CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc
Europe

DOI: 10.1002/chem.201200265

Lower Ligand Denticity Leading to Improved Thermodynamic and Kinetic Stability of the Gd^{3+} Complex: The Strange Case of OBETA**

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Polyaminocarboxylic ligands are widely employed in several applications involving the formation of stable metal ion complexes.[1] Extremely high stability is required for complexes used in biomedical studies and clinical applications in which paramagnetic or radioactive metal ions could be released by dissociation processes exerting toxic effects.[2] The search for efficient ligands for the chelation of trivalent lanthanide ions (Ln^{3+}) is particularly active since complexes of these ions are used as probes for several diagnostic or therapeutic techniques. Well-known examples include Gd^{3+} for magnetic resonance imaging (MRI),[3] Eu^{3+} and Tb^{3+} for luminescence assays[4] and radioactive isotopes ($^{153}Sm^{3+}$, $^{166}Ho^{3+}$, $^{177}Lu^{3+}$) for therapeutic applications.[5] Paramagnetic Gd^{3+} complexes represent a real challenge in this task because high stabilities are usually obtained with octa- or nonadentate ligands. The most common coordination numbers of Gd^{3+} complexes in solution are 8 and 9, and thus, such ligands reduce the space available for the coordination of one or more water molecule(s) ($q=1$, 2) required to attain high relaxivities. Ligands with lower denticities often result in higher relaxivities and reduced stabilities.[3a] A compromise is normally attained with octadentate DOTA- or DTPA-like complexes, although we recently demonstrated that properly designed heptadentate ligands can combine excellent stability profiles[6] with superior relaxivities for the corresponding Gd^{3+} complexes.[7]

During an undergoing screening of heptadentate ligands directed to the formation of Gd^{3+} complexes for MRI applications, we became interested in the acyclic ligand OBETA (2,2′-oxybis(ethylamine)-*N*,*N*,*N*′,*N*′-tetraacetic acid). OBETA (Figure 1) can be considered as the lower homologue of the better known congener EGTA[8,9] and its first preparation was described in the patent literature in the early 1950s.[8] Later, scattered studies on its complexation properties towards different metal ions appeared, including a polarographic study on Ln^{3+} complexes evidencing a significant affinity topping in the middle of the lanthanide series.[10] Specifically, the complex stability was shown to be highest with Gd^{3+}, and most surprisingly was even higher than that of the corresponding complex with the octadentate analogue EGTA. This remarkable trend could be extremely useful for the development of highly efficient Gd^{3+}-based MRI contrast agents (CAs) in view of the above-cited considerations on denticity and the number of coordinated water molecules. Nevertheless, for a safe in vivo use, the kinetic inertness of Gd^{3+} complexes is known to be equally imperative; thermodynamic and kinetic stability need to coexist in order to avoid unwanted toxicity effects.

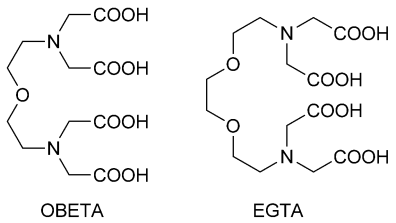

Figure 1. The polyaminocarboxylic ligand OBETA and its congener EGTA.

We were then prompted to gain a deeper insight into the chelating properties of the ligand OBETA and the relaxometric behavior of its Gd^{3+} complex. In this work we report a careful redetermination of the thermodynamic properties of selected Ln^{3+} complexes undertaken with more modern and accurate potentiometric techniques. In addition, we carried out a detailed study on the dissociation rates of the MRI-relevant Gd^{3+} chelate and finally performed a prelimi-

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[**] OBETA = 2,2′-oxybis(ethylamine)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201200265.

nary evaluation of its paramagnetic properties in order to ascertain its potential as an MRI contrast agent.

OBETA was prepared from bis(2-chloroethyl)ether in four steps, involving its conversion to bis(2-aminoethyl)ether through a Gabriel synthesis and subsequent alkylation with *tert*-butyl bromoacetate and final removal of the protecting ester groups. Alternatively, the intermediate bis(2-aminoethyl)ether is commercially available from TCI. A detailed complexometric analysis was then conducted through careful pH-potentiometric titrations. In a first stage the ligand protonation constants were obtained by allowing the determination of five $\log K_i^H$ values very similar to those reported for EGTA (Table S1 in the Supporting Information). These values were combined with the results acquired from parallel titrations in the presence of selected Ln^{3+} ions, thereby providing the corresponding stability constants. The results are reported in Table 1 and are compared with the corresponding data for EGTA and DTPA.

Table 1. Stability constants of lanthanide complexes of OBETA, EGTA and DTPA.

$\log K_{ML}$	OBETA		EGTA		DTPA[b]
I	0.1 M KCl	0.1 M KNO_3[a]	0.1 M KCl	0.1 M KNO_3[a]	0.1 M KCl
La^{3+}	16.89 (0.02)	16.29	15.60 (0.01)	15.84	19.48
Nd^{3+}	18.39 (0.01)	17.81	16.77 (0.01)	16.28	21.60
Gd^{3+}	19.37 (0.01)	18.21	17.66 (0.01)	17.50	22.46
Ho^{3+}	18.93 (0.01)	18.17	18.10 (0.01)	17.90	22.79
Lu^{3+}	17.93 (0.01)	17.92	18.67 (0.01)	18.48	22.44

[a] Ref. [10]. [b] Ref. [11].

The stability constants obtained from the potentiometric analyses are significantly higher than those resulting from previously reported polarographic determination; the stability trend is confirmed and shows a clear preference for Gd^{3+} compared to the late and early members of the lanthanide series. In addition, it is quite unexpected to verify that the heptadentate ligand OBETA exceeds the octadentate EGTA in complex stability through most of the lanthanide series from La^{3+} to Ho^{3+}. Since the total basicity (Table S1 in the Supporting Information) and the charge of the donor atoms of deprotonated OBETA and EGTA ligands are very similar, the higher stability of $Ln(OBETA)^-$ complexes can be explained by the optimal coordination geometry of the donor atoms wrapping around the Gd^{3+} ions. On the other hand, the $\log K$ value of $Gd(OBETA)^-$ is even higher than that of $Gd(DTTA\text{-}Me)^-$ (H_4DTTA-Me = *N*,*N*′-[(methylimino)-bis(ethane-2,1-diyl)]bis[*N*-(carboxymethyl)glycine], $\log K_{Gd(DTTA-Me)} = 18.6$),[12] in spite of the fact that a nitrogen atom is generally considered a better donor than an ether oxygen. The high value of $\log K_{GdOBETA}$ is of considerable interest for MRI due to the stability requirements imposed by in vivo applications.

Nevertheless, a comprehensive stability evaluation must include kinetic studies of the rate of decomplexation. Thus, the rate of the transmetallation reaction of $Gd(OBETA)^-$ with physiologically relevant competitor ions (Cu^{2+} and Zn^{2+}) was determined. The half-lives ($t_{1/2}$) of $Gd(OBETA)^-$ and $Gd(EGTA)^-$, calculated under physiological conditions, are reported in Table 2 and are compared with those of other Gd complexes with acyclic polyaminocarboxylate ligands, DTPA-BMA, DTPA and BOPTA. The kinetic stabilities of the complexes are characterized either by the rate of dissociation measured in HCl (0.1 M) or by the rate of metal exchange reaction occurring with the endogenous Zn^{2+} or Cu^{2+} ions.[13–15] Interestingly, $Gd(OBETA)^-$ shows a 26.5-times longer half-life than $Gd(EGTA)^-$, as a consequence of the slow proton (k_1, Table S2 in the Supporting Information) and even slower metal-assisted (k_3^{Cu} and k_3^{Zn}, Table S2) dissociation pathways. It is worth noting that the decomplexation half-life of $Gd(OBETA)^-$ is even longer than those of clinically relevant Gd^{3+} complexes, such as Gd-(DTPA-BMA) and $Gd(DTPA)^{2-}$; this indicates a striking kinetic inertness towards transmetallation reactions involving endogenous Cu^{2+} and Zn^{2+} ions. These findings are of particular significance for possible in vivo MRI applications.

Table 2. The pseudo-first-order rate constants (k_{obs}^s) and half-life ($t_{1/2}$) for the dissociation of the complexes $[Gd(OBETA)]^-$, Gd(DTPA-BMA) and $Gd(DTPA)^{2-}$ at pH 7.4, $[Cu^{2+}] = 1\times10^{-6}$ M and $[Zn^{2+}] = 1\times10^{-5}$ M ($k_{obs}^s = k_1[H^+] + k_3^{Cu}[Cu^{2+}] + k_3^{Zn}[Zn^{2+}]$; $t_{1/2} = \ln 2/k_{obs}^s$; 0.1 M KCl, 25 °C).

	$10^6\times k_{obs}^s$ [s^{-1}]	$t_{1/2}$ [h]
$Gd(OBETA)^-$	0.94	205
$Gd(EGTA)^-$	25.1	7.7
Gd(DTPA-BMA) (1.0 M KCl)[a]	1.21	158
$Gd(DTPA)^{2-}$ (1.0 M KCl)[b]	1.51	127
$Gd(BOPTA)^{2-}$ (0.15 M NaCl)[c]	1.14	169

[a] Ref. [13]. [b] Ref. [14]. [c] Ref. [15].

Once the thermodynamic and kinetic stability of $Gd(OBETA)^-$ was established, a detailed ^{1}H NMR relaxometric study in aqueous solution was carried out. The relaxivity of $Gd(OBETA)^-$ was measured to be 7.2 and 6.5 $mM^{-1}s^{-1}$ at 20 and 60 MHz, respectively (298 K, pH 7). These values overcome those of $Gd(EGTA)^-$[16] and of clinically used Gd-based CAs by more than 50%; this clearly indicates the presence of two water molecules in the inner coordination sphere of the metal ion. The $^{20}r_1$ value compares also very well with that of related $q = 2$ complexes (Figure 2). The hydration number of the complex was independently determined by luminescence lifetime measurements on the related Eu^{3+} complex. The hydration number, q_{Eu}, found for $Eu(OBETA)^-$ was 1.8 ± 0.1 and is fully in agreement with the relaxometric results.

Stable Gd^{3+} chelates with higher hydration state are important for the development of MRI probes optimized for the high magnetic field strength associated with the new generation of scanners.[19] Increasing the hydration number partially offsets the decrease of relaxivity that occurs at high field in the case of small chelates and of macromolecular systems. However, it has been often observed that the two bound water molecules of $q = 2$ Gd^{3+} complexes are displaced by bidentate oxoanions of biological relevance (e.g., lactate, citrate, oxalate, carbonate) with formation of *outer*

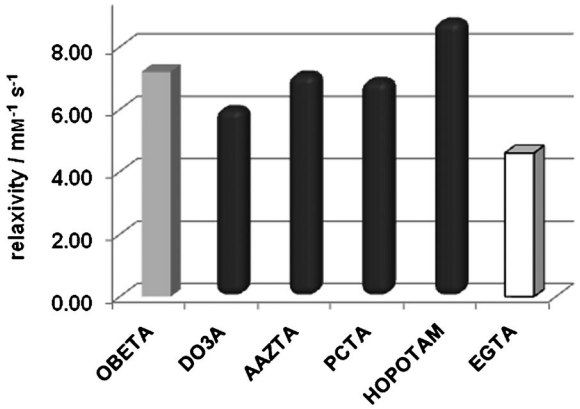

Figure 2. Plot of the proton relaxivity, r_1, for selected Gd complexes at 20 MHz and 298 K. Data are taken from ref. [17] (DO3A, PCTA), ref. [6a] (AAZTA), ref. [18] (HOPOTAM) and ref. [16] (EGTA).

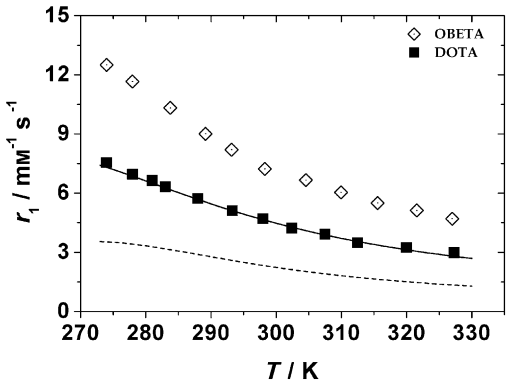

Figure 3. Temperature dependence of the proton relaxivity (20 MHz) of $Gd(OBETA)^-$ and $Gd(DOTA)^-$. The lower dashed curve represents the inner sphere contribution to r_1 for $Gd(DOTA)^-$, limited by the long residence lifetime of the coordinated water for $T < \sim 285$ K.[3b]

sphere ($q = 0$) ternary complexes.[20] Analogously, donor groups of human serum albumin (e.g., aspartate or glutammate residues) can replace the inner sphere water molecules to give low relaxivity values in serum.[21] Only in very few cases this process does not occur, the most relevant examples being the complexes of HOPO-type ligands, AAZTA and aDO3A[22] (aDO3A = 1,4,7-tris[(4′-(carboxy)-1′-carboxybutyl]-1,4,7,10-tetraazacyclododecane).

In the presence of a 20-fold molar excess of lactate, citrate and phosphate the relaxation rate of a solution of $Gd(OBETA)^-$ (0.5 mM) decreases by 6, 4 and 12%, respectively, at 20 MHz and 310K; this indicates a very weak tendency to ternary complex formation. In addition, the constant relaxivity over the pH range 4 to 10 demonstrates that carbonate anions dissolved in the aerated aqueous solution do not replace the bound water molecules by chelating Gd^{3+}. A final demonstration of the stability of $Gd(OBETA)^-$ was gained by measuring the relaxivity value in Seronorm®, a medium that simulates a physiological background. A solution of the complex (1.0 mM) in Seronorm® showed an R_1 value of 9.0 s^{-1} (20 MHz, 298K), which is slightly higher than that in pure water; this is likely because of the higher viscosity affecting both rotation and water diffusion. In addition, the R_1 value of a 1.4 mM solution of the complex in Seronorm® was measured at 20 MHz and 310 K over a period of 24 h and no changes were detected (Figure S4 in the Supporting Information). This result represents a clear evidence of the stability of the complex in a simulated physiological environment. The other key property that must be associated with an effective MRI probe is the fast rate of water exchange.[3] At pH 7, the relaxivity of $Gd(OBETA)^-$ increases exponentially with lowering temperature (Figure 3), following the typical behavior of systems in the fast-exchange regime (exchange lifetime, τ_M, is much shorter than the longitudinal relaxation time of the bound water protons, T_{1M}), such as $Gd(EGTA)^-$ or Gd(HOPOTAM). Thus, the process of water exchange will not represent a limiting factor for the relaxivity of $Gd(OBETA)^-$ and of its macromolecular derivatives.

The apparent contradiction resulting from the increase in the stability of Ln^{3+} complexes by decreasing the ligand denticity from EGTA to OBETA cannot be simply related to the omission of one donor atom, even if the latter is a relatively weak coordinating ethereal oxygen atom. It is likely that the denticity reduction triggers a radical change in the donor atom arrangement around the coordination geometry of the lanthanide ion. The coordination behavior of EGTA towards lanthanide ions is well-known; crystallographic studies of different $Ln(EGTA)^-$ complexes,[9] combined with solution NMR spectroscopy studies[16] or molecular dynamics calculations[23] evidence a change in the metal ion coordination number (and consequently in the hydration) from 10 to 9 and finally to 8 along the lanthanide series. This trend is accompanied by concomitant transitions from a coordination polyhedron approximating a bicapped square antiprism for lighter lanthanides to a monocapped square antiprism, and then to a tricapped trigonal prism around Eu^{3+}, and ends with an undefined octacoordinated polyhedron for Yb^{3+} and Lu^{3+} complexes, although in some examples the cationic counterion may shift slightly these loose boundaries.[24]

Preliminary DFT calculations performed on $[Gd(OBETA)(H_2O)_2]^-$ advocate for a nonacoordinated metal ion with a distorted tricapped trigonal prism (TTP) geometry (Figure 4). Furthermore, preliminary NMR spectroscopy experiments on $[Ln(OBETA)(H_2O)_2]^-$ suggest a highly dy-

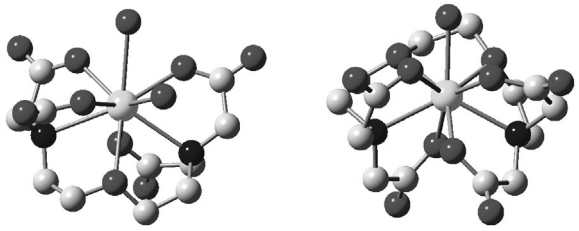

Figure 4. Minimum energy conformation obtained from preliminary calculations for $[Gd(OBETA)(H_2O)_2]^-$ (left) and $[Gd(EGTA)(H_2O)]^-$ (right).

namic behavior of the acetate groups and the oxydiethylene bridge of the OBETA molecules. Comparison with the calculated structure of $[Gd(EGTA)(H_2O)]^-$ (Figure 4), built on the structural data gained from the molecular dynamics calculation by Yerly et al.[23] shows that these complexes share the preference for the TTP coordination geometry, with nitrogen atoms and a water molecule occupying the three capping positions. Nevertheless, the more significant difference is found in the position of the backbone oxyethylenic bridges, the length and denticity of which dictates the switch between two different arrangements. Indeed, the oxyethylenic bridge of EGTA is placed close to the water molecule located in the capping position whereas in the corresponding OBETA complex the bridge is opposite to the water molecule. DFT calculations performed on the $[Gd(OBETA)(H_2O)_2]^-$ and $[Gd(EGTA)(H_2O)]^-$ systems provide very similar bond distances of the metal coordination environment in both complexes (Table S3 in the Supporting Information). This result suggests that the lower stability of EGTA complexes is related to the important degree of flexibility of the long spacer connecting the two amine nitrogen atoms of the ligand, whereas OBETA is better suited to efficiently wrap around to the metal ion as a consequence of its shorter oxydiethylene bridge.

In conclusion, complexes of Ln^{3+} ions with the heptadentate ligand OBETA were investigated in terms of solution equilibria and relaxometric properties. The decrease of the denticity of the ligand (from 8 to 7) surprisingly results in an increase of the thermodynamic stability of the Ln^{3+} complexes as compared with the corresponding $Ln(EGTA)^-$ complexes. For the Gd^{3+} complex a remarkable $\Delta\log K_{GdOBETA-GdEGTA}$ of 1.71 was observed and indicates the high affinity of OBETA towards this metal ion. Since the nature and charge of the donor atoms are not at the origin of the high stability of $Gd(OBETA)^-$, a possible alternative explanation could be found in the level of preorganization of the chelator. In EGTA the linker connecting the two terminal amine nitrogen atoms is longer and more flexible, whereas OBETA presents a better preorganized geometry for the coordination to the metal ion. In addition, preliminary results indicate that the kinetic inertness of $Gd(OBETA)^-$ towards the transmetallation reaction with Cu^{2+} and Zn^{2+} ions is significantly higher than not only $Gd(EGTA)^-$ but also Gd(DTPA-BMA) and $Gd(DTPA)^{2-}$. The metal chelate has two water molecules in its inner coordination sphere with a short residence lifetime. These water molecules are not displaced by dissolved carbonate at high pH values or by oxoanions at physiological concentration. The unexpected combination of thermodynamic and kinetic stability and of relaxometric properties candidates $Gd(OBETA)^-$ as a very promising scaffold for the development of novel and efficient MRI contrast agents.

Additional work will be necessary to assess the subtle reasons underlying the higher stabilities and the different selectivity shown by this heptadentate chelating agent towards Ln^{3+} ions. This will include necessarily detailed structural and computational studies. Meanwhile, the preparation of $Gd(OBETA)^-$-based MRI contrast agents (lipophilic derivatives, multimers, nanosized systems, bifunctional agents) will be undertaken in order to explore the potential of this interesting paramagnetic system.

Experimental Section

Materials: The chemicals used for the experiments were of the highest analytical grade. The $LnCl_3$ solutions were prepared by dissolving Ln_2O_3 (99.9% Fluka) in HCl (6 M) and evaporating the excess acid. The concentration of $LnCl_3$, $ZnCl_2$ and $CuCl_2$ stock solutions were determined by complexometric titration with standard EDTA disodium solution, with the use of Xylenol Orange ($LnCl_3$, $ZnCl_2$) and murexide ($CuCl_2$) indicators. The concentration of the OBETA and EGTA were determined by pH-potentiometric titrations in the presence and absence of a 40-fold excess of Ca^{2+}. The pH-potentiometric titrations were made with standardized 0.2 M KOH.

Tetra-*tert*-butyl 2,2′-oxybis(ethylamine)-*N*,*N*,*N*′,*N*′-tetraacetate (OBETA-*t*Bu$_4$): Potassium carbonate (5.12 g, 37 mmol, 5 equiv) was added to a solution of *O*-(2-aminoethyl)-ethanolamine (770 mg, 7.4 mmol) in acetonitrile (10 mL). The solution was cooled to 0 °C and *tert*-butyl bromoacetate (4.56 mL, 31.1 mmol, 4.2 equiv) was added dropwise. The solution was then stirred at room temperature for 48 h and the reaction was monitored by TLC. Inorganic salts were filtered off and the solvent was evaporated. The crude product was purified by gravimetric column chromatography (petroleum ether/ethyl acetate 8:2→7:3) to afford OBETA-*t*Bu$_4$ as clear light-yellow oil (3.01, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K): $\delta = 3.50$ (t, $J = 6.0$ Hz, 4H), 3.41 (s, 8H), 2.85 (t, $J = 5.8$ Hz, 4H), 1.38 ppm (s, 36H); ^{13}C NMR ($CDCl_3$, 75.4 MHz, 298 K): $\delta = 172.1$ (C), 82.1 (C), 71.5 (CH_2), 57.9 (CH_2), 54.7 (CH_2), 29.5 ppm (CH_3); MS (ESI+) calcd for $C_{28}H_{52}N_2O_9$ 560.4; found: 561.3 [$M+H^+$].

2,2′-Oxybis(ethylamine)-*N*,*N*,*N*′,*N*′-tetraacetic acid (OBETA): OBETA-*t*Bu$_4$ (2.99 g) was dissolved in trifluoroacetic acid (15 mL). The solution was stirred at room temperature, overnight. Volatiles were evaporated and the product was purified by dissolution in MeOH and precipitation with diethyl ether. This procedure was repeated three times to afford 1.68 g (94% yield) of OBETA as white powder. ^{1}H NMR (D_2O, 300 MHz, 298 K): $\delta = 4.06$ (s, 8H), 3.76 (bt, $J = 3.8$ Hz, 4H), 3.54 ppm (bt, $J = 4.0$ Hz, 4H); ^{13}C NMR (D_2O, 75.4 MHz, 298 K): $\delta = 171.2$ (C), 67.2 (CH_2), 58.7 (CH_2), 58.0 ppm (CH_2); IR (KBr): $\tilde{\nu} = 2969.9$, 1737.1, 1365.6, 1217.0, 1229.8, 1091.2, 885.4 cm^{-1}; MS (ESI+) calcd for $C_{12}H_{20}N_2O_9$ 336.1; found: 359.1 [$M+Na^+$], 337.1 [$M+H^+$].

Equilibrium measurements: All the equilibrium measurements were made at a constant ionic strength maintained with KCl (0.1 M) at 25 °C. For determining the protonation constants of EGTA and OBETA two parallel pH-potentiometric titrations were made with KOH (0.2 M) in ligand (0.005 M) solutions. The stability constants and protonation constants of complexes $Ln(EGTA)^-$ and $Ln(OBETA)^-$ were determined by direct pH-potentiometric titration (0.002 M Ln^{3+} and 0.002 M ligand solutions). For the calculation of the $\log K_{ML}$ ($K_{ML} = [ML]/[M][L]$) and $\log K_{MLHi}$ ($K_{MLHi} = [MLH_i]/[MLH_{i-1}][H^+]$, $i = 0$) values, the data obtained in the pH range 1.7–5.5, reporting the volume of the base added versus pH were used. The pH-potentiometric titrations were carried out in the pH range 1.7–11.7 with a Metrohm 785 DMP Titrino workstation with the use of a Metrohm-6.0233.100 combined electrode. The titrated solution (10 mL) was thermostated at 25 °C and stirred under N_2. For the calibration of the pH meter, potassium hydrogen phthalate (pH 4.005) and borax (pH 9.177) buffers were used. For the calculation of the H^+ concentration from the measured pH values, the method proposed by Irving et al. was used.[25] A 0.01 M HCl (0.1 M KCl) solution was titrated with 0.2 M KOH and the difference between the measured and calculated pH values was used to calculate the $[H^+]$ from the pH values measured in the titration experiments. For the calculation of the equilibrium constants the PSEQUAD program was used.[26]

Kinetic measurements: The rates of the metal exchange reactions of Gd(EGTA)$^-$ and Gd(OBETA)$^-$ with Cu^{2+} and Zn^{2+} were studied by spectrophotometry and relaxometry. The metal exchange reaction between Gd(OEBTA)$^-$, Gd(EGTA)$^-$ and Cu^{2+} were followed by the formation of the Cu^{2+} complexes at 320 nm with a Cary 1E spectrophotometer. The concentration of the Gd(EGTA)$^-$ and Gd(OBETA)$^-$ complex was 1×10^{-4} M, whereas the concentration of Cu^{2+} was 10- to 40-times higher, in order to guarantee pseudo-first-order conditions. The progress of the transmetallation with Zn^{2+} was followed by measuring the water proton relaxation rates ($1/T_1$) of the samples with a Bruker MQ-20 spectrometer operating at 20 MHz. The longitudinal relaxation time was measured by the "inversion recovery" method (180°– τ –90°) by using ten different τ values. The measurement were made with 1×10^{-3} M Gd(OBETA)$^-$ solution whereas the concentration of the Zn^{2+} was 0.010, 0.020, 0.030 and 0.040 M in order to guarantee pseudo-first-order conditions and 70–80% conversions. The relaxivity of Gd(OBETA)$^-$ ($r_1=7.2\ mM^{-1}s^{-1}$) differs considerably from that of Gd^{3+} ($r_1=13.0\ mM^{-1}s^{-1}$) at 25 °C and 20 MHz. The temperature was maintained at 25 °C and the ionic strength of the solutions was kept constant with KCl (0.1 M). The exchange rates were studied in the pH range 3.3–6.0 by using 1,4-dimethylpiperazine (pH range 3.3–4.1), *N*-methylpiperazine (pH range 4.1–5.2) and piperazine ($5.2<pH<6$) buffers (0.010 M). The pseudo-first-order rate constants (k_{obs}) were calculated by fitting the absorbance and relaxivity data with the use of Equation (1):

$$A_t = (A_0 - A_p)e^{-k_{obs}t} + A_p \quad (1)$$

where A_t, A_0 and A_p are the absorbance or water proton relaxation rates at times t, t_0 and at equilibrium, respectively.

^{1}H relaxivity: The water proton longitudinal relaxation rates as a function of temperature (20 MHz) were measured with a Stelar Spinmaster Spectrometer FFC-2000 (Mede, PV, Italy) with about 0.5–1.5 mM aqueous solutions in nondeuterated water. The exact concentrations of gadolinium were determined by measurement of bulk magnetic susceptibility shifts of a *t*BuOH signal on a Bruker Avance III spectrometer (11.7 T). The ^{1}H T_1 relaxation times were acquired by the standard inversion recovery method with typical 90° pulse width of 3.5 μs, 16 experiments of 4 scans. The reproducibility of the T_1 data was ±5%. The temperature was controlled with a Stelar VTC-91 airflow heater equipped with a calibrated copper-constantan thermocouple (uncertainty of ±0.1 °C).

Computational details: All calculations were performed by employing hybrid DFT with the B3LYP exchange-correlation functional,[27] and the Gaussian 09 package (Revision A.02).[28] Full geometry optimizations of the $[Gd(OBETA)(H_2O)_2]^-$ system were performed in aqueous solution (IEFPCM)[29] by using the small-core Stuttgart–Bonn RECP an associated (14s13p10d8f)/[10s8p5d4f] segmented valence basis set[30] for Gd and the 6-31G(d) basis set for C, H, N and O atoms. Additional optimizations in aqueous solution were performed on the $[Gd(OBETA)(H_2O)_2]^-$ and $[Gd(EGTA)(H_2O)]^-$ systems by using the 6-31G(d) basis set for ligand atoms and the large-core RECP and related [5s4p3d]-GTO valence basis set for Gd.[31] A comparison of the both distances of the Gd environment in $[Gd(OBETA)(H_2O)_2]^-$ shows that both the large-core and small-core approaches provide very similar molecular geometries. The stationary points found on the potential energy surfaces as a result of the geometry optimizations have been tested to represent energy minima rather than saddle points through frequency analysis.

Acknowledgements

Financial support from Regione Piemonte (PIIMDMT and NANO-IGT Projects) is gratefully acknowledged. Zs.B. thanks the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 and TÁMOP 4.2.2./B-10/1-2010-0024, project implemented through the New Hungary Development Plan, co-financed by the European Social Fund and the European Regional Development Fund, for financial support of this work. This research was performed within the framework of the EU COST Action D38. The authors are indebted to Centro de Supercomputación de Galicia for providing the computer facilities.

Keywords: contrast agents · kinetics · lanthanides · magnetic resonance imaging · thermodynamics

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Received: January 24, 2012
Published online: May 21, 2012

