Synthesis and Physicochemical Characterization of a New Gd Complex and its Eu Analogue, Suitable Bimodal Contrast Agents for MRI and Optical Imaging

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Introduction:

The synthesis and the *in vitro* physico-chemical properties of the Gd(III) and Eu(III) complexes of [2,6-pyridinediylbis (methylenenitrilo)] tetraacetic acid (PMN tetraacetic acid, figure 1) for a potential use as bimodal contrast agents for MRI and optical imaging are reported.

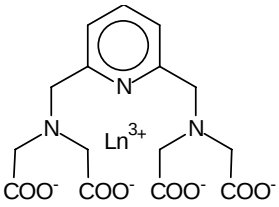

figure 1

Methods:

The ligand was obtained by hydrolysis of the tetraester prepared by reaction of 2,6-dibromomethylpyridine with t-butyl iminodiacetate (1). It was then complexed with $GdCl_3$ or $EuCl_3$. The water residence time of the Gd-complex was obtained from the analysis of the temperature dependence of the transverse relaxation of water O-17. The number of coordinated water molecules (q) in the Gd-complex was determined by the O-17 chemical shift of water resonance (AMX 300 Bruker, 7.05T). The NMRD profile was recorded on a relaxometer Stelar (Mede, Italy). The q value of the Eu complex was measured by luminescence. The proton relaxivity of the Gd-complex was measured as a function of the magnetic field (0.24mT to 7.05T) and its stability versus transmetallation by Zn ions was checked by proton relaxometry.

Results:

Gd-complex: O-17 chemical shift analysis shows the presence of 2 coordinated water molecules. The proton relaxivity is increased as compared to Magnevist, Omniscan or Dotarem (at 310 K, r_1= 5.69 and 5.03 s^{-1} mM^{-1} at 20 and 60 MHz respectively) as a result of the presence of 2 water molecules in the first coordination sphere (figure 2). The water residence time is short (35 ns at 310 K) and close to the optimal value. The possible interaction of the complex with serum albumin was tested. No binding of the Gd-complex to HSA could be detected.

The stability was first tested in a phosphate buffer (pH=7). No significant change of the relaxivity was observed. The possible transmetallation process was then assessed by the measurement of the evolution of the proton longitudinal paramagnetic relaxation rate (R_1^P) of a phosphate buffer containing an equimolar amount of Gd-complex and Zn ions (2.5 mM). The stability versus Zn(II) ions is comparable to that of Gd-DTPA-BMA

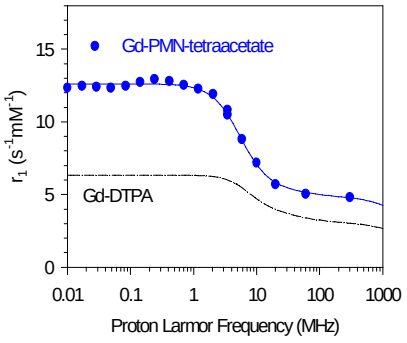

Figure 2

Eu-complex: the excitation at 270 nm in water leads to an emission characteristic of Eu ion (an intense band at 616 nm and additional bands at 580 nm, 592 nm, 651 nm, 686 nm and 699 nm) with a lifetime equal to 400 µs. The comparison with the data obtained in heavy water confirms the presence of two water molecules in the first coordination sphere of the complex. The luminescence intensities of the Eu-complex with various concentrations of EDTA allowed the estimation of the stability constant (log K_{cond} was found to be 15.0). Experiments in phosphate buffer and in presence of bidentate coordinating anions (citrate, carbonate, bicarbonate) showed the kinetic inertness of the Eu-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 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. The Φ_{tot} value is within the actual range of commercially available di-aquo Eu complexes.

Conclusion: The Gd and Eu complexes of PMN-tetraacetic acid show interesting properties either for MRI or for optical imaging: for the Gd-complex, a high proton relaxivity and an acceptable stability in physiological medium; for the Eu-complex, energy transfer from pyridine to europium and a luminescence lifetime long enough to avoid the overlap with biological background. The introduction of functional groups on the ligand for coupling to biological materials is in progress.

Reference : (1) Mukkala, V.M. et al., Helv. Chim. Acta, 1992, 75, 1621