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Interaction of Biphenyl-Functionalized Eu²⁺-Containing Cryptate with Albumin: Implications to Contrast Agents in Magnetic Resonance Imaging

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Abstract

The influence of albumin on the efficacy of a Eu²⁺-containing complex capable of interacting with human serum albumin (HSA) was investigated at different field strengths (1.4, 3, 7, 9.4, and 11.7 T). Relaxometric measurements indicated that the presence of albumin at higher field strengths (>3 T) did not result in an increase in the relaxivity of the Eu²⁺ complex, but a relaxation enhancement of $171 \pm 11\%$ was observed at 1.4 T. Titration experiments using different percentages (2, 4.5, 6, 10, 15, and 25% w/v) of HSA and variable-temperature ¹⁷O NMR measurements were performed to understand the effect of albumin on the molecular properties of the biphenyl-functionalized Eu²⁺ complex that are relevant to magnetic resonance imaging.

Keywords

cryptate; relaxivity; magnetic resonance imaging; contrast agent; europium; albumin

1. Introduction

Magnetic resonance imaging (MRI) is a noninvasive imaging modality that can be used to study anatomical structures [1], and the use of ultra-high field strength (> 7 T) magnets enables faster acquisition of MR images with increased spatial resolution [2–4]. However, common contrast agents such as low-molecular-weight Gd³⁺-containing complexes become less efficient at ultra-high field strengths relative to lower field strengths [5]. Unlike these Gd³⁺-based contrast agents, Eu²⁺-containing cryptates have higher relaxivity values at ultra-high field strengths (7 and 9.4 T) than at lower field strengths (1.4 and 3 T) [6, 7].

We hypothesized that the relaxivity of Eu²⁺-containing cryptates could be increased by using strategies that improve the relaxivity of Gd³⁺-based agents. One of these strategies is the slowing of molecular-tumbling rate that can be influenced by covalent or noncovalent interactions with macromolecules including proteins [1,8–27]. Human serum albumin (HSA), the most abundant protein in plasma with concentration of ~670 μM or 4.5% (w/v) [16], noncovalently binds to some contrast agents that contain lipophilic moieties [16–25]. McMurry and coworkers reported an increase in relaxivity of over 200% as a result of the noncovalent interaction of albumin with a Gd³⁺-containing complex functionalized with biphenyl [25]. Because of the work with Gd³⁺ and the ability to influence the properties of

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Eu^{2+} with functionalized cryptands [28], we hypothesized that a cryptand with a biphenyl group would produce a Eu^{2+} complex that interacts with albumin to result in an increase in relaxivity.

To test our hypothesis, we synthesized biphenyl-modified Eu^{2+} -containing cryptate **1** (Fig. 1), and measured the relaxivity of cryptate **1** in the presence and absence of HSA at different field strengths (1.4, 3, 7, 9.4, and 11.7 T). We compared the resulting relaxivity values to estimates calculated using the Solomon–Bloembergen–Morgan equations [29]. We also illustrated the effect of albumin on the contrast-enhancing ability of **1** using phantom images acquired at 7 T. Finally, we performed variable-temperature ^{17}O NMR experiments that probed the molecular basis of the relaxivity values of complex **1** in the presence and absence of HSA.

2. Material and methods

2.1. Materials

Commercially available chemicals were of reagent-grade purity or better and were used without further purification unless otherwise noted. Biphenyl-functionalized cryptand and cryptate **1** were prepared following a published procedure [6].

2.2. Concentrations

Samples for inductively coupled plasma mass spectrometry (ICP–MS) were diluted using aqueous nitric acid (2% v/v). Standard solutions were prepared by serial dilution of a Eu standard (High-Purity Standards). ICP–MS measurements were conducted on a PE Sciex Elan 9000 ICP–MS instrument with a cross-flow nebulizer and Scott-type spray chamber.

2.3. Preparation of HSA samples

Stock solutions of HSA (30% w/v) and (35% w/v) were prepared by dissolving 300 and 350 mg of HSA, respectively in 1 mL of 1× degassed phosphate buffered saline (PBS). An aliquot of the stock HSA solution was added to a solution of **1** in degassed PBS to produce the desired concentrations of **1** and HSA.

2.4. Preparation of Sr^{2+} analogue of **1**

A degassed aqueous solution of SrCl_2 (1 equiv, 41.2 mM) was mixed with a degassed aqueous solution of the biphenyl cryptand (2 equiv, 34.2 mM). The resulting mixture (1 mM SrCl_2 and 2 mM cryptand) was stirred for 12 h at ambient temperature under Ar. Degassed PBS (10×) was added to make the entire reaction mixture 1× in PBS, and stirring was continued for 30 min. The concentration of Sr in the resulting solution was verified by ICP–MS.

2.5. T_1 and relaxivity measurements

Longitudinal relaxation times, T_1 were measured using standard recovery methods with a Bruker Minispec mq 60 (1.4 T) at 60 MHz and 37 °C; a Varian Unity 400 (9.4 T) at 400 MHz and 20 and 37 °C; and a Varian 500S (11.7 T) at 500 MHz and 20 and 37 °C. The slope of a plot of $1/T_1$ vs concentration of Eu was used to calculate relaxivity. Four to six different concentrations of Eu (0–10 mM) were used, and measurements were repeated 3–9 times with independently prepared samples.

Susceptibility weighted imaging (SWI) was performed at 3 (Siemens TRIO) and 7 T (ClinScan) using volume coils. The acquisition parameters were as follows: $T_R = 37$ ms, $T_E = 5.68$ – 31.18 ms, and resolution = $0.5 \times 0.5 \times 2$ mm³ for 3 T; and $T_R = 21$ ms, $T_E = 3.26$ –

15.44 ms, and resolution = $0.27 \times 0.27 \times 2 \text{ mm}^3$ for 7 T. Multiple flip angles (5, 10, 15, 20, 25, and 30°) were used in the SWI experiments to allow for the determination of longitudinal relaxation time, T_1 [30]. MR images were processed using SPIN software (SVN Revision 1751). Matlab (7.12.0.635 R2011a) was used to generate effective transverse relaxation time, T_2^* , and corrected T_1 maps. The $1/T_1$ values from the corrected T_1 maps were plotted vs the concentration of Eu in the samples to calculate longitudinal relaxivities, r_1 , as previously reported [6].

2.6. Variable-temperature ^{17}O NMR experiments

Variable-temperature ^{17}O NMR measurements of solutions of **1**–HSA (1 mM 125% HSA) and its Sr^{2+} analogue (1 mM Sr^{2+} –cryptate, 25% HSA) in degassed PBS (pH 7.4) were obtained on a Varian–500S (11.7 T) spectrometer. ^{17}O -enriched water (10% H_2^{17}O , Cambridge Isotope Laboratories, Inc.) was added to samples to yield 1% ^{17}O enrichment. Transverse ^{17}O relaxation rates were measured at 15, 25, 30, 35, 40, 45, and 50 °C. The Sr^{2+} analogue of **1**–HSA was used as diamagnetic reference. A/\hbar was fixed to $-4.1 \times 10^{-6} \text{ rad/s}$ [31]. The least-squares fits of the ^{17}O NMR relaxation data were calculated using Origin software (8.0951 B951) following published procedures [32].

3. Results and discussion

Biphenyl-functionalized cryptate **1** was designed based on the structure of Gd^{3+} -based contrast agents that interact with HSA. These Gd^{3+} -containing albumin-binding contrast agents contain lipophilic aryl groups such as the biphenyl moiety that enable them to interact with HSA [16–25]. Therefore, we expected that the lipophilic biphenyl moiety in cryptate **1** would enable investigation of the effect of albumin binding on the relaxivity of Eu^{2+} at different field strengths.

The relaxivity, r_1 of **1** in PBS in the presence and absence of HSA was measured at multiple field strengths, and because not all of the magnets were capable of variable-temperature measurements, we separated data into Fig. 2a and Fig. 2b so that measurements obtained at each temperature can be compared. In the absence of HSA, the r_1 of **1** at 20 °C is higher at 7 and 9.4 T than at 3 T suggesting that this complex is more efficient at ultra-high field strengths than at lower field strengths (Fig. 2a). However, we found that the r_1 values at 3 and 11.7 T were not different (Student *t* test). Moreover, the relaxivity of **1** as a function of field strength is similar to other Eu^{2+} -containing cryptates in the same conditions [7]. In the presence of HSA (4.5% w/v), the relaxivity of **1** decreased by $24.8 \pm 0.6\%$ to $40.2 \pm 0.2\%$ at field strengths of 3, 7, 9.4, and 11.7 T (Fig. 2a). However, at 37 °C and 1.4 T, the r_1 of **1** in the presence of albumin was $171 \pm 11\%$ higher than in the absence of HSA (Fig. 2b). An increase of 218% in relaxivity (measured at 37 °C and 0.47 T MHz) was reported by McMurry and coworkers for a Gd^{3+} -containing complex functionalized with biphenyl in the presence of albumin [25]. McMurry's report and the relaxation data of **1** at 1.4 T indicate that the observed relaxation enhancement of **1** in the presence of HSA is likely due to the interaction of the biphenyl moiety of Eu^{2+} cryptate with albumin [33]. However, the relaxivity of **1** was $15.2 \pm 0.5\%$ and $49.8 \pm 0.2\%$ lower in the presence of HSA at 37 °C and 9.4 T and 11.7 T, respectively. The observed decreasing trend in relaxivity of **1** in the presence of HSA at 37 °C as a function of field strength is consistent with the trend obtained by calculating the relaxivity of a slowly rotating Eu^{2+} -containing cryptate at multiple field strengths using the Solomon–Bloembergen–Morgan equations (Fig. 2b and Supplementary material).

To better understand the effect of albumin on the relaxivity of **1** titration experiments were performed using different percentages (2, 4.5, 6, 10, 15, and 25% w/v) of HSA while

keeping the concentration (1 mM) of cryptate **1** constant. In preparation of **1**, two equivalents of the biphenyl cryptand were used for each equivalent of Eu^{2+} to facilitate metalation of the biphenyl cryptand; therefore, the samples 2, 4.5, 6, and 10% HSA have an excess of biphenyl relative to HSA ($\text{HSA}/\mathbf{1} < 1$ except 10% HSA sample) and samples with 15 and 25% HSA have a subcess of biphenyl relative to HSA ($\text{HSA}/\mathbf{1} > 1$). The MR images (Fig. 3a) of **1** with $\text{HSA}/\mathbf{1}$ ratios less than one have higher signal intensities as compared to the images of samples with $\text{HSA}/\mathbf{1}$ ratios greater than 1. To quantify this observation, the proton longitudinal relaxation rate, $1/T_1$, of the sample with the lowest $\text{HSA}/\mathbf{1}$ ratio is $102.7 \pm 0.3\%$ higher than the sample with the highest $\text{HSA}/\mathbf{1}$ ratio and $47.0 \pm 0.2\%$ higher than the other sample (15% HSA) that has an excess of HSA relative to biphenyl (Fig. 3b). The difference in signal intensity and $1/T_1$ values among the samples indicate that the presence of albumin affects the relaxivity of complex **1** and does not translate to a higher signal intensity and higher efficacy of **1** at ultra-high fields. These observations support the relaxivity measurements described in Figure 2.

To explain the observations of relaxivity, we performed variable-temperature ^{17}O NMR measurements using a sample of **1** in PBS and **1** in PBS with 25% HSA. We chose 25% HSA because the majority of **1** was expected to be bound to albumin at this percentage [25], and the ^{17}O NMR data obtained from this sample would be representative of the molecular properties of cryptate **1** that is bound to HSA. The molecular properties that influence relaxivity that were studied using variable-temperature ^{17}O NMR spectroscopy were the residence lifetime of bound water molecules, τ_m^{298} , the water-exchange rate, $k_{ex}^{298} = 1/\tau_m^{298}$; and the number of inner-sphere water molecules, q (Table 1). While the residence lifetime of bound water of cryptate **1** was not different (Student t test) in the presence and absence of albumin, the number of inner-sphere water molecules was reduced from two to one upon addition of albumin indicating that the observed decrease in relaxivity for cryptate **1** in the presence of albumin at 3, 7, 9.4, and 11.7 T is likely caused by the displacement of inner-sphere water molecules from the Eu^{2+} complex. Moreover, the residence lifetime of bound water was not different in the presence or absence of albumin suggesting that protein side chains do not hinder the remaining bound water molecule from exchanging with the bulk solvent. With the water-exchange rate and the number of inner-sphere water molecules of **1** in the presence of HSA remaining constant at different field strengths, the relaxivity enhancement observed at 1.4 T likely can be attributed to the enhanced contribution of rotational correlation rate at this field strength.

4. Conclusions

The relaxation profile of Eu^{2+} -containing cryptate **1** in the absence of albumin shows that cryptate **1** is more efficient at higher field strengths (7 and 9.4 T) than at lower field strengths (1.4 and 3 T). MR images and $1/T_1$ values of samples with different $\text{HSA}/\mathbf{1}$ ratios suggest that possible interaction of **1** with HSA occurs, but this interaction does not lead to an increase in relaxivity of Eu^{2+} at higher field strengths (> 3 T). However, in the presence of albumin, an increase in relaxivity of **1** is only observed at 1.4 T likely due to decreased rotational correlation rate values, and a decrease in relaxivity was observed at 3, 7, 9.4, and 11.7 T because of a decreased water-coordination number. These data are in agreement with calculations made using the Solomon–Bloembergen–Morgan equations. Studies using different lengths of linkers between the biphenyl and cryptate could provide a good insight on the influence of albumin interaction on the relaxivity of cryptate **1**. Also, binding a Eu^{2+} -containing cryptate to different sizes of macromolecules could provide a better understanding on the effect of macromolecular binding on the relaxivity of Eu^{2+} -containing cryptates at higher fields. These studies are currently underway in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ICP-MS	inductively coupled plasma mass spectrometry
HAS	human serum albumin
MRI	magnetic resonance imaging
PBS	phosphate buffered saline

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/>

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33. To confirm that the increase in relaxivity was not due to nonspecific binding of the cryptate with HSA, we repeated the measurement at 1.4 T using Eu^{2+} [2.2.2]cryptate (without biphenyl) and observed a relaxivity value of $3.31 \pm 0.07 \text{ mM}^{-1}\text{s}^{-1}$.

Highlights

The influence of albumin on a Eu^{2+} -containing complex was investigated

Relaxivity increased at 1.4 T relative with albumin

Relaxivity decreased at 3, 7, 9.4, and 11.7 T with albumin

The number of inner-sphere water molecules decreased in the presence of albumin

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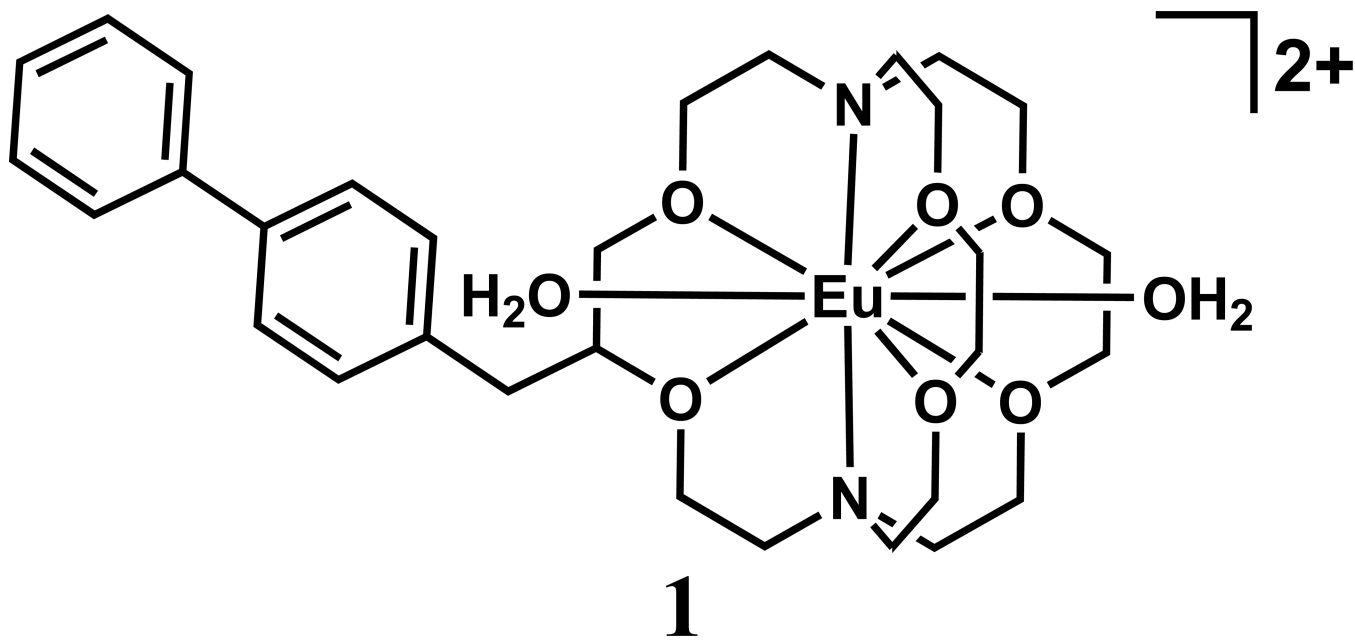


Fig. 1.
Structure of biphenyl-functionalized Eu²⁺-containing complex **1**.

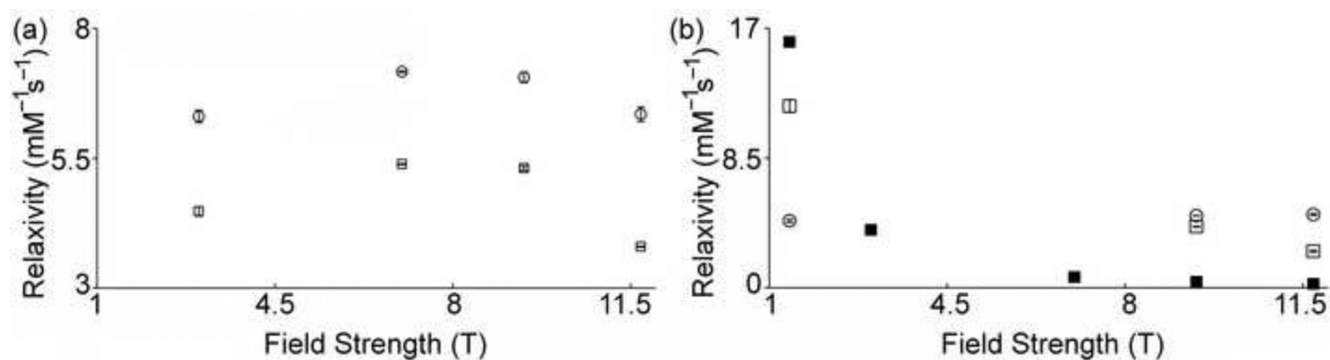


Fig. 2.

(a) Proton longitudinal relaxivity (20 °C, pH = 7.4) of biphenyl-functionalized cryptate **1** in the absence (○) and presence (□) of HSA as a function of magnetic field strength. (b) Proton longitudinal relaxivity (37 °C, pH = 7.4) of biphenyl-functionalized cryptate **1** in the absence (○) and presence (□) of HSA as a function of magnetic field strength. Simulated relaxivity values (■) of a slowly rotating Eu^{2+} -containing complex at 37 °C. Values at 1.4, 3, 7, and 11.7 T without HSA are from reference 6. HSA concentration was 4.5% (w/v). Error bars represent standard error of the mean of 3–9 independently prepared samples.

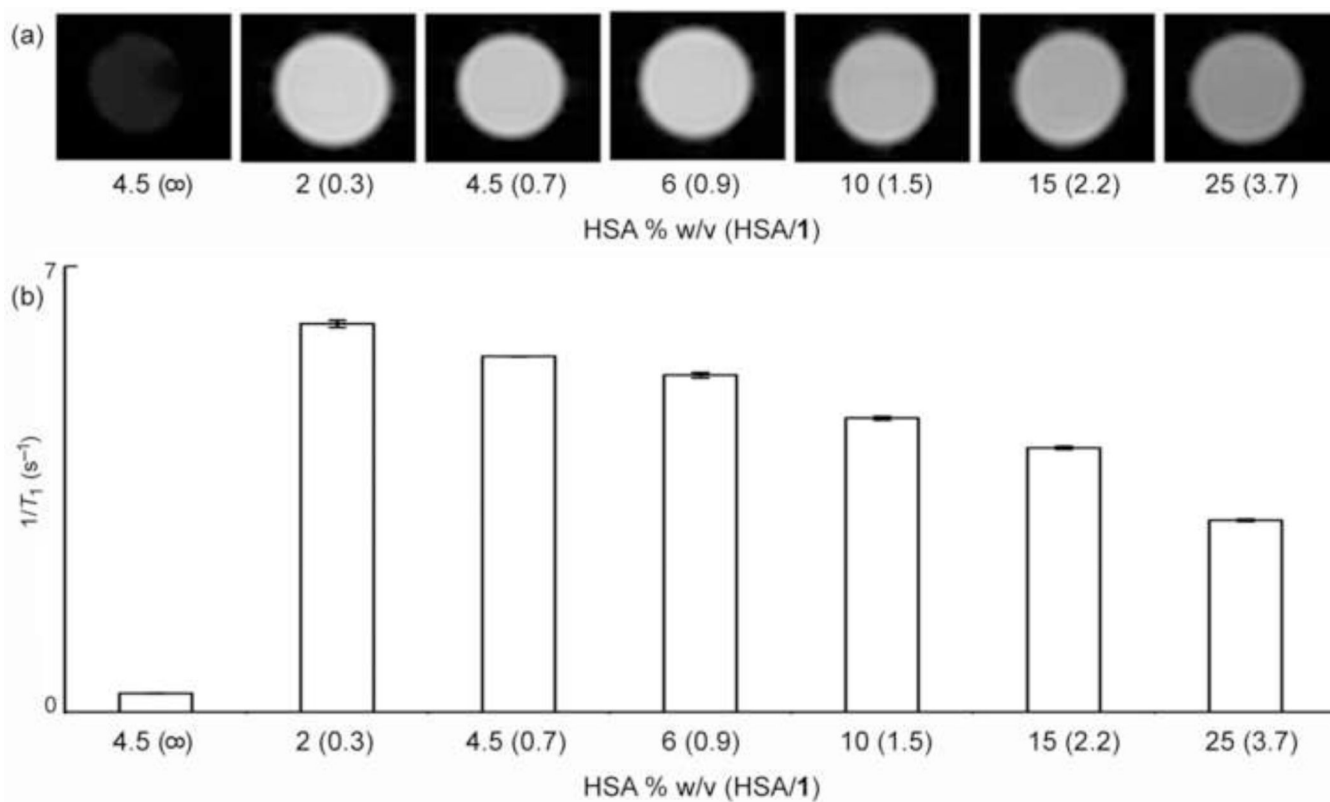


Fig. 3.

(a) T_1 -weighted MR images of albumin-containing solutions of biphenyl-functionalized cryptate **1** at 7 T and 20 °C. The diameter of the tubes that were used for imaging was 6 mm. Imaging parameters were $T_R = 21$ ms; $T_E = 3.26$ ms; and resolution = $0.27 \times 0.27 \times 2$ mm³. (b) Proton longitudinal relaxation rate (20 °C, pH = 7.4) as a function of % HSA at 7 T. The concentration of cryptate **1** was 1 mM. Error bars represent standard error of the mean of three samples. ^ano cryptate.

Table 1Results of ^{17}O NMR experiments of cryptate **1** with and without albumin.

	1 ^a	1-HSA ^b
τ_m^{298} (ns)	48 ± 4	40 ± 7
K_{ex}^{298} (10^8s^{-1})	0.21	0.25
q	2	1
ΔH (kJ/mol)	70 ± 2	62 ± 8

^aReference 6,^bHSA concentration was 25% (w/v)