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In vitro characterization of the Gd complex of [2,6-pyridinediylbis(methylene nitrilo)] tetraacetic acid (PMN-tetraacetic acid) and of its Eu analogue, suitable bimodal contrast agents for MRI and optical imaging

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Abstract—Gd and Eu complexes of PMN-tetraacetic acid show interesting properties either for MRI or for optical imaging; that is, for the Gd-complex, a high proton relaxivity with favorable water residence time; for the Eu-complex, a luminescence lifetime of 400 μs at room temperature compatible with the use of time-resolved luminescence technique. Both complexes have a good stability in physiological medium.

Molecular imaging requires molecular probes able to interact with specific targets and characteristic of the imaging technique. For magnetic resonance imaging (MRI) the presence of a paramagnetic or superparamagnetic center is required[1] whereas a fluorescent probe is necessary for optical imaging.[2]

In this work, we report the in vitro physicochemical properties of the Gd (III) and the Eu (III) complexes of the [2,6-pyridinediylbis(methylene nitrilo)] tetraacetic acid (PMN-tetraacetic acid) with the aim of using them as contrast agents for MRI and optical imaging respectively.

The ligand synthesized as previously described[3,4] was complexed with $GdCl_3$ or $EuCl_3$ (Fig. 1).

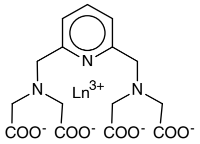

Figure 1. Chemical structure of the Ln–PMN tetraacetate complexes.

The efficiency of paramagnetic complexes as MRI contrast agents depends on several parameters related to the outer- and innersphere contributions to the water proton relaxation rates.[5–7] Among these, the number of coordinated water molecules (q) and their residence time (τ_M) can be measured by oxygen-17 NMR.[8–11] The rotational correlation time (τ_R) and the electronic relaxation properties are extracted from the analysis of the Nuclear Magnetic Relaxation Dispersion (NMRD) profiles.

The number q was obtained by comparing the chemical shifts induced on the O-17 NMR resonance of Gd–DTPA solution (for which $q = 1$) and the measured value for the Gd–PMN tetraacetate and resulted in a value of $q = 2$.[12]

The τ_M value was obtained by analysis of the transverse relaxivity of water oxygen resonance as a function of temperature (Fig. 2a).[8–11] This procedure allows for the determination of: (i) $A/\hbar$, the hyperfine coupling constant between the oxygen nucleus of bound water

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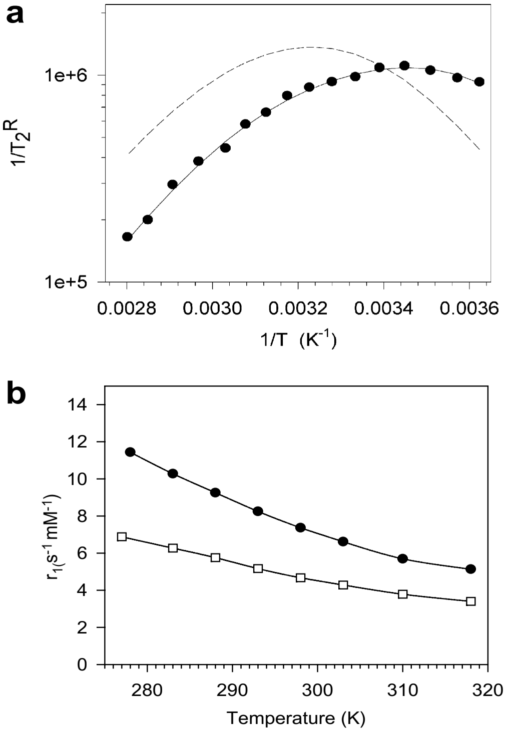

Figure 2. (a) Temperature dependence of the reduced transverse paramagnetic relaxation rate of O-17 of Gd–PMN tetraacetate solution at 7.05 T ($1/T_2^R = (1/T_2^{obs} - 1/T_2^{dia}) * 55.55/[\text{Gd-complex}]$). The fitted data of Gd–PMN tetraacetate (plain line) were obtained with the following parameters: $\Delta H^{\#} = 52.2 \pm 0.7$ kJ/mol, $\Delta S^{\#} = 66 \pm 0.5$ J/mol K, $B = 8.13 \pm 0.2\ 10^{20}\ s^{-2}$, $\tau_V^{298} = 14.8 \pm 0.3$ ps, $E_v = 5.31 \pm 2.30$ kJ/mol, $A/\hbar = -4.17 \pm 0.03\ 10^6\ \text{rad s}^{-1}$, and $q = 2$. The curve of Gd–DTPA has been added for comparison (dashed line). (b) Temperature dependence of the proton relaxivity of the Gd–PMN tetraacetate complex (closed circles) at 20 MHz. The curve of Gd–DTPA has been added for comparison (open squares).

molecules and the gadolinium(III) ion; (ii) the parameters describing the electronic relaxation times of gadolinium(III), that is, the correlation time modulating the electronic relaxation, τ_V, its activation energy, E_v, and a parameter related to the mean square of the zero-field splitting energy, B ($B = 2.4\Delta^2$); and (iii) parameters related to the water exchange, that is, the enthalpy ($\Delta H^{\#}$) and entropy ($\Delta S^{\#}$) of the process. The value of τ_M calculated for Gd–PMN tetraacetate at 310 K is short (35.2 ± 3 ns) and agrees with the decrease of the proton relaxivity measured at 0.47 T when temperature increases (Fig. 2b).

The efficacy of an MRI contrast agent is measured by its proton relaxivity (r_1 or r_2), defined as the relaxation rate increase induced by one millimole per liter of paramagnetic ions. The proton relaxivities at 310 K and 20 and 60 MHz (two clinically relevant magnetic field strengths) were equal to 5.69 and 5.03 s^{-1} mM^{-1}, respectively. These values are significantly greater as compared to Gd–DTPA ($r_1 = 3.8$ s^{-1} mM^{-1} at 20 MHz and 3.4 s^{-1} mM^{-1} at 60 MHz) and corroborate the presence of two water molecules in the first coordination sphere.

Table 1. Parameters obtained from the theoretical adjustment of the proton NMRD profile ($T = 310$ K)

Complexes	τ_M (ns)[b]	τ_R (ps)	τ_{SO} (ps)[c]	τ_V (ps)
Gd–PMN tetraacetate	35.2 ± 3	56 ± 1	205 ± 15	12.9 ± 3.8
Gd-DTPA[a]	143 ± 25	54 ± 1.4	87 ± 3	25 ± 3

[a] From Ref. 13.

[b] Fixed to the value obtained by O-17 relaxometry.

[c] τ_{SO} is the electronic relaxation time at zero field and can be related to B and τ_v ($\tau_{SO} = (5\ B\ \tau_v)^{-1}$).

The NMRD profile, which represents the evolution of the proton relaxivity with the Larmor frequency, was analyzed with the classical model taking into account the innersphere and the outersphere mechanisms (Table 1).[5–7] The fitted value of the rotational correlation time of Gd–PMN tetraacetate ($\tau_R = 56 \pm 1$ ps) is in good agreement with the expected value for a complex of this size.

The possible interaction of the complex with serum albumin was tested (Fig. 3). No obvious binding of the Gd-complex to HSA could be detected. The slight increase of the paramagnetic relaxation rates results from the increase in viscosity as already observed for other contrast agents.[13]

The thermodynamic and the conditional stability constants of the Gd-complex were previously reported ($\log K_{\text{therm}} = 18.6$, $\log K_{\text{cond}} = 16.4$ at pH 7.3).[14]

The in vivo stability of contrast agents is an important issue since the complete elimination of an administered contrast agent can require several hours or even some days. The stability was first tested in a phosphate buffer (pH = 7). No significant change of the relaxivity was observed, indicating that the complex is stable in this medium. The possible transmetallation process was then assessed by a technique based on the measurement of the evolution of the proton longitudinal paramagnetic relaxation rate (R_1^P) of a buffer solution (phosphate buffer, pH 7) containing 2.5 mM of Gd-complex and 2.5 mM of ZnCl$_2$. The relative R_1^P value at time t, $R_1^P(t)/R_1^P(0)$, allows to estimate the extent of the transmetallation process (Fig. 4).[13,15] After approximately 500 min, $R_1^P(t)/R_1^P(0)$ was of the order of 50% and was

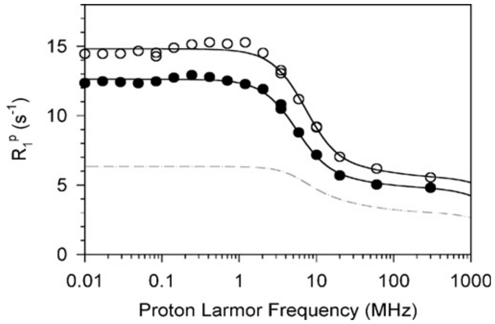

Figure 3. Proton NMRD profiles showing the paramagnetic relaxation rates of 1 mM Gd–PMN tetraacetate in water (closed circles) and in a solution containing 1 mM of the Gd-complex and HSA 4% (open circles). The curve of Gd–DTPA in water has been added for comparison (dashed line). The temperature is equal to 310 K.

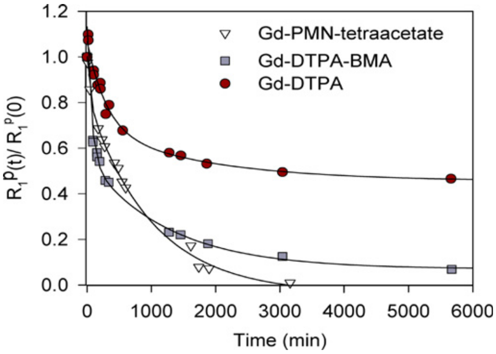

Figure 4. Assessment of the stability of the Gd-complex with respect to Zn(II) transmetallation.

close to zero after 3000 min. This complex is thus less stable than Gd–DTPA regarding the zinc transmetallation process, but during the first 17 h its behavior is nevertheless rather similar to that of Gd–DTPA–BMA, the commercial Omniscan.

Luminescence properties of the Eu-complex were investigated in various aqueous media. The excitation through the ligand π–π* transition ($\lambda_{exc} = 270$ nm) in H_2O solution at room temperature led to an emission characteristic of the Eu^{3+} ion (an intense band at 616 nm ($^5D_0 \rightarrow {}^7F_2$) and additional bands at 580 nm ($^5D_0 \rightarrow {}^7F_0$), 592 nm ($^5D_0 \rightarrow {}^7F_1$), 651 nm ($^5D_0 \rightarrow {}^7F_3$), 686 nm and 699 nm ($^5D_0 \rightarrow {}^7F_4$)) with a lifetime equal to 400 μs in good agreement with the value reported by Mukkala et al.[3] (Fig. 5). This lifetime increases substantially in D_2O solution (2.35 ms), caused by the well-known sensitivity of the Eu^{3+} luminescence toward solvent hydroxyl groups.[16] Comparison of lifetimes in H_2O and D_2O allowed an assessment of the hydration state of the Eu-complex, estimated using the Horrocks analysis.[17] The value of q (the number of metal-bound water molecules) using this analysis is 1.96, and is in perfect agreement with that obtained by analysis of the O-17 chemical shifts of water in the solution of Gd-complex.

The luminescence excitation spectrum corresponds well with the ground state absorption spectrum, confirming that the pyridine chromophore acts as a light-absorbing center for collecting UV photons and transferring them to the europium center. The values of the overall quantum yield (Φ_{tot}) obtained upon ligand excitation and efficiency of the sensitization process (η_{sens}) were determined to be 2.8% and 31%, respectively.[18] The Φ_{tot}-value is modest but fully remains within the actual range of commercially available di-aquo Eu complexes.[19]

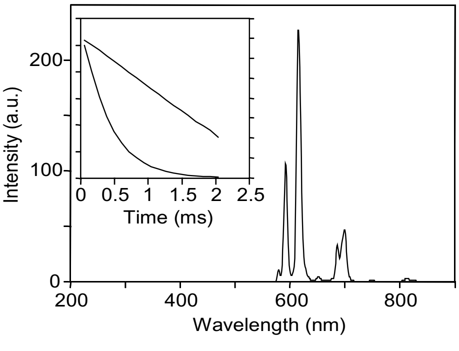

Figure 5. Emission ($\lambda_{exc} = 270$ nm) of the Eu-complex solution (2.1×10^{-5} M in H_2O). The insert shows the excited-state lifetime: luminescence decay curve (bottom plot) and ln(intensity) versus time (upper plot).

As far as the stability of the Eu-complex is concerned, no change in luminescence data (luminescence intensity and lifetime, emission profile) of the complex in H_2O and in buffers commonly used in bioanalytical chemistry, including phosphate buffer (0.05 M, pH 7.4), was observed after several days at room temperature. These results confirm the data obtained on the Gd-complex (see above). Further experiments also showed the kinetic inertness of the Eu-complex toward bioactive oxyanions. When the anionic Eu-complex was incubated for several days in the presence of a 10-fold excess of bidentate coordinating anions such as citrate, carbonate or bicarbonate, its photophysical properties were almost identical with those obtained in water. These results highlight the use of anionic europium complexes *vs.* cationic ones.[20] The luminescence intensities of the Eu-complex (2.1×10^{-4} M) at pH 7.0 with various concentrations of EDTA were also measured. When the complex was challenged with a 5- and 10-fold excess of EDTA, a dissociation of 50% and 63.5% was observed after 2 days (Fig. 6). From these experiments and by using the Verhoeven analysis,[21] log K_{cond} (pH 7.0) was found to be 15.0. This value compares well with those reported for Gd–PMN tetraacetate (log K_{cond} = 16.4 at pH 7.3)[14] and for Gd–DTPA–BMA (log K_{cond} = 14.9 at pH 7.4)[22] and suggests that Ln-complexes derived from PMN tetraacetate are suitable for application in bioanalytical assays.

The lanthanide complexes of the PMN-tetraacetic acid thus present interesting properties for both MRI and optical imaging. In fact the Ln-complexes have a relatively good stability in physiological environment. The Gd-complex has a high proton relaxivity and a value of τ_M^{310} which approaches the optimal value required to obtain high relaxivity once the chelate is bioconjugated to macromolecules. Moreover, an efficient energy transfer from the pyridine to the metal occurs in the

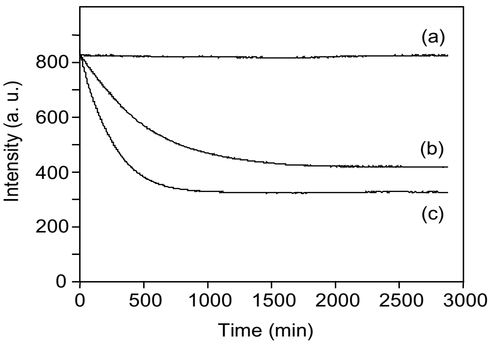

Figure 6. Luminescence (excited at 270 nm, monitored at 616 nm) of a solution of the Eu-complex in H_2O: (a) no added EDTA, (b) and (c) five and ten molar equivalent of EDTA added, respectively.

Eu-complex and the luminescence lifetime of the complex is long enough to avoid the overlapping with biological background. The introduction of functional groups in the ligand for allowing a coupling to biological materials is presently underway in our laboratories.

Acknowledgments

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