.35±0.02	4.83±0.01	4.44±0.01
		(4.00±0.03)	(3.98±0.05)	(4.07±0.01)
	$[MH_2L]/[MHL][H]$	3.8±0.1	2.57±0.01	
		(3.65±0.04)	(2.62±0.05)	(2.82±0.07)
	[ML]/[ML(OH)][H]	10.46±0.01	13.06±0.01	12.41±0.01
Ni(II)	[ML]/[L][M]	20.5±0.2	20.2±0.2	20.0±0.1
		(20.03±0.07)	(20.82±0.01)	(19.91±0.03)
		[17.25]	[15.75]	[15.26]
	[MHL]/[ML][H]	4.59±0.02	4.55±0.07	4.34±0.02
		(3.51±0.03)	(4.17±0.05)	(4.12±0.03)
	$[MH_2L]/[MHL][H]$	3.21±0.08	3.2±0.2	3.07±0.06
		(4.72±0.03)	(2.72±0.05)	(3.31±0.03)
	[ML]/[ML(OH)][H]	10.87±0.01	12.91±0.01	10.88±0.01
Zn(II)	[ML]/[L][M]	18.7±0.1	18.04±0.05	17.6±0.1
		(21.05±0.01)	(19.42±0.02)	(16.27±0.01
		[18.90]	[14.42]	[15.81]
	[MHL]/[ML][H]	5.33±0.01	4.57±0.02	4.28±0.01
		(4.19±0.03)	(4.07±0.05)	(4.25±0.03)
	$[MH_2L]/[MHL][H]$	3.96±0.02		
		(3.54±0.03)	(2.60±0.05)	
	[ML]/[ML(OH)][H]	10.62±0.01	13.91±0.01	9.95±0.01
Cu(II)	[ML]/[L][M]	22.72±0.04	22.49±0.03	21.87±0.06
		(22.21±0.01)	(21.53±0.04)	(21.60±0.03)
		[19.06]	[17.29]	[18.60]
	[MHL]/[ML][H]	4.45±0.03	4.27±0.02	3.91±0.01
		(4.30±0.03)	(3.85±0.05)	(3.68±0.03)
Cu(II)	$[MH_2L]/[MHL][H]$	3.92±0.03		2.50±0.01
		(3.61±0.03)	(2.36±0.05)	(2.90±0.03)
	[ML]/[ML(OH)][H]	10.54±0.01	10.33±0.01	11.71±0.01

[a] Determined by direct continuous potentiometric titration. [b] Parenthetic values reported by Delgado and Frausto Da Silva [3]: 0.100 M tetramethylammonium nitrate (TMAN), 25.0 °C, for complexes of DOTA; 0.100 M KNO_3, 25.0 °C, for complexes of TRITA and TETA. [c] Bracketed values reported by Stetter and Frank [2]: 0.100 M KCl, 20.0 °C, for complexes of DOTA, TRITA and TETA. [d] Determined by competition with EDTA (L′). Log protonation constants for EDTA [9], $\mu = 0.100$ M, 25.0 °C; 10.17, 6.11, 2.68, 2.0. Stability constants [9] for Cu(II)–EDTA: log $K_{ML'} = 18.78$, log $K_{MHL'} = 3.1$, log $K_{MH_2L'} = 2.0$.

been accounted for. The log value of the protonation constants for the Cu(II) macrocyclic complexes of DOTA, TRITA and TETA were determined, respectively, to be log K^H_{MHL} = log $[MHL]/[ML][H] = 4.45, 4.27, 3.91$; log $K^H_{MH_2L}$ = log $[MH_2L]/[MHL][H] = 3.92, \sim 2, 2.50$. The log protonation constants for the hydroxo–Cu(II) macrocyclic complexes ($K^H_{ML} = [ML]/[ML(OH)][H]$) are 10 or higher. The values given do not show discernable trends based on stability constants, ligand or metal ion.

Species distribution curves for the ternary Cu(II)–DOTA–EDTA complex system (Fig. 6) show that Cu(II)–EDTA is formed to the extent of 67% of the total Cu(II) present at p[H] 4.5 and decreases at higher p[H] while Cu(II)–DOTA is formed in increasing proportions beginning at p[H] 3.5 and becomes the only complex present above p[H] 10. The Cu(II) ion crosses over between EDTA and DOTA at p[H] 6.7 at which both complexes account for 50% of the metal ion species. The cross-over points for EDTA with Cu(II)–TRITA and Cu(II)–TETA (not shown) are p[H] 7.2 and 7.8, respectively.

In the present work, there are clear trends in stability constants for the bivalent metal complexes with the tetraazamacrocyclic ligands. The order of stability for each metal complex with Cu(II), Co(II), Ni(II) and Zn(II) is DOTA>TRITA>TETA. Reversals of stability constants for these metal complexes do not appear in the present work. The variation

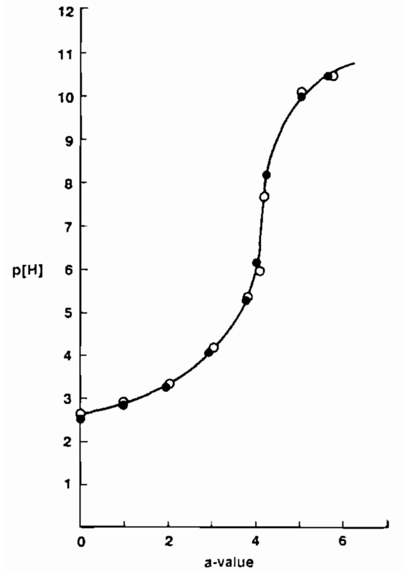


Fig. 5. Equilibrium p[H] profile of the ternary solution containing equivalent amounts of Cu(II), DOTA and EDTA. Equilibrium was approached from the forward direction (●) and the reverse direction (○). $[Cu]_T = [DOTA]_T = [EDTA]_T = 2.00 \times 10^{-3}$ M ($\mu = 0.100$ M (KCl), 25.0 °). a = mmol base/mmol ligand.

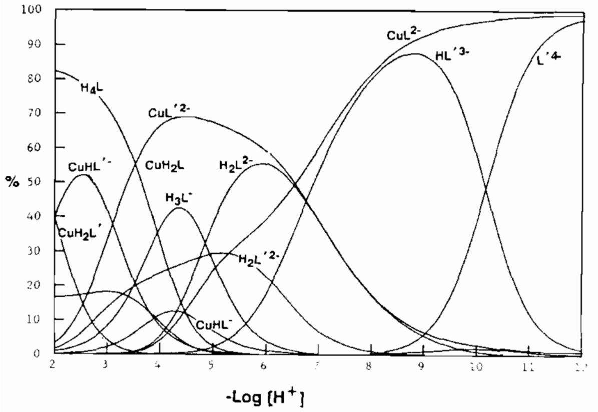


Fig. 6. Species distribution curves for a solution containing equimolar Cu(II), DOTA (L) and EDTA (L'); $[Cu]_T = [L]_T = [L']_T = 2.00 \times 10^{-3}$ M, ($\mu = 0.100$ M (KCl), 25.0 °C). Protonation constants and stability constants appear in Tables 1 and 4.

of stabilities for each ligand with metal ion follows the same order as that generally observed for the first transition series, Co(II) < Ni(II) < Cu(II) > Zn(II), with the relative values for Co(II) and Zn(II) somewhat variable.

Discussion

Ligand protonation constants

For DOTA, TRITA and TETA the observed pattern of two high (K^H_1 and K^H_2) and two low (K^H_3 and K^H_4) protonation constants reflects the pattern of protonation of the parent tetraazamacrocyclic ligands (Table 2; refs. 13–17). For the parent ligands, the first two protonation constants are high and indicate that the two charges reside on two of the ring nitrogen atoms. However, coulombic repulsions preclude further protonation of the parent ring as indicated by the low and relatively unreliable values for the third and fourth protonation constants. Therefore, the existence of the third and fourth protonation constants in the measurable range (log $K^H_n = 2.5$–4.8) for acetate derivatives of these tetraaza ligands suggests that the third and fourth protonations may be due in part to carboxylate protonation, as well as to the important coulombic contribution of the more negative charges of the ligand molecule. The third and fourth protonations, therefore, may be considered as involving a number of microspecies resulting from both nitrogen and oxygen protonation.

In the tri- and tetra-protonated acid forms of DOTA, TRITA and TETA, the protonated carboxylate groups probably enter into hydrogen-bonding with the unprotonated aza nitrogens. Additional protons probably bind to the remaining carboxylate groups, for which there would be only weak affinity. Although the fifth and sixth stepwise protonation constants (log K^H_5 and log K^H_6) have been reported [3] for DOTA and TETA, these protonation constants have values near or below 2, in the range of less accurate potentiometric measurements [8]. Similar protonation sequences have been interpreted by Desreux *et al.* [1] and Ascenso *et al.* [18] on the basis of coulombic arguments, supported by evidence of proton NMR shifts for the methylene protons as a function of pD. The exact solution conformations of DOTA, TRITA and TETA cannot be discerned because the shifts do not change in a predictable manner as pD is varied, as would be expected if several microspecies are involved. Effects of hydrogen-bonding between the protonated nitrogens and the carboxylate oxygens are unclear [1, 18], and structures for the metal-free forms of DOTA, TRITA and TETA are not yet available.

The agreement among the protonation constants of DOTA is reasonably good for those that were measured by Delgado and Frausto Da Silva [3] in KNO_3 (11.22 ± 0.03, 9.68 ± 0.01, 4.37 ± 0.01, 4.36 ± 0.01) and also in tetramethylammonium nitrate (12.09 ± 0.04, 9.68 ± 0.01, 4.55 ± 0.01, 4.13 ± 0.01), but Delgado and Frausto Da Silva did not measure the protonation constants of TRITA and TETA in tetramethylammonium nitrate to determine possible formation of a potassium complex with these additional ligands. Also, Stetter and Frank [2] did not consider the possibility of potassium ion complex formation with DOTA in 0.100 M KCl. Nevertheless, the agreement between the results of the two different research groups for the determination of the protonation constants of DOTA, TRITA and TETA in KNO_3 and KCl electrolytes is reasonably good.

A third set of protonation constants for DOTA and TETA was reported by Desreux *et al.* [1] who used 1.00 M KNO_3 electrolyte. Good agreement was observed with the results of Delgado and Frausto Da Silva and Desreux *et al.* for values of the protonation constants of TETA, but their values for DOTA in the two different concentrations of KNO_3 differ markedly, especially with respect to $K^H{}_1$, because of K^+ binding by L^{4-}. Delgado and Frausto Da Silva pointed out that the agreement between their work in 0.100 M KNO_3 and that of Desreux *et al.* in 1.00 M KNO_3 was improved after recalculation for a medium of 0.100 M ionic strength.

Metal binding constants

Table 4 contains the literature determinations for the stability constants of the metal complexes of Cu(II), Co(II), Zn(II) and Ni(II) with the tetraazamacrocyclic tetraacetates. The stability constants reported by Delgado and Frausto Da Silva [3] are in most cases higher than those reported by Stetter and Frank [2] whereas the stability constants reported in this present work usually have intermediate values. The determinations reported by Delgado and Frausto Da Silva [3] are based on data that were taken over equilibration periods that did not usually last longer than 3 min. However, it is not clear why many of the stability constants reported by the Delgado group are so high. Also, Delgado and Frausto Da Silva reported a reversal of the stability constants for the Ni(II)–TRITA complex (20.82) in relation to the stability of the Ni(II)–DOTA complex (20.03). This reversal may have come about as a result of the short equilibration periods that were used. A reversal of the log stability constant for the Zn(II)–TRITA complex (14.42) relative to the log stability constant of the Zn(II)–TETA complex (15.81) was reported by Stetter and Frank [2], but the time allowed for reaching equilibrium was not mentioned in their paper.

Arrangement of donor groups in metal complexes

The potentiometric determinations for DOTA, TRITA and TETA show a break at $a = 4$ for all 1:1 transition metal complexes (Figs. 1–3) indicating the availability of all eight donor groups of these ligands for metal ion coordination. Of these eight donors, probably only six donor groups participate in octahedral complex formation, and it is suggested that these consist of two carboxylate oxygen atoms symmetrically spaced and four ring nitrogen atoms. It is impossible to predict from thermodynamic data the orientation of the carboxylate groups in solution but it seems logical, from symmetry and energy considerations, that two coordinated carboxylate groups be derived from acetate substituents in *trans* positions, and that the remaining acetates extend outward away from the metal centers to maximize solvation.

Support for this prediction of solution structure comes from the X-ray crystal structures [19] of the isomorphous $NiH_2(DOTA)$ and $CuH_2(DOTA)$ complexes for which four nitrogen atoms of the macrocycle and two *trans* carboxylate groups are bound to the central metal cation resulting in a distorted *cis*-octahedral geometry. Two additional pendant acetate groups are protonated and uncoordinated. The two coordinated nitrogen atoms bound to the coordinated acetates are *trans* to each other. The axial positions of the metal complex are occupied by the remaining two nitrogen atoms which are attached to the uncoordinated carboxylate groups. No other crystal structures of mononuclear complexes of tetraazamacrocyclic tetraacetate ligand complexes of bivalent metal ions have been described.

Trends in stability constants

Trends in the stabilities of the metal complexes of the alkaline earth and bivalent transition metal ions with the tetraazamacrocyclic tetraacetates may be interpreted in terms of the chelate rings formed with the macrocyclic ions within the donor nitrogens. In Fig. 7, the variation of log K_{ML} is shown with the size of the macrocyclic ring or the size of the

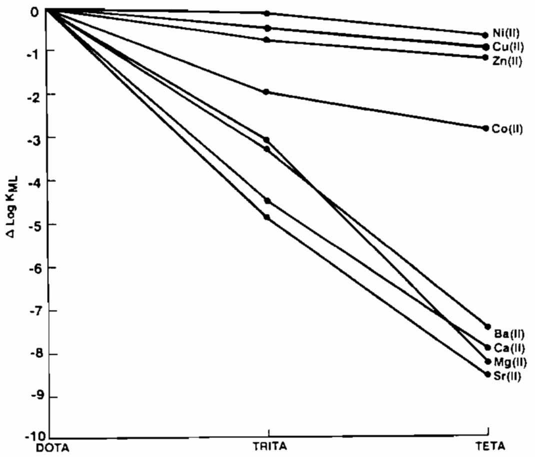


Fig. 7. Variation of log stability constants (log K_{ML}) with chelate ring size in the macrocyclic series, DOTA, TRITA and TETA relative to the DOTA complexes. Numbers of six-membered chelate rings are DOTA, 0; TRITA, 1; TETA, 2. All remaining chelate rings are five-membered.

chelate ring in the series of three ligands [12]AneN$_4$-ac$_4$ to [14]AneN$_4$-ac$_4$. The changes in log stability constants shown are relative to those for the metal complexes of [12]AneN$_4$-ac$_4$, (DOTA), for each metal ion. Relatively small differences are observed for the metal binding constants of the small metal ions such as Ni(II), Zn(II), Co(II) and Cu(II). The large metal cations such as Ba(II), Ca(II) and Sr(II) show large differences in binding constants within the series of tetraaza-tetracarboxylate ligands. The behavior of the Mg(II) ion as a large size metal ion is an anomaly which probably reflects different, and probably weaker, coordination of a very small metal ion with a large macrocyclic ring.

These data were interpreted in a manner similar to that of Thom *et al.* [20] who applied molecular mechanics to the study of size–fit interactions of the parent [12]AneN$_4$ and [14]AneN$_4$ tetraazamacrocyclic ligands with a variety of metal cations. Thom *et al.* [20] reported that the size of the macrocyclic ring appears to provide a selectivity pattern where the [14]AneN$_4$ ligand ring favors smaller metal ions with a maximum ionic radius range of about 2.0 to 2.1 Å, whereas the smaller [12]AneN$_4$ ligand ring favors larger metal size ions over a broad range of ionic radii [21]. These patterns were explained by the conformations of the macrocyclic complexes which were assumed to minimize strain energy [22] and also by the size of the metal chelate rings formed [20, 23]. An increase in the size from five- to six-membered chelate rings provides a change in the bond angle of the M–N bond length such that the smaller size metal ions are favored by the smaller 'bite' angles of the six-membered chelate rings.

The pattern of selectivity of metal complexes with tetraazamacrocyclic tetraacetates is interpreted on the basis of the principle that six-membered chelate rings impart lower stability to complexes formed with larger metal ions. The octahedral complexes of TETA contain two six-membered chelate rings and four five-membered chelate rings and, consequently, have lower stability constants than those of either DOTA or TRITA, which have six five-membered chelate rings and five five-membered chelate rings, respectively. These results are in conformity with the principles pointed out by Hancock and Martell [24] that larger metal ions are more effectively coordinated by smaller chelate rings.

These principles of size–fit interactions may also explain why the K$^+$ ion forms a complex with DOTA but not with TRITA and TETA. The K–DOTA complex is apparently favored by an interaction of the K$^+$ ion with DOTA (in the L^{4-} form) since DOTA can better accommodate large size ions than TRITA or TETA.

Acknowledgements

E. T. Clarke expresses thanks and appreciation to the Cooley's Anemia Foundation for a Postdoctoral Fellowship. This work was also supported by US Public Health Service, National Heart, Lung and Blood Institute, Grant No. HL-42780.

References

1 J. F. Desreux, E. Merciny and M. R. Loncin, *Inorg. Chem., 20* (1981) 987.
2 H. Stetter and W. Frank, *Angew. Chem., Int. Ed. Engl., 15* (1976) 686.
3 R. Delgado and J. J. R. Frausto Da Silva, *Talanta, 29* (1982) 815.
4 J. R. Desreux, *Inorg. Chem., 19* (1980) 1319.
5 J. R. Richman and T. J. Atkins, *J. Am. Chem. Soc., 96* (1974) 2268.
6 L. Fabbrizzi, *J. Chem. Soc., Dalton Trans.,* (1979) 1857.
7 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals,* Pergamon, New York, 1966.
8 A. E. Martell and R. J. Motekaitis, *Determination and Use of Stability Constants,* VCH, New York, 1989.
9 F. J. Rossotti and H. Rossotti, *J. Chem. Educ., 42* (1965) 375.
10 G. Schwarzenbach and M. Flaschka, *Complexometric Titrations,* Methuen, London, 1969.
11 R. J. Motekaitis and A. E. Martell, *Can. J. Chem., 50* (1982) 2403.
12 R. M. Smith and A. E. Martell, *Critical Stability Constants,* Vol. 6, Plenum, New York, 1989.
13 M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.,* (1976) 2403.
14 M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.,* (1977) 2269.
15 M. Kodama and E. Kimura, *J. Chem. Soc., Dalton Trans.,* (1976) 1720.
16 A. P. Leugger, L. Hertli and T. A. Kaden, *Helv. Chim. Acta, 61* (1978) 2296.
17 M. Micheloni, A. Sabatini and P. Paoletti, *J. Chem. Soc., Perkins Trans. II,* (1985) 828.
18 J. R. Ascenso, R. Delgado and J. J. R. Frausto Da Silva, *J. Chem. Soc., Perkins Trans. II,* (1978) 781.
19 A. Riesen, M. Zehnder and R. A. Kaden, *Helv. Chim. Acta, 69* (1986) 2067.
20 V. J. Thom, C. C. Fox, J. A. Boeyens and R. D. Hancock, *J. Am. Chem. Soc., 106* (1984) 5947.
21 V. J. Thom, G. D. Hosken and R. D. Hancock, *Inorg. Chem., 24* (1985) 3378.
22 Y. Hung, L. Y. Martin, S. C. Jackels, A. M. Tait and P. H. Busch, *J. Am. Chem. Soc., 99* (1977) 4029.
23 R. D. Hancock, R. Bhaven, C. A. Wagner and G. D. S. Hosken, *Afr. J. Chem., 39* (1986) 2238.
24 R. D. Hancock and A. E. Martell, *Comments Inorg. Chem., 6* (1988) 237.