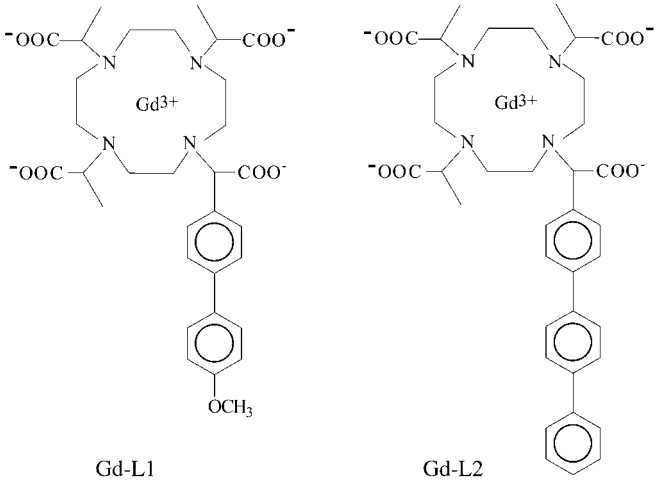
New Insights for Pursuing High Relaxivity MRI Agents from Modelling the Binding Interaction of Gd^{III} Chelates to HSA

Silvio Aime,*[a] Eliana Gianolio,[a] Dario Longo,[a] Roberto Pagliarin,[b] Clara Lovazzano,[b] and Massimo Sisti[c]

It was recognized early on that the relaxivity of a Gd^{III} complex at 0.5–1 T can be strongly enhanced if its molecular correlation time is lengthened by linking it to a slowly moving macromolecule.[1,2] In this context, a huge amount of attention has been devoted in the past decade to the study of systems able to form noncovalent adducts with serum albumin, which also has the advantage of yielding systems that remain confined in the blood vessels.[2–5] Theory foresees the attainment of relaxivities $>100\ mM^{-1}s^{-1}$ for macromolecular monoaquo Gd chelates characterized by a molecular reorientation time of 10–30 ns.[2,6] However, in spite of a number of investigated systems, relaxivities of such magnitude for Gd chelates bound to HSA have never been found. One major limiting factor has been recognized to be the occurrence of an insufficiently fast exchange rate of the coordinated water (τ_M).[7,8]

Therefore a primary requisite for pursuing high relaxivity for a Gd-L/HSA adduct is the occurrence of a relatively fast exchange of the inner-sphere water molecule, that is, τ_M has to be in the range of tens of nanoseconds at room temperature.[2,4,9] Within macrocyclic systems, Gd–DOTMA ((1*R*,4*R*,7*R*,10*R*)-α,α′,α″,α‴-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,1,10-tetraacetic acid [10] satisfies this condition ($\tau_M = 68$ ns).[2]

On this basis, we synthesised two Gd–DOTMA-like complexes with the expectation that they would show high binding affinity to HSA, faster exchange of the coordinated water and overall rigidity of the coordination cage, a property that has been associated with a long electronic relaxation time[11] and is a further requisite for the attainment of very high relaxivity.



Gd-L1 Gd-L2

L1 and L2 were synthesised, with a methoxybiphenyl or a triphenyl group, respectively, on the DO3MA-terbutylester. The synthetic pathways were designed in order to yield the pure *RRRR* forms, as clearly assessed by the number of resonances detected in the ^1H NMR spectra of Eu-L1 and Eu-L2 complexes.

Gd^{III} complexes were synthesised by adding stoichiometric amounts of the ligands and $GdCl_3$ and stirring at 60 °C for 24 h. The excess of free Gd was removed by filtering the basic solutions with 0.2 μm syringe filters. The orange xylenol UV-spectrophotometric method was used to check the absence of free Gd^{III} ions.

The ^1H relaxivities of GdL1 and GdL2 are 6.0 and 6.4 $mM^{-1}s^{-1}$, respectively at 298 K and 20 MHz. These values are higher than that of the parent Gd–DOTMA complex ($r_{1p} = 3.8\ mM^{-1}s^{-1}$).[10,12] Probably the hydrophobic moieties promote

[a] *Prof. S. Aime, Dr. E. Gianolio, Dr. D. Longo*
Dipartimento di Chimica IFM e Centro per il Molecular Imaging
Università di Torino
via P. Giuria 7, 10125 Torino (Italy)
Fax: (+39) 011-670-7855
E-mail: silvio.aime@unito.it

[b] *Dr. R. Pagliarin, Dr. C. Lovazzano*
Dipartimento di Chimica Organica e Industriale
Università di Milano
viale Venezian 21, 20133 Milano (Italy)

[c] *Prof. Dr. M. Sisti*
Dipartimento di Scienze Chimiche e Ambientali
Università degli Studi dell'Insubria
Via Valleggio 11, 22100 Como (Italy)

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

  DOI: 10.1002/cbic.200400364 *ChemBioChem* **2005**, *6*, 818–820

some kind of reversible association that causes an increase in the molecular correlation time, which in turn is responsible for yielding a r_1 value significantly larger than one would expect on the basis of M_W increase alone. The τ_M value of Gd-L1 had been accurately determined by VT ^{17}O NMR T_2 measurements[13] as 65 ns at 298 K, that is, not too different from the parent Gd–DOTMA and still in the range of the optimal values for our purpose. Gd-L2 was not soluble enough for ^{17}O NMR measurements.

Next, binding to HSA was investigated by titrating solutions of Gd-L1 and Gd-L2 (0.1 mM, buffer phosphate) with HSA and measuring the relative relaxation enhancement. Analysis of the obtained titration curves (Supporting Information) gave one binding site with $K_a = 2.7 \times 10^3$ and $9.5 \times 10^4\ M^{-1}$ for Gd-L1 and Gd-L2, respectively. Competitive binding assays with substrates known to tightly bind to HSA (e.g., Ibuprofen, Warfarin)[14] allowed us to decide that the binding site of Gd-L1 and Gd-L2 is site II (subdomain IIIA). In a typical competition assay, a solution containing a Gd-L and HSA (in a ratio such that the Gd-L/HSA adduct is $>50\%$) undergoes a T_1 measure (at 20 MHz and 298 K) in the presence of increasing amounts of the competitive ligand. A decrease of R_{1obs} is an indication of competition between the chelate and the added substrate. In Figure 1, the

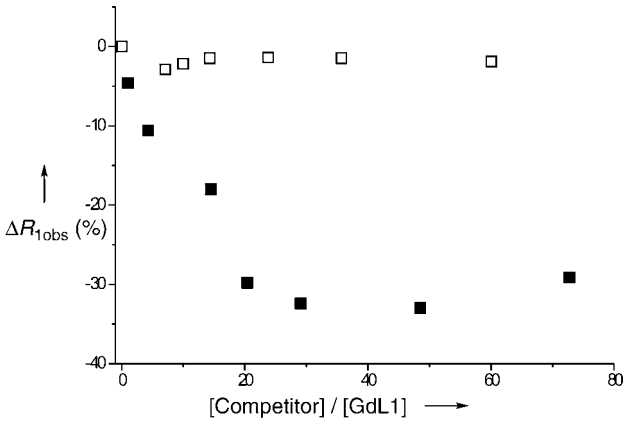


Figure 1. *Relaxometric competitive-binding studies performed on the GdL1/HSA adduct in the presence of Ibuprofen (■) and Warfarin (□) at 20 MHz, 298 K and neutral pH.*

results for the competition-binding assay between Gd-L1 and two classical HSA binding substrates is reported. Only in the case of Ibuprofen (typical Sudlow's site II ligand) was a marked decrease in R_{1obs} observed, whereas no competition effect is observed in the case of Warfarin (typical Sudlow's site I ligand). The same behaviour was observed in the case of the Gd-L2 complex.

As the structural of site II is well characterised, it has been possible to pursue accurate molecular modelling studies of the docking interaction of Gd-L1 and Gd-L2 complexes at HSA by using the Molecular Operating Environment (MOE) programme package.[15]

Docking of GdL1 with fatted HSA gives only one solution, in which the phenylmethoxy group is located well inside the hydrophobic pocket, and the Gd-cage is just outside its rim at a binding distance with the Glu492 residue (Figure 2A). The outer aromatic ring is sandwiched between Leu387 and Arg410, whereas the methoxy group points towards the hydrophobic region of the binding pocket formed by Phe409, Leu430, Val433, Ala449,Leu453 and Leu457 The binding interaction of Gd-L1 is further strengthen because one of the four acetate arms of the Gd cage can form a hydrogen bond with the side chain of Lys414.

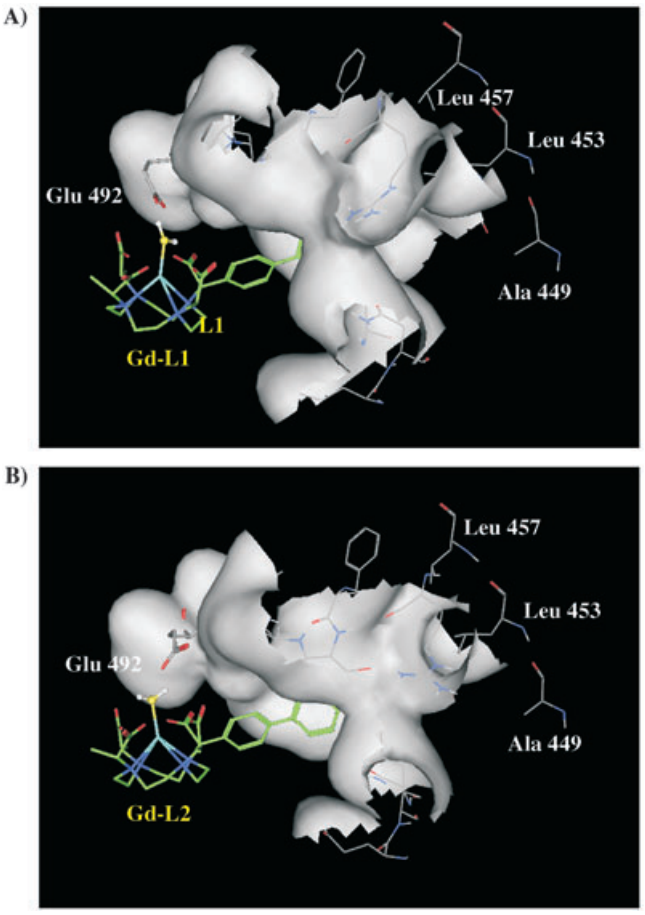


Figure 2. *Results of docking procedure of Gd-L1 and Gd-L2 to fatted HSA (PDB code: 1bj5). Gd complexes are rendered as sticks and with carbon atoms in green, residue atoms as lines and with carbon atoms in dark grey. Nonpolar hydrogens are omitted for clarity, residues involved in the interaction with Gd complexes are labelled. Inner-sphere water molecules are in yellow.*

For Gd-L2, the longer hydrophobic moiety causes the central aromatic ring to be sandwiched between Leu387 and Arg410. Thus the coordination cage is spaced further from the surface of the protein than Gd-L1. Even so, one of the acetate arms can form a hydrogen bond with the amidic group of Val493 (Figure 2B). In short, the main result from the docking work-up is that the coordination cage of Gd-L1 is more embedded in the hydration layer of the protein surface than Gd-L2.

The findings from molecular modelling (MM) studies strongly support the different behaviour shown by Gd-L1 and Gd-L2 as far the exchange of their coordinated water when the complexes are bound to HSA is concerned. In fact, binding to HSA

causes "quenching" of the exchange of the inner-sphere water of GdL1, as clearly shown by the low ^{17}O NMR R_{2p} values (Supporting Information), which are almost superimposable on those observed for the HSA solution in the absence of any paramagnetic complex. Analogous "quenching" of the exchange rate of the coordinated water upon binding a paramagnetic chelate to HSA were previously reported.[16] The combined use of relaxometry and docking simulations confirms that blocking the water-exchange process in Gd-L1 is likely to be a consequence of the hydrogen-bond formation between the Glu492 residue and the coordinated water, if not the replacement of such a water following direct coordination of the Glu carboxylate moiety to the Gd^{III} ion. Thus the relaxivity increase shown by the GdL1/HSA adduct ($r_{1p}=35\ mM^{-1}s^{-1}$) reflects contributions coming from water molecules in the hydration layers and exchangeable protons on the surface of the protein in close proximity to the binding site of the paramagnetic complex.

On the other hand, the results obtained for the Gd-L2/HSA adduct show that the increased size of the triphenyl moiety allows the Gd-chelate to protrude far from the Glu492 residue, so that it is unable to interfere with the exchange of the coordinated water molecule. Unfortunately, the low solubility of Gd-L2 prevents the obtainment of meaningful ^{17}O NMR R_2 data to directly support this conclusion. However, the higher ^1H r_{1p} value shown by the Gd-L2/HSA adduct ($r_{1p}=43.5\ mM^{-1}s^{-1}$, with respect to the corresponding value for the Gd-L1/HSA system) is fully consistent with the conclusions drawn from the docking experiments. Further support for this view has been gained by measuring the ^1H r_{1p} relaxivity as a function of temperature. It has been found that the r_{1p} of the Gd-L2/HSA adduct decreases exponentially as the temperature increases, as expected when the observed relaxivity is not affected by long τ_M values (see Supporting Information).

Comparison of the $1/T_1$ NMRD profiles of the GdL1/HSA and Gd-L2/HSA adducts fully supports the above conclusions (Figure 3 and Supporting Information). The relaxivity peak of the Gd-L2/HSA adduct is much lower than the value one would have expected for a system with one inner-sphere water molecule, $\tau_R \sim 30$ ns and τ_M of 65 ns. The observed profile can be fitted by using a τ_R value of 1.2 ns, that is, 25 times lower than that of HSA. As shown by MM studies, the triphenyl moiety is tightly bound in the hydrophobic cavity of site II in subdomain IIIA of HSA and only local low-amplitude oscillations appear possible. We surmise that the shorter τ_R of the magnetic vector between the Gd^{III} ion and the protons of the coordinated water is actually the result of the superposition of the internal rotation of the coordinated water on the overall molecular reorientation.[17] In this case, the target of the theoretically foreseen r_1 values $>100\ mM^{-1}s^{-1}$ (at 20 MHz) pose a new challenge to chemists on how to conjugate a fast exchange rate of the coordinated water with its blockage inside the coordination cage of the Gd^{III} ion.

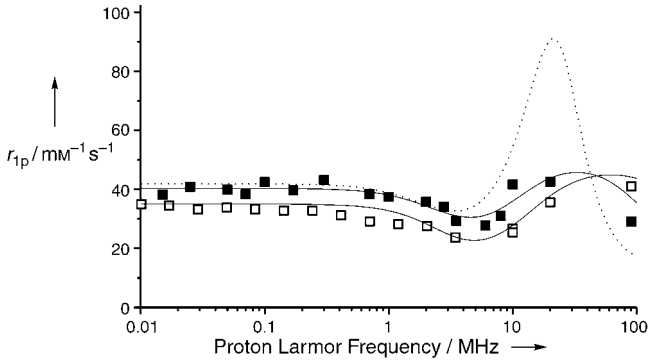


Figure 3. *NMRD profiles of GdL1/HSA (□) and Gd-L2/HSA (■) adducts measured at 298 K and neutral pH. The dotted line represents the simulated curve obtained with the same fitting parameters of Gd-L2/HSA adduct but lengthening τ_R to 30 ns.*

Keywords: albumin · chelates · drug delivery · high relaxivity · imaging agents · molecular modeling

[1] P. Caravan, N. J. Cloutier, M. T. Greenfield, S. A. McDermid, S. U. Dunham, J. W. M. Bulte, J. C. Amedio, R. J. Looby, R. M. Supkowski, W. D. Horrocks, T. J. McMurry, R. B. Lauffer, *J. Am. Chem. Soc.* **2002**, *124*, 3152.
[2] S. Aime, M. Botta, M. Fasano, E. Terreno, *Chem. Soc. Rev.* **1998**, *27*, 19.
[3] P. Caravan, J. J. Ellison, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[4] A. E. Merbach, E. Tóth, *The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, Wiley, Chichester, **2001**.
[5] P. Caravan, M. T. Greenfield, X. D. Li, A. D. Sherry, *Inorg. Chem.* **2001**, *40*, 6580.
[6] L. Vander Elst, S. Laurent, H. M. Bintoma, R. N. Muller, *MAGMA*, **2001**,*12*, 135.
[7] S. Aime, M. Chiaussa, G. Digilio, E. Gianolio, E. Terreno, *J. Biol. Inorg. Chem.* **1999**, *4*, 766.
[8] S. Aime, M. Botta, S. Geninatti Crich, G. B. Giovenzana, R. Pagliarin, M. Piccinini, M. Sisti, E. Terreno, *J. Biol. Inorg. Chem.* **1997**, *2*, 470.
[9] S. Hajela, M. Botta, S. Giraudo, J. D. Xu, K. N. Raymond, S. Aime, *J. Am. Chem. Soc.* **2000**, *122*, 11 228.
[10] R. S. Ranganathan, N. Raju, H. Fan, X. Zhang, M. F. Tweedle, J. F. Desreux, V. Jacques, *Inorg. Chem.* **2002**, *41*, 6856.
[11] F. Yerly, A. Borel, L. Helm, A. E. Merbach, *Chem. Eur. J.* **2003**, *9*, 5468.
[12] P. Caravan, J. J. Ellison, T. J. Mc Murry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293.
[13] D. H. Powell, O. M. Ni Dhubhghaill, D. Pubanz, L. Helm, Y. S. Lebedev, W. Schlaepfer, A. E. Merbach, *J. Am. Chem. Soc.* **1996**, *118*, 9333.
[14] T. J. Peters, *All About Albumin, Biochemistry, Genetics and Medical Applications*, Academic Press, San Diego, CA, **1996**.
[15] MOE ver. 2004.03 Chemical Computing group Inc.
[16] 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.
[17] F. A. Dunand, A. Borel, A. E. Merbach, *J. Am. Chem. Soc.* **2002**, *124*, 710.

Received: October 13, 2004
Published online on March 24, 2005

