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Kinetics of the Exchange Reactions between $Gd(DTPA)^{2-}$, $Gd(BOPTA)^{2-}$, and Gd(DTPA-BMA) Complexes, Used As MRI Contrast Agents, and the Triethylenetetraamine-Hexaacetate Ligand

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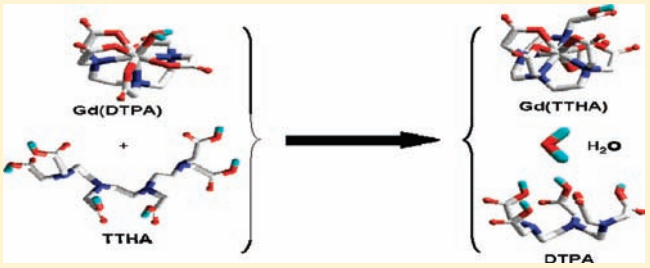
Supporting Information

ABSTRACT: The kinetics of ligand exchange reactions occurring between the Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) complexes, used as contrast agents in MRI, and the ligand TTHA, have been studied in the pH range 6.5−11.0 by measuring the water proton relaxation rates at 25 °C in 0.15 M NaCl. The rates of the reactions are directly proportional to the concentration of TTHA, indicating that the reactions take place with the direct attack of the $H_iTTHA^{(6-i)-}$ (i = 0, 1, 2 and 3) species on the Gd^{3+} complexes, through the formation of ternary intermediates. The rates of the exchange reactions of the neutral Gd(DTPA-BMA) increase when the pH is increased from 6.5 to 9, because the less protonated $H_iTTHA^{(6-i)-}$ species can more efficiently attack the Gd^{3+} complex. The rates of the exchange reactions of $[Gd(DTPA)]^{2-}$ and $[Gd(BOPTA)]^{2-}$ also increase from pH 8.5 to 11, but from 6.5 to 8.5 an unexpected decrease was observed in the reaction rates. The decrease has been interpreted by assuming the validity of general acid catalysis. The protons from the $H_iTTHA^{(6-i)-}$ species (i = 2 and 3) can be transferred to the coordinated DTPA or BOPTA in the ternary intermediates when the dissociation of the Gd^{3+} complexes occurs faster. The kinetic inertness of Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) differs very considerably; the rates of the ligand exchange reactions of Gd(DTPA-BMA), thus the rates of its dissociation, are 2 to 3 orders of magnitude higher than those of Gd(DTPA) and Gd(BOPTA). The rates of the ligand exchange reactions increase with increasing concentration of the endogenous citrate, phosphate, or carbonate ions at a pH of 7.4, but the effect of citrate and phosphate is negligible at their physiological concentrations. The increase in the reaction rates at the physiological concentration of the carbonate ion is significant (20−60%), and the effect is the largest for the Gd(DTPA-BMA) complex.



INTRODUCTION

The complexes of gadolinium(III) formed with the octadentate aminopolycarboxylate ligands DTPA, BOPTA, and DTPA-BMA (Scheme 1) are clinically used as contrast agents in magnetic resonance imaging (MRI) to improve the image contrast (H_5DTPA = diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid, H_5BOPTA = 4-carboxyl-5,6,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecane-13-oic acid, H_3DTPA-BMA = DTPA-bis(methylamide)

The contrast agents, injected intravenously, after the distribution in the extracellular space of the body, are eliminated predominantly through the kidneys with a half-time of about 1.5 h.[1−3] The elimination of Gd(BOPTA) occurs partially through the hepatobiliary system because of the presence of the lipophilic benzyloxymethyl group. Since both the free Gd^{3+} and the ligands (H_iL) are toxic, the Gd(III) complexes used as contrast agents must have high thermodynamic stability and kinetic inertness.[5,6] In spite of the strict requirements, numerous experimental data obtained by both animal and human studies indicate that the excretion of Gd^{3+} from the body is not full.[3,6−10] The amount of retained Gd^{3+} is generally very low because the elimination of the contrast agents is much faster than their dissociation, which is necessary for the deposition of Gd^{3+}.[7,8] However, the excretion of Gd^{3+} chelates is slower from the body of patients with chronic kidney disease (e.g., dialyzed patients), when the half-time of elimination is about 30−40 h.[3] In these cases, the amount of retained Gd^{3+} can be higher, which may lead to the development of a newly discovered disease referred to as Nephrogenic Systemic Fibrosis (NSF). NSF has been observed most frequently in those cases when the contrast agent used was Gd(DTPA-BMA), which has a relatively lower stability constant.[3,11,12] Because of the discovery of NSF, the knowledge of the physicochemical properties of contrast agents, first of all that of the rate of dissociation, became highly important. The in vivo dissociation of the Gd^{3+} chelates is presumably followed by the reaction of the free Gd^{3+} with some endogenous ligands (e.g., citrate, lactate, etc.), while the free aminopolycarboxylate ligand reacts with the endogenous metal ions, like Zn^{2+}, Cu^{2+}, or Ca^{2+}. The dissociation of Gd^{3+} complexes can take place

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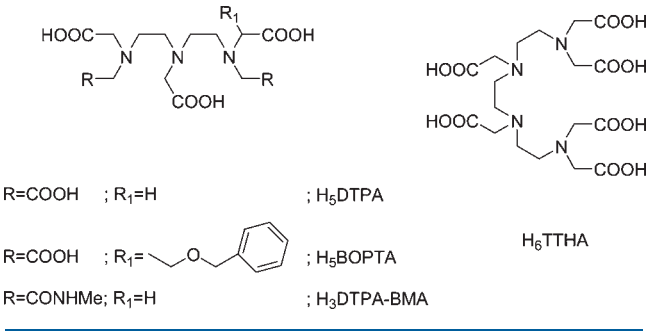
Scheme 1. Formulas of the Ligands



spontaneously and with the assistance of protons, or with the direct attack of the endogenous metals or ligands on the Gd^{3+} complex. Of these reactions, the spontaneous and proton assisted dissociation of complexes is generally very slow at physiological pH. The kinetic inertness of Gd^{3+} chelates has been characterized and compared with the proton assisted dissociation rates determined in 0.1 M HCl.[3] More detailed information was obtained for the kinetics of dissociation in the studies on the rates of metal exchange reactions, occurring between the Gd^{3+} complexes and Eu^{3+}, Cu^{2+}, or Zn^{2+} ions in the pH range 3−6. The circumstances of these studies were far from the physiological conditions; moreover, the role of the small endogenous ligands in the reactions has not been investigated.[13−17] The possible role of ligand exchange reactions in the in vivo dissociation of the Gd^{3+} aminopolycarboxylates has not been studied, probably because the stability constants of the Gd^{3+} complexes formed with the small endogenous ligands are relatively low. However, the issue was raised that some of the high molecular mass proteins, first of all transferrin, may play some role in the dissociation of Gd^{3+} chelates by displacing the aminopolycarboxylate ligand, but the rates of such ligand exchange reactions have not been studied.[9,18]

Ligand exchange reactions can take place during the spectrophotometric determination of Ca^{2+} in serum, if the samples were taken within the first 12−24 h after MRI examinations, performed with Gd(DTPA-BMA) (Omniscan) as a contrast agent. For the determination of Ca^{2+} *o*-cresolphtaleine complexone or methylthymol blue is used, which can displace the DTPA-BMA ligand in the Gd(DTPA-BMA) complex, leading to an apparent decrease in the serum Ca^{2+} level.[19,20] Similar interference was not observed when $Gd(DOTA)^-$ (H_4DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) or $Gd(DTPA)^{2-}$ was used as a contrast agent, because the stability constants of these complexes are significantly higher than that of Gd(DTPA-BMA).

The experiments reported by Puttagunta et al. show that the ligand exchange reaction between Gd(DTPA-BMA) and DTPA at 20 mM concentrations, at a pH of 7.4, can take place with the release of about 85% of DTPA-BMA within 2 h. The kinetics of the exchange reactions were not investigated.[21]

Recently, a nanostructure silica material, functionalized with the 1-hydroxy-2-pyridinone ligand, was proposed as a sorbent to remove Gd^{3+} from the blood of dialyzed patients, examined by MRI with the use of a Gd^{3+}-containing contrast agent. The 1-hydroxy-2-pyridinone, bound to the silica, can remove the Gd^{3+} from the Gd(DTPA-BMA) complex. This reaction can also be regarded as a ligand exchange reaction.[22]

In order to obtain information about the kinetics and mechanisms of the ligand exchange reactions of the Gd^{3+} aminopolycarboxylates, we have recently studied the rates of reactions taking place between the Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) complexes and the ligand TTHA (Scheme 1; H_6TTHA = triethylenetetraamine-hexaacetic acid) as model reactions. The stability constant of $Gd(TTHA)^{3-}$ (log K_{GdL} = 23.83) is higher than that of $Gd(DTPA)^{2-}$ (log K_{GdL} = 22.46), $Gd(BOPTA)^{2-}$ (log K_{GdL} = 22.59), or Gd(DTPA-BMA) (log K_{GdL} = 16.85),[23,24] and as the species distribution calculations show, the exchange reactions take place practically completely at pH > 6.5 in the presence of 10-fold TTHA excess. (The charges of ligands and complexes will be used only, when it is necessary.) These ligand exchange reactions could be studied in a broad pH range (6.5 < pH < 11) where the hydrolysis of Gd^{3+} did not take place because of the presence of a ligand excess. The effect of the presence of small endogenous ligands (citrate, phosphate, and carbonate) on the rates of ligand exchange reactions was also investigated in order to obtain information about the role of these ligands in the dissociation of Gd^{3+} complexes.

EXPERIMENTAL SECTION

Materials. The $GdCl_3$, $EuCl_3$, and $LaCl_3$ stock solutions were prepared by dissolving Gd_2O_3, Eu_2O_3, and La_2O_3 (99.9%, Fluka) in 6 M HCl, and the excess acid was evaporated off. The concentration of the lanthanide(III) (Ln(III)) stock solutions was determined by complexometric titration using a standardized Na_2H_2EDTA solution in the presence of xylenolorange as an indicator. The stock solutions of DTPA (Fluka), BOPTA (Bracco Imaging S.p.A.), DTPA-BMA (Bracco Imaging S.p.A.), and TTHA (Fluka) were prepared by dissolving the solid ligands in double distilled water, and the ligand concentration was determined by pH-potentiometry on the basis of titration curves obtained in the absence and presence of a 50-fold excess of Ca^{2+}. GdL, EuL, and LaL complexes were prepared by mixing the ligand stock solutions with an equimolar amount of $GdCl_3$, $EuCl_3$, or $LaCl_3$ solution, and the pH was set to about 6 by NaOH. A slight excess of ligand (0.5%) was used to ensure the complete complexation of Ln^{3+}. Phosphate, citrate, hydrogen carbonate, and NaCl stock solutions were prepared from analytical grade sodium salts in double distilled water.

pH Potentiometry. The pH potentiometric titrations were performed with standardized NaOH solution at 25 °C at a constant ionic strength (0.15 M NaCl). The samples (10 mL) were stirred with a magnetic stirrer, while a constant N_2 flow was bubbled through the solutions. A Metrohm 6.0233.100 combined electrode was used to measure the pH. The pH meter was calibrated using standard KH-phtalate (pH = 4.004) and borax (pH = 9.177) buffers. The potentiometric measurements were carried out with the use of a computer-controlled Methrom 702 SM Titrino automatic buret. The H^+ ion concentrations have been calculated from the measured pH values using the method suggested by Irving et al.[25]

NMR Measurements. ^1H and ^{13}C NMR spectra of La(DTPA-BMA) and Eu(DTPA) were recorded on a Bruker Avance 500 MHz spectrometer in the temperature range 274−363 K in the absence and presence of ligand excess, respectively. The pH of the samples was adjusted to 7.4. The concentrations of La(DTPA-BMA) and EuDTPA were 0.1 M, while the concentrations of DTPA-BMA and a DTPA excess were varied between 0.25 and 0.5 M.

Kinetic Studies. The relaxivity value of Gd(TTHA) (r_1 = 3.1 mM^{-1} s^{-1}) differs considerably from those of Gd(DTPA) (r_1 = 6.0 mM^{-1} s^{-1}), Gd(DTPA-BMA) (r_1 = 5.5 mM^{-1} s^{-1}), and Gd(BOPTA) (r_1 = 6.9 mM^{-1} s^{-1}). (The relaxivity r_1 (mM^{-1} s^{-1}) is the increase in the water proton relaxation rates ($1/T_1$) observed when the concentration of the Gd^{3+} complex increases by 1 mmol). The progress of the ligand exchange reactions between the Gd^{3+} complexes formed

with the DTPA derivatives (GdL) and H_iTTHA (H_iX) in the absence and presence of the endogenous anions was followed by measuring the water proton relaxation rates ($1/T_1$) of the samples with an MS-4 NMR spectrometer (Institute Jozef Stefan, Ljubljana) at 9 MHz or a Bruker minispec mq20 NMR analyzer at 20 MHz. The longitudinal relaxation times were measured by the inversion recovery method (180°−τ−90°) by using 8−10 different τ values. The concentration of the GdL complexes in the samples was generally 1.0 mM.

For studying the effect of TTHA on the reaction rates, the concentration of TTHA was varied between 5 and 40 mM, while the pH of the samples was maintained constant (pH = 7.4) by a noncoordinating buffer, HEPES. In the pH-dependent measurements, the pH of the samples was varied in a broad pH range (6.5−11.0), when the concentration of TTHA was 10 mM.

In the samples containing the different endogenous anions, the concentration of TTHA was 2.0 mM, the pH was adjusted to 7.4, the citrate, phosphate, and hydrogen carbonate concentrations were varied between 0 and 20 mM and 0−40 mM, respectively.

The kinetic measurements were started (t = 0 s) by mixing the calculated volume of the GdL complex with a solution containing the ligand TTHA or the ligand TTHA and the Na salt of the corresponding endogenous anion. The GdL complex and the TTHA solutions were prethermostated at 25 °C. All kinetic measurements were carried out at 25 °C in a 0.15 M NaCl solution to maintain a constant ionic strength. The first-order rate constants (k_{obs}) have been calculated with the use of eq 1:

$$r_{1t} = (r_{10} - r_{1e}) \exp(-k_{obs}t) + r_{1e} \quad (1)$$

where r_{10}, r_{1t}, and r_{1e} are the measured relaxivity values at the start, at time t, and at equilibrium of the reaction, respectively.

RESULTS

The ligand exchange reactions taking place between the Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) complexes and the chelating agent TTHA can be described by eq 2:

$$GdL + H_iTTHA^{(6-i)-} = Gd(TTHA)^{3-} + H_jL + (i-j)H^+ \quad (2)$$

where the charges of the species GdL and H_jL are not indicated due to the different charges of the ligands.

The rates of the ligand exchange reactions of $Gd(DTPA)^{2-}$ and $Gd(BOPTA)^{2-}$ have been studied in the pH range 6.5−11. The reactions between Gd(DTPA-BMA) and TTHA were found to be relatively fast at pH > 9, so the rates of these reactions had been studied from pH 6.5 to 9. The study of the reaction rates was performed at pH ≥ 6.5, because at pH < 6.5, the protonated $Gd(HTTHA)^{2-}$ complex could be formed (the protonation constant of $Gd(TTHA)^{3-}$ is log K_{GdHX} = 4.73[23]), which has a higher relaxivity than $Gd(TTHA)^{3-}$.

The rate of the reaction 2 has been studied in the presence of TTHA excess, in order to ensure pseudo-first-order conditions. Since the reactions are first-order in regard to the complexes GdL, the rates of the ligand exchange can be expressed as follows:

$$-\frac{d[GdL]}{dt} = k_p[GdL] \quad (3)$$

where k_p is a pseudo-first-order rate constant and [GdL] is the concentration of the Gd(DTPA), Gd(BOPTA), or Gd(DTPA-BMA).

In order to obtain the rate law for the ligand exchange reactions, the pseudo-first-order rate constants have been determined at different TTHA and hydrogen ion concentrations. The k_p values determined at physiological pH by the variation of the TTHA concentration are presented in Figure 1.

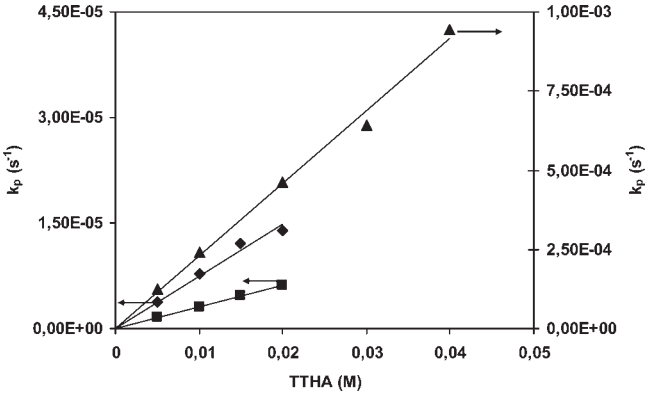


Figure 1. Dependence of the k_p values, characterizing the rates of the exchange reactions of Gd(DTPA) (♦), Gd(BOPTA) (■), and Gd(DTPA-BMA) (▲) on the TTHA concentration (pH = 7.4, [GdL] = 1 × 10^{-3} M, 25 °C, 0.15 M NaCl).

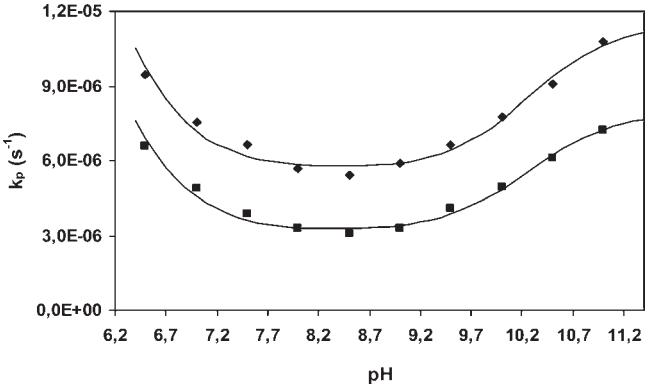


Figure 2. pH dependence of the k_p values characterizing the rates of exchange reactions between Gd(DTPA) (♦), Gd(BOPTA) (■), and TTHA ([GdL] = 1 × 10^{-3} M, [TTHA] = 1 × 10^{-2} M, 25 °C, 0.15 M NaCl).

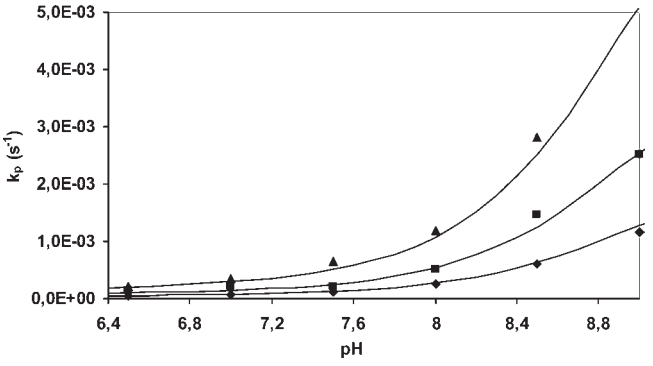


Figure 3. pH dependence of the k_p values characterizing the rates of exchange reactions between Gd(DTPA-BMA) and TTHA ([GdL] = 1 × 10^{-3} M; [TTHA] = 5 × 10^{-3} M (♦), 1 × 10^{-2} M (■), and 2 × 10^{-2} M (▲); 25 °C; 0.15 M NaCl).

The ligand TTHA is present in different protonated forms in the pH range investigated. The study of the reaction rates as a function of pH gives information about the role of the differently protonated $H_iTTHA^{(6-i)-}$ species in the exchange reactions. The k_p values obtained at different pH's are shown in Figures 2 and 3,

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Table 1. Rate Constants Characterizing the Ligand Exchange Reactions between the Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) Complexes and TTHA (25 °C, 0.15 M NaCl)

	Gd(DTPA)	Gd(BOPTA)	Gd(DTPA-BMA)
k_X (M^{-1} s^{-1})	$(1.16 \pm 0.05) \times 10^{-3}$	$(7.9 \pm 0.4) \times 10^{-4}$	
k_{HX} (M^{-1} s^{-1})	$(5.9 \pm 0.4) \times 10^{-4}$	$(3.5 \pm 0.3) \times 10^{-4}$	0.58 ± 0.04
k_{H2X} (M^{-1} s^{-1})	$(5.6 \pm 0.2) \times 10^{-4}$	$(3.1 \pm 0.1) \times 10^{-4}$	$(1.1 \pm 0.1) \times 10^{-2}$
k_{H3X} (M^{-1} s^{-1})	$(2.2 \pm 0.2) \times 10^{-3}$	$(1.8 \pm 0.2) \times 10^{-3}$	

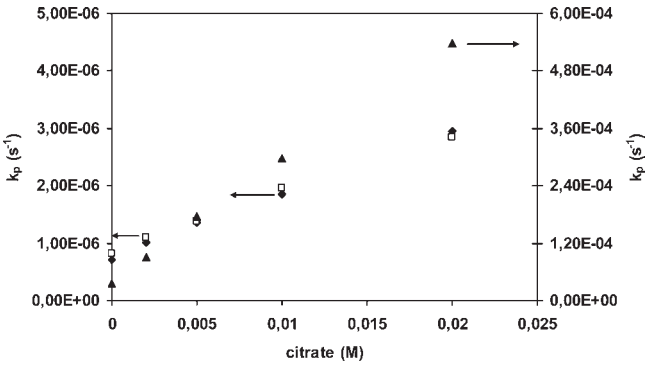

Figure 4. Dependence of the k_p values on the citrate concentration for the exchange reactions of Gd(DTPA) (♦), Gd(BOPTA) (□), and Gd(DTPA-BMA) (▲) (pH = 7.4, [GdL] = 1×10^{-3} M, [TTHA] = 2×10^{-3} M, 25 °C, 0.15 M NaCl).

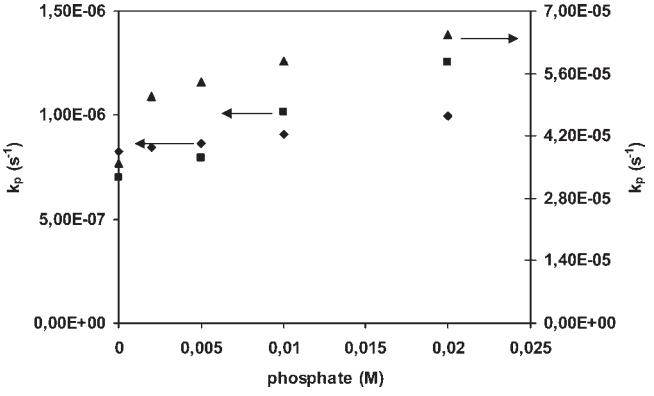

Figure 5. Dependence of the k_p values on the phosphate concentration for the exchange reactions of Gd(DTPA) (♦), Gd(BOPTA) (■), and Gd(DTPA-BMA) (▲) (pH = 7.4, [GdL] = 1×10^{-3} M, [TTHA] = 2×10^{-3} M, 25 °C, 0.15 M NaCl).

where the squares, triangles, and diamonds indicate the experimental k_p data, while the curves show the k_p values calculated with the use of the obtained rate constants (Table 1).

The effect of the endogenous citrate, phosphate, and carbonate ions on the rates of ligand exchange reactions has been studied at physiological pH. At pH = 7.4, the citrate is present as a fully deprotonated cit^{3-} ligand, while for the phosphate and carbonate, the species HPO_4^{2-} and HCO_3^- predominate, respectively. The rate constants, k_p, determined as a function of the total citrate, phosphate, and carbonate concentrations are presented in Figures 4−6.

For obtaining easily observable rate effects for the endogenous citrate, phosphate, and carbonate ions, a lower TTHA excess was used (2 mM), but in these cases the pseudo-first-order rate

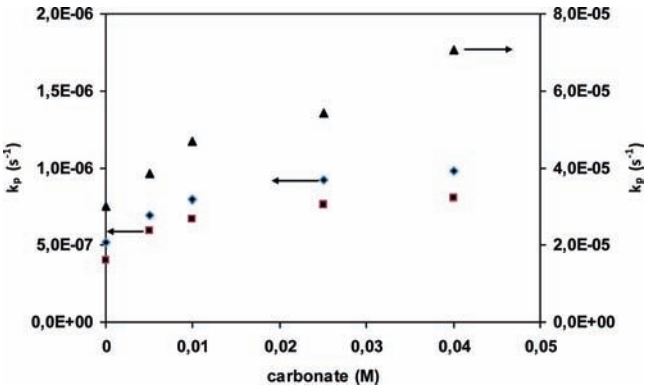

Figure 6. Dependence of the k_p values on the carbonate concentration for the exchange reactions of Gd(DTPA) (♦), Gd(BOPTA) (■), and Gd(DTPA-BMA) (▲) (pH = 7.4, [GdL] = 1×10^{-3} M, [TTHA] = 2×10^{-3} M, 25 °C, 0.15 M NaCl).

constants were calculated from the data, obtained for the first 40−50% conversion of the reactions.

DISCUSSION

The exchange reactions taking place between the complexes Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) and the ligand TTHA are very slow at pH values around 7.5−8.5, as is seen from the rate data presented in Figures 2 and 3. The half-life of the exchange reaction for the Gd(DTPA) calculated from the k_p value obtained at pH = 8.5 is 36 h, so the reactions had to be followed for a few days.

The ligand exchange reactions in principle can take place with the spontaneous and proton assisted dissociation of complexes, which is followed by fast reaction between the free Gd^{3+} and TTHA. The other possible pathway is the direct attack of the TTHA on the Gd^{3+} complex, when the TTHA displaces the DTPA, BOPTA, or DTPA-BMA ligands from the GdL complexes.

As seen in Figure 1, the k_p values characterizing the rates of ligand exchange reactions are directly proportional to the total concentration of TTHA. The linear dependence of the first-order rate constants on the TTHA concentration shows that the exchange reactions occur with the participation of the TTHA, presumably with the direct attack of the differently protonated $H_iTTHA^{(6-i)-}$ species on the GdL complexes. On the basis of the direct proportionality between the k_p values and the total concentration of TTHA ($[TTHA]_t$), the first order rate constants can be expressed as follows:

$$k_p = k_0 + k_L[TTHA]_t \quad (4)$$

where k_0 is characteristic for the spontaneous and proton assisted dissociation of the GdL complexes, while k_L characterizes the

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rates of reactions, occurring with the direct encounter of the $H_iTTHA^{(6-i)-}$ species and the GdL complexes. The k_0 and k_L values have been calculated from the k_p and $[TTHA]_t$ values, presented in Figure 1, using the least-squares method. The k_0 values are very low, lower than the calculated errors, so the term k_0 in eq 4 can be neglected. The rates of the proton assisted dissociation of the GdL complexes can be calculated from the rate data known from the results of the kinetic studies on the metal exchange reactions, taking place between the GdL complexes and the Eu^{3+} ion.[15,16,24] The pseudo-first-order rate constants, calculated for the proton assisted dissociation of Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) at a pH of 6.5 are 1.8×10^{-7} s^{-1}, 1.4×10^{-7} s^{-1}, and 4.0×10^{-6} s^{-1}, respectively.[15,16,24] However, the k_p values obtained for the ligand exchange reactions for Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) at a pH of 6.5 and $[TTHA]_t$ of 1×10^{-2} M are 7.6×10^{-6} s^{-1}, 5.1×10^{-6} s^{-1}, and 1.3×10^{-4} s^{-1}, respectively. The comparison of these rate data shows that the proton assisted dissociation of complexes is relatively slow, even at the highest H^+ concentration (the contribution of these pathways is 2.4%, 2.8%, and 3.1%, respectively), so it has practically no role in the ligand exchange reactions at pH > 6.5 and in the presence of a TTHA excess.

The k_L (M^{-1} s^{-1}) values, calculated for the GdL complexes (eq 4 and Figure 1), which characterize the efficiency of the attack of the $H_iTTHA^{(6-i)-}$ species on the Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) at a pH of 7.4, were found to be $(7.4 \pm 0.9) \times 10^{-4}$ M^{-1} s^{-1}, $(3.1 \pm 0.2) \times 10^{-4}$ M^{-1} s^{-1}, and $(2.3 \pm 0.03) \times 10^{-2}$ M^{-1} s^{-1}, respectively. These rate data, similarly to the k_p values, reveal that the behavior of Gd(DTPA) and Gd(BOPTA) in the ligand exchange reactions differs considerably from that of Gd(DTPA-BMA). The k_L values, thus the rates of reactions of Gd(DTPA-BMA), are more than 2 orders of magnitude higher than those of Gd(DTPA) and Gd(BOPTA).

The pH dependence of the rates of the ligand exchange reactions also differs considerably for the negatively charged $Gd(DTPA)^{2-}$ and $Gd(BOPTA)^{2-}$ and for the neutral Gd(DTPA-BMA). Figures 2 and 3 show the pseudo-first-order rate constants as a function of pH. The k_p values obtained for the reactions of $Gd(DTPA)^{2-}$ and $Gd(BOPTA)^{2-}$ vary according to minimum curves, with minima at a pH of about 8.5. The trend of the k_p values determined for the reactions of Gd(DTPA-BMA) is different. The k_p values slightly increase in the pH range 6.5−7.5, then at pH> 7.5 the rate constants increase abruptly. At pH > 9, the reactions are too fast to be followed by measuring the proton relaxation rates. A comparison of the rate data, presented in Figures 2 and 3, also shows that the rates of the exchange reactions of Gd(DTPA-BMA) are about 2 to 3 orders of magnitude higher than those of the Gd(DTPA) and Gd(BOPTA).

To understand the mechanisms of the ligand exchange reactions and to interpret the difference in the behavior of the negatively charged and neutral complexes, we have to take into account the structure of the complexes and the flexibility of the coordinated ligands. The structure of the GdL complexes in solution is similar to those found by ^1H and ^{13}C NMR studies.[26−28] The ligands are coordinated in octadentate fashion, and the ninth coordination site of Gd^{3+} is occupied by a water molecule. This water exchanges fast with the bulk water, which is of primary importance for the relaxation effect of the Gd^{3+} complexes. This coordination site is presumably very important in the ligand exchange, because as a first step of the reaction, a carboxylate group of the attacking $H_iTTHA^{(6-i)-}$ can be coordinated to this site, when a ternary intermediate is formed. The stability of this intermediate is very low, and the dissociation of TTHA from the latter is very probable. However, in a favorable case, due to the intramolecular rearrangement of the donor atoms in the complex, a second and then further donor atom of TTHA can be coordinated to the Gd^{3+}, and slowly, step by step, the whole coordinated L ligand is displaced by the TTHA, which leads to the formation of the Gd(TTHA) complex.

There are probably several reasons why the ligand exchange reactions of Gd(DTPA-BMA) are considerably faster than the similar reactions of Gd(DTPA) and Gd(BOPTA). One of the possible reasons is the stronger electrostatic interaction between the uncharged Gd(DTPA-BMA) and $H_iTTHA^{(6-i)-}$ in comparison to the interaction between the $Gd(DTPA)^{2-}$ or $Gd(BOPTA)^{2-}$ and $H_iTTHA^{(6-i)-}$. (The Gd(DTPA-BMA) has a partial positive charge because of the coordination of the two uncharged amide oxygen atoms.[29])

The other reason leading to the faster ligand exchange reaction of Gd(DTPA-BMA) is probably the higher rate of the intramolecular rearrangements of its donor atoms.[28] The rearrangements may result in the transitional presence of free coordination site(s) on the complexed Gd^{3+}, when the attack of the $H_iTTHA^{(6-i)-}$ species on the complex can be more efficient.

The rates of the intramolecular rearrangements have been studied for several $Ln(DTPA)^{2-}$ and Ln(DTPA-BPA) complexes by ^1H and ^{13}C NMR spectroscopy in a broad temperature range (0−100 °C; DTPA-BPA = DTPA-bis(propylamide)).[26−28] Both in the Ln(DTPA) and Ln(DTPA-BPA) complexes, the inversion of the three nitrogen atoms of the diethylenetriamine backbone is precluded. In the Ln(DTPA) complexes, the middle nitrogen is chiral, while in the Ln(DTPA-BPA) complexes, all three of the nitrogen atoms are chiral. Consequently, the Ln(DTPA) complexes have two isomers, which were detected by ^1H NMR spectroscopy for the paramagnetic complexes at low temperatures.[26,27] For the DTPA-bis(amide) derivative complexes, the formation of eight isomers is expected, and in the ^{13}C NMR spectrum of Nd(DTPA-BPA), the signals of the eight isomers could be observed.[28] At lower temperatures, slow exchange was observed between the different isomers. The rates of isomerization increased with the increase of temperature, and both the ^1H and ^{13}C signals broadened and coalesced at higher temperatures. The NMR studies have shown that two exchange processes occur in the complexes: (i) the relatively rapid racemization of the middle nitrogen atom, associated with the interconversions of the two gauche conformations of the ethylenediamine groups, and (ii) the slow racemization at the terminal nitrogens, which can take place with the decoordination of a nitrogen and its neighboring acetate and amide oxygens. The latter process is very slow for the Ln(DTPA) complexes, because the iminodiacetate (IMDA) groups are strongly bound to the Gd^{3+}, so this process cannot be observed in the temperature range studied. For the Ln(DTPA-BPA) complexes, the inversion of both the middle and the terminal nitrogens was observed by ^{13}C NMR spectroscopy, because the amide oxygen−Ln^{3+} bond is weaker.[28]

In the ^{13}C NMR spectra of La(DTPA-BMA) (Figure 7), the broadening of the signals can also be observed with the increase in temperature. A similar broadening effect can be observed in the presence of a DTPA-BMA ligand excess, when the exchange between the coordinated and free ligands is slow (the signals are separated). In this study, we used a DTPA-BMA excess instead of TTHA, because in the La(DTPA-BMA)−DTPA-BMA system, where the exchange takes place between the coordinated and free DTPA-BMA ligands, the investigation by NMR spectroscopy is simpler. It can be assumed that the mechanisms of the slow

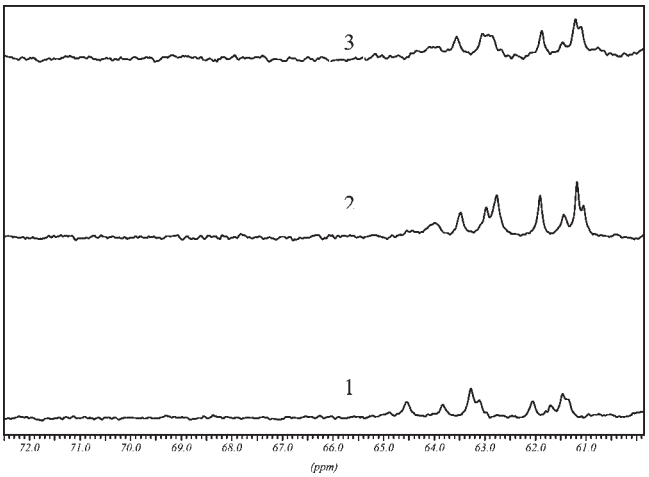

Figure 7. Part of the ^{13}C NMR spectra of 0.1 M La(DTPA-BMA) at 35 °C (1) and 50 °C (2) and that in the presence of a 0.4 M DTPA-BMA excess at 35 °C (3) (pH = 6.8, ethanol was used as internal standard).

ligand exchange reaction between La(DTPA-BMA) and DTPA-BMA are similar to those occurring between the La(DTPA-BMA) and TTHA, so in the presence of a DTPA-BMA excess, a second DTPA-BMA can be coordinated to the La^{3+}, and in the intermediate formed, the second ligand can slowly displace the other ligand. In the presence of a DTPA-BMA excess, the signals of the La(DTPA-BMA) broaden, which indicates the increase in the rate of the mobility of the donor atoms. In Figure 7, the signals at about 64 ppm belong to the acetate methylene carbons, so the broadening indicates the increase of the rate of the racemization of the terminal nitrogens.[28] The increase in the rate of decoordination of the terminal iminodiacetate methylamide groups may result in the increase of the ligand exchange processes in both the La(DTPA-BMA)−DTPA-BMA and Gd(DTPA-BMA)−TTHA systems.

The increase in the rates of the intramolecular rearrangements has been detected for the Eu(DTPA) in the presence of a DTPA excess by ^{1}H NMR spectroscopy. At low temperatures (0−5 °C), the proton spectra of Eu(DTPA) consist of 18 signals, which average to nine signals at high temperatures (70−100 °C).[26,27] In this temperature range, the increase of temperature results in the narrowing of the signals, which indicates the increase in the rates of the intramolecular rearrangements. In the spectrum of 0.1 M Eu(DTPA), recorded at 85 °C (Figure S1, Supporting Information), the half-widths of the signals observed at −9.13, −1.35, and 7.13 ppm are 135, 65, and 138 Hz, respectively. (The signals at −9.13 and −1.35 ppm belong to the terminal acetate methylene, while that at 7.13 ppm belongs to the ethylene protons.[27]) At 90 °C, the signals are narrower; their half-widths are 95, 51, and 114 Hz, respectively. In the presence of a 0.4 M DTPA excess, the signals of Eu(DTPA) at 85 °C are narrower than in the absence of DTPA; the widths are 118, 51, and 135 Hz, respectively. These data also indicate that in the presence of free DTPA, because of the encounter of the complexes and the free ligands, the rates of the intramolecular rearrangements in Eu(DTPA) are higher, and it can be assumed that the presence of TTHA in the solution results in a similar effect.

The rates of the ligand exchange reactions of Gd(DTPA) and Gd(BOPTA) vary according to minimum curves as a function of the pH (Figure 2). To understand the pH dependence of the k_p values, we have to know the concentration of the attacking $H_iTTHA^{(6-i)-}$ species at different pH values. To calculate the species distribution, the protonation constants of the TTHA have been determined in 0.15 M NaCl, because at such ionic strengths, there were no data in the literature. The protonation constants determined are as follows: log K_1^H = 9.99 (0.003); log K_2^H = 9.11 (0.003); log K_3^H = 6.00 (0.005); log K_4^H = 4.02 (0.006); log K_5^H = 2.76 (0.007); log K_6^H = 2.15 (0.01). The protonation constants obtained are lower than those determined in 0.1 M KCl, because the stability constants of the Na^+−TTHA complexes are expectedly higher than those of the K^+−TTHA species. The species distribution calculations show that in the pH range 6.5−11 the species H_3X^{3-}, H_2X^{4-}, HX^{5-}, and X^{6-} are present, but the concentration of the H_3X^{3-} species is low and negligible at pH > 7.5

The attack of the $H_iTTHA^{(6-i)-}$ species on the Gd^{3+} complexes is expected to be more efficient, if one of its IMDA groups is deprotonated. The infrared and ^{1}H NMR spectroscopic studies on the protonation scheme of TTHA indicate that the first three protons protonate three nitrogen atoms of the amine backbone. A deprotonated IMDA group is present in the species X^{6-}, HX^{5-}, and H_2X^{4-}, but the ratio of the H_3X^{3-} species, bearing a deprotonated IMDA group, is low.[30]

The inspection of the rate data presented in Figure 2 show that the k_p values do not vary considerably between pH 6.5 and 11. This finding suggests that the reactivities of the predominating TTHA species (H_2X^{4-}, HX^{5-}, and X^{6-}) do not differ considerably. The increase in the k_p values from pH 8.5 to 11 can be interpreted as the increase of the concentration of the TTHA species containing less protons (HX^{5-} and X^{6-}), which can more efficiently attack the Gd^{3+} complexes. By considering this statement, it is difficult to explain the decrease in the k_p values from pH 6.5 to 8.5. For interpreting these data, we have to assume that in the ternary intermediates formed by the encounter of the Gd^{3+} complexes and TTHA, the protons from the protonated H_3X^{3-} and H_2X^{4-} species can be transferred to the coordinated DTPA and BOPTA, which accelerates the displacement of these ligands by the TTHA. The realization of these reaction pathways means the general acid catalysis in these reactions is valid, which was observed earlier in the dissociation reactions of the amine and aminopolycarboxylate complexes.[31,32]

In the ligand exchange reactions of Gd(DTPA-BMA), the k_p values increase with the increase of pH (Figure 3). The stronger interaction between the Gd(DTPA-BMA) and $H_iTTHA^{(6-i)-}$ species and the higher rate of the intramolecular rearrangements in the complex make the ligand exchange reactions relatively rapid, so the validity of the general acid catalysis cannot be observed.

On the basis of these considerations, the rates of the ligand exchange reactions can be expressed as follows:

$$-\frac{d[GdL]}{dt} = (k_{H3X}[H_3X] + k_{H2X}[H_2X] + k_{HX}[HX] + k_X[X])[GdL] \quad (5)$$

where k_{H3X}, k_{H2X}, k_{HX}, and k_X are the rate constants characterizing the rates of the ligand exchange reactions taking place with the attack of the TTHA species H_3X^{3-}, H_2X^{4-}, HX^{5-}, and X^{6-}, respectively. By comparing eqs 3 and 5, the pseudo-first-order rate constants can be given by eq 6:

$$k_p = k_{H3X}[H_3X] + k_{H2X}[H_2X] + k_{HX}[HX] + k_X[X] \quad (6)$$

By fitting the k_p values (Figures 2 and 3) to eq 6, the k_{HiX} rate constants have been calculated and are presented in Table 1.

By the calculation of the rate constants, k_{HiX}, the concentration of the protonated H_iX species was figured out with the use of the protonation constants of TTHA. By calculating the k_{HiX} rate constants, characterizing the reaction of the Gd(DTPA-BMA), the lowest errors were obtained, when in eq 6 the second and third terms were considered only. (In the pH range 6.5−9, the concentration of the species H_3X^{3-} and X^{6-} is low, and so the reactions with the H_2X^{4-} and HX^{5-} species are more efficient.)

The rate constants k_X, k_{HX}, k_{H2X}, and k_{H3X}, obtained for the ligand exchange reactions of Gd(DTPA) and Gd(BOPTA), do not differ considerably, probably because in each of the X^{6-}, HX^{5-}, and H_2X^{4-} species, a free IMDA group is available;[30] however, the proton transfer from the H_3X^{3-} and H_2X^{4-} species to the GdL complex can take place efficiently. The k_{H2X} and k_{HX} rate constants determined for the reaction of Gd(DTPA-BMA) are considerably higher than those obtained for the Gd(DTPA) and Gd(BOPTA), which also indicates that the ligand exchange reactions of Gd(DTPA-BMA) occur significantly faster.

The pseudo-first-order rate constants, k_p, increase with the increase in the concentration of the endogenous citrate, phosphate, and carbonate ligands, as seen in Figures 4−6. The increase in the reaction rates may be the result of the formation of low stability ternary complexes between the endogenous ligands and the GdL species (first of all with CO_3^{2-} ions), which accelerates the intramolecular rearrangements and, so, the dissociation of the complexes.[33] The other possibility is the proton transfer from the HPO_4^{2-}, $H_2PO_4^-$, or HCO_3^- ions to the coordinated L ligand, which also increases the dissociation rate of the Gd^{3+} complexes; that is, the general acid catalysis is valid.[30,32] The increase in the reaction rates is significant for the citrate (and probably for the other oxycarboxylic and dicarboxylic acids, present in the blood plasma; Figure 4). In the presence of phosphate, the increase in the rates of the exchange reactions of Gd(DTPA) and Gd(BOPTA) is very low, while in the case of Gd(DTPA-BMA), the effect is larger (Figure 5). Carbonate ions increase the reaction rates only slightly, but since the concentration of the carbonate in the blood plasma is larger (25 mM), its effect is observable under biological conditions. The k_p values determined for Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) in the presence of 25 mM carbonate at a pH of 7.4 are larger by 37%, 20%, and 63%, respectively. The kinetics of the ligand exchange reactions in the presence of endogenous ligands was not studied in detail. The data obtained indicate that the rates of dissociation of the Gd^{3+} complexes used as MRI contrast agents are practically not affected by the presence of citrate and phosphate in the plasma, because of their low concentrations, but carbonate ions may slightly increase the rate of dissociation. However, phosphate ions can increase the rates of the dissociation of Gd^{3+} complexes in those experiments, where phosphate buffers of a high concentration are used to study the rates of the reactions. In such experiments, the presence of phosphate can result in an apparent increase in the reaction rates, in particular, for the reaction of the Gd(DTPA-BMA) complex.[34]

CONCLUSIONS

The Gd(DTPA), Gd(BOPTA), and Gd(DTPA-BMA) complexes, used as contrast agents in MRI, undergo exchange reactions with multidentate ligands, like TTHA. The dissociation of Gd^{3+} complexes through the ligand exchange reactions occurs significantly faster at a pH of 7.4 than the dissociation via the proton assisted pathway. The ligand exchange reactions take place with the direct attack of the $H_iTTHA^{(6-i)-}$ species on the Gd^{3+} complexes, through the formation of ternary intermediates. The formation of ternary intermediates is related to the intramolecular rearrangements, occurring in the Gd^{3+} complexes. The rate of the rearrangements is higher in Gd(DTPA-BMA) (it is indicated by NMR studies), and so the rates of the ligand exchange reactions of this complex are about 2 to 3 orders of magnitude higher than those of the Gd(DTPA) and Gd(BOPTA). The lower kinetic inertness of Gd(DTPA-BMA) is consistent with the experiences, showing that it dissociates faster than Gd(DTPA) or Gd(BOPTA), so the amount of retained Gd^{3+} in the living systems can be higher when Gd(DTPA-BMA) (Omniscan) is used as a MRI contrast agent.

The rates of the ligand exchange reactions of Gd(DTPA-BMA) increase with the increase in pH from 6.5 to 9, because the attack of the less protonated $H_iTTHA^{(6-i)-}$ species via the formation of the ternary intermediates is more efficient. The increase of the rates of the exchange reactions of Gd(DTPA) and Gd(BOPTA) from pH 8.5 to 11 can be interpreted similarly, but on the contrary, the increase in pH from 6.5 to 8.5 results in a decrease in the rates of the reactions. This unexpected trend has been interpreted by assuming the validity of the general acid catalysis, which has a lower effect at a higher pH. The proton(s) from the $H_iTTHA^{(6-i)-}$ species can be transferred to the coordinated DTPA or BOPTA, when the dissociation of these ligands from the Gd^{3+} complex is more probable.

The ligand exchange reactions take place more quickly in the presence of the endogenous citrate, phosphate, and carbonate ions at a pH of 7.4. The rates of the exchange reactions increase with the increase in the concentration of these ions, but the effect of the citrate and phosphate is very low at their physiological concentrations. Phosphate ions may have a significant effect on the reaction rates, particularly on that of the Gd(DTPA-BMA), when the kinetic studies are carried out in a phosphate buffer. The effect of the carbonate ions on the reaction rates is easily measurable, because of its higher physiological concentration, when the increase in the rates of the exchange reactions is the largest for Gd(DTPA-BMA).

ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectra of 0.1 M Eu(DTPA). This material is available free of charge via the Internet at http://pubs.acs.org.

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