DOI: 10.1002/chem.200500129

A Multinuclear NMR Relaxometry Study of Ternary Adducts Formed between Heptadentate Gd^{III} Chelates and L-Lactate

Enzo Terreno,[a] Mauro Botta,[b] Patrizia Boniforte,[a] Chiara Bracco,[c] Luciano Milone,[a] Bruna Mondino,[a] Fulvio Uggeri,[d] and Silvio Aime*[a]

Abstract: Dramatic relaxation enhancements of L-lactate resonances have been observed upon formation of ternary adducts with Gd^{III} complexes of heptadentate DO3A and DO3A-like ligands (DO3A = 1,4,7,10-tetraazaciclododecane-1,4,7-triacetic acid). Detailed ^{1}H and ^{17}O NMR relaxometry investigations allow us to obtain structural, dynamic and thermodynamic information on the ternary complexes in which L-lactate acts as a bidentate ligand replacing two water molecules in the inner coordination sphere of the Gd^{III} ion. It has been found that the exchange rate of the coordinated L-lactate is modulated by the structural and electronic properties of the parent Gd-heptacoordinated macrocyclic chelate. In addition to the characterisation of the relaxation behaviour of the ^{1}H methyl resonance of L-lactate, this study has been extended to its ^{13}C isomer (fully enriched at the three positions) and to the trifluoro-L-lactate. The obtained results may be relevant to the development of relaxation agents able to promote the relaxation enhancement of specific substrates detectable by in vivo magnetic resonance spectroscopy.

Keywords: gadolinium · lactate · macrocyclic ligands · NMR spectroscopy · relaxometry

Introduction

Nowadays, it is well established that relaxometry is the technique of choice for studying Gd^{III} complexes. In fact, through the measurement of ^{1}H and ^{17}O relaxation times of the solvent water nuclei, it is possible to obtain relevant information on the structure and dynamics of Gd^{III} complexes in aqueous solutions. These studies are of fundamental importance in the assessment of the potential of a given Gd^{III} complex as contrast agent for magnetic resonance imaging (MRI) applications.[1] Actually, the basic property for such applications is represented by the *relaxivity*, that is, the relaxation enhancement of solvent water protons promoted by the paramagnetic complex at 1 mM concentration. In the last two decades, an impressive amount of work has been carried out aimed at elucidating the relationship between the determinants of the water relaxation enhancement and the structural and dynamic characteristics of the Gd^{III} complex.[2]

Conversely, not much has been done in the field of the relaxation enhancement of substrates other than water that may replace water molecule(s) in the inner coordination sphere of the Gd^{III} ion. In view of the current applications of the in vivo magnetic resonance spectroscopy, which gives information about the distribution of low-molecular-weight metabolites, we decided it would be of interest to undertake a detailed relaxometry study of L-lactate, which is a metabolite of high relevance in the diagnosis of important diseases.[3–5]

As the most common coordination number of Gd^{III} ion is nine and L-lactate acts as a bidentate ligand, the formation of ternary complexes can be pursued with Gd^{III} chelates and heptacoordinate ligands.[6–10]

In this paper, we report the results of relaxometry investigations on the ternary adducts formed between L-lactate (and its fluorinated analogue) and a series of heptacoordi-

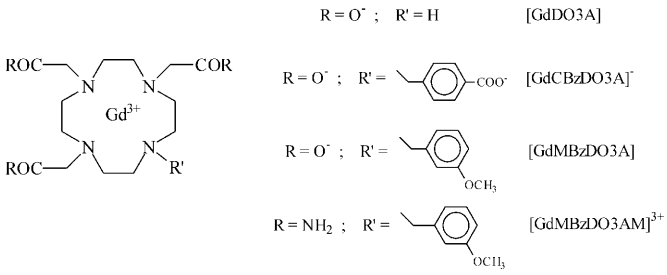
[a] Dr. E. Terreno, Dr. P. Boniforte, Prof. L. Milone, Dr. B. Mondino, Prof. S. Aime
Dipartimento di Chimica I.F.M.
Università degli Studi di Torino
Via P. Giuria 7, 10125 Torino (Italy)
Fax: (+39) 011-670-7855
E-mail: silvio.aime@unito.it

[b] Prof. M. Botta
Dipartimento di Scienze e Tecnologie Avanzate
Università del Piemonte Orientale "Amedeo Avogadro"
C. Borsalino, 54, 15100 Alessandria (Italy)

[c] C. Bracco
Laboratorio Integrato di Metodologie Avanzate
BioIndustry Park del Canavese, Via Ribes, 5
10010 Colleretto Giacosa (TO) (Italy)

[d] Dr. F. Uggeri
Imaging S.p.A, Via E. Folli, 50–20134
Milano (Italy)

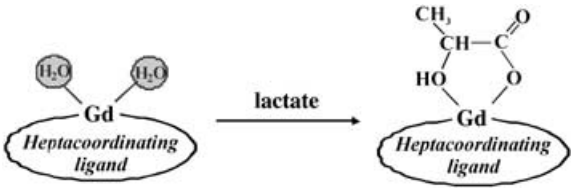
nate macrocyclic Gd^{III} complexes: [Gd(DO3A)], [Gd(MBzDO3A)], [Gd(CBzDO3A)]$^-$ and [Gd(MBzDO3AM)]$^{3+}$.



The structure of the metal complexes considered herein allows the assessment of the role played by the residual electric charge ([Gd(MBzDO3A)] vs [Gd(MBzDO3AM)]$^{3+}$) and by the substitution at the macrocyclic nitrogen site ([Gd(DO3A)] vs [Gd(MBzDO3A)] and [Gd(CBzDO3A)]$^-$) on the formation and dynamic of the resulting ternary adducts.

Results and Discussion

^{1}H and ^{17}O water relaxation: In principle, the binding of L-lactate to Gd^{III} complexes with heptadentate ligands may involve the replacement of the two inner coordination sphere water molecules as sketched in Scheme 1.



Scheme 1.

Therefore, relaxometry measurements, which are known to be sensitive to the number of metal bound water (q), provide a reliable route to investigate the formation of ternary complexes between [Gd(DO3A)] (and related derivatives) and L-lactate.[8,11] The r_1 values (10 MHz, pH 6.5 and 298 K) measured for the Gd^{III} complexes investigated herein are reported in Table 1. In spite of the relatively high number of parameters that control the r_1 value, the relaxivity at 10 MHz of structurally similar Gd^{III} complexes is basically proportional to q and to the reorientation correlation time of the complex, τ_R.[2]

The relaxivity of [Gd(DO3A)] is significantly lower than that of other chelates. This result can be mainly ascribed to a shorter τ_R value (or eventually to a slightly longer distance between Gd^{III} and the coordinated water protons), rather than a decrease of q, because it has been demonstrated that this complex is present in aqueous solution under the predominant form of the bis-aquo species.[12,13] Moreover, the higher r_1 values measured for [Gd(MBzDO3A)], [Gd(CBzDO3A)]$^-$ and [Gd(MBzDO3AM)]$^{3+}$ may also reflect an anisotropic effect on the molecular reorientational motion caused by the introduction of a bulky substituent at the secondary amine site of the macrocycle.

Table 1. Relaxivities at 10 MHz (for the free and L-lactate-bound complexes), binding constants with L-lactate and water exchange rates of the heptadentate Gd^{III} complexes investigated in this work obtained from ^{1}H and ^{17}O water relaxometry (298 K, pH 6.5).

	r_1 [$s^{-1}mM^{-1}$]	$^{298}k_{ex}^{W}$ [$\times 10^6\ s^{-1}$]	K_A	r_1^b [$s^{-1}mM^{-1}$]
[Gd(DO3A)]	6.95	13.3 ± 1.4	150 ± 10	3.2
[Gd(CBzDO3A)]$^-$	10.5	21.7 ± 2.1	1300 ± 80	4.0
[Gd(MBzDO3A)]	9.50	31.2 ± 1.8	1500 ± 100	3.9
[Gd(MBzDO3AM)]$^{3+}$	8.95	10.1 ± 1.4	8500 ± 450	3.7

Figure 1 reports the temperature dependence of the paramagnetic contribution to the transverse relaxation rate of ^{17}O water in the presence of the four Gd^{III} complexes.

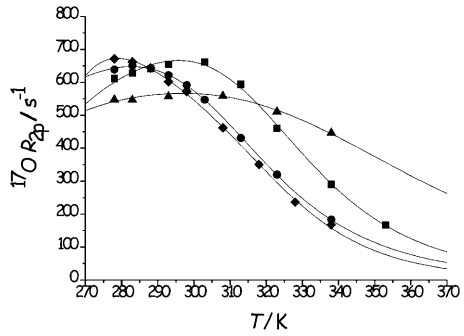


Figure 1. Plots of the paramagnetic contribution to the ^{17}O water transverse relaxation rate (7 T) versus temperature for 20 mM aqueous solutions (pH 6.5) of [Gd(DO3A)] (squares), [Gd(MBzDO3A)] (circles), [Gd(CBzDO3A)] (diamonds), and [Gd(MBzDO3AM)]$^{3+}$ (triangles).

The similar values observed for ^{17}O R_{2p} maxima support the view that the Gd^{III} ion in the four complexes has an analogous hydration state. However, the difference in the shape of the curves suggests that the coordinated water molecules have different exchange rates, k_{ex}^{W}. In fact, the quantitative analysis of these data according to the available theory,[2] and upon assuming that the two water molecules display the same exchange rate, provided the $^{298}k_{ex}^{W}$ values listed in Table 1.

The value obtained for the parent compound [Gd(DO3A)] is in excellent agreement with the previously published data ($(11.0(\pm 1.0) \times 10^6\ s^{-1})$.[12] Interestingly, the water exchange rates for [Gd(MBzDO3A)] and [Gd(CBzDO3A)]$^-$ are slightly higher, as already observed for other N-functionalised DO3A derivatives,[8,14,15] whereas the presence of three neutral coordinating amide groups in [Gd(MBzDO3AM)]$^{3+}$ yields a decrease of the exchange rate. This last result indicates that the effect of the residual electric charge on k_{ex}^{W} in macrocyclic heptadentate chelates is

much less marked with respect to the corresponding octadentate DOTA-like complexes, which adopt square-antiprismatic geometry.[16–18]

Upon titrating a solution of [Gd(DO3A)] (and related derivatives) with L-lactate, the changes in the observed water proton relaxation rate give information on the formation of the ternary complexes.[11] In fact, each point in the diagram reported in Figure 2 represents the weighted average of the contributions arising from the bis-aquo complex and the L-lactate adduct.

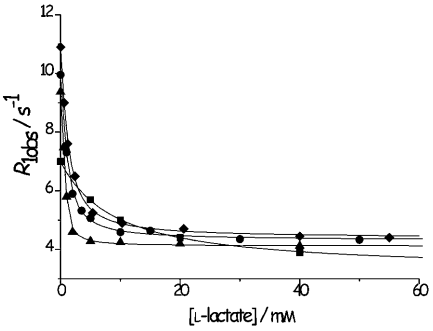


Figure 2. Plots of the longitudinal relaxation rate of ^{1}H water protons versus concentration of L-lactate for 1 mM aqueous solutions of [Gd(DO3A)] (squares), [Gd(MBzDO3A)] (circles), [Gd(CBzDO3A)] (diamonds), and [Gd(MBzDO3AM)]$^{3+}$ (triangles) (10 MHz, 298 K, pH 7).

The affinity towards L-lactate for the Gd^{III} complexes examined in this work follows the order [Gd(MBzDO3AM)]$^{3+}$ > [Gd(MBzDO3A)], [Gd(CBzDO3A)]$^-$ > [Gd(DO3A)] (see Table 1). The higher affinity displayed by the amide derivative is likely to be a result of the stronger electrostatic interactions between the anionic substrate and the positively charged complex. As far as the other neutral, on the coordination cage, complexes are concerned, the affinity of [Gd(MBzDO3A)] and [Gd(CBzDO3A)]$^-$ is significantly higher, approximately one order of magnitude, than the parent [Gd(DO3A)].

The relaxivity values (r_1^b) of the ternary adducts, listed in Table 1, fall in the range 3.2–4.0 s^{-1} mM^{-1}, consistent with the absence of water in the inner coordination sphere of the metal.

Exchange rate of L-lactate in the ternary adducts: Having estimated the thermodynamic stability (K_A) of the binding interaction between L-lactate and the heptadentate Gd^{III} complexes, we went on to investigate the kinetic properties of the ternary adducts.

Analogously to the exchange process for Gd^{III}-bound water molecules, the assessment of the residence lifetime of the anionic substrate at the metal site can be carried out by measuring the temperature dependence of the paramagnetic contribution to the longitudinal relaxation rate of a given nucleus of the substrate molecule (R_{1p}^S).

In analogy to the procedure followed in the analysis of the relaxation rates of water protons,[2] R_{1p}^S term is given by Equation (1), in which the superscript S refers to a given nucleus of the substrate molecule (e.g. the methyl group of L-lactate), obs is its relaxation rate measured in a solution containing the Gd^{III} complex, dia is the corresponding relaxation rate measured in a solution devoid of the paramagnetic agent, and the terms "is" and "os" refer to the contributions to the relaxation rate arising from substrate molecules in the inner and in the outer sphere of the metal ion, respectively.

$$R_{1p}^S = R_1^{S\text{-}obs} - R_1^{S\text{-}dia} = R_{1p}^{S\text{-}is} + R_{1p}^{S\text{-}os} \quad (1)$$

In contrast to the case of water protons, one may safely assume that the outer sphere contribution of the substrate is negligible and, consequently, Equation 1 reduces to give Equation (2) in which $[Sub]_{bound}/[Sub]_{total}$ is the molar fraction of the substrate bound to the Gd^{III} complex, T_{1M}^S is the relaxation rate of the substrate nucleus when the substrate is bound to the complex and τ_M^S is the residence lifetime of the substrate at the metal centre.

$$R_{1p}^S; R_{1p}^{S\text{-}is} = \frac{[Sub]_{bound}}{[Sub]_{total}} \frac{1}{T_{1M}^S + \tau_M^S} \quad (2)$$

Of course, the molar fraction of the bound substrate is dependent on the total concentration of the paramagnetic complex ([Gd(L)]) and on the affinity constant K_A [Eq. (3)].

$$[Sub]_{bound} = \frac{K_A[Gd(L)] + K_A[Sub]_{total} + 1 - \sqrt{(K_A[Gd(L)] + K_A[Sub]_{total} + 1)^2 - 4K_A{}^2[Gd(L)][Sub]_{total}}}{2K_A} \quad (3)$$

For relatively fast-tumbling systems (rotational mobility $\tau_R < 500$ ps) and at the magnetic field strength of 7 T, the extreme narrowing conditions ($\omega_H^2\tau_R^2 \ll 1$) are met and, therefore, T_{1M}^S is given by Equation (4) in which K^{DIP} is a constant accounting for the dipolar interaction between the unpaired electrons of Gd^{III} ion and the nuclear spins of the substrate (e.g., 3.887×10^{-42} m^{6}s^{-2} for proton), r_S is their distance, and τ_R^S is the rotational correlation time of the ternary adduct.

$$(T_{1M}^S)^{-1} = \frac{1}{5} \frac{K^{DIP}\tau_R^S}{r_S^6} \quad (4)$$

Since the determination of τ_M^S involves the analysis of the temperature dependence of R_{1p}^S, it is necessary to consider the temperature dependence of all the involved parameters [Eq. (5) and (6)].

$$(\tau_j^S)_T^{-1} = \frac{(\tau_j^S)_{298}^{-1} T}{298.15} \exp\left[\frac{\Delta H_j}{R}\left(\frac{1}{298.15} - \frac{1}{T}\right)\right] \quad (5)$$

$$\ln K_A = -\frac{\Delta H_{add}}{RT} + \frac{\Delta S_{add}}{R} \quad (6)$$

In Equations (5) and (6) $j = M$ (residence lifetime) or R (rotational mobility), ΔH_j is the activation enthalpy for the corresponding process, and ΔH_{add} and ΔS_{add} represent the enthalpy and the entropy for the binding equilibrium, respectively.

First of all, the temperature dependence of the binding affinity between L-lactate and the heptadentate Gd^{III} complexes has been determined by measuring the proton relaxation enhancements as function of temperature in the range 280–320 K. The data, fitted to the van't Hoff equation [Eq. (6)], yielded the results reported in Table 2. The thermodynamic data indicate that the formation of the ternary adducts is, as expected, an exothermic, enthalpy-driven process.

Table 2. Thermodynamic data related to the formation of the ternary adducts with L-lactate.

	ΔH_{add} [kJ mol^{-1}]	ΔS_{add} [J mol^{-1} K]
[Gd(DO3A)]	-12.9 ± 1.5	-2.2 ± 0.2
[Gd(CBzDO3A)]$^-$	-35.9 ± 1.4	-63.0 ± 2.9
[Gd(MBzDO3A)]	-36.5 ± 2.5	-62.4 ± 3.2
[Gd(MBzDO3AM)]$^{3+}$	-45.2 ± 2.9	-76.5 ± 4.1

As the exploitation of the relaxation enhancement of L-lactate resonance is in the field of spectroscopic MRI, it is worth assessing the relaxation behaviour of the various nuclei.

In Figure 3 the temperature dependence of the relaxation rate of methyl protons, $R_1^{Me\text{-}is}$, obtained for solutions containing 10 mM of L-lactate and 1 mM of the heptadentate Gd^{III} complexes, is reported.

First of all, it has to be noted that the $R_1^{Me\text{-}is}$ values are sensibly higher than the typical water proton relaxivities reported for Gd^{III} complexes. This finding relies on the fact that the inner sphere paramagnetic contribution to R_1 is directly related to the molar fraction of nuclei bound to the

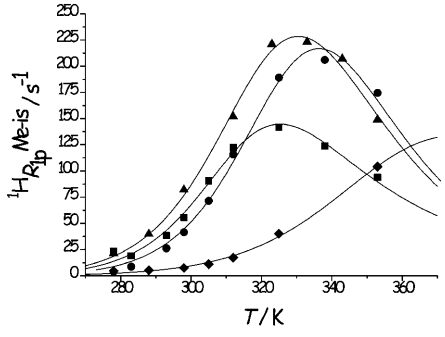


Figure 3. Plots of the paramagnetic contribution to the ^{1}H-longitudinal relaxation rate (7 T) of methyl protons of L-lactate (10 mM) versus temperature for 1 mM aqueous solutions (pH 7) of [Gd(DO3A)] (squares), [Gd(MBzDO3A)] (circles), [Gd(CBzDO3A)] (triangles), and [Gd(MBzDO3AM)]$^{3+}$ (diamonds).

Gd^{III} centre and this value is equal to approximately 3.6×10^{-5} in the case of water protons (1 mM of a bis-aquo Gd^{III} complex), but about three orders of magnitude higher in the case of L-lactate (e.g., it gives a value of 5.8×10^{-2} for a 1 mM solution of [Gd(DO3A)] and 10 mM solution of L-lactate at 298 K).

Figure 3 shows that the temperature dependence of $R_1^{Me\text{-}is}$ is characterised by a bell-shaped profile. This behaviour reflects the opposite temperature dependence of T_{1M}^S and τ_M^S; the former term decreases upon increasing the temperature (owing to the shortening of τ_R^S), whereas the latter increases. On this basis, the $R_{1p}^{Me\text{-}is}$ enhancement upon increasing temperature indicates that τ_M^{Me} is larger than T_{1M}^{Me} [see Eq. (2)], whereas the decrease of $R_{1p}^{Me\text{-}is}$ corresponds to the occurrence of the $T_{1M}^{Me} > \tau_M^{Me}$ condition. Qualitatively, for systems endowed with similar T_{1M}^S values as those investigated herein, the higher the temperature at which the maximum $R_{1p}^{Me\text{-}is}$ value is recorded, the longer the residence lifetime of the substrate. The fitting of the data, according to the set of equations reported above, confirmed this qualitative conclusion and yielded the parameters listed in Table 3 (in analogy to water molecules the residence lifetime of L-lactate is expressed as exchange rate, $k_{ex}^{Me} = 1/\tau_M^{Me}$).

Table 3. Exchange rates of L-lactate and mean distance between methyl protons and Gd^{III} ion in the ternary adducts.

	$^{298}k_{ex}^{Me}$ [s^{-1}]	r^{Me} [Å]
[Gd(DO3A)]	1200 ± 70	4.78 ± 0.04
[Gd(CBzDO3A)]$^-$	920 ± 55	4.70 ± 0.04
[Gd(MBzDO3A)]	490 ± 35	4.75 ± 0.04
[Gd(MBzDO3AM)]$^{3+}$	90 ± 5	4.75 ± 0.04

The exchange rate of L-lactate at the Gd^{III} site is significantly slower than that of the water molecules, as expected on the basis of its bidentate interaction with the metal centre. Actually, the lability of the substrate appears to be inversely related to the binding affinity. The distances between the methyl protons of L-lactate and the Gd^{III} centre obtained from our fitting (4.75–4.78 Å) are in good agreement to what is observed in the X-ray structure of a ternary adduct in which the same substrate is bound to an Yb^{III} complex of a triamide DO3A derivative (an average of 4.87 Å for the three methyl protons).[19] This confirms that the anionic substrate "bites" the metal centre with the carboxylate group at the equatorial position and the OH group in the capping axial position. In Figure 4 the structure for the [Gd(DO3A)(L-lactate)] adduct obtained in silico is reported.

Ternary adducts with ^{13}C-enriched L-lactate: We extended the study to other nuclei of L-lactate substrate. Whereas the detection of the CH proton is difficult, either as a result of the multiplicity due to its coupling to methyl protons or because its resonance frequency is quite close to the resonance of water protons, the measurement of the relaxation rate of ^{13}C resonances for the fully ^{13}C-enriched L-lactate is straight-

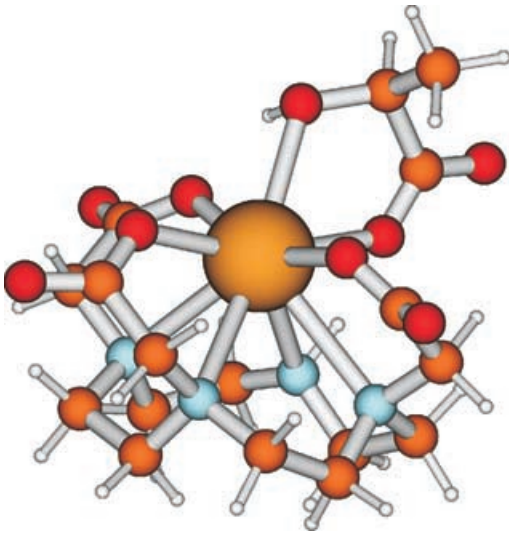


Figure 4. Structure of [Gd(DO3A)(L-lactate)] adduct obtained in silico.

forward. The temperature dependence of the relaxation rate of three ^{13}C resonances of L-lactate in the presence of [Gd(CBzDO3A)]$^-$ is shown in Figure 5.

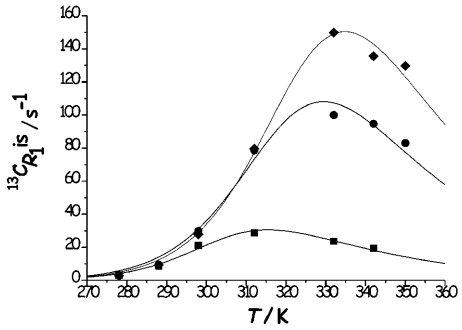


Figure 5. Plots of the paramagnetic contribution to the ^{13}C longitudinal relaxation rate (7 T) of L-lactate (10 mM) versus temperature for 1 mM aqueous solution of [Gd(CBzDO3A)] (pH 7): $-CH_3$ (squares), $-CH$ (circles), $-COO^-$ (diamonds).

A qualitative inspection into the results reported in Figure 5 indicates that the $^{13C}R_{1p}^{is}$ values for the methyl carbon are significantly smaller than those ones measured for the protons of the same chemical moiety. Since $^{13C}r^{Me} < {}^{1H}r^{Me}$, this observation means that the ratio $^{1H}K^{DIP}/{}^{13C}K^{DIP}$ is larger than $(^{1H}r^{Me}/{}^{13C}r^{Me})^6$. Actually, the quantitative analysis of such data yielded a $^{13C}r^{Me}$ value of 4.0 Å versus 4.75 Å obtained for $^{1H}r^{Me}$ (these values have been obtained under the assumption that the reorientational correlation times for Gd–H(methyl) and Gd–C(methyl) vectors are equal).

On the other hand, the larger $^{13C}R_{1p}^{is}$ values measured for the α-carbon atom and for the carboxylate group are due to the shorter ^{13C}r values (3.0 and 2.75 Å, respectively).

It is worth noting that the ^{13C}r values obtained from our analysis are only slightly shorter (ca. 12%) than the distances observed in the X-ray structure of a related system,[18] thus confirming again the good reliability of this approach for assessing the structural properties of these adducts.

Concerning $^{13C}k_{ex}$, the obtained values at 298 K are in a relatively good agreement to those ones previously determined from the ^1H measurements on the same ternary adduct: 920 s^{-1} from the methyl protons, 885 s^{-1} from the methyl carbon atom, 1150 s^{-1} from the α-carbon atom and 1040 s^{-1} from the carboxylate group.

Interestingly, and in contrast to the case of methyl protons, the differences in the temperature at which $^{13C}R_{1p}^{is}$ maxima occur have now to be associated with different $^{13C}T_{1M}^{is}$ values rather than different exchange rates.

Ternary adducts with trifluoromethyl-L-lactate: In addition to ^1H and ^{13}C detection, our study has also considered ^{19}F relaxation through the use of trifluoromethyl-L-lactate. Whereas L-lactate and its ^{13}C-enriched form have the same affinity towards a given Gd^{III} complex, it is expected that the presence of the trifluoro residue may affect the binding strength between the two interacting species. For this reason, we measured the K_A values for the adducts formed by trifluoro-L-lactate with [Gd(DO3A)] and [Gd(MBzDO3A)]. Actually, although the relative affinity trend is maintained, the binding affinity of the trifluoro derivative is noticeably less than L-lactate (Table 4).

Table 4. Binding constant, exchange rate and mean ^{19}F–Gd^{III} distance determined for ternary adducts with trifluoro-L-lactate.

	K_A	$^{298}k_{ex}^{CF_3}$ [s^{-1}]	r^{CF_3} [Å]
[Gd(DO3A)]	9±3	21600±2200	5.21±0.08
[Gd(MBzDO3A)]	90±20	7700±800	5.7±0.09

Likely, this is the result of the strong electron-withdrawing ability of fluorine atoms that weakens the coordinating ability of the oxygen donor atoms.

The temperature dependence of $^{19F}R_{1p}^{CF_3\text{-}is}$ of trifluoro-L-lactate measured for solutions containing [Gd(DO3A)] and [Gd(MBzDO3A)] is shown in Figure 6.

The highest ^{19}F relaxation rates are reached at lower temperatures (about 300 K) with respect to the ^1H (ca. 330 K) and ^{13}C (315 K for methyl carbon atom) experiments with L-lactate. This observation can be accounted for in terms of either a longer $^{19F}T_{1M}^{CF_3}$ or a short $\tau_M^{CF_3}$. The ^{19}F nucleus has a magnetogyric ratio 6% lower than ^1H and, consequently $^{19F}K^{DIP}$ is only 12% lower than $^{1H}K^{DIP}$. This means that $^{19F}T_{1M}^{CF_3}$ should not be much lower than $^{1H}T_{1M}^{Me}$ and, therefore, the data reported in Figure 6 suggest that $\tau_M^{CF_3} \ll \tau_M^{Me}$. Actually, the fitting of the experimental data (carried out, in this case, without considering the temperature dependence of K_A) confirmed this view yielding $k_{ex}^{CF_3}$ values (Table 4) much higher than k_{ex}^{Me}.

Interestingly, the direct correlation between K_A and k_{ex} is still maintained with a $k_{ex}^{CF_3}/k_{ex}^{Me}$ ratio almost equal to $K_A^{Me}/K_A^{CF_3}$ for both Gd^{III} complexes.

Though the $^{19F}T_{1M}^{CF_3}$ values are rather short and partly quenched by $\tau_M^{CF_3}$, $^{19F}R_{1p}^{CF_3\text{-}is}$ values are not particularly high if

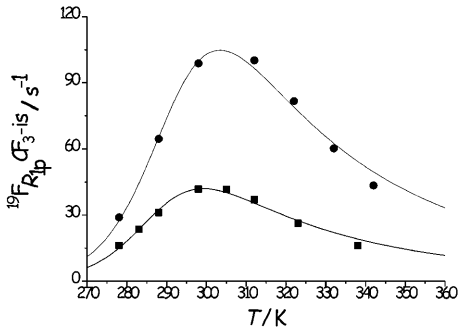


Figure 6. Plots of the paramagnetic contribution to the ^{19}F longitudinal relaxation rate (7 T) of trifluoro-L-lactate (10 mM) versus temperature for 1 mM aqueous solution (pH 7) of [Gd(DO3A)] (squares) and [Gd(MBzDO3A)] (circles).

compared to the corresponding values measured for methyl protons. This observation is the result of the lower affinity shown by the trifluoro derivative that reduces considerably the molar fraction of substrate molecule bound to the metal centre.

The distances between Gd^{III} ion and the fluorine atoms of trifluoro-L-lactate in the ternary adduct are longer than the corresponding values for methyl protons as a consequence of their increased atomic size.

Conclusion

L-Lactate is a commonly in vivo detectable substrate in ^1H NMR spectroscopy as it may be present at millimolar concentration in living organisms. Its concentration is taken as reliable marker of a limited oxygen supply to cellular metabolism. The availability of paramagnetic agents able to promote a dramatic enhancement of the relaxation rates of L-lactate nuclei may be very useful to improve the detection of this metabolite. Moreover, the design of agents endowed with specific intracellular distribution may provide new important insights on the underlying pathway of formation and use of L-lactate at cellular level.

Experimental Section

Chemicals: All the reagents and the inorganic salts were purchased from Sigma–Aldrich and used without further purification. DO3A ligand (DO3A = 1,4,7,10-tetraazaciclododecane-1,4,7-triacetic acid) was kindly provided by Bracco Imaging S.p.A. (Milan, Italy). ^{13}C-Enriched L-lactate was purchased from Isotec Inc.

Synthesis of the ligands: MBzDO3A (MBzDO3A = 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-triacetic acid]) and MBzDO3AM (MBzDO3AM = 10-[(3-methoxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-tris-[(aminocarbonyl)methyl]) ligands were synthesised as reported in the literature.[11]

CBzDO3A (CBzDO3A = 10-[(4-carboxyphenyl)methyl]-1,4,7,10-tetraazaciclododecane-1,4,7-triacetic acid]) ligand was synthesised as follows:

Cyclen (1,4,7,10-tetraazacyclododecane, 0.22 mol) was N-alkylated with 4-bromomethyl benzoic acid (0.045 mol, solvent water, room temperature, overnight). The solution, brought to neutral pH with HCl 5%, was concentrated and most of the unreacted cyclen was removed as solid by adding ethanol. Then, the alcoholic solution was evaporated and the solid residue, still containing some cyclen, was purified by liquid chromatography (solid phase Duolite® C20 MB, eluent: water up to pH 7 and then ammonia 2 M). The monoalkylated cyclen was obtained in 85% yield (11.8 g).

The monoalkylated cyclen (5 g, 0.016 mol) was dissolved in water, and bromoacetic acid (0.068 mol) was added dropwise. Then, the pH of the solution was brought up to 10 by adding NaOH 10 N. After heating (50–60 °C, overnight), the solution was filtered and acidified with HCl 10% up to pH 2.5. The final product was purified by liquid cromatography. A first eluition (solid phase: Amberlite® XAD 1600, eluent: water) was carried out in order to separate the product and bromide ions from secondary reaction products. Then, a second eluition (solid phase: Duolite® C20 MB, eluent: water up to pH 7 and then ammonia 2 M) was performed from separate the halide ions from the product. CBzDO3A was obtained in 74.3% yield (5.7 g). ^1H NMR (D_2O, *t*BuOH as reference): $\delta = 7.85$ (d, $J = 8.1$ Hz, 2H), 7.55 (d, $J = 8.1$ Hz, 2H), 3.9 (s, 3H), 2.9–3.7 ppm (m, 24H); MS (MALDI-TOF): *m*/*z* calcd for $C_{22}H_{32}N_4O_8$: 480.5; found: 479.1.

Synthesis of the Gd^{III} complexes: The Gd^{III} complexes were synthesised in water at room temperature by adding equimolar amounts of $GdCl_3 \cdot 6H_2O$ and a given heptacoordinating ligand. The pH was monitored during the synthesis in order to keep it in the 6–8 range. The final concentration of the metal complex was checked by NMR spectroscopy by means of the Evans' method.[20]

Water proton relaxivity measurements: The water proton longitudinal relaxation times were measured at 298 K and 0.235 T (Proton larmor frequency 10 MHz) by using a Stelar fast field cycling (FFC) relaxometer (Stelar, Mede, PV, Italy) installed at the "Laboratorio Integrato di Metodologie Avanzate", Bioindustry Park del Canavese (TO, Italy). The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper-constantan thermocouple (uncertainty of $0.1 \pm$ °C).

Water ^{17}O relaxation measurements: Variable-temperature ^{17}O NMR measurements were recorded on a Bruker Avance 300 (7 T) spectrometer, equipped with a 5 mm probe. Typical experimental settings were: spectral width 30 000 Hz, pulse width 10 μs (90°), acquisition time 10 ms, 512 scans and no sample spinning. Aqueous solutions of the paramagnetic complexes (at pH 6.5) containing 2.6% of water enriched in the ^{17}O isotope (Yeda, Israel) were used. The observed transverse relaxation rates (R_2) were calculated from the signal width at half height.

T_1 measurements on L-lactate and trifluoro-L-lactate: The ^1H, ^{13}C (^1H decoupled) and ^{19}F longitudinal relaxation times of L-lactate and trifluro-L-lactate were measured on a Bruker Avance 300 (7 T) spectrometer by means of the usual inversion–recovery pulse sequence.

Acknowledgements

We thank MIUR, EMIL (European Molecular Imaging Laboratories) and DiMI (Diagnostic Molecular Imaging) European networks for financial support. This work was carried out under the auspices of EC D-18 COST Action "Lanthanide Chemistry for Diagnosis and Therapy".

[1] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293–2352.
[2] É. Tóth, L. Helm, A. E. Merbach, in *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging* (Eds.: A. E. Merbach, É. Tóth), Wiley, Chichester, **2001**, pp. 45–119.
[3] O. Henriksen, S. Weislander, F. Gierris, K. M. Jensen, *Acta Radiol.* **1991**, *32*, 95–100.
[4] a) M. Castillo, L. Kwock, in *Neuroimaging Clinics of North America*, *Vol. 8*, (Ed.: M. Castillo), Saunders, Philadelphia, **1998**, pp. 733–752; b) T. L. Richards, S. R. Dager, S. Posse, *Neuroimaging Clinics of North America*, *Vol. 8*, (Ed.: M. Castillo), Saunders, Philadelphia, **1998**, pp. 823–834.

[5] W. Grodd, I. Krägeloh-Mann, U. Klose, R. Santer, *Radiology* **1991**, *181*, 173–180.
[6] D. Parker, R. S. Dickins, H. Puschmann, C. Crossland, J. A. K. Howard, *Chem. Rev.* **2002**, *102*, 1977–2010.
[7] S. Aime, M. Botta, V. Mainero, E Terreno, *Magn. Reson. Med.* **2002**, *47*, 10–13.
[8] M. Botta, S. Aime, A. Barge, G. Bobba, R. S. Dickins, D. Parker, E. Terreno, *Chem. Eur. J.* **2003**, *9*, 2102–2109.
[9] S. Aime, D. Delli Castelli, F. Fedeli, E. Terreno, *J. Am. Chem. Soc.* **2002**, *124*, 9364–9365.
[10] R. S. Dickins, A. S. Batsanov, J. A. K. Howard, D. Parker, H. Puschmann, S. Salamano, *Dalton Trans.* **2004**, *1*, 70–80.
[11] E. Terreno, M. Botta, F. Fedeli, B. Mondino, L. Milone, S. Aime, *Inorg. Chem.* **2003**, *42*, 4891–4897.
[12] S. Aime, M. Botta, S. Geninatti Crich, G. B. Giovenzana, R. Pagliarin, M. Sisti, E. Terreno, *Magn. Reson. Med.* **1998**, *36*, S200-S208.
[13] E. Toth, O. M. Ni Dhubhghaill, G. Besson, L. Helm, A. E. Merbach, *Magn. Reson. Med.* **1999**, *37*, 701–708.
[14] S. Aime, E. Gianolio, E. Terreno, G. B. Giovenzana, R. Pagliarin, M. Sisti, G. Palmisano, M. Botta, M. P. Lowe, D. Parker, *J. Biol. Inorg. Chem.* **2000**, *5*, 488–497
[15] E. Terreno, P. Boniforte, M. Botta, F. Fedeli, L. Milone, A. Mortillaro, S. Aime, *Eur. J. Inorg. Chem.* **2003**, *19*, 3530–3533.
[16] S. Aime, A. Barge, M. Botta, D. Parker, A. S. de Sousa, *J. Am. Chem. Soc.* **1997**, *119*, 4767–4768.
[17] S. Aime, A. Barge, J. I. Bruce, M. Botta, J. A. K. Howard, J. M. Moloney, D. Parker, A. S. de Sousa, M. Woods, *J. Am. Chem. Soc.* **1999**, *121*, 5762–5771.
[18] S. R. Zhang, Z. Kovacs, S. Burgess, S. Aime, E. Terreno, A. D. Sherry, *Chem. Eur. J.* **2001**, *7*, 288–296.
[19] R. S. Dickins, S. Aime, A. S. Batsanov, A. Beeby, M. Botta, J. Bruce, J. A. K. Howard, C. S. Love, D. Parker, R. D. Peacock, H. Puschmann, *J. Am. Chem. Soc.* **2002**, *124*, 12697–12705.
[20] D. M. Corsi, C. Platas-Iglesias, H. van Bekkum, J. A. Peters, *Magn. Reson. Chem.* **2001**, *39*, 723–726.

Received: February 4, 2005
Published online: July 12, 2005

