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

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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)- (\(K = 8200 \pm 810 \text{ M}^{-1}\)) and (S)-enantiomers (\(K = 7710 \pm 460 \text{ M}^{-1}\)).

The use of gadolinium(III)-based contrast agents in magnetic resonance imaging (MRI) continues to increase. 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. 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 \(t_R\), 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).

We have sought to address this issue by designing a new ligand for Gd(III) incorporating a rigid, chiral, hydrophobic 3,5-dihydro-4H-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 luminescence 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 butyl groups in TFA to yield the pro-ligand, which was then reacted with EuCl\(_3\)-6\(\text{H}_2\text{O}\) to yield (R)- or (S)-3.

\(^1\text{H}\) NMR spectra of (R)-3 and (S)-3 were identical, and typical 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 antiprismic coordination geometry (the so-called M isomer). 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 antiprismic 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). A 60:40 mixture (non-assignable) of the two diastereomers appears in solution, corresponding to (e.g. for (R)-3) (R)-\(\Delta(\lambda\lambda\lambda\lambda)\) and (R)-\(\Lambda(\delta\delta\delta\delta)\) stereoisomers. (S)-3 shows an identical spectrum, as the enantiomers of (R)-3 square antiprismic geometries are observed, i.e. (S)-\(\Delta(\delta\delta\delta\delta)\) and (S)-\(\Lambda(\lambda\lambda\lambda\lambda)\) 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 (\(\lambda_{\text{max}} = 383\) nm), however, emission is also seen (at about the same intensity) from europium (\(\sim 575-710\) nm for \(\Delta\lambda = 0\) to \(\Delta\lambda = 4\) transitions). This emission is sensitized via energy transfer from the binaphthyl chromophore. Luminescent lifetime measurements in \(\text{H}_2\text{O}\) and \(\text{D}_2\text{O}\) enable the hydration state

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

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Eu(III) emission is noted, \( q \) giving a value of \( q = 0.95 \)
(see supporting information). The excitation spectrum shifts by
4.5% HSA, the nature of the emission spectrum changes markedly
spectra of \( \lambda_{ex} \). However, a dramatic
in the presence (black) and absence (grey) of 4.5% HSA.
binaphthyl chromophore to Eu(III) in the presence of protein. The
emission intensity is due to more efficient energy transfer from the
clearly seen in Fig. 2. It is most likely that this large increase in
switched on when the complex is bound to HSA. This can be
in moving from an aqueous environment to the hydrophobic
excited state energy will also have lowered and thus be better
matched with the \( ^{2}D_{0} \) excited state of Eu (17240 cm
the Eu(III) luminescence is essentially
\[ \lambda_{em} = 614 \text{ nm} \] and emission spectra of 3 (330–725 nm, 0.1 mM, pH 7.4, PBS, \( \lambda_{ex} = 320 \text{ nm} \)).

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 (\( \lambda_{max} = 303 \text{ nm} \) in PBS and
\( \lambda_{max} = 323 \text{ nm} \) 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
\( \lambda_{em} = 320 \text{ nm} \) excites the tail of the tryptophan absorption
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 \( ^{2}D_{0} \) excited state of Eu (17240 cm
the change in absorption maximum in moving from an aqueous environment to the hydrophobic
environment of the HSA binding site is expected. The enhance-
ment of Eu luminescence is not due to a change in hydration state

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 (\( q = 0.82 \), \( \tau_{H2O} = 0.75 \text{ ms} \), \( \tau_{D2O} = 2.52 \text{ ms} \)).

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
\( K = 8200 \pm 810 \text{ M}^{-1} \) 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 (\( K = 7710 \pm 460 \text{ M}^{-1} \)). 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 re-
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 com-
plexes 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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Fig. 1 Excitation (260–345 nm monitoring \( \lambda_{em} = 614 \text{ nm} \)) and emission spectra of 3 (330–725 nm, 0.1 mM, pH 7.4, PBS, \( \lambda_{ex} = 320 \text{ nm} \)).

Fig. 2 Eu(III) emission spectra of 3 (0.1 mM, pH 7.4, PBS, \( \lambda_{ex} = 320 \text{ nm} \)) 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, \( \lambda_{ex} = 320 \text{ nm} \)).
Notes and references


