The Rates of the Exchange Reactions between $[Gd(DTPA)]^{2-}$ and the Endogenous Ions Cu^{2+} and Zn^{2+}: A Kinetic Model for the Prediction of the In Vivo Stability of $[Gd(DTPA)]^{2-}$, Used as a Contrast Agent in Magnetic Resonance Imaging

L. Sarka,[a] L. Burai,[a][b] and E. Brücher*[a]

Abstract: The kinetic stability of the complex $[Gd(DTPA)]^{2-}$ (H_5DTPA = diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid), used as a contrast-enhancing agent in magnetic resonance imaging (MRI), is characterised by the rates of the exchange reactions that take place with the endogenous ions Cu^{2+} and Zn^{2+}. The reactions predominantly occur through the direct attack of Cu^{2+} and Zn^{2+} on the complex (rate constants are $0.93 \pm 0.17\,M^{-1}s^{-1}$ and $(5.6 \pm 0.4) \times 10^{-2}\,M^{-1}\,s^{-1}$, respectively). The proton-assisted dissociation of $[Gd(DTPA)]^{2-}$ is relatively slow ($k_1 = 0.58 \pm 0.22\,M^{-1}s^{-1}$), and under physiological conditions the release of Gd^{3+} predominantly occurs through the reactions of the complex with the Cu^{2+} and Zn^{2+} ions. To interpret the rate data, the rate-controlling role of a dinuclear intermediate was assumed in which a glycinate fragment of DTPA is coordinated to Cu^{2+} or Zn^{2+}. In the exchange reactions between $[Gd(DTPA)]^{2-}$ and Eu^{3+}, smaller amounts of Cu^{2+} and Zn^{2+} and their complexes with the amino acids glycine and cysteine have a catalytic effect. In a model of the fate of the complex in the body fluids, the excretion and the "dissociation" of $[Gd(DTPA)]^{2-}$ are regarded as parallel first-order processes, and by 10 h after the intravenous administration the ratio of the amounts of "dissociated" and excreted $[Gd(DTPA)]^{2-}$ is constant. From about this time, 1.71 % of the injected dose of $[Gd(DTPA)]^{2-}$ is "dissociated". The results of equilibrium calculations indicate that the Gd^{3+} released from the complex is in the form of Gd^{3+}-citrate.

Keywords: chelates · contrast agents · exchange reactions · gadolinium · kinetics

Introduction

The complex $[Gd(DTPA)]^{2-}$ (H_5DTPA = diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid) is the first contrast agent clinically used to increase the proton relaxation rates in magnetic resonance imaging (MRI). As a hydrophilic compound, the intravenous administration of $[Gd(DTPA)]^{2-}$ is followed by its distribution into the extracellular and intravascular spaces, and it has a rapid renal clearance.[1, 2] The half-time of excretion in the case of rats is about 0.3 h, while for humans it is approximately 1.6 h.[1, 3] Since the dissociation of $[Gd(DTPA)]^{2-}$ results in the formation of highly toxic free Gd^{3+} and DTPA, both the high stability constant (K_{GdL}) and the low rate of dissociation (at physiological pH) therefore play important roles in the safe use of the complex.

In spite of the enormous number of clinical applications, there are some concerns about the possible in vivo dissociation of $[Gd(DTPA)]^{2-}$. Some experiments indicated that the excretion of the complex from the body of mice was not complete.[4–6] It was also observed that after an MRI investigation, the biodistribution of radioactive ^{67}Ga was unusual in a patient; this was interpreted as the presence of free Gd^{3+} which had resulted from the dissociation of $[Gd(DTPA)]^{2-}$.[7] The in vivo dissociation of the complex may take place spontaneously through reaction with protons or endogenous metal ions such as Cu^{2+} and Zn^{2+} (Ca^{2+} does not compete with Gd^{3+}, since $K_{GdL} >> K_{CaL}$). In an in vitro study, Tweedle et al. found that in a phosphate buffer, at relatively high concentrations of $[Gd(DTPA)]^{2-}$ and Cu^{2+} or Zn^{2+}, a significant amount of Gd^{3+} was displaced by Cu^{2+} or Zn^{2+}.[8]

To understand the in vivo fate of $[Gd(DTPA)]^{2-}$ and to interpret its incomplete excretion, some species distribution calculations were carried out on the basis of a plasma model.[4, 9] The calculations indicated the release of about 1×10^{-3} mM Gd^{3+} in an equilibrium solution at a dose of 0.1 mmol kg^{-1} of $[Gd(DTPA)]^{2-}$.[4] However, because of the

[a] Prof. E. Brücher, L. Sarka, L. Burai
Department of Inorganic and Analytical Chemistry
lajos Kossuth University, Debrecen 4010 (Hungary)
Fax: (+36) 52-489667
E-mail: ebrucher@tigris.klte.hu

[b] L. Burai
Current address: Institute of Inorganic and Analytical Chemistry, University of Lausanne
BCH, 1015 Lausanne (Switzerland)

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slow dissociation and rapid excretion of the complex, the solution excreted is far from equilibrium.

To estimate the amount of Gd^{3+} released, the rate of dissociation of $[Gd(DTPA)]^{2-}$ should be known. In a study of the tissue distribution, Wedeking et al. found a correlation between the rate of dissociation measured in 0.1M HCl and the long-term deposition of Gd^{3+} in the whole body.[5] For the exchange reactions between several $Ln(DTPA)]^{2-}$ complexes (Ln= La, Ce, Nd, Ho, Lu, and Y) and Ln^{3+} ions, the rate laws are known,[10–13] but the reactions of $[Gd(DTPA)]^{2-}$ with Ln^{3+}, Cu^{2+} or Zn^{2+} ions have not been studied.

To obtain more information on the role of the kinetic stability of $[Gd(DTPA)]^{2-}$ in biological systems, we have studied the rates of the exchange reactions that take place between the complex and the endogenous ions Cu^{2+} and Zn^{2+}. Although the concentration of Cu^{2+} in the plasma is very low, it has been found to be kinetically very active in the exchange reactions of the aminopolycarboxylate complexes of transition metals.[14] For comparison, the kinetics of exchange reactions between $[Gd(DTPA)]^{2-}$ and Eu^{3+} were also studied, and a simplified plasma model was used to carry out species distribution calculations.[15]

Results and Discussion

The species distribution calculations predict the release of a small amount of Gd^{3+} into the plasma at equilibrium.[4, 9] In the displacement of Gd^{3+}, the most important role is played by the most abundant Zn^{2+}, but in the equilibrium calculations only the formation of $[Zn(DTPA)]^{3-}$ was considered [Eq. (1)].[4, 9]

$$GdL^{2-}+Zn^{2+} \rightleftharpoons ZnL^{3-}+Gd^{3+} \quad (1)$$

The equilibrium constant for this reaction is very low, $K_e = 6.76 \times 10^{-5}$, since the stability constants are $\log K_{GdL} = 22.46$ and $\log K_{ZnL} = 18.29$.[16] It is known, however, that Zn^{2+} forms a dinuclear complex, $[Zn_2(DTPA)]^-$, and the formation equilibrium [Eq. (2)], is characterized by the stability constant $\log K_{Zn2L} = 4.48$.[16] Thus, the equilibrium constant for the Equation (3) is $K_e = 2.04$, this predicts the formation of some $[Zn_2(DTPA)]^-$, even in the presence of comparable amounts of $[Gd(DTPA)]^{2-}$ and Zn^{2+}.

$$ZnL^{3-}+Zn^{2+} \rightleftharpoons Zn_2L^- \quad (2)$$

$$GdL^{2-}+2Zn^{2+} \rightleftharpoons Zn_2L+Gd^{3+} \quad (3)$$

With the use of a simplified equilibrium model, in which only the competition between Zn^{2+} and Gd^{3+} was taken into account [Eqs. (1) and (3)], we carried out a species distribution calculation. The model involved only those ligands which maybe of some importance in the formation of complexes with Gd^{3+} (0.1 mM) and Zn^{2+} (0.05 mM). The ligands used were as follows: DTPA (0.1 mM), gly (1.0 mM), asp (0.07 mM), hys (0.08 mM), cys (0.04 mM), succinate (0.04 mM), lactate (2.0 mM), and citrate (0.1 mM)[9, 17] (gly represents all the simple α-amino acids). The calculations indicated the presence of Gd^{3+} in the forms of $[Gd(DTPA)]^{2-}$ (90.7 %) and [Gd(cit)] (9.2 %). The complexes formed with Zn^{2+} in larger amounts are $[Zn_2(DTPA)]^{2-}$ (19.1 %), [Zn(hys)] (17.8 %), [Zn(cys)] (45.3 %) and [Zn(cit)] (12.4 %). These equilibrium data also indicate the partial displacement of Gd^{3+} by Zn^{2+}, when the formation of [Gd(cit)] is particularly important.

To characterize the kinetic stability of $[Gd(DTPA)]^{2-}$, the rates of the exchange reactions [Eqs. (1) and (3)] were studied with the use of Zn^{2+}, Cu^{2+}, or Eu^{3+} as exchanging metal ions. In the presence of excess of the exchanging ions the rate of the reactions can be expressed as shown in Equation (4). The

$$-d[GdL]_t/dt = k_{obs}[GdL]_t \quad (4)$$

value k_{obs} is a pseudo-first-order rate constant, and $[GdL]_t$ is the total concentration of the complex. The rates of the exchange reactions were studied at different concentrations of the exchanging ions at various pH values. The rate constant k_{obs} obtained are presented in Figures 1–3.

The k_{obs} values exhibit a significant difference in the reactions that take place with the participation of Eu^{3+}, Zn^{2+}, and Cu^{2+}. The exchange proceeds much faster with Cu^{2+} or Zn^{2+} than with Eu^{3+}. However, the reaction between the complex and Eu^{3+} strongly depends on the pH of the solutions, while the reactions with Cu^{2+} and Zn^{2+} are practically independent of pH. The trend observed in the k_{obs} values in Figure 1 is quite surprising, since at higher H^+ concentrations increase of the concentration of Eu^{3+} results in a decrease in k_{obs}. Similar findings were observed in the exchange reactions of some other $[Ln(DTPA)]^{2-}$ complexes

Abstract in Hungarian: *A mágneses rezonanciás képalkotás során kontrasztnövelő anyagként használt $[Gd(DTPA)]^{2-}$ komplex kinetikai stabilitását az endogén Cu^{2+} és Zn^{2+} ionokkal lefolyó cserereakcióinak a sebessége határozza meg. A reakciók döntően a Cu^{2+} és Zn^{2+} ionoknak a komplexen történő közvetlen támadásával folynak le. A sebességi állandók értéke $0{,}93 \pm 0{,}17\,M^{-1}s^{-1}$ és $(5{,}6 \pm 0{,}4) \times 10^{-2}\,M^{-1}s^{-1}$. A $[Gd(DTPA)]^{2-}$ protonkatalizált disszociációjának sebessége pH = 7 körül ezekhez viszonyítva elhanyagolható (a sebességi állandó $0{,}58 \pm 0{,}22\,M^{-1}s^{-1}$), így a komplex szabad Gd^{3+} képződésével járó reakcióiban a Cu^{2+} és Zn^{2+} ionokkal lefolyó reakcióknak van döntő szerepe. A Cu^{2+} és Zn^{2+} ionokkal lefolyó cserereakciók közben kétmagvú komplexek képződnek, melyekben a DTPA egy glicinát csoportja koordinálódik a Cu^{2+} és Zn^{2+} ionokhoz. A $[Gd(DTPA)]^{2-}$ és az Eu^{3+} közötti cserereakciót a Cu^{2+} vagy Zn^{2+} ionok katalizálják, a katalitikus hatás glicin illetve cisztein jelenlétében is érvényesül. A testfolyadékokba injektált $[Gd(DTPA)]^{2-}$ sorsának modellezésére a komplex szervezetből történo kiürülését és a kis koncentrációban jelenlévő Cu^{2+} és Zn^{2+} ionokkal lefolyó cserereakcióit kinetikailag elsőrendu reakcióként kezeljük. Az injektálást követő 10 óra eltelte után a komplex vesén keresztül történő kiürülését jellemző sebességi állandó és az endogén fémekkel lejátszódó reakciók sebességi állandói összegének viszonya állandó. Ekkor az injektált komplex 1,71 %-a lép reakcióba, és így a Gd^{3+} 1,71 %-a marad vissza a szervezetben. Az egyensúlyi számítások szerint a $[Gd(DTPA)]^{2-}$ komplexből a cserereakciók révén szabaddá váló Gd^{3+} mint Gd^{3+}-citrát van jelen a testfolyadékokban.*

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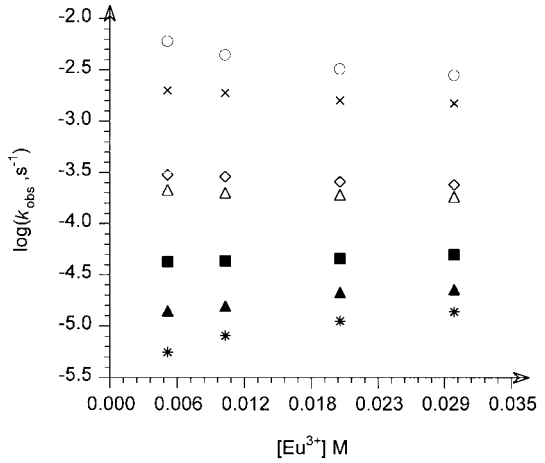

Figure 1. Plots of log k_{obs} versus Eu^{3+} concentration for the reaction between [Gd(DTPA)]$^{2-}$ and Eu^{3+}. Concentration of [Gd(DTPA)]$^{2-}$ = 5×10^{-4} M; pH = 3.67 (○), 3.86 (×), 4.30 (◇), 4.33 (△), 4.75 (■), 5.08 (▲), 5.38 (*) (25 °C, 1.0 M KCl).

with Eu^{3+}[13] and also in the reaction between [Cd(CDTA)]$^{2-}$ and Pb^{2+}[18] (H_4CDTA = *trans*-diaminocyclohexane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid). This unusual phenomena can be interpreted by considering the contribution of every single reaction that takes place along the different pathways. As may be seen in Figure 1, k_{obs} increases significantly with an increase in the H^+ concentration, particularly at lower [Eu^{3+}]. The dependence of k_{obs} on the H^+ concentration can be expressed by Equation (5) which indicates that the exchange can take

$$k_{obs} = k_0 + k'[H^+] + k''[H^+]^2 \quad (5)$$

place by proton-independent and proton-assisted pathways, presumably with the formation and dissociation of monoprotonated and diprotonated complexes. However, both k_0 and k' increase with an increase in the concentration of Eu^{3+}, which is characteristic of direct attack of Eu^{3+} on the complex, by the formation of dinuclear complexes. The formation of dinuclear [Ln(DTPA)]Eu^+ complexes was assumed earlier,[13] and the complex [Nd_2(DTPA)]$^+$ was detected by ^{1}H NMR spectroscopy.[19] The concentration of dinuclear species increases with an increase in the concentration of Eu^{3+}, when the concentration of the monoprotonated species [Gd(HDTPA)]$^-$ decreases ([Eu^{3+}] ≫ [H^+]). As a result, the rate of proton-assisted dissociation of the complex also decreases, which explains the unusual dependence of the exchange rate on the concentration of Eu^{3+}.

By taking all the possible reaction pathways into account, the rate of exchange between [Gd(DTPA)]$^{2-}$ and the exchanging metal ions (M^{2+}) can be expressed as shown in Equation (6), where [GdHL] and [GdLM] are the concen-

$$-d[GdL]_t/dt = k_{GdHL}[GdHL] + k^H_{GdHL}[GdHL][H^+] + k_{GdLM}[GdLM] + k^M_{GdHL}[GdHL][M] \quad (6)$$

trations of the protonated and dinuclear complexes, respectively. If we take into account the total concentration of the complex ($[GdL]_t = [GdL] + [GdHL] + [GdLM]$), the equations defining the stability constants of the protonated and dinuclear complexes ($K_{GdHL} = [GdHL]/[GdL][H^+]$ and $K_{GdLM} = [GdLM]/[GdL][M]$) and Equation (4), the rate constant k_{obs} can be expressed as follows in Equation (7).[13] The

$$k_{obs} = \{k_1[H^+] + k_2[H^+]^2 + k^M_3[M] + k^M_4[M][H^+]\}/\{1 + K_{GdHL}[H^+] + K_{GdLM}[M]\} \quad (7)$$

rate constants, k_1 ($= k_{GdHL}K_{GdHL}$) and k_2 ($= k^H_{GdHL}K_{GdHL}$) are characteristic of the reactions that occur by dissociation of the monoprotonated and diprotonated complexes (the reactions between the free ligand and Eu^{3+}, Cu^{2+} or Zn^{2+} are very fast). The reactions which involve direct attack of the exchanging metal ion on the complex and the protonated complex are characterised by the rate constants k^M_3 ($= k_{GdLM}K_{GdLM}$) and k^M_4 ($= k^M_{GdHL}\ K_{GdHL}$), respectively.

The protonation constant K_{GdHL} was determined by pH-potentiometric titration of [Gd(DTPA)]$^{2-}$ with HCl, and the value $K_{GdHL} = 100 \pm 14$ was obtained. With this K_{GdHL} value, the term $K_{GdHL}[H^+]$ in the denominator of Equation (7) can be neglected, since $K_{GdHL}[H^+] << 1$ in the pH range investigated.

The k_{obs} values obtained for the reaction between the complex and Eu^{3+} were fitted to Equation (7), the rate constants calculated are as follows: $k_1 = 0.58 \pm 0.22\ M^{-1}s^{-1}$; $k_2 = (9.7 \pm 0.6) \times 10^4\ M^{-2}s^{-1}$; $k^{Eu}_3 = (4.9 \pm 0.8) \times 10^{-4}\ M^{-1}s^{-1}$; $k^{Eu}_4 = 40 \pm 20\ M^{-2}s^{-1}$ and $K_{GdLEu} = 20 \pm 5$.

The rates of the exchange reactions between [Gd(DTPA)]$^{2-}$ and Cu^{2+} or Zn^{2+} strongly increase with increase of the concentration of the exchanging metal ions. However, the k_{obs} values are practically independent of pH, as is seen in Figures 2 and 3. These findings indicate that the

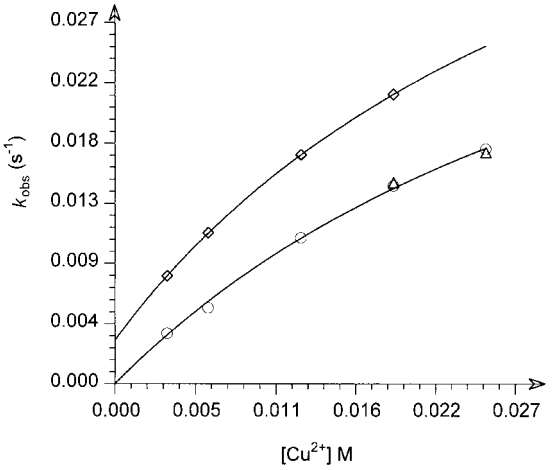

Figure 2. Plots of k_{obs} versus Cu^{2+} concentration for the reaction between [Gd(DTPA)]$^{2-}$ and Cu^{2+}. Concentration of [Gd(DTPA)]$^{2-}$ = 5×10^{-4} M; pH 5.20 (○), 4.92 (△) (25 °C, 1.0 M KCl); pH 5.21 (◇) (37 °C, 1.0 M KCl).

exchanges predominantly occur through direct attack of the Cu^{2+} and Zn^{2+} ions on the complex, and the reactions that take place through the dissociation of protonated complexes [Eq. (6)] are practically negligible in the pH range investigated (and also at higher pH). For a description of the dependence of k_{obs} on the concentration of Cu^{2+} or Zn^{2+}, Equation (7) can be used in the form shown in Equation (8), where k_0 is the rate of proton-assisted dissociation of the complex ($k_0 = k_1[H^+] + k_2[H^+]^2$). The values of k_0, k^M_3 and

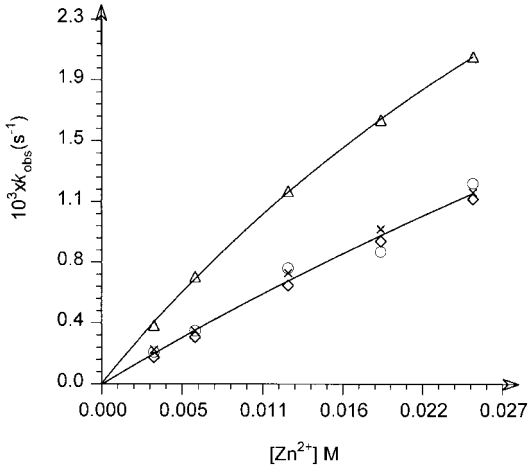

Figure 3. Plots of k_{obs} versus Zn^{2+} concentration for the reaction between $[Gd(DTPA)]^{2-}$ and Zn^{2+}. Concentration of $[Gd(DTPA)]^{2-} = 5 \times 10^{-4}$ M; pH 4.62 (○), 4.80 (×), 5.51 (◇) (25 °C, 1.0 M KCl); pH 5.21 (△) (37 °C, 1.0 M KCl).

K_{GdLM} were calculated by fitting the k_{obs} data shown in Figures 2 and 3 to Equation (8). The rate constants k_3^M and

$$k_{obs} = \{k_0 + k_3^M[M]\}/\{1 + K_{GdLM}[M]\} \quad (8)$$

the stability constants K_{GdLM} obtained for the exchange reactions are compared in Table 1. The value of k_0 could be calculated only with appreciable error, since $k_0 \ll k_3^M[M]$ in the numerator of Equation (8).

Table 1. The rate constants k_3^M and the stability constants K_{GdLM} of dinuclear complexes.

Exchanging metal ion	25 °C, 1.0 M KCl		37 °C, 1.0 M KCl	
	k_3^M ($M^{-1}s^{-1}$)	K_{GdLM}	k_3^M ($M^{-1}s^{-1}$)	K_{GdLM}
Eu^{3+}	$(4.9 \pm 0.8) \times 10^{-4}$	20 ± 5	–	–
Cu^{2+}	0.93 ± 0.17	13 ± 9	1.59 ± 0.03	28 ± 1
Zn^{2+}	$(5.6 \pm 0.4) \times 10^{-2}$	7 ± 3.7	0.11 ± 0.01	13 ± 5.6

However, the value of k_0 is known from the study of the exchange reaction between $[Gd(DTPA)]^{2-}$ and Eu^{3+}. A comparison of the rate constants k_3^M indicates that endogenous Cu^{2+} and Zn^{2+} are much more efficient in the substitution of Gd^{3+} from the complex than Eu^{3+}. During these exchange reactions, a dinuclear intermediate is formed, in which the functional groups of the ligand are gradually transferred from the Gd^{3+} to the attacking metal ion, this has already been assumed for the exchange reactions of the aminopolycarboxylate complexes of 3d transitional metals.[20] The stability constants of the dinuclear complexes formed are relatively low (Table 1). The stability constant values suggest that, in the dinuclear complexes formed in the equilibrium reactions, only a carboxylate group is coordinated to the exchanging metal ion. However, for an interpretation of the higher kinetic activities of Cu^{2+} and Zn^{2+}, it must be assumed that during the gradual transfer of the ligand the dinuclear reaction intermediate, which is highly important in the rate-determining step, has a higher stability constant for Cu^{2+} or Zn^{2+} than for Eu^{3+}. Such an intermediate can be formed when a glycinate moiety of DTPA is coordinated to the attacking metal ion. The stability constants of the glycinate (X) complexes of Cu^{2+} and Zn^{2+} ($\log K_{CuX} = 8.13$ and $\log K_{ZnX} = 4.96$) are significantly higher than that of Eu^{3+} ($\log K_{EuX} = 3.5$).[21] Since the Cu^{2+}–N and Zn^{2+}–N bonds are stronger than the Eu^{3+}–N bond, the transfer of additional functional groups of the ligand proceeds with a higher probability in the Cu^{2+}–glycinate and Zn^{2+}–glycinate-containing intermediates than in an intermediate with a Eu^{3+}–glycinate fragment.

Since the reaction between $[Gd(DTPA)]^{2-}$ and Cu^{2+} or Zn^{2+} proceeds much faster than the proton-assisted dissociation of the complex, if $[Cu(DTPA)]^{3-}$ and $[Zn(DTPA)]^{3-}$ are not inert, the exchange reactions of $[Gd(DTPA)]^{2-}$ can be expected to occur faster in the presence of Cu^{2+} or Zn^{2+}. Such a phenomenon has been observed in the reaction between $[Ni(EDTA)]^{2-}$ and Zn^{2+}, which occurs faster on Cu^{2+} catalysis, because Cu^{2+} ions are about 6000 times more efficient than Zn^{2+} ions in the displacement of Ni^{2+} in $[Ni(EDTA)]^{2-}$.[14]

To obtain information on the possible catalytic effects of Cu^{2+} and Zn^{2+} in the dissociation of $[Gd(DTPA)]^{2-}$, the rate of the exchange reaction between $[Gd(DTPA)]^{2-}$ (5×10^{-4} M) and Eu^{3+} (5×10^{-3} M) was studied in the presence of various amounts of Cu^{2+} or Zn^{2+}. In order to approach the in vivo conditions, where exchangeable Cu^{2+} and Zn^{2+} are present in the form of complexes with amino acids,[17] some of the samples studied also contained glycine or cysteine. The pseudo-first-order rate constants obtained in these experiments are presented in Table 2. The rate data shown in Table 2

Table 2. Pseudo-first order rate constants k_{obs} characterizing the reaction between $[Gd(DTPA)]^{2-}$ (5×10^{-4} M) and Eu^{3+} (5×10^{-3} M) in the absence and presence of Cu^{2+}, Zn^{2+}, glycine, and cysteine (pH = 5.10, 25 °C, 1.0 M KCl).

$10^5 \times [Cu^{2+}]$ M	$10^3 \times$ [gly] M	$10^5 \times k_{obs}$ (s^{-1})
0	0	1.1
0.62	0	1.5
1.25	0	1.6
2.5	0	2.1
2.5	1.0	1.9
$10^4 \times [Zn^{2+}]$ M	$10^3 \times$ [cys] M	
0	0	1.1
1.0	0	1.2
2.5	0	1.9
5.0	0	5.0
5.0	1.0	4.9
5.0	2.0	4.9
10.0	0	9.5
10.0	2.0	8.5
10.0	4.0	7.2

indicate that the exchange reaction between $[Gd(DTPA)]^{2-}$ and Eu^{3+} proceeds faster in the presence of Cu^{2+} or Zn^{2+}. The catalytic effect of Cu^{2+} is particularly high, since the rate constant doubles when the amount of Cu^{2+} is only 5% of that of $[Gd(DTPA)]^{2-}$. Surprisingly, the presence of glycine or cysteine, that is the formation of Cu^{2+}–glycine and Zn^{2+}–cysteine complexes, has practically no influence on the rate-increasing effects of Cu^{2+} and Zn^{2+}. (Complex formation between Eu^{3+} and glycine or cysteine is not significant at pH 5.[22])

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The experimental data presented in Figures 2 and 3 and Table 2 indicate that Cu^{2+} and Zn^{2+} may substitute Gd^{3+} in $[Gd(DTPA)]^{2-}$. The concentrations of exchangeable Cu^{2+} and Zn^{2+} in the plasma model were estimated to be 1×10^{-6} M and 1×10^{-5} M, respectively.[17] With these concentrations, the pseudo-first-order rate constants k_{obs} were calculated $k_{obs} = k_3^M[M^{2+}]$, since the other terms in Equation (8) can be neglected. The k_{obs} values obtained for the reactions with Cu^{2+} and Zn^{2+} at 25 °C (and 37 °C) are 9.3×10^{-7} s^{-1} (15.9×10^{-7} s^{-1}) and 5.6×10^{-7} s^{-1} (11.0×10^{-7} s^{-1}), respectively. At physiological pH (pH 7.4), the contribution of the proton-assisted dissociation of $[Gd(DTPA)]^{2-}$ [$k_{obs} = k_1[H^+]$ from Eq. (7)] to the exchange rate is only $k_{obs} = 2.31 \times 10^{-8}$ s^{-1} (25 °C). These data indicate that, in the exchange reactions of $[Gd(DTPA)]^{2-}$ that occur in the body fluids, the reactions with Cu^{2+} and Zn^{2+} are much more important than the proton-assisted dissociation of the complex.

With the use of the sum of the k_{obs} values ($k_{obs}^s = 15.1 \times 10^{-7}$ s^{-1} at 25 °C and $k_{obs}^s = 27.1 \times 10^{-7}$ s^{-1} at 37 °C), it is possible to calculate the concentration of $[Gd(DTPA)]^{2-}$ that "dissociates" during a given period of time after intravenous administration (the "dissociation" of $[Gd(DTPA)]^{2-}$ is the result of the direct attack of Cu^{2+} and Zn^{2+} on the complex). For the calculation, an average dose of 0.1 mmol kg^{-1} was taken into account. The complex is distributed into the extracellular spaces and 3 min after the injection its concentration in the plasma was found to be 0.66 mM (31 % of the total dose).[3] If the excretion of $[Gd(DTPA)]^{2-}$ from the body is not taken into account, the amount of the decayed complex 24 h after the injection at 25 °C would be 12.2 % of the dose (since there are no data for the average concentration of $[Gd(DTPA)]^{2-}$ in the extracellular space, for the calculation we used the total dose). However, the injected $[Gd(DTPA)]^{2-}$ is rapidly eliminated from the body. The half-time of excretion was found to be 1.6 h[3] (at 37 °C) and it can be characterized by a first-order rate constant of $k_e = 0.433$ h^{-1}. The excretion of $[Gd(DTPA)]^{2-}$ from the body and the "dissociation" of the complex can be regarded as parallel first-order reactions. For such reactions, the ratio of the concentrations of the products is equal to the ratio of the first-order rate constants.[23] In the case of the "dissociation" and elimination of $[Gd(DTPA)]^{2-}$, the ratio of the amounts of the "dissociated" and eliminated complex is equal to $k_{obs}^s/k_e = 1.74 \times 10^{-2}$ (at 37 °C). This result shows that the ratio of the amounts of "dissociated" and excreted complex at any time is constant. Since $k_{obs}^s \ll k_e$, after 5–6 half-times of excretion ($t_{1/2} = 1.6$ h), the amount of $[Gd(DTPA)]^{2-}$ "dissociated" is 1.71 % of the injected dose.

Comparison of the rate data obtained here for the "dissociation" of $[Gd(DTPA)]^{2-}$ and the results found by Weinmann et al. for the rate of elimination of the complex from the human body[3] allows the conclusion that, about 10 h after intravenous administration, the amount of Gd^{3+} released is about 1.71 % of the injected dose. This result is much lower than the error limit in the results of the pharmacokinetic study of $[Gd(DTPA)]^{2-}$, in which it was found that within 24 h 91(±13)% of the injected dose left the human body.[3] However, our result is comparable with the results of Wedeking et al.[5] and Harrison et al.,[6] who found the whole body retention of Gd^{3+} from $[Gd(DTPA)]^{2-}$ in mice 24 h after the injection was about 1.3–1.4 %. In the equilibrium calculations based on the plasma model, the amount of Gd^{3+} released was underestimated, because the formation of the dinuclear $[Zn_2(DTPA)]$ was not taken into account.[4, 9]

The small amount of Gd^{3+} released is probably in the form of Gd^{3+}–citrate, which can also be partly excreted. The ligand DTPA formed on the "dissociation" of $[Gd(DTPA)]^{2-}$ is presumably in the form of the species $[Cu(DTPA)]^{3-}$, $[Zn(DTPA)]^{3-}$ (or a dinuclear species) and $[Ca(DTPA)]^{3-}$. Owing to the very low concentrations of exchangeable Cu^{2+} and Zn^{2+} in the body fluids, the rates of the exchange reactions between $[Gd(DTPA)]^{2-}$ and these endogenous ions do not increase significantly and $[Gd(DTPA)]^{2-}$ can be used as a safe contrast agent in MRI.

Conclusions

The exchange reactions between $[Gd(DTPA)]^{2-}$ and the endogenous Cu^{2+} and Zn^{2+} ions occur predominantly through the direct attack of the metal ions on the complex. Copper(II) and zinc(II) ions are much more efficient in displacing Gd^{3+} from the complex than lanthanide(III) ions such as Eu^{3+}. Thus Zn^{2+} and Cu^{2+} exhibit a catalytic effect in the exchange reaction between $[Gd(DTPA)]^{2-}$ and Eu^{3+}.

The in vitro studies predict that under physiological conditions, the "dissociation" of $[Gd(DTPA)]^{2-}$ occurs by reactions with the exchangeable Cu^{2+} and Zn^{2+} in body fluids, and the proton-assisted dissociation of the complex, which was assumed to be the most important pathway, plays a negligible role.

The excretion of $[Gd(DTPA)]^{2-}$ from the body and the decay of the complex, both of which occur with the participation of Cu^{2+} and Zn^{2+} ions, can be regarded as parallel first-order processes. This suggests, a constant ratio for the amounts of "dissociated" and excreted $[Gd(DTPA)]^{2-}$. With the use of the kinetic data presented here, the amount of the complex "dissociated" in body fluids can be calculated at any time after the intravenous administration.

Experimental Section

The chemicals used in the experiments were of the highest analytical grade. The $GdCl_3$ solution was prepared from Gd_2O_3 of 99.9 % purity (Fluka) by dissolution in excess HCl, which was evaporated off. The concentrations of the metal ions in the $GdCl_3$, $EuCl_3$, $CuCl_2$, and $ZnCl_2$ solutions were determined by complexometric titration with standardized Na_2H_2EDTA solution. H_5DTPA (Fluka) was purified by recrystallization from water. The $K_2[Gd(DTPA)]$ solution was prepared by mixing equivalent amounts of $GdCl_3$ and K_3H_2DTPA solutions and the pH was set at 5–5.5 with KOH.

The exchange reactions between $[Gd(DTPA)]^{2-}$ and Eu^{3+} or Cu^{2+} were studied with a Cary 1E spectrophotometer at 260 and 300 nm, respectively. The reaction of $[Gd(DTPA)]^{2-}$ with Zn^{2+} was monitored by measuring the longitudinal relaxation rates ($1/T_1$) of water protons at 9 MHz, with an MS4 relaxometer (Institut Jozef Stefan, Ljubljana), by the inversion recovery method. The relaxation rates measured in 1 mM Gd^{3+} aqueous and $[Gd(DTPA)]^{2-}$ solutions were 18.2 mM^{-1}s^{-1} and 7.7 mM^{-1}s^{-1} at 25 °C and 15.6 mM^{-1}s^{-1} and 5.3 mM^{-1}s^{-1} at 37 °C, respectively. The rates of reactions were studied in thermostated cells and sample holders at 25 °C and 37 °C. The pseudo-first-order rate constant (k_{obs}) values were determined with the use of the Equation (9), where A_0, A_t, and A_e are the measured absorbance

$$A_t = A_e + (A_0 - A_e)e^{-k_{obs}t} \quad (9)$$

or relaxation rate values at the start, at time t and at equilibrium of the reaction. The concentration of $[Gd(DTPA)]^{2-}$ was 5×10^{-4} M, while the concentration of the exchanging metal ions was varied between 3×10^{-3} M and 3×10^{-2} M at each pH applied. The reaction does not go to completion even in the presence of the largest Zn^{2+} excess. In order to avoid the difficulties with the treatment of a complicated reversible reaction ($[Zn(DTPA)]^{3-}$ and $[Zn_2(DTPA)]^{-}$ are formed), for calculation of k_{obs} values we used the rate data obtained until the conversion in the reaction was about 50–60% and the reformation of $[Gd(DTPA)]^{2-}$ was not significant. To maintain a constant pH, a H_3BO_3 (0.01M) (−)-mannitol (0.04M) buffer was used. The ionic strength of the solutions was kept constant (1.0M KCl). For the pH measurements, a Radiometer pHM 85 pH-meter was used with a combination electrode, PHC 2406. The equilibrium calculations were carried out with the computer program PSEQUAD.[15]

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