J.M. Tennant, D.A. Barkhuizen, M. Raghoo and G.E.M. Maguire,
S. Afr. J. Chem., 2006, **59**, 86–89,
<http://journals.sabinet.co.za/sajchem/>.

APTA: A Simple Ligand for Lanthanide Energy Transfer Luminescence in Aqueous Solution

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Received 2 June 2006; revised 3 July 2006; accepted 25 July 2006.

ABSTRACT

Amino-phenol triacetic acid (apta) has been evaluated as a potential sensitizing ligand for energy transfer luminescence to lanthanide ions in aqueous media at pH 7.2. Photophysical data including quantum yields (Φ) excitation wavelength (265 nm), emission wavelength (544 nm) and excited state lifetimes (τ_{water} = 0.83 ms) for the 1:1 complex are reported. The number of water molecules coordinated to the metal ion has been calculated using isotopic experiments and these data were compared with the octadentate ligand bis-amino-phenol tetraacetic acid (bapta). Apta demonstrates excellent discrimination between Tb^{3+} and Eu^{3+} ions and apta is the simplest ligand reported thus far capable of functioning as an electronic energy transfer sensitizing moiety.

KEYWORDS

Apta, terbium, sensitizer, luminescence.

1. Introduction

Over the past 15 years there has been a renewed interest in lanthanide coordination chemistry, especially the behaviour of complexes in aqueous solution.[1–3] The photophysical properties of these complexes have been shown to have significant usage in a number of scientific fields. Long-lived lanthanide luminescence has been widely employed in analytical methods, including time resolved immunoassay.[4] The interest in using this form of emission as a signalling mechanism arises from the fact that this method has several advantages over molecular organic fluorescence systems, including millisecond lifetimes and relatively narrow-band emissions.[5] As a result, new ligands with the necessary physicochemical requirements have been pursued.[6] Although capable of binding a number of metals, apta has been demonstrated to be selective towards magnesium ions under biological conditions.[7] For this reason it has been incorporated in the structure of fluorescent sensor devices capable of monitoring real-time Mg^{2+} activity in sub-cellular domains.[8] A photo-induced electron transfer (P.E.T.) fluorescent-based sensor that responds to the presence of Mg^{2+} ions by 'switching on' its emission process upon binding of the metal ion has also been reported.[9] More recently we reported the x-ray structure of the corresponding *t*-butyl ester derivative.[10] Apta can also be employed as a chelating agent for other cations such as zinc and aluminum and it is this flexibility which led us to investigate its potential as a lanthanide ligand.[11] A photophysical investigation had been carried out on the calcium selective ligand bis-amino-phenol tetraacetic acid (bapta).[12,13] Owing to the similarity of the structures of bapta and apta, we decided to examine the possibility of this latter ligand being capable of acting as an 'antenna' for the sensitization of terbium atomic luminescence (Fig. 1).

2. Experimental

The luminescence experiments were performed using a Perkin-Elmer LS 50B spectrometer. The band pass was 10 nm for both excitation and emission outputs. The luminescence measurements were carried out in 1.0 cm quartz cells. The spectra were uncorrected. A Metrohm 744 pH meter was used for all pH measurements and calibrated using standard Merck buffers. NMR characterization was performed with a Varian Gemini 300 MHz spectrometer. All chemicals were purchased from Sigma-Aldrich Fluka and were used without further purification. Each stock solution of Eu^{3+} and Tb^{3+} (0.001 M) was prepared by dissolving the trichloride (99.5%) in doubly distilled water or D_2O (99.5%). The ligand stock solution (0.001 M) was made up in water or D_2O (99.5%). Buffer solutions (0.1 M) were prepared from morpholinepropanesulfonic acid (MOPS), tris(hydroxy-methyl)aminomethane (Tris), and sodium acetate in either water or deuterium oxide. The pH meter was calibrated for standard buffer solutions in the pH experiments. The meter was then corrected for pD values using equation 1.[14]

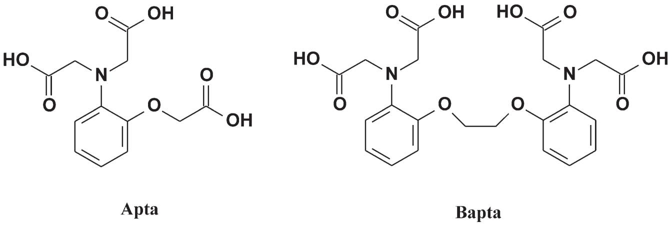
$$\mathrm{pD} = \mathrm{pH}_{\mathrm{meas}} + 0.40 \qquad (1)$$

The apta ligand was prepared as reported.[15] The apta salt was obtained by hydrolysis of the *t*-butyl ester in methanol using three equivalents of KOH. Bapta was synthesized using a previously reported method.[16] The bapta salt was obtained by hydrolysis of the methyl ester in methanol using four equivalents of KOH.

^{1}H NMR for apta: (300 MHz, $CDCl_3$): 6.81–6.92 (4*H*, m, ArH), 4.52 (2*H*, s, OCH_2), 4.05 (4*H*, s, NCH_2), 1.44 (9*H*, s, *t*-butyl), 1.40 (s, 18*H*, *t*-butyl) ppm. M.p. = 339–340 K.

^{1}H NMR for bapta: (300 MHz, $CDCl_3$): 6.78–6.91 (8*H*, m, ArH), 4.23 (4*H*, s, OCH_2), 4.13 (8*H*, s, NCH_2), 3.54 (12*H*, s, OMe) ppm. M.p. = 360–262 K.

The solutions for the luminescence studies were prepared



Apta **Bapta**

Figure 1

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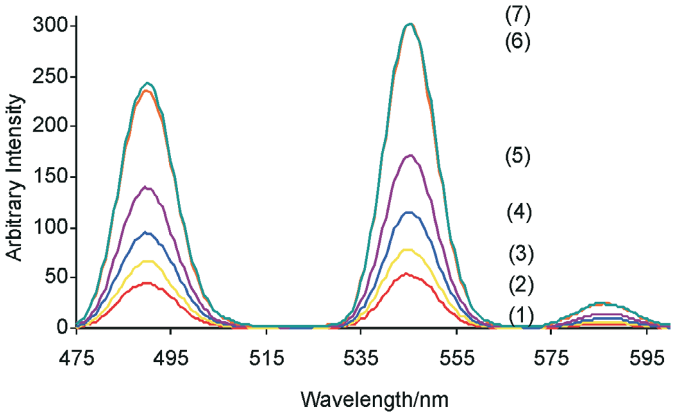


Figure 2 Variation in luminescence emission as a function of the molar ratio of [apta]:[Tb^{3+}] at pH 7.2 ([MOPS] = 1 × 10^{-2} M, λ_{ex} 265 nm, [apta] = 1 × 10^{-5} M). [apta]:[Tb^{3+}]; (1) 1:0, (2) 1:0.2, (3) 1:0.4, (4) 1:0.6 (5) 1:0.8, (6) 1:1, (7) 1:2.

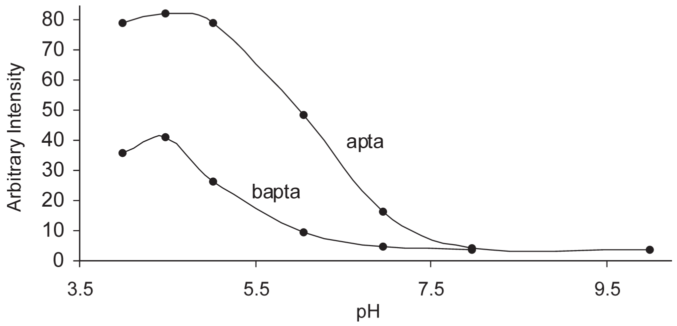


Figure 3 Variation in luminescence emission for the complexes [Tb^{3+}]:[apta] and [Tb^{3+}]:[bapta] over a range of pH. ([MOPS], [sodium acetate], [Tris-HCl] = 1 × 10^{-2} M, λ_{ex} 265 nm ([apta] = [Tb^{3+}] = 1 × 10^{-5} M) and λ_{ex} 270 nm ([bapta] = [Tb^{3+}] = 1 × 10^{-5} M).

independently in the following manner. Aliquots of appropriate volumes of the buffers and ligand solutions were added to a 10 mL volumetric flask. An aliquot of the lanthanide solution was then added and the pH adjusted to 7.2. The final complex concentration in solution was 10^{-5} M. The final buffer concentration was 10^{-3} M. All quantum yield and lifetime experiments were performed under these conditions. For the metal-dependent experiments the lanthanide ion was added in increasing concentrations to each solution. For the pH-dependent experiments the initial pH of the solution was 2.5 and this was changed incrementally using KOH to pH 10. All experiments were carried out at ambient temperature (298 K).

3. Results

3.1. Luminescence Response

In the control experiment the apta ligand was excited at λ = 265 nm. The fluorescence emission was recorded. The intensity of this response diminished in the presence of terbium cations. Using the mole ratio method[17] the luminescence emission response for terbium ions was measured in the presence of apta. Increasing the ligand concentration to a hundred-fold excess afforded no further increase in the maximum emission intensity. Figure 2 shows an example of the emission response recorded for the terbium-apta complex in aqueous solution at pH 7.2. The lanthanide luminescence intensity increases as a function of the terbium ion concentration until an equimolar concentration is achieved. Three peaks corresponding to *f–f* transitions occurring at λ = 490 nm ($^5D_4 \rightarrow {}^7F_6$), 544 nm ($^5D_4 \rightarrow {}^7F_5$) and 587 nm ($^5D_4 \rightarrow {}^7F_4$) were observed.[18] The delayed luminescence results demonstrated that the origin of the emission response arises from a triplet energy-transfer mechanism.

The same procedures were repeated while employing europium as the target metal. No luminescence emission was observed for the corresponding lanthanide complex in these experiments. This result parallels that reported for work carried out on bapta.[13] There are a number of reasons for this latter result. First, because the excited state of the europium cation is lower in energy than that of terbium, the europium cation is more sensitive to quenching by coordinated water oscillators. Second, it may be due to a charge transfer interaction between the europium ion and the aromatic moiety, which would facilitate a non-radiative decay pathway for the excited electrons.

3.2. pH Response

The protonation state can have a profound effect on the ability of the ligand to facilitate the formation of the triplet state.[19] Ideally we would like the maximum quantum yield to occur at around pH 7.0. The luminescence emission characteristics of the terbium–apta and terbium–bapta complexes as functions of the pH in the aqueous solution were evaluated. Figure 3 shows the intensity of the terbium-apta and -bapta complexes over an appropriate pH range. At high pH the emission intensity is low; this is most probably due to the formation of terbium hydroxide species. As the pH is lowered the metal is able to complex the 'free' ligand. As the pH is lowered still further (below 7) the protonation of the uncomplexed ligand lone pair begins to takes place (the pK_a of apta is 5.5).[9] The intensity of the emission achieves a maximum at pH 4.7. The pK_a of bapta is 6.36 and thus the increase in lanthanide luminescence intensity occurs at a higher pH than that demonstrated by the apta ligand. It can be seen that for both bapta and apta at very low pH the carboxylic residues can become protonated. This event causes a reduction in binding of the metal to the ligand and hence this would also lead to a diminished emission output.

3.3. Quantum Yield and Lifetime Evaluation

The relative quantum yield for the Tb-apta ligand at pH 7.2 was calculated by direct comparison with $TbCl_3$ solution at equimolar concentrations.[20,21] The photophysical properties of these energy-transfer complexes are affected by a number of factors. A major component of this is governed by the ability of the ligand to protect the lanthanide excited state from vibronic deactivation by the hydroxyl group vibrations present in the associated water molecules in solution.[22] Lanthanide ions have larger radii than alkaline earth metals and tend to require high coordination numbers. Ligands previously reported in the literature typically have between six and twelve chelating sites.[23] Replacement of the water molecules by multiple coordination sites of the ligand usually results in an increase in emission intensity. Apta has a maximum of only five coordinating sites so it would be reasonable to anticipate that the emission intensity would be on average less than that observed for other ligands. To semi-quantify the number of associated water molecules in close proximity to the Tb-apta complex, lifetime measurements of the excited state in water and deuterium oxide were undertaken. Equation 2 describes the calculation reported in the literature, which has been established to rationalize the number of coordinated water molecules.[24,25]

$$q = A(k_{\mathrm{H_2O}} + k_{D_2O}) \tag{2}$$

where *A* is a constant for the lanthanide, which has a value of 4.2 for terbium, and *k* is equal to 1/τ. Variations on this formula have been reported.[23] As is typical for these experiments, the lifetime

Table 1 A comparison of the physical data observed for apta and bapta terbium complexes in aqueous media.

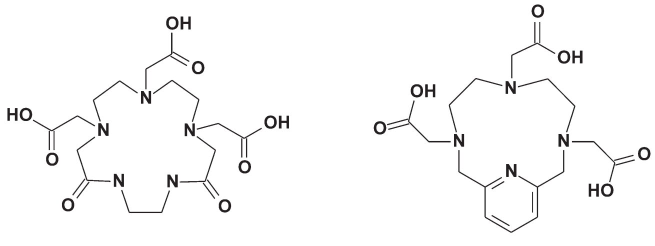
	Tb-apta complex (λ_{ex} = 265 nm, λ_{em} = 544 nm)	Tb-bapta complex (λ_{ex} = 270 nm, λ_{em} = 544 nm)
Quantum yield, Φ	0.009	0.026 [a]
Lifetime, $\tau(H_2O)$/ms	0.83	1.73 [a]
Lifetime, $\tau(H_2O)$/ms	1.52	3.44 [a]
Number of water molecules	2.3	0.5 [a]

[a] Values were obtained from reference [13].

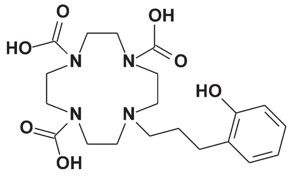
of the complex excited state was longer in D_2O than in H_2O. For the terbium-apta complex a value of 2.3 water molecules was obtained. This figure, as expected, exceeds that reported for bapta and other octadentate ligands.[26] The observed physical parameters for the apta and bapta ligands are compared in Table 1.

4. Discussion

The apta ligand demonstrates typical activity for an electronic energy transfer ligand. The maximum emission pH result appears to be similar to that reported for other amino-phenyl ether systems and in this case the maximum at pH below 5 for both ligands probably arises from the protonation of the nitrogen lone pair of the free ligands in equilibrium with the kinetically labile metal complex. The luminescence quantum yield observed for apta is lower than that for other chelating ligands, but this can be explained by the reduced number of binding sites present in the molecule. It is apparent from our results that in apta the lanthanide cations are not as well protected from the deactivating nature of the surrounding water hydroxyl vibrational interference. It should be noted, however, that the accuracy of these isotopic labelling experiments has been reported to be ± 20%[23] or 0.5 water molecules.[27] We can however compare the result obtained for the apta ligand with examples from the literature. Figure 4 shows the structures of three molecules which have tri-acetic acid ligating sites and two or more coordinating neutral atoms. The Eu^{III} DTPA-eam, Tb^{III} PCTA and Gd^{III} propyl-phenyl DO3A complexes were reported to have 2.3, 2.4 and 2.0 water molecules associated with the complexes respectively.[28,29,30] These results are similar in value to those we obtained. No quantum yields however were reported for these examples.



DTPA-eam PCTA



1-[3-(2-hydroxyphenyl)]-propyl DO3A

Figure 4

Although the overall photophysical performance (e.g. quantum yield), for the apta ligand is not of the same magnitude as those of previously reported sensitizers, the novelty of this molecule arises from the fact that first, it functions at all, and second, its synthesis is facile. The study has demonstrated, however, that when examining the suitability of organic ligands as triplet energy sensitizing agents, aromatic amine derivatives are not ideal targets. As far as we can ascertain apta appears to be the simplest lanthanide ligand capable of functioning as an energy-transfer sensitizing moiety in a 1:1 complex ratio.[31]

Acknowledgements

G.E.M.M. would like to acknowledge funding received for this work from the National Research Foundation of South Africa (Economic Growth and Development Fund, Gun 2053369). The authors would like to thank Dr H.G. Kruger (University of KwaZulu-Natal) and Professor A.P. de Silva (Queen's University of Belfast) for their helpful discussions.

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31 Ligands such as 2,6-pyridinecarboxylic acid have been widely reported but these molecules tend to form complexes with unequal ratios, e.g. $Eu(DPA)_3^{3-}$ (see A.L. Jenkins and G.M. Murray, *Anal. Chem.,* 1996, **68**, 2974–2980).