Thermodynamic Stability and Kinetic Inertness of MS-325, a New Blood Pool Agent for Magnetic Resonance Imaging

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Received October 10, 2000

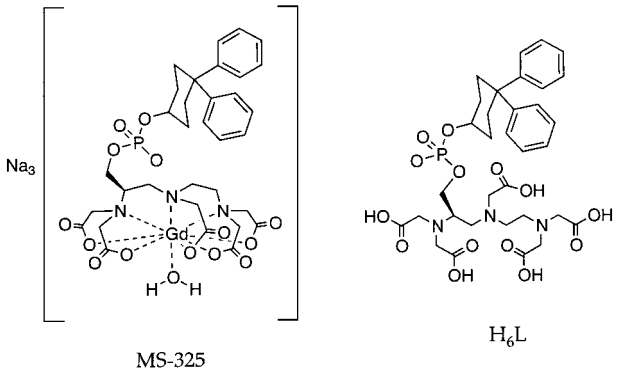
Stability constants were measured for complexes formed between a modified DTPA ligand and the metal ions Gd(III), Eu(III), Fe(III), Ca(II), Cu(II), and Zn(II) at 25 °C in 0.1 M $NaClO_4$. The gadolinium complex of this ligand is MS-325, a novel blood pool contrast agent for magnetic resonance imaging currently undergoing clinical trials. Stability constants were determined by 4 different methods: direct pH titration, pH titration with competition by EDTA, competition with DTPA using an HPLC-MS detection system, and competition with Eu(III) by monitoring equilibrium by luminsecence spectroscopy. The 1:1 stability constants, log β_{101}, are the following: Gd, 22.06 (23.2 in 0.1 M Me_4NCl); Eu, 22.21; Fe, 26.66; Ca, 10.45; Cu, 21.3; Zn, 17.82. The exchange kinetics of the Gd complex, MS-325, with the radioactive tracer ^{152,154}Eu were studied at 25 °C in 0.1 M $NaClO_4$. The exchange reaction has acid-dependent and acid-independent terms. The rate expression is given by the following: $R = k_a[GdL][H]^2 + k_b[GdL][Gd][H] + k_c[GdL][Gd]$. The rate constants were determined to be the following: $k_a = 1.84 \times 10^6\ M^{-2}min^{-1}$, $k_b = 2.87 \times 10^3\ M^{-2}min^{-1}$, $k_c = 3.72 \times 10^{-3}\ M^{-1}min^{-1}$. MS-325 is 2−3 times more stable than GdDTPA at pH 7.4 and is 10−100 times more kinetically inert.

Introduction

It has been over 10 years since $[Gd(DTPA)(H_2O)]^{2-}$ has been approved for use as a contrast agent for magnetic resonance imaging (MRI). The field of aqueous lanthanide coordination chemistry has expanded and matured considerably in that time, including research spurred by the need to understand the physicochemical and biological properties of gadolinium contrast agents. The wealth of information obtained from these studies has enabled the rational design of new contrast agents.[1,2]

MS-325 is an example of a rationally designed contrast agent (Chart 1).[3,4] It possesses a lipophilic diphenylcyclohexyl group which provides a strong noncovalent binding interaction with the blood plasma protein human serum albumin (HSA). Binding reversibly to HSA serves three purposes. First, it targets the complex to the blood pool allowing selective enhancement of arteries and veins. Second, protein binding slows down the molecular tumbling time of the complex, providing a 5−10-fold increase in relaxation enhancement as compared to $[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist) in plasma.[3] Finally, albumin binding increases the half-life of the drug in vivo, which allows the radiologist time to image multiple body regions and to employ pulse sequences which give high-resolution images.

Chart 1



MS-325 H_6L

It is critical that the gadolinium complex be excreted intact for both safety and efficacy reasons. The dissociated Gd(III) ion and the unchelated ligand are both generally much more toxic than the complex. The Gd(III) ion comprises the magnetic core of the drug; loss of Gd(III) eliminates the signal generation function of the drug.

MS-325 was designed using the $[Gd(DTPA)(H_2O)]^{2-}$ (Magnevist) core which has an excellent human safety record.[5,6] It was expected that substitution on the ethylene backbone would not adversely affect the thermodynamic stability nor the kinetic inertness of the complex. Indeed, there was reason to believe

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10.1021/ic001117r CCC: $20.00
Published on Web 03/24/2001

that a substituted DTPA may form an even more stable and more inert complex with Gd(III).[7] To define quantitatively the change in stability/inertness upon adding a phosphodiester group containing a bulky lipophilic substituent, an investigation of the stability and kinetic inertness of MS-325 was undertaken.

This paper reports measurements of the stability constants and kinetic parameters of MS-325. The stability constants of the complexes formed by the ligand and the metal ions Gd(III), Fe(III), Zn(II), Cu(II), and Ca(II) have been determined by several methods. These involved pH titration (Ca(II) and Zn(II)), potentiometric titration using EDTA as a competitor ligand (Gd(III), Cu(II)), luminescence spectroscopy of Eu(III) as a competitor metal ion (Gd(III)), and an HPLC-MS method with DTPA as a competitor ligand (Fe(III)). Although the processes in the human body may not be at equilibrium, these data allow the prediction of which endogenous metal ions, if any, could displace Gd(III) in vivo, based upon thermodynamic equilibrium parameters. As the rate of transmetalation could prove more critical to the integrity of a drug than its stability constant, the rate of dissociation of Gd(III) from MS-325 was measured over the pH range 4−6 using ^{152,154}Eu(III) as a radiotracer and the rate law was determined.

Experimental Section

The ligand, H_6L (Chart 1), was synthesized as the *R* isomer by Mallinckrodt Medical Inc. and is a chemically stable and moderately hygroscopic white powder. The composition of the material used for the experiments was determined by a combination of elemental analysis and Karl Fischer titration. Anal. Calc (found) for $C_{33}H_{44}N_3O_{14}P\cdot H_2O$: C, 52.45 (52.04); H, 6.13 (6.11); N, 5.56 (5.50); P, 4.09 (4.06); H_2O, 2.38 (2.35). To determine if the solid had absorbed additional moisture since the time of elemental analysis, a Karl Fischer titration was performed on the solid immediately prior to its use in this study. The new analysis gave a water content of 4.13% which would give $C_{33}H_{44}N_3O_{14}P\cdot 1.75H_2O$, with an effective molecular weight of 769.3. Metal oxides CaO (99.95%), ZnO (99.999%), and Gd_2O_3 (99.99%) were obtained from Alfa Aesar. $FeCl_3\cdot 6H_2O$, 97% and $CuCl_2$, 99+% (both ACS reagent grade), were obtained from Aldrich.

Titration pH measurements of the ligand in the absence and presence of Gd(III), Fe(III), Cu(II), Ca(II), and Zn(II) were performed with a Fisher Accumet 25 pH meter equipped with an Orion Ross combination semi-micro electrode. The electrode was calibrated before each titration by titrating a known amount of standardized $HClO_{4(aq)}$ or $HCl_{(aq)}$ with standardized NaOH or Me_4NOH solution at an ionic strength of 0.1 M using $NaClO_4$ or Me_4NCl as the inert electrolyte. A plot of mV (measured) versus pH (calculated) gave a working slope and intercept so that pH could be read as $-\log[H^+]$ directly. In this report, pH refers to the hydrogen ion concentration and not activity. A Metrohm automatic buret (Dosimat 665) was used for the NaOH additions, and the buret and pH meter were interfaced to a PC such that each titration was automated using the program TITRATE.[8] The temperature of each solution, maintained in a covered, water-jacketed vessel, was kept constant at 25.0 ± 0.1 °C by a Fisher Isotemp 901 circulating bath. The ionic strength was kept constant at 0.10 M $NaClO_4$ (or 0.10 M Me_4NCl). Nitrogen, after passage through 30% NaOH, was bubbled through the solutions to exclude carbon dioxide.

Distilled deionized water (Nanopure, Barnstead) was used for all solutions. Solutions of the ligand were prepared by dissolving a weighed quantity into a known volume of 0.1 M $NaClO_4$. The concentration was calculated based on the effective molecular weight of the complex and was confirmed by titration. There are two inflections in the ligand titration curve and two equivalents of hydroxide were required to span these two inflections. Perchlorate stock solutions of Gd(III), Ca(II), and Zn(II) were prepared by dissolving a known amount of the oxide in a slight excess of perchloric acid and diluting to a known volume. Because hydrolysis of these metal ions occurs at pH > 5, the excess acid concentration was determined directly by titration with standard NaOH and analysis by Gran's method.[9] Ferric and cupric chloride solutions were prepared by dissolving the hydrated chloride salts. The metal ion concentration (Fe(III), Cu(II)) was determined by loading an aliquot of the metal ion solution onto a cation exchange column (H^+ form, Amberlite) and titrating the liberated acid. Sodium hydroxide solutions (0.1 M) were prepared from dilution of 50% NaOH with freshly boiled, distilled deionized water that had been saturated with argon. The base solutions were standardized against potassium hydrogen phthalate. The amount of carbonate present in the NaOH solutions was estimated from Gran plots[9] and was always less than 1%. Acid solutions were standardized against standard NaOH.

The ligand solutions (1−2 mM) were titrated with NaOH over a pH range from 2 to 11 collecting about 110 data points per titration. The titration data was fit to a model of a ligand with five ionizable groups using the program BEST.[10] The value of pK_w was fixed at 13.78 for all analyses.[11] Equimolar metal/ligand solutions were titrated (110 data points per titration) over the pH range 2−11 with NaOH for Ca(II) and Zn(II), and the stability constants were determined by analysis of the titration curve with BEST.[10] The Ca(II) data was fit to a model containing two metal−ligand species: CaL and CaHL. The Zn(II) data was modeled with three metal−ligand species: ZnL, ZnHL, and ZnH_2L. For Gd(III), Fe(III), and Cu(II), the fraction of free metal ion was insignificant at pH 2, so the stability constant could not be determined directly by titration. However, deprotonation constants of the metal complexes were determined from direct titration. For Gd(III), only one species was present over the entire pH range: GdL. For Fe(III), there were four species present: FeH_2L, FeHL, FeL, and FeL(OH). For the copper system four species were observed: CuH_3L, CuH_2L, CuHL, and CuL.

To determine the formation constant for GdL and CuL, competition titrations with EDTA were performed. For Gd(III), a ratio of 1 Gd(III)/1 L/2 EDTA was employed. For Cu(II), the ratio was 1 Cu(II)/1 L/1 EDTA. Under these conditions the metal ion is partitioned between L and EDTA, and the rate of transmetalation is fast enough between pH 2−4 for the method to be viable. The pH reading stabilized within minutes; however, care was taken to ensure that there was no slow pH drift due to slow transmetalation kinetics. The data were fit by the program BEST using the literature values for MEDTA stability constants.[11] Binuclear complexes of the form $[Cu_2L]$ were excluded from the model since the ligand(s) was always in excess of the total Cu(II).

Transmetalation in the Fe(III)−L system was too slow for a ligand competition study to be monitored by pH change. In this instance a chromatographic method was employed. Six solutions were prepared containing 1 equiv L/0.9 equiv Fe(III)/0.7−1.3 equiv DTPA. The pH varied over the range 3.4−4.4. After equilibration for one month, aliquots of each solution were injected onto a weak anion exchange column on an LC-MS and eluted with a pH 6.8 NH_4OAc buffer. Species were detected by a mass spectrometer operating in single negative ion detection mode. Under these conditions, the FeDTPA complex elutes at 3.33 min and the FeL complex elutes at 4.46 min. The ion response of each ion was calibrated by measurement of standard solutions of FeL and FeDTPA. Under these conditions it was possible to directly monitor the distribution of Fe(III). Since the pH of each solution was measured, the amount of [FeHDTPA] and [FeDTPA] and [FeHL] and [FeL] could be calculated from the protonation constants of these metal complexes. Knowledge of the ligand protonation constants allowed determination of the concentration of deprotonated DTPA and L at each pH which were used with the appropriate mass balance equation to determine the FeL formation constant.

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The stability constant for GdL was also determined by competition with Eu(III), using the luminescence intensity of the Eu(III) in the method of Horrocks and co-workers.[12−14] The formation constant of EuL was determined by a ligand−ligand competition method using EDTA as the competitor ligand.[14] The $^5D_0 \rightarrow {}^7F_0$ excitation spectra of Eu(III) in the EuEDTA and EuL complexes were measured. For the determination of the stability constant, 10 solutions were prepared containing equimolar quantities of Eu(III) and L (at a concentration of ca. 10^{-5} M). To these solutions, varying quantities of EDTA were added and the spectrum measured by exciting selectively the Eu(III) $^7F_0 \rightarrow {}^5D_0$ transition in the 578−581 nm region and monitoring the "hypersensitive" emission $^5D_0 \rightarrow {}^7F_0$ at 614 nm. The excitation of Eu(III) was achieved using a Nd:YAG laser coupled with a dye laser pump. The spectra, corrected to constant laser power, were deconvoluted (Gaussian-Lorentian) and processed using the program SQUAD.[15] The experiment was repeated twice using a formation constant for EuEDTA of $\log K_{ML} = 17.29$.[11]

$^5D_0 \rightarrow {}^7F_0$ excitation spectra were taken of solutions containing equimolar amounts of Eu(III) and L (at concentrations of about 10^{-4} M), with and without Gd(III). The concentration of Gd(III) was adjusted to give an intensity of the Eu(III) luminescence equal to half of the intensity obtained in absence of Gd(III). The luminescence intensity was taken at the maximum (100 pulses/point, 40 ns/pulse, laser power 20 mJ) and corrected to constant laser power. All the solutions were buffered at pH 4.00 with acetate buffer. The ionic strength was maintained at 0.1 M using $NaClO_4$. These experiments were repeated three times, and for each experiment the excitation intensity was taken as the average of duplicate measurements.

The procedure for determining the rate of dissociation was the same as described previously.[16] Solutions containing $Gd(ClO_4)_3$ and GdL (0.5−2 mM) in acetate buffer (20 mM) were prepared at an ionic strength of 0.1 M $NaClO_4$. Solutions were prepared at different [H] and different [Gd]/[GdL] ratios. To a given solution, 100 μL of $^{152,154}Eu(ClO_4)_3$ (Oak Ridge National Laboratories, 2500 cpm/μL) was added. At fixed times during the first two reaction half-lives a 1.00 mL aliquot was withdrawn and passed through a column of cation-exchange resin (Dowex 50W X4, 50−100 mesh) which had been equilibrated with acetate buffer of the same pH as the reaction mixture. The column was rinsed with 3.00 mL of buffer, and after a period of at least 10 reaction half-lives, two 1.00 mL aliquots were removed. One was passed through the resin to give an infinite time point, and the second was diluted with 3 mL buffer to give the total activity.

Results

Stability Constants. The M/L = 1:1 titration curves are shown in Figure 1 and the corresponding stability constants are listed in Tables 1 and 2. All of the metal ion complexes show titration curves depressed relative to the titration curve for the ligand, reflecting complex formation. The deviation between the ligand titration curve and the metal + ligand curves indicate that the weakest complex is formed between Ca(II) and L, while Fe(III), Cu(II), Zn(II), and Ca(II) form protonated complexes between pH 3−6. The depression of the Fe(III) titration curve at high pH is indicative of formation of a ternary hydroxo complex.

Titration data from Figure 1 were used to determine metal complex protonation constants of Fe(III) and Cu(II). The stability constant β_1^H (eq 1) can be determined directly whereas

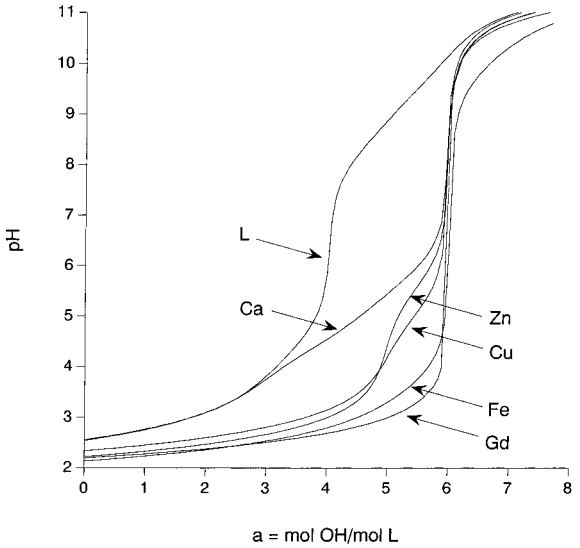

Figure 1. Observed pH versus *a* (mol OH^-/mol L) at 1 M/1 L ratios, temp = 25 °C, μ = 0.1 M $NaClO_4$, [M] = [L] ≈ 1.5 mM.

Table 1. Protonation Constants of L and DTPA[a]

protonation constant	L[b] 0.1 M $NaClO_4$	DTPA[b] 0.1 M $NaClO_4$	L[b] 0.1 M Me_4NCl	DTPA 0.1 M Me_4NCl
[HL]/[H][L]	9.56 (0.09)	10.00 (0.06)	11.15 (0.12)	10.71
$[H_2L]/[H][HL]$	8.31 (0.09)	8.63 (0.06)	8.62 (0.06)	8.64
$[H_3L]/[H][H_2L]$	4.41 (0.09)	4.13 (0.18)	4.51 (0.09)	4.28
$[H_4L]/[H][H_3L]$	2.92 (0.03)	2.90 (0.03)	2.96 (0.06)	2.6
$[H_5L]/[H][H_4L]$	2.43 (0.15)	2.46 (0.09)	2.37 (0.20)	2.0

[a] All values are from this work, except for DTPA in Me_4NCl (ref 11). I = 0.1 M ($NaClO_4$ or Me_4NCl); t = 25 °C. [b] Values in parentheses are 3σ.

the constant β_{111} (eq 2) cannot because there is insufficient free aqua ion under these conditions. The equations are

$$M + L + H \rightleftharpoons MHL \qquad \beta_{111} = \frac{[MHL]}{[M][L][H]} \tag{2}$$

The stability constant β_{111} can be determined if the formation constant, β_{101}, is known where

$$M + L \rightleftharpoons M \qquad \beta_{101} = \frac{[ML]}{[M][L]} \tag{3}$$

The formation constant β_{101} can be determined by competition with a competitor ligand, L′, via eq 4:

$$ML + L' \rightleftharpoons ML' + L \qquad K_{comp} = \frac{[ML'][L]}{[ML][L']} \tag{4}$$

from a knowledge of $K_{ML'}$. The competing ligand must have a similar affinity for M so that under the conditions employed there are measurable amounts of ML, ML′, L, and L′, allowing K_{comp} to be determined with accuracy.

For Gd(III), Eu(III), and Cu(II), EDTA was chosen as a competitor ligand, and the ratio of EDTA/L was varied such that there were appreciable amounts of both ML and ML′. The stability constants of L with Gd(III) and Cu(II) were determined by titrating a mixture of M, L, and EDTA. Since the protonation constants of H_6L and H_4EDTA are known, as is the formation

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Table 2. Stability Constants Determined in This Study and Their DTPA Analogs from Ref 11[a]

equilibrium	log β (ML)[b]	log β (MDTPA)[b]	method
[CaL]/[Ca][L]	10.45 (0.06)	10.75	direct pH titration
[CaHL]/[H][CaL]	5.66 (0.02)	6.11	direct pH titration
[ZnL]/[Zn][L]	17.82 (0.09)	18.29	direct pH titration
[ZnHL]/[H][ZnL]	5.60 (0.02)	5.60	direct pH titration
[ZnH_2L]/[H][ZnHL]	2.54 (0.03)	3.06	direct pH titration
[CuL]/[Cu][L]	21.3 (0.3)	21.4	competition with EDTA, titration
[CuHL]/[H][CuL]	5.16 (0.08)	4.80	direct pH titration
[CuH_2L]/[H][CuHL]	2.88 (0.10)	2.96	direct pH titration
[CuH_3L]/[H][CuH_2L]	2.26 (0.20)	2.56	direct pH titration
[FeL]/[FeL(OH)][H]	9.88 (0.10)	9.66	direct pH titration
[FeL]/[Fe][L]	26.66 (0.12)	28.0	competition with DTPA, LC-MS
[FeHL]/[H][FeL]	3.57 (0.03)	3.56	direct pH titration
[FeH_2L]/[H][FeHL]	1.28 (0.09)	not reported	direct pH titration
[GdL]/[Gd][L]	22.06 (0.02)	22.39	competition with EDTA, titration
[GdL]/[Gd][L]	22.11 (0.20)	22.39	competition with Eu, luminescence
[GdL]/[Gd][L][c]	23.2 (0.6)	22.39	competition with EDTA, titration
[EuL]/[Eu][L]	22.21 (0.20)	22.39	competition with EDTA, luminescence

[a] $I = 0.1$ M $NaClO_4$; $t = 25$ °C. [b] Values in parentheses are 3σ. [c] $I = 0.1$ M Me_4NCl.

constant for M(EDTA), the observed [H^+] can be used to obtain β_{101} for ML. The FeL stability constant was determined by competition with DTPA. Under the conditions employed, the Fe(III) was partioned between four species, FeL, FeHL, FeDTPA, and FeHDTPA. The concentrations of (FeL + FeHL) and (FeDTPA + FeHDTPA) were directly determined from HPLC-MS. The protonation constants of each metal complex were known from prior titrations.

For Eu(III), the concentrations of the complexes Eu(EDTA) and EuL were directly measured from deconvolution of the Eu(III) emission spectra. From the pH and knowledge of the ligand deprotonation constants, [L] and [L′] could be computed. Thus each luminsecence experiment allowed the direct measurement of the equilibrium in eq 4. Knowledge of the EuEDTA stability constant allowed the computation of the EuL stability constant.

An alternative method to ligand−ligand competition used a competing metal ion. Equation 5 describes the equilibrium for the competition of Eu(III) and Gd(III) for L:

$$\mathrm{EuL} + \mathrm{Gd} \rightleftharpoons \mathrm{GdL} + \mathrm{Eu} \qquad K = \frac{[\mathrm{GdL}][\mathrm{Eu}]}{[\mathrm{EuL}][\mathrm{Gd}]} = \frac{\beta_{101}^{\mathrm{Gd}}}{\beta_{101}^{\mathrm{Eu}}} \qquad (5)$$

The Eu speciation is obtained from the luminescence spectra, and the concentrations of the Gd containing species can be obtained from the mass balance equations. From the values of K (eq 5) and $\beta_{101}{}^{\mathrm{Eu}}$, $\beta_{101}{}^{\mathrm{Gd}}$ can be calculated.

Kinetics. The rate of transmetalation between GdL and Eu was measured using ^{152,154}Eu as a tracer. For the exchange reaction (eq 6), the tracer level

$$^{152,154}\mathrm{Eu} + \mathrm{GdL} \rightleftharpoons \mathrm{Gd} + {}^{152,154}\mathrm{EuL} \qquad (6)$$

concentrations of Eu and EuL vary significantly, but this level of reaction has insignificant effect on the macro Gd and GdL concentrations. For isotopic exchange, the rate, R, of the reaction is given by eq 7, where X denotes the activity of the sample at a given time, t, and X_e is the activity at equilibrium.[17] By plotting

$$\ln\left(1 - \frac{X}{X_e}\right) = -R\left(\frac{[\mathrm{Gd}] + [\mathrm{GdL}]}{[\mathrm{Gd}][\mathrm{GdL}]}\right)t \qquad (7)$$

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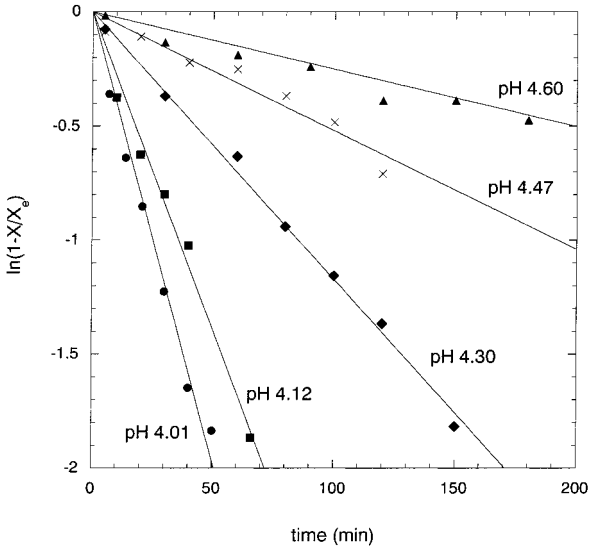


Figure 2. The change in radioactivity ($\ln(1 - X/X_e)$) versus time for the GdL/^{152,154}Eu system at various pH levels. [Gd] = [GdL] = 1 mM.

the left-hand side of eq 7 vs t, the rate R can be obtained from the slope. Figure 2 shows the results of the exchange experiments.

Experiments were conducted also at constant concentrations of GdL and Gd, but with variable pH. In the pH range from 3.9 to 4.6 a second order dependence upon [H^+] was found. The dependence on free ion and/or complex concentration was checked at constant pH (4.3) and with varying concentrations of GdL and Gd. The rate was independent of [$Gd_{(aq)}{}^{3+}$] but first order in [GdL]. The rate equation for the pH range 3.9 to 4.6 is given by eq 8.

$$R = k_a[\mathrm{GdL}][\mathrm{H}]^2 \qquad (8)$$

When the pH was increased (≥5), the order with respect to H decreased to ca. 1.5 indicating the presence of an additional acid-dependent term in the rate equation. The data in the pH range from 5 to 6 were analyzed by subtracting the rate defined by eq 8 from the observed rate. A plot of this corrected rate, R_{corr}, vs [H] (at constant [Gd] and [GdL]) gave a straight line with a nonzero intercept indicating terms that were first and zero order with respect to [H]. Both these terms were found to have a first order dependence upon the concentrations of both GdL and Gd. Consequently, the overall rate equation found,

Table 3. Rate Constants Determined in This Study and Comparative Literature Values[a]

system	k_a (10^{-6} M^{-2} min^{-1})	k_b (10^{-3} M^{-2} min^{-1})	k_c (10^{3} M^{-1} min^{-1})	ref
GdL/^{152,154}Eu	1.84 (0.13)[b]	2.87 (0.2)[b]	3.72 (0.4)[b]	this work
Y−DTPA/Y	10.5	51.5	240	23
Nd−DTPA/Nd	110	340	350	24
Ce−DTPA/Ce	300	not reported	1600	25

[a] The rate expression in eq 9 applies to all four studies. $I = 0.1$ M $NaClO_4$; $t = 25$ °C. [b] Values in parentheses are 3σ.

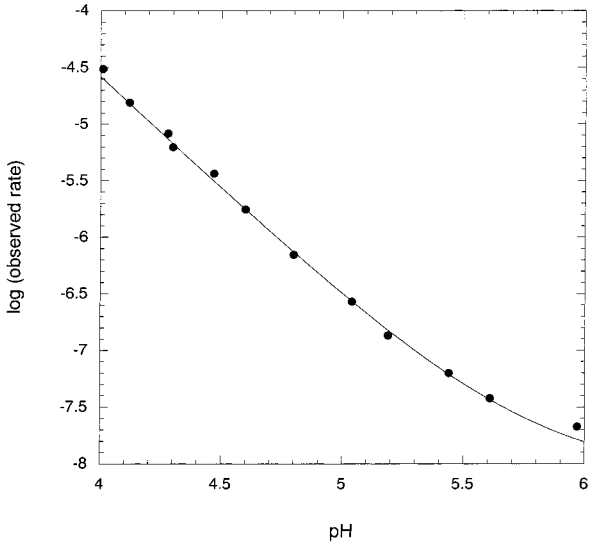


Figure 3. Logarithm of the observed rate as a function of pH. The solid line is the calculated rate based upon the rate eq 9 and the rate constants in Table 3.

under these conditions, is given by eq 9.

$$R = k_a[\mathrm{GdL}][\mathrm{H}]2 + k_b[\mathrm{GdL}][\mathrm{Gd}][\mathrm{H}] + k_c[\mathrm{GdL}][\mathrm{Gd}] \quad (9)$$

The values of the rate constants are given in Table 3. Figure 3 shows a plot of observed rate vs pH with the line defined by eq 9.

Discussion

Stability Constants. The ligand, L, behaves similarly to DTPA in terms of its protonation constants. However, the first two protonation constants are significantly depressed in comparison to DTPA when titrated in sodium perchlorate (0.1 M) medium. This could be either an electron withdrawing effect of the phosphodiester moiety or an enhanced binding of sodium ion by L. The protonation constants were redetermined in tetramethylammonium chloride medium (0.1 M). In this medium, L is more basic than DTPA (Table 1). This suggests that the depression of the protonation constants of L in $NaClO_{4\ (aq)}$ is because of better sodium ion binding by L relative to DTPA.

Figure 4 shows speciation diagrams for the five metal ions studied. The protonated species could be expected for a ligand with eight potential donor atoms coordinating to metal ions which have typical coordination numbers less than eight (with the exception of Gd(III)). Indeed, the metal complexes implied by the titration data of the various metals with L have direct counterparts with DTPA. The only exception to this is FeH_2L (not reported in Martell and Smith's compendium[11]) which is formed under very acidic conditions. A crystal structure of the dimeric structure $[FeH_2DTPA]_2$ has been reported.[18] The protonated GdL complex was not observed under the conditions employed in this study but has been reported for $[Gd(HDTPA)]^-$.[11]

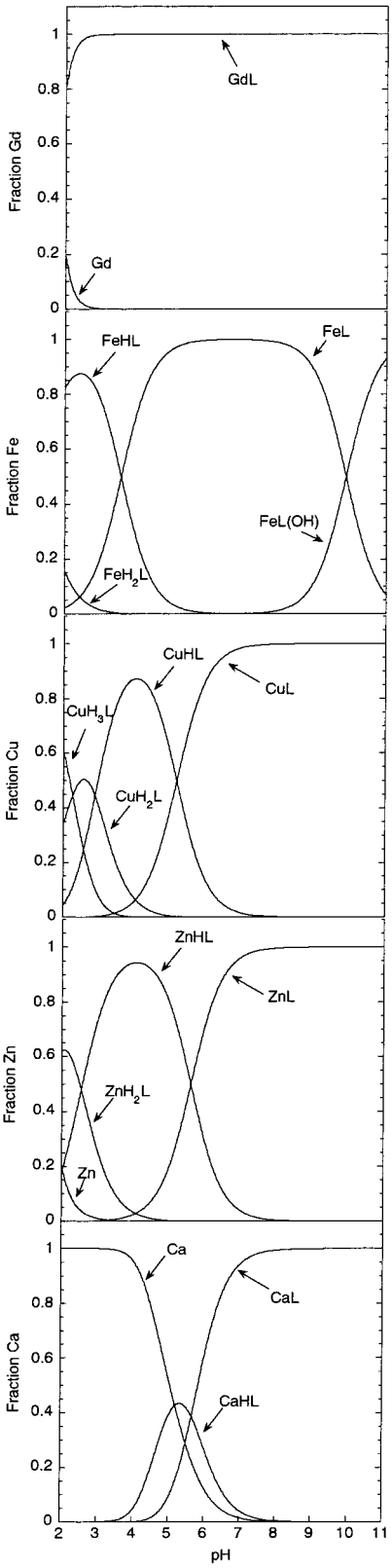


Figure 4. Speciation diagrams for Ca(II), Zn(II), Cu(II), Fe(II), and Gd(III) with L, drawn at [M] = [L] = 1 mM.

(18) Finnen, D. C.; Pinkerton, A. A.; Dunham, W. R.; Sands, R. H.; Funk, M. O., Jr. *Inorg. Chem.* **1991**, *30*, 3960−3964.

Table 4. pM Values Calculated at pH 7.4 (10 mM L_{tot}/1 mM M_{tot}) for ML Systems Described in This Study, along with Those of MDTPA and Various DTPA Derivatives for Comparison

metal ion	pM (M−L)[a]	pM (M−DTPA)[b]	pM (M−LDTPA)[c]	pM (BOPTA)[d]	medium
Ca(II)	8.34	7.72			0.1 M $NaClO_4$
Zn(II)	15.71	15.58			0.1 M $NaClO_4$
Cu(II)	19.18	18.57			0.1 M $NaClO_4$
Fe(III)	24.54	24.97			0.1 M $NaClO_4$
Eu(III)	20.09	19.43			0.1 M $NaClO_4$
Gd(III)	19.94	19.43			0.1 M $NaClO_4$
Gd(III)	19.20	18.90			0.1 M Me_4NCl
Gd(III)		19.00	18.70	19.40	0.1 M KCl

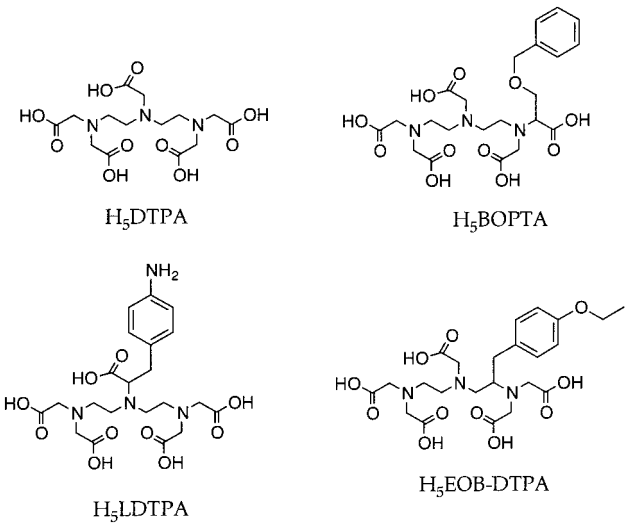
[a] Calculated from this work. [b] Ref 11. [c] Ref 21. [d] Ref 20.

A comparison of the stability constants in Table 2 shows that the DTPA stability constants are consistently higher than the stability constants of L with the same metals in sodium perchlorate medium. This result is in good agreement with the first two protonation constants also being lower for L in sodium perchlorate medium, relative to DTPA. To compare the sequestering ability of DTPA and L under standard conditions at physiological pH (7.4) and in various ionic media, pM values were calculated (Table 4). The pM value is $-\log[M]_{free}$ calculated at a ratio of 10 mM total ligand to 1 mM total metal ion at pH 7.4; the larger the pM, the smaller the amount of free metal ion present. The pM values were calculated from the stability constants in Table 2 and the protonation constants in Table 1. The pM values in Table 4 serve to rank the complexing abilities of various DTPA-like ligands at pH 7.4. Not surprisingly, the pM values are very similar for a given metal ion with the various DTPA-like ligands. However, Table 4 shows that L is a slightly better complexing agent than DTPA at pH 7.4 for all the metals studied with the exception of iron(III).

Tables 2 and 4 show that the only metals that compete with Gd(III) for L from a thermodynamic viewpoint are Fe(III) and Cu(II). However, both of these cations exist in very low concentrations in vivo because they are sequestered by native iron and copper proteins. The more bioavailable Zn(II) and Ca(II) ions have much lower affinities for L at pH 7.4.

Introduction of a functional group on the ethylenediamine backbone appears to slightly augment thermodynamic stability. This may be explained by the steric effect of the bulky substituents constraining the ligands into a chelating conformation.[19] Comparing pM values (Table 4) for $[GdL]^{3-}$ and $[Gd(DTPA)]^{2-}$ in either 0.1 M $NaClO_4$ or 0.1M Me_4NCl shows that GdL is 2−3 times more stable than $[Gd(DTPA)]^{2-}$ at pH 7.4. This is also true for substitution on one of the acetate arms, e.g., Gd−BOPTA (Chart 2),[20] where the substituted DTPA is slightly more stable. However, for LDTPA (where the central nitrogen acetate arm is substituted), the pM value with Gd(III) is lower.[21] Muller et al.[22] also give qualitative evidence that GdL is more thermodynamically stable than $[Gd(DTPA)]^{2-}$ or $[Gd(EOB\text{-}DTPA)]^{2-}$ at pH 7.4. The studies described here were performed at the standard temperature of 25 °C in order to compare the data to DTPA and other DTPA derivatives. It is likely that the same trends in stability will be observed at the physiological temperature of 37 °C.

Chart 2



Kinetics. The rate law found for the GdL/^{152,154}Eu system is the same as that for the Y-DTPA/Y[23] and Nd-DTPA/Nd[24] isotopic exchange systems. The Ce-DTPA/Ce isotopic exchange rate has also been reported[25] and the rate expression given is similar but lacks the second term which is first order with respect to [H]. These rate constants are listed in Table 3 to compare with those for GdL/^{152,154}Eu. Brücher and Laurenczy[26] reported a kinetic study on the rate of transmetalation of DTPA complexes of La, Nd, Ho, and Lu by Eu. They found that the rate of transmetalation was slower as the ionic radius of the lanthanide decreased. From this report, one would expect the rate of self-exchange between Gd-DTPA/Gd to lie somewhere between Nd and Y.

The mechanism for this isotope exchange reaction is likely the same as was proposed previously.[27] The mechanism involves two rate determining reaction pathways: in one pathway hydrogen ion catalysis is responsible for the rate determining step, while in the second path the rate is determined by addition of the competing metal ion to the complex; both result in dissociation of the complex and release of the original metal ion.

The GdL system (MS-325) is more kinetically inert to substitution than any of the previously reported examples. There is precedent for this in the work of McMurry et al.[7] who showed that substitutions on the diethylenetriamine backbone slowed

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the rate of acid-catalyzed dissociation of yttrium derivatives of DTPA. In addition, a recent report by Muller et al.[22] provides qualitative evidence that MS-325 (GdL) is more inert to transmetalation by a Zn/PO_4^{3-} slurry than $[Gd(DTPA)]^{2-}$. These authors also showed that GdL is more inert to complex formation by a cocktail of ATP, phosphocreatine, and inorganic phosphate than both $[Gd(DTPA)]^{2-}$ and $[Gd(EOB\text{-}DTPA)]^{2-}$. The increased kinetic inertness of MS-325 (GdL) compared to $[Gd(DTPA)]^{2-}$ likely stems from the bulky substituent on the ethylenediamine backbone[27] which hinders the unwrapping of the ligand about the metal ion.

Conclusion

MS-325 ($[GdL]^{3-}$) was shown to be more thermodynamically stable and kinetically inert to metal ion substitution than Magnevist ($[Gd(DTPA)]^{2-}$). The phosphodiester substituent on the ethylenediamine backbone apparently serves to predispose the ligand to binding and at the same time represents a steric impediment to dissociation of the complex. Thus, chemical modification which promotes albumin binding and enhances relaxivity also confers desirable chemical stability in the gadolinium complex.

Acknowledgment. Dr. William Cacheris is kindly acknowledged for providing a copy of the program TITRATE, and the FSU Laser Lab is kindly acknowledged for the use of its facilities.

IC001117R