Relaxometric characterization of functionalized derivatives of $[Gd(AAZTA)(H_2O)_2]^-$ with aminoacids

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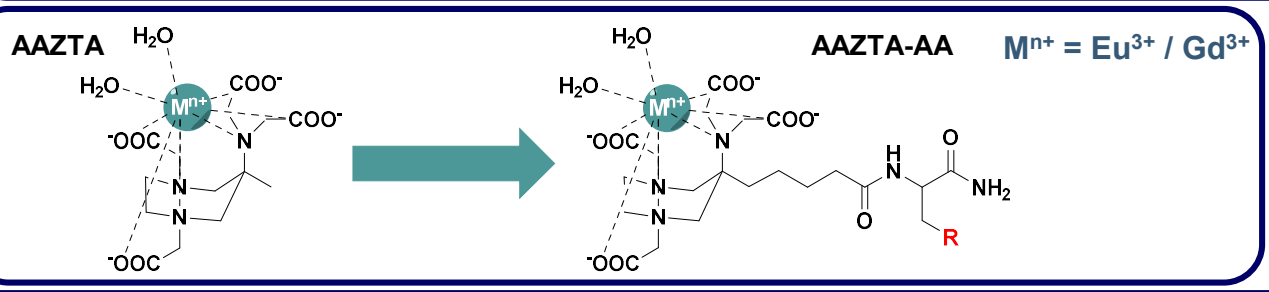
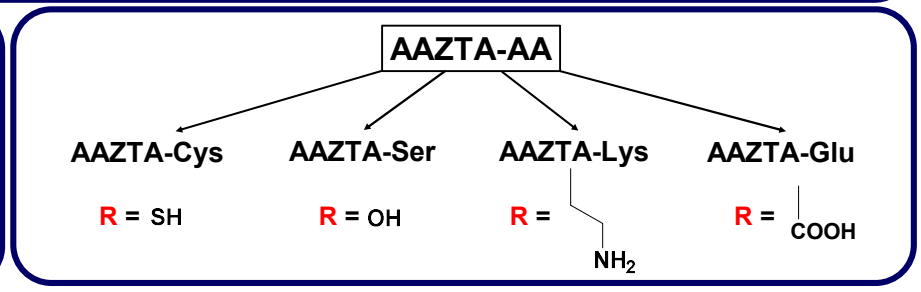
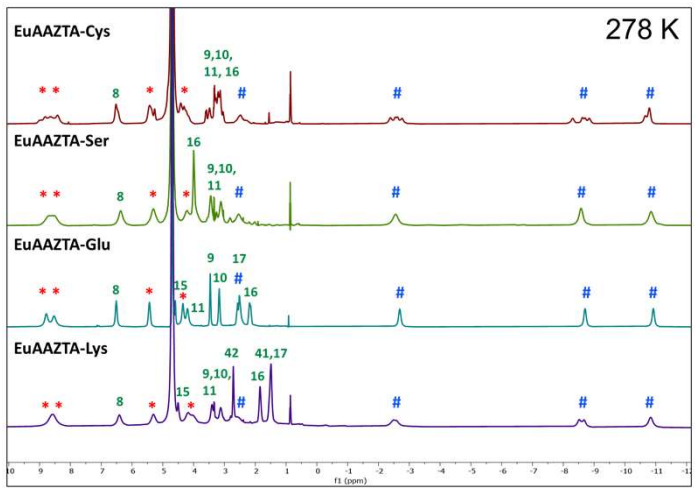
Introduction

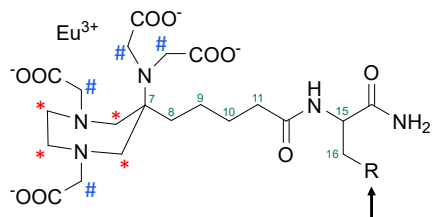
The $[Gd(AAZTA)(H_2O)_2]^-$ complex (AAZTA = 6-amino-6-methylperhydro-1,4-diazepine tetra acetic acid) is a platform of great interest for the design of new innovative MRI probes, due to its remarkable magnetic properties, thermodynamic stability, kinetic inertness, and high chemical versatility [1,2]. We developed in collaboration with the University of Torino some derivatives functionalized with amino acid residues (AAZTA-AA) with different molecular weight and charge. Three main reasons led to the choice of including amino acid residues in the ligand structure: (i) to evaluate the increase in efficacy (relaxivity) at the imaging fields (> 1 T) associated with the increase in the rotational correlation time; (ii) to promote non-covalent interactions with protein structures in biological environments, hence forming supramolecular adducts with high relaxivity; (iii) to study the properties of model compounds prior to synthesis of derivatives containing polypeptide residues for molecular imaging applications. The Eu(III) chelates were characterized by ^{1}H NMR and time-resolved photoluminescence in order to obtain structural information and determine the hydration state of the metal ion, while the relaxometric properties of the Gd(III) chelates were analysed in order to determine their molecular parameters, which describe the paramagnetic relaxation mechanism. These were accurately assessed by simultaneous fitting of the ^{1}H NMRD profiles (in the 0.01-120 MHz range) and the ^{17}O transverse relaxation rates (R_2) and shift (Dw) measured at 11.7 T and at different temperatures [3].

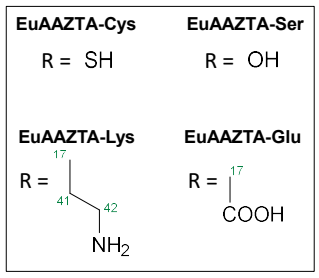




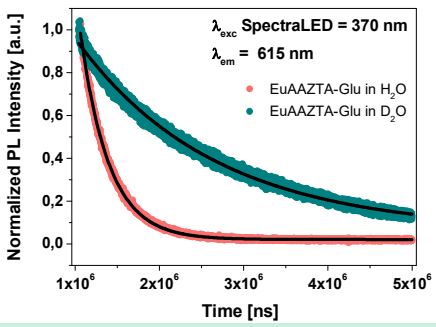
High resolution ^{1}H NMR







Time-resolved photoluminescence



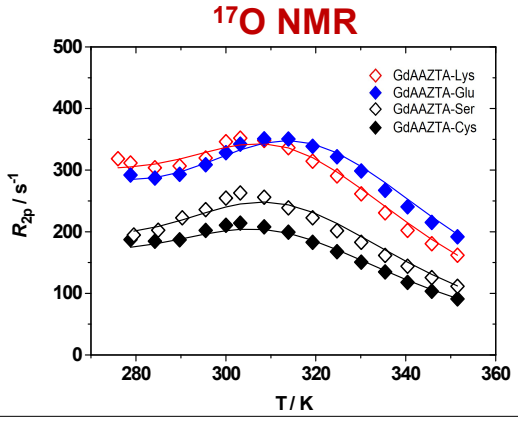
The Eu(III) chelates were characterized by ^{1}H NMR and time-resolved photoluminescence in order to obtain structural information and determine the hydration state (***q***) of the metal ion:

- From the ^{1}H NMR data, it is possible to assert the absence of isomeric forms of the coordination cage, as the typical separation of signals due to the interaction with the shift reagent Eu(III) is not detected for the peaks belonging to the latter.
- From the time-resolved photoluminescence it was possible to establish the lifetime of the excited state $^5D_0$ in H_2O and in D_2O for the Eu^{3+} complexes: applying the Parker *et al.* [5] equation, the values of ***q*** obtained for the EuAAZTA-AA were approximatively 2, as found for GdAAZTA [1].

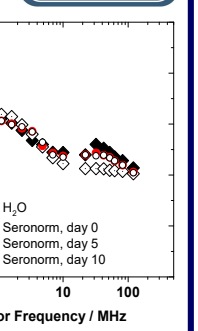
Through simultaneous fitting of the ^{1}H NMRD and the ^{17}O NMR data, the molecular parameters responsible for the relaxation process were accurately established:

- GdAAZTA-AAs show remarkable relaxivity values, higher than the parent complex GdAAZTA. This is led by the increase of molecular weight of the complexes due to the aminoacidic functionalization, which leads to an increase of the reorentetional correlation time (τ_R);
- The GdAAZTA-Ser complex shows a high relaxivity ($^{298}r_1$ 62 MHz = 12.7 mM^{-1} s^{-1}) despite the lowest molecular weight among the complex studied as a result of a significant *second sphere* contribution, attributable to the presence of interacting water molecules through hydrogen bonds with the polar side chain of the residue [4];
- The water residence lifetime (τ_M) values among the GdAAZTA-AA complexes are similar, indicating that the exchange rate of the inner sphere water molecules does not depend on the various aminoacidic residues functionalizations.

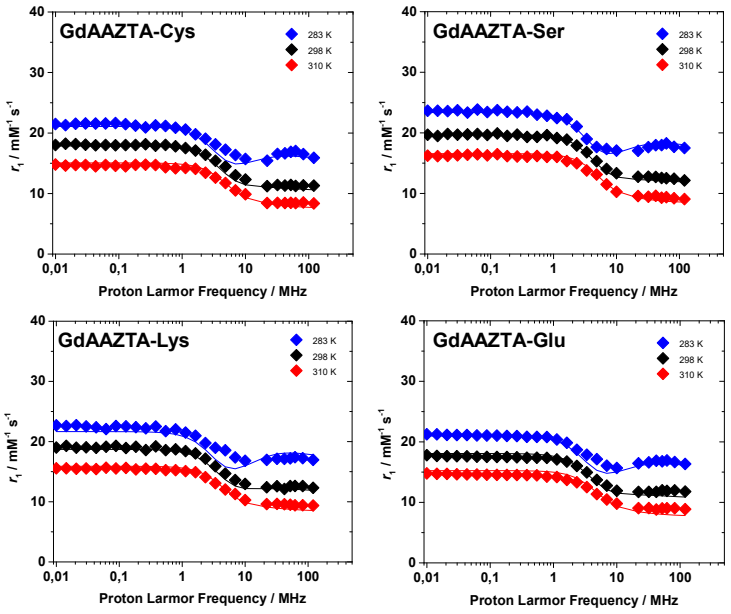
^{17}O NMR



GdAAZTA-AA in reconstituted human serum



^{1}H NMRD profiles



Parameter	R = Ser (MM=723 g/mol)	R = Cys (MM=739 g/mol)	R = Lys (MM=765 g/mol)	R = Glu (MM=767g/mol)	GdAAZTA [1] (MM=550 g/mol)
$^{298}r_1$ (62 MHz) (mM^{-1}s^{-1})	12.7 ± 0.2	11.2 ± 0.2	12.6 ± 0.2	11.9 ± 0.1	7.0
Δ^2 / 10^{19} s^{-2}	2.56 ± 0.30	2.30 ± 0.22	2.40 ± 0.26	2.30 ± 0.23	2.15
τ_V / ps	30.0 ± 1.0	31.0 ± 1.2	30.0 ± 0.9	30.0 ± 0.9	31.0
τ_M/ ns	206 ± 4	190 ± 3	219 ± 5	245 ± 7	90.0
ΔH_M / kJ mol^{-1}	30.9 ± 0.8	29.0 ± 1.0	29.8 ± 0.9	29.2 ± 0.9	/
τ_R / ps	115 ± 4	121 ± 3	140 ± 3	127 ± 6	74
$^{SS}\tau_R$ / ps	115 ± 7	/	/	/	/
$A_O/\hbar$ /10^{6}rad s^{-1}	-3.8 ± 0.3	-3.8 ± 0.2	-3.8 ± 0.3	-3.9 ± 0.2	-3.8

The best fit was obtained by fixing the following patameters: E_V = 1.0 kJ mol^{-1}, q = 2, r_{GdH} = 3.0 Å, a_{GdH} = 4.0 Å, ^{298}D = 2.24 · 10^5 cm^2 s^{-1}. For the GdAAZTA-Ser complex, assuming a *second sphere* contribution, ^{SS}q = 1 and ^{SS}r = 3.5 Å were also fixed.

By comparing the ^{1}H NMRD profiles of GdAAZTA-AA complexes in aqueous solution with those in reconstituted human serum, a marked difference at high magnetic fields is detectable: this increment in the relaxation rate values can be explained by a marked interaction of the complexes with serum proteins contained in the biological medium, with the formation of high molecular weight supramolecular adducts. Furthermore, no significant differences of the profiles collected are seen over time, suggesting a high chemical stability of GdAAZTA-AA complexes in the biological medium.

Conclusion and acknowledgments

GdAAZTA-AA show improved relaxometric properties as compared to GdAAZTA, mostly due to a longer rotational correlation time associated with higher molecular size. Thanks to their high relaxivity values, stability and kinetic inertness, these chelates can be considered good model systems for the design of MRI probes containing polypeptide residues for molecular imaging applications.

This work was carried out in collaboration with the Molecular Imaging Center of the University of Torino, which provided with the synthesis and purification of AAZTA-AA ligands.

References

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