COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Eu^{II}-cryptate with optimal water exchange and electronic relaxation: a synthon for potential pO_2 responsive macromolecular MRI contrast agents†

László Burai, Rosario Scopelliti and Éva Tóth*

Ecole Polytechnique Fédérale de Lausanne, ICMB, BCH, Lausanne, Switzerland.
E-mail: eva.jakabtoth@epfl.ch; Fax: 41 21 693 9875; Tel: 41 21 693 9878

Received (in Cambridge, UK) 11th July 2002, Accepted 28th August 2002
First published as an Advance Article on the web 18th September 2002

The cryptate $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ displays several interesting features with respect to pO_2 responsive MRI contrast agent applications: it is relatively stable against oxidation, it has two inner sphere water molecules, and the water exchange and electron spin relaxation rates are in the optimal range to attain high proton relaxivities, provided the rotation is also optimized.

Current developments in medical magnetic resonance imaging aim to visualize the physical–chemical state of tissues.[1] The oxygen partial pressure, pO_2, is a particularly significant parameter to address. It plays an important role in cellular metabolic processes, and its anomalies indicate many pathologies. Complexes containing a redox active metal site have been proposed as pO_2 responsive MRI contrast agents. Such complexes may cause different proton relaxation enhancement depending on the redox state of the metal, itself determined by the oxygen partial pressure of the biological environment.

In addition to Mn^{II}/Mn^{III} chelates as proposed by Aime *et al.*,[2] Eu^{II}/Eu^{III} complexes could also function as pO_2 responsive MRI contrast agents. The reduced Eu^{II} state is isoelectronic with Gd^{III}, thus efficiently enhances proton relaxation, whereas the oxidized Eu^{III} state is a poor relaxing agent. The few Eu^{II} complexes studied so far in aqueous medium were too unstable against oxidation.[3,4] An eventual biomedical application of the Eu^{II}/Eu^{III} system would require the relative stabilization of the reduced state. It implies that the coordinating ligand has to favour Eu^{II} to Eu^{III}, which can be achieved through a good size match between a macrocyclic ligand and the Eu^{II} ion.

The cryptand 2.2.2 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) is known to have an appropriate cage for Sr^{II} and thus for the similar size Eu^{II}. Indeed, the $Eu^{II}(2.2.2)^{2+}$ cryptate is the most redox stable Eu^{II} chelate known so far.[5]‡ Based on the redox potentials of the cryptate and the aqua ion,[5] the thermodynamic stability for $Eu^{II}(2.2.2)^{2+}$ is revealed to be seven orders of magnitude higher than for the corresponding Eu^{III} cryptate. Here we report the parameters that influence the proton relaxation enhancement, such as the rate and mechanism of water exchange, rotation and electron spin relaxation for $Eu^{II}(2.2.2)^{2+}$. To our knowledge, this is the first ten-coordinate Eu^{II} complex described in aqueous solution. Besides the eight donor atoms of the ligand, there are two inner sphere water molecules ($[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$) yielding higher proton relaxivity§ with respect to monohydrated complexes.

In the lack of suitable crystals of $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ for X-ray analysis, we determined the structure of the corresponding Sr cryptate.¶ Sr^{II} is very similar in size to Eu^{II}, and it has been proved in several cases that the crystals of Sr^{II} and Eu^{II} complexes are isomorphic.[4,6] In aqueous solution they also adopt identical structures, according to XAFS.[7] As shown in Fig. 1, the encapsulated metal ion is ten-coordinate, bound to the six oxygens and two nitrogens of the cryptand and to two additional oxygens, one from a water and one from a triflate counter ion. In aqueous solution this triflate is replaced by a second inner sphere water, as proved by ^{17}O NMR on the Eu^{II} analogue. The coordination polyhedron is trigonal bicapped. The metal ion is in the middle between the two, almost parallel planes of coordinating oxygens: −1.322(2) Å from the O2–O3–O6, and +1.337(2) Å from the O1–O4–O5 plane, both planes being almost parallel to the O7–Sr1–O8 plane (6.1° and 9.6°). The $Sr–O_{water}$ distance is 2.618(3) Å, identical to that in $[Sr(DTPA)(H_2O)]^{3-}$ and Sr^{2+}·aq. Remarkably, the metal–nitrogen and metal–cryptand oxygen distances are about 0.1 Å longer than in Sr(ODDA), an 18-membered macrocycle.[4] This is the consequence of the rigid, preorganized cryptand structure.

The $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ cryptate was prepared by mixing an electrochemically reduced Eu^{II}-triflate stock solution with 2.2.2 (Fluka) dissolved in 0.1 M TRIS buffer, in a Schlenk tube under nitrogen.[3] The measurements were done with the exclusion of oxygen.

Variable temperature ^{17}O transverse and longitudinal relaxation rates and chemical shifts were measured at 9.4 T for

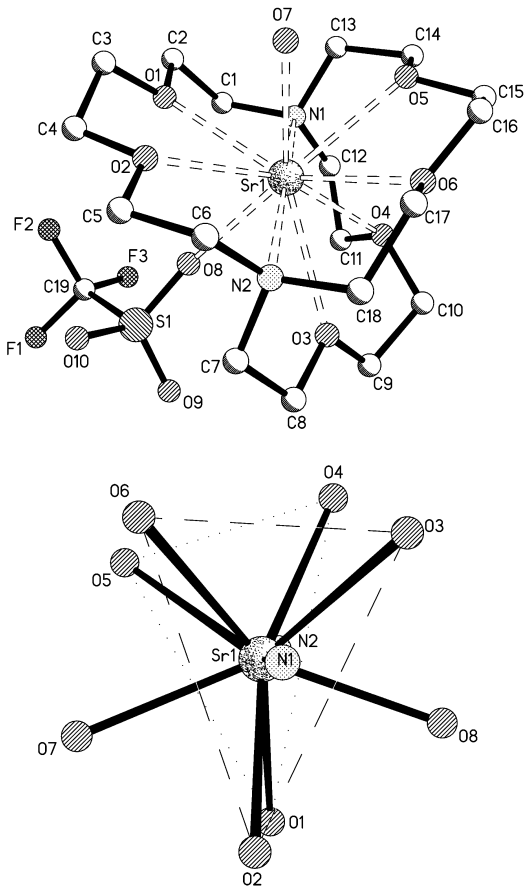

Fig. 1 Crystal structure of $Sr(2.2.2)(H_2O)(CF_3SO_3)_2$, showing only the cation.

† Electronic supplementary information (ESI) available: crystal data and structure refinement, bond lengths and angles for $[Sr(2.2.2)(H_2O)Cr_3SO_3)]$. ^{17}O relaxation rates, electronic relaxation rates and proton relaxivities for $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ solution. See http://www.rsc.org/suppdata/cc/b2/b206709a/

DOI: 10.1039/b206709a

$[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ with $[Sr^{II}(2.2.2)(H_2O)_2]^{2+}$ as diamagnetic reference. Proton relaxivities as a function of the magnetic field and transverse electron spin relaxation rates at 0.34 T were obtained at different temperatures for $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$. The experimental data were analysed simultaneously with the Solomon–Bloembergen–Morgan theory to yield parameters describing water exchange, rotation and electron spin relaxation.[3,4,8] The fitted parameters are given in Table 1 and the fitted data shown in Fig. 2.

The value of the ^{17}O scalar coupling constant, $A/$, is typical of Eu^{II} chelates ($A/$ ranges from $-(3.3–4.3) \times 10^6$ rad s^{-1})[3,4] and proves unambigously the presence of two inner sphere water molecules. These two water molecules may be non-equivalent in terms of exchange, however, the ^{17}O NMR data do not allow to get information on this or to determine the individual rates. Therefore the water exchange rate, k_{ex}, in Table 1 is an average value. It is the lowest rate of all Eu^{II} chelates studied so far,[3,4] which is probably the consequence of a higher positive charge of $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$, though it is still much higher than k_{ex} on the majority of Gd^{III} complexes.[8] The water exchange proceeds via an interchange mechanism ($\Delta V^{\ddagger} = +0.93 \pm 0.03$ cm^3 mol^{-1}, determined by variable pressure ^{17}O NMR, Fig. 2), implying that the incoming water molecule also participates in the rate-determining step.

The proton relaxivity of Gd^{III}-based MRI contrast agents, mainly of macromolecular ones, is limited by slow water exchange.[8] Eu^{II} complexes in general have much faster water exchange due to the larger size and smaller charge of Eu^{II} with respect to Gd^{III}. For $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$, k_{ex} is in the optimal range to attain high proton relaxivities.

The EPR line widths of $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ at X-band are narrower by a factor of 8–10 than those of *e.g.* $[Eu^{II}(DTPA)(H_2O)]^{3-}$,[3] indicating a remarkably slow electron spin relaxation. It is comparable to that on the Eu^{II}.aqua ion, which itself has slower electronic relaxation than Gd^{III}·aq.[9] For $[Eu^{II}(DTPA)(H_2O)]^{3-}$, proton relaxivity is limited by fast electron spin relaxation (a phenomenon never observed for Gd^{III} complexes), thus the low relaxation rate of the cryptate seems particularly significant.

In overall, a ten-coordinate Eu^{II} chelate with two inner sphere water molecules was characterized in aqueous solution with respect to redox responsive MRI contrast agent applications. In contrast to previously described Eu^{II} chelates, $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$ possesses several beneficial features: (i) it is the most stable Eu^{II} complex against oxidation, (ii) its two inner sphere water molecules allow for increased proton relaxivity, (iii) its water exchange rate is in the optimal range to attain high relaxivities, and (iv) its electron spin relaxation is slow enough not to limit proton relaxivity. Given these properties, this cryptate can be considered as a synthon to build macromolecular MRI contrast agents that are capable of reporting on the partial oxygen pressure of the surrounding tissue. The macromolecular structure will allow a slow rotation which, together with the optimal water exchange and electron spin relaxation can lead to high relaxivities for the MRI active Eu^{II} state. As a consequence, when used as a redox on/off switch, the oxidation of Eu^{II} to Eu^{III} will lead to a larger decrease in relaxivity, which translates to a more significant signal intensity difference on the MR image.

We acknowledge the Swiss National Science Foundation and the Federal Office for Education and Sciences for research grants. This work was performed within COST D18.

Table 1 Parameters obtained from ^{17}O NMR, EPR and NMRD data

	$[Eu^{II}(DTPA)(H_2O)]^{3-a}$	$[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$
$k_{ex}^{298}/10^9$ s^{-1}	1.3	0.31 ± 0.10
$\Delta H^{\ddagger}$/kJ mol^{-1}	26.3	30.6 ± 5.4
$\Delta S^{\ddagger}$/J $mol^{-1}K^{-1}$	+18.4	+20.5 ± 1.1
$\Delta V^{\ddagger}$/cm^3 mol^{-1}	+4.5	+0.93 ± 0.03
$A/10^6$ rad s^{-1}	−3.5	−4.1 ± 0.6
τ_R^{298}/ps	74	90.3 ± 14.2[b]
E_R/kJ mol^{-1}	18.9	17.9 ± 5.8
τ_v^{298}/ps	13.6	17.0 ± 1.9[c]
$\Delta^2/10^{20}$ s^{-2}	1.7	0.21 ± 0.03

[a] Ref. 3 simultaneous fit of ^{17}O NMR and NMRD data. [b] Rotational correlation time of the Eu^{II}–O_{water} vector. [c] The activation energy for τ_v was fixed to 1 kJ mol^{-1}.

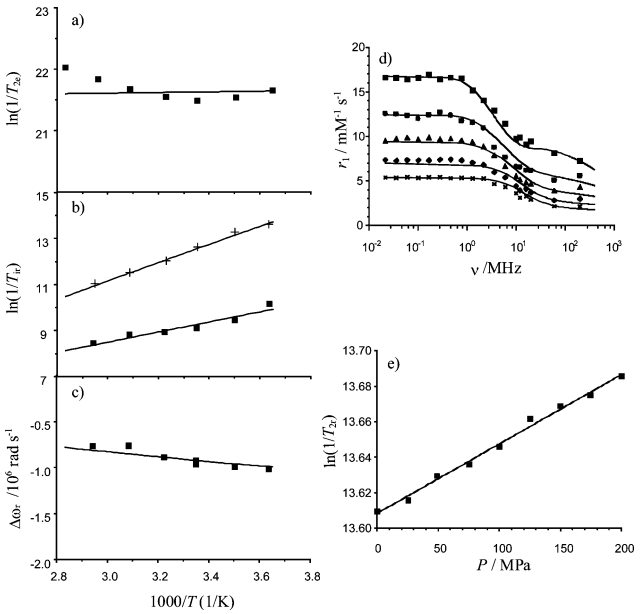

Fig. 2 Temperature dependence of (a) transverse electronic relaxation rates at 0.34 T, (b) longitudinal (■) and transverse (+) ^{17}O relaxation rates, (c) ^{17}O chemical shifts at 9.4 T, d) ^1H NMRD profiles at 5 °C, 15 °C, 25 °C, 37 °C and 50 °C from top to bottom, and (d) pressure dependence of transverse ^{17}O relaxation rates at 9.4 T for $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$.

Notes and references

‡ $E_{1/2}$ = −0.21, −0.63, −0.82, 1.35 V *vs.* Ag/AgCl, for $[Eu^{II}(2.2.2)(H_2O)_2]^{2+}$, $[Eu(H_2O)_8]^{2+}$, $[Eu^{II}(ODDA)(H_2O)]$, $[Eu^{II}(DTPA)(H_2O)]^{3-}$, respectively.

§ Proton relaxivity directly describes the efficiency of an MRI contrast agent. It is defined as the paramagnetic enhancement of the water proton relaxation rate, referred to 1 millimolar concentration.

¶ *Crystal data* for $Sr(2.2.2)(H_2O)(CF_3SO_3)_2$: $C_{20}H_{38}F_6N_2O_{13}S_2Sr$, M = 780.26, monoclinic, space group Cc (no. 9), a = 17.729(4), b = 11.889(2), c = 16.456(2) Å, β = 116.462(10)°, V = 3105.2(10) Å^3, Z = 4, μ = 1.969 mm^{-1}, T = 143(2) K, Mo-Kα radiation, λ = 0.71070 Å, 10850 reflections collected, 5660 independent reflections, (R_{int} = 0.0294), 5263 observed reflections [$I > 2\sigma(I)$], R_1 [$I > 2\sigma(I)$] = 0.0477, wR_2 (all data) = 0.1203, Absolute structure parameter 0.027(6). CCDC reference number 190053. See http://www.rsc.org/suppdata/cc/b2/b206709a/ for crystallographic data in CIF or other electronic format.

1 V. Jacques and J. F. Desreux, *"New Classes of MRI Contrast Agents" in Topics in Current Chemistry, Vol. 221: Contrast Agents I. Magnetic Resonance Imaging*, ed. W. Krause, Springer-Verlag, Berlin, 2002.
2 S. Aime, M. Botta, E. Gianolio and E. Terreno, *Angew. Chem., Int. Ed.*, 2000, **39**, 747.
3 S. Seibig, É. Tóth and A. E. Merbach, *J. Am. Chem. Soc.*, 2000, **122**, 5822.
4 L. Burai, É. Tóth, S. Seibig, R. Scopelliti and A. E. Merbach, *Chem. Eur. J.*, 2000, **6**, 3761.
5 E. L. Yee, O. A. Gansow and M. J. Weaver, *J. Am. Chem. Soc.*, 1980, **102**, 2278.
6 P. Starynowicz, *Polyhedron*, 1995, **14**, 3573.
7 G. Moreau, L. Burai, R. Scopelliti, L. Helm, J. Purans, A. E. Merbach, submitted.
8 É. Tóth, L. Helm and A. E. Merbach, *"Relaxivity of Gadolinium(III) Complexes: Theory and Mechanism" in The Chemistry of Contrast Agents in Medical Magnetic Resonance Imaging*, ed. É. Tóth and A. E. Merbach, Wiley, Chichester, 2001, p. 45.
9 P. Caravan, É. Tóth, A. Rockenbauer and A. E. Merbach, *J. Am. Chem. Soc.*, 1999, **121**, 10403.