A binaphthyl-containing Eu(III) complex and its interaction with human serum albumin: a luminescence study†

Jacqueline Hamblin, Neil Abboyi and Mark P. Lowe*

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On binding to human serum albumin (HSA), the Eu(III) luminescent emission enhancement of a complex containing a binaphthyl chromophore enables the determination of binding constants, showing no chiral discrimination for the (*R*)- (K = 8200 ± 810 M^{-1}) and (*S*)-enantiomers (K = 7710 ± 460 M^{-1}).

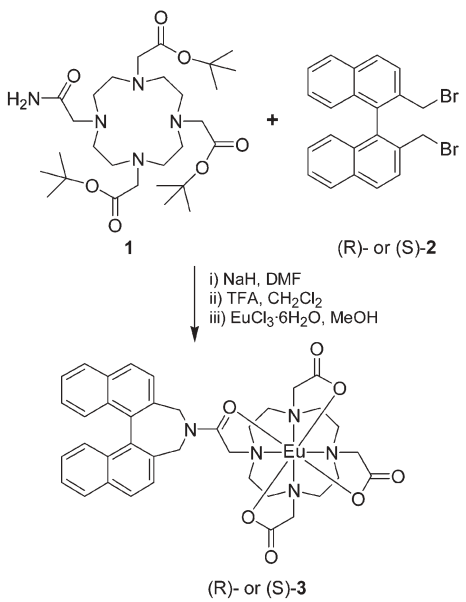
The use of gadolinium(III)-based contrast agents in magnetic resonance imaging (MRI) continues to increase.[1] The intensity of the signal in MRI is dependent on the relaxation rates of water molecules, which are increased in the presence of paramagnetic ions such as Gd(III). Contrast agents directed towards vascular imaging have been developed by incorporation of hydrophobic moieties into Gd(III)-based contrast agents.[2] This enables non-covalent binding to hydrophobic sites on the protein human serum albumin (HSA). The resultant compartmentalization, and relaxation rate enhancement brought about by slowing down the rate of tumbling (lengthening τ_R, the rotational correlation time) achieves this goal. However, the expected maximum relaxation rate enhancements of HSA-bound Gd(III) complexes is rarely observed, often due to independent rotations of the bound complex (and also in some cases, due to slower water exchange rates).[1,3] We have sought to address this issue by designing a new ligand for Gd(III) incorporating a rigid, chiral, hydrophobic 3,5-dihydro-4*H*-dinaphth[2,1-*c*:1′,2′-*e*]azepine moiety into a DOTA-based ligand. The rigidity of this binding group, coupled with the amide link is designed to minimize independent rotation of the Gd(III)-complex once bound to HSA.

Spectroscopic techniques that are inaccessible to Gd(III) are available for its neighbors in the periodic table. In particular the luminescence behavior of Eu(III) complexes provides a valuable structural probe for corresponding Gd(III) analogues. Herein, we report the luminescent properties of the (*R*)- and (*S*)-enantiomers of **3**, and their binding affinities for HSA, determined by the luminescent enhancement of the HSA-bound complex.

Complexes (*R*)- and (*S*)-**3** were synthesized from the corresponding enantiopure 2,2′-bis[bromomethyl]-1,1′-binaphthyl (**2**) as shown in Scheme 1. The monoamide derivative of DOTA, **1** was reacted with **2** in the presence of NaH in DMF, followed by removal of ^{t}butyl groups in TFA to yield the pro-ligand, which was then reacted with $EuCl_3 \cdot 6H_2O$ to yield (*R*)- or (*S*)-**3**.

^{1}H NMR spectra of (*R*)-**3** and (*S*)-**3** were identical, and typical[4] of DOTA-like complexes of Eu(III), showing resonances in the range −20 to + 40 ppm. The resonances shifted to highest frequency (34 to 40 ppm) correspond to four of the eight axial ring hydrogens. Resonances in this range are indicative of the square antiprismatic coordination geometry (the so-called M isomer).[4] Due to the non-symmetrical nature of the ligand, four resonances are expected in this range. However, in both (*R*)- and (*S*)-**3** two sets of four (*i.e.* eight in total) resonances are noted, corresponding to *two* diastereomers of the square antiprismatic geometry. This is a result of the chirality of the binaphthyl moiety, which is sufficiently remote that little influence is exerted on the helicity of the complex (often in the presence of chiral groups, one single diastereomer is formed in preference).[4] A 60 : 40 mixture (non-assignable) of the two diastereomers appears in solution, corresponding to (*e.g.* for (*R*)-**3**) (*R*)-Δ(λλλλ) and (*R*)-Λ(δδδδ) stereoisomers. (*S*)-**3** shows an identical spectrum, as the enantiomers of (*R*)-**3** square antiprismatic geometries are observed, *i.e.* (*S*)-Λ(δδδδ) and (*S*)-Δ(λλλλ) respectively.

(*R*)- and (*S*)-**3** show interesting luminescence behavior (Fig. 1). In phosphate buffered saline (PBS) at pH 7.4, excitation of the binaphthyl chromophore results in typical fluorescent emission from binaphthyl (λ_{max} = 383 nm), however, emission is also seen (at about the same intensity) from europium (~575–710 nm for ΔJ = 0 to ΔJ = 4 transitions). This emission is sensitized *via* energy transfer from the binaphthyl chromophore. Luminescent lifetime measurements in H_2O and D_2O enable the hydration state



Scheme 1

† Electronic supplementary information (ESI) available: Synthesis, ^{1}H NMR spectrum (**3**), luminescence spectra, binding constant determination. See http://www.rsc.org/suppdata/cc/b4/b415464a/

*mplowe@le.ac.uk

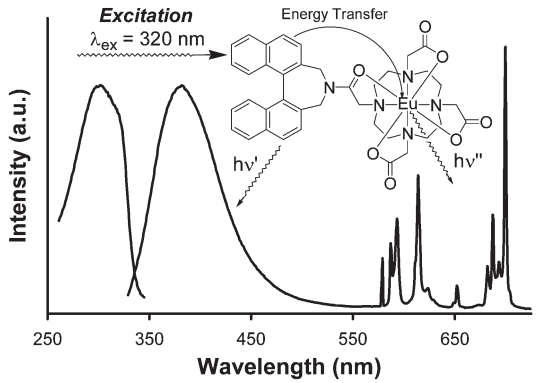


Fig. 1 Excitation (260–345 nm monitoring λ_{em} = 614 nm) and emission spectra of **3** (330–725 nm, 0.1 mM, pH 7.4, PBS, λ_{ex} = 320 nm).

(q) of the complex to be calculated,[5] giving a value of q = 0.95 (τ_{H_2O} = 0.57 ms, τ_{D_2O} = 1.41 ms). The presence of one inner-sphere water molecule is vital if the Gd(III) analogue is to be used as a contrast agent.

In the presence of HSA, at imaging concentrations (0.1 mM **3**, 4.5% HSA), the nature of the emission spectrum changes markedly (see supporting information). The excitation spectrum shifts by ~20 nm to longer wavelength (λ_{max} = 303 nm in PBS and λ_{max} = 323 nm in PBS + 4.5% HSA). The fluorescence from the binaphthyl is now essentially quenched; the major emission in this region corresponds to the solitary tryptophan residue in the protein (λ_{ex} = 320 nm excites the tail of the tryptophan absorption band). However, a dramatic ~6.5-fold increase in intensity of Eu(III) emission is noted, *i.e.* the Eu(III) luminescence is essentially switched on when the complex is bound to HSA. This can be clearly seen in Fig. 2. It is most likely that this large increase in emission intensity is due to more efficient energy transfer from the binaphthyl chromophore to Eu(III) in the presence of protein. The shift in wavelength of the excitation spectrum of the HSA-bound complex indicates a lowering of the energy of the singlet excited state of the chromophore, it is therefore expected that the triplet excited state energy will also have lowered and thus be better matched with the $^5D_0$ excited state of Eu (17240 cm^{-1}) leading to more efficient energy transfer. The change in absorption maximum in moving from an aqueous environment to the hydrophobic environment of the HSA binding site is expected. The enhancement of Eu luminescence is not due to a change in hydration state

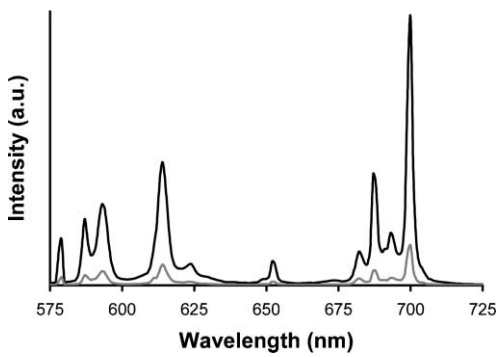


Fig. 2 Eu(III) emission spectra of **3** (0.1 mM, pH 7.4, PBS, λ_{ex} = 320 nm) in the presence (black) and absence (grey) of 4.5% HSA.

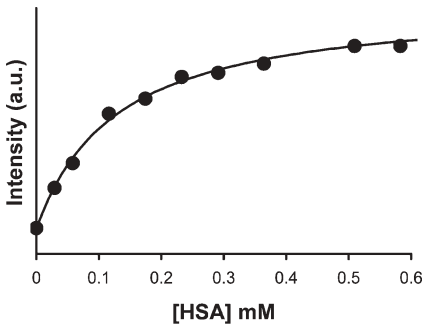


Fig. 3 Determination of the binding constant for (*R*)-**3** to HSA (0.01 mM **3**, pH 7.4, PBS, λ_{ex} = 320 nm).

from q = 1 to q = 0. Luminescent lifetime measurements confirm that the complex remains in possession of an inner-sphere water molecule on binding to HSA (q = 0.82, τ_{H_2O} = 0.75 ms, τ_{D_2O} = 2.52 ms).

The Eu(III) luminescence enhancement on binding to HSA was used to determine the binding affinities of (*R*)- and (*S*)-**3** for HSA. The binding curve for (*R*)-**3** is shown in Fig. 3. A binding constant $K = 8200 \pm 810\ M^{-1}$ is observed for (*R*)-**3**. The data fits well to a 1 : 1 binding isotherm; it is common to observe only one strong affinity site on HSA for such complexes.[1,2]

Some differentiation was anticipated between the (*R*)- and (*S*)-enantiomers of **3**, when binding to HSA. However, rather surprisingly, the (*S*)-enantiomer has essentially the same binding affinity ($K = 7710 \pm 460\ M^{-1}$). It has previously been demonstrated that (*R*)- and (*S*)-enantiomers of the contrast agent Gd-EOB-DTPA have very different affinities for HSA, however for **3** no chiral discrimination was observed.[6]

Contrast agents have been shown to bind to regions in HSA denoted sites I and II (in sub-domains IIA and IIIA respectively).[1,2,7] Site I contains the solitary tryptophan residue of the protein, and, as the fluorescence from this residue is not perturbed upon binding of **3**, it is likely that **3** binds to site II. The binding constants obtained for (*R*)- and (*S*)-**3** indicate ~83% of the complexes are bound to the protein at MR imaging concentrations.

In conclusion, we have developed a ligand system for Gd(III) bearing a rigid hydrophobic group, designed to bind to HSA. We have demonstrated *via* luminescent studies on the corresponding Eu(III) complex a relatively high affinity for HSA for these complexes. The luminescent emission from Eu(III) is dramatically enhanced on binding to the protein, and no chiral discrimination is observed for (*R*)- or (*S*)-**3**. Relaxation rate measurements on the corresponding Gd(III) complex are underway, as are modifications to the ligand structure designed to enhance the binding affinity for HSA, and to increase water exchange rates thereby maximizing the relaxation rate enhancement of the bound complex. This will be achieved by incorporation of negatively charged residues on the periphery of the ligand, the surface of the protein being replete with positively charged lysine residues.

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Jacqueline Hamblin, Neil Abboyi and Mark P. Lowe*
Department of Chemistry, University of Leicester, University Road, Leicester, UK LE1 7RH. E-mail: mplowe@le.ac.uk

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