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Porphyrin sensitization of circularly polarised near-IR lanthanide luminescence: enhanced emission with nucleic acid binding†

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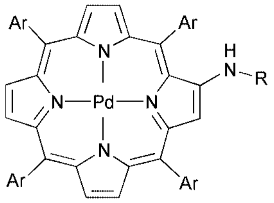
A palladium porphyrin has been covalently linked to a chiral lanthanide complex and effectively sensitises near-IR emission from Nd and Yb; sensitisation is enhanced in the absence of oxygen and in the presence of a nucleic acid.

The use of 'antennae' chromophores (such as substituted aryl,[1] bipyridyl[2] and phenanthridyl[3] groups) to sensitize lanthanide emission is now well established, particularly for lanthanide complexes which emit in the visible region of the spectrum, *e.g.* Eu(III) and Tb(III). The triplet energies of porphyrins (typically 12 000–17 000 cm^{-1}) render them suitable chromophores for sensitization of the relatively low-lying excited state(s) of lanthanide ions such as Yb(III) (10 200 cm^{-1}) and Nd(III) (11 360 cm^{-1}). Lanthanides with emission bands in the near-IR region of the spectrum have a variety of potential applications, including time-resolved luminescence imaging,[4] beyond the range of biological absorption or emission (*e.g.* from NADPH, haematoporphyrin). Previous work has demonstrated that Yb–porphyrin complexes possess rapid rates of energy transfer ($>10^7\ \text{s}^{-1}$) from the porphyrin antennae to the bound Yb ion[5] and the Yb emission intensity is primarily limited by quenching of the $^2F_{5/2}$ excited state involving vibrational energy transfer to bound OH oscillators.[6] In these cases, the Yb emission is insensitive to oxygen quenching of the intermediate excited porphyrin triplet. When an Yb ion is further away from the sensitising chromophore, the energy transfer step is less efficient and deactivation of the intermediate triplet state by oxygen may compete. Palladium porphyrin complexes possess particularly long-lived triplet states[7] and are subject to quite efficient oxygen quenching in polar media. Tetracationic Pd–porphyrins have been shown to bind to DNA[8] and the intercalation suppresses oxygen quenching, enhancing the phosphorescence by factors of up to 20.

With this background in mind, we have prepared a set of Pd–porphyrin conjugates which are linked to a well defined cationic, chiral lanthanide moiety. The strongly helical tetra-amide complexes of Eu, Tb and Yb, incorporating an intercalating phenanthridinium moiety, have recently been shown to bind to DNA stereoselectively and with a factor of 50 in GC *vs.* AT base-pair differentiation.[9] The aim of the work described herein was to explore the sensitising ability of the proximate Pd–porphyrin chromophore for Nd and Yb emission and the effect of oxygen in the presence and absence of a simple oligonucleotide.

2-Aminoporphyrin **1** was prepared following the method described by Crossley and King.[10] Amide coupling of **1** with chloroacetic acid, using standard amide coupling conditions, followed by Pd complexation afforded the corresponding porphyrin chloroacetamide, which was then treated with an excess of KI in THF–MeCN to give the porphyrin iodoacetamide **2**. Reaction of **2** with an excess of cyclen in THF at 50 °C gave an almost quantitative yield of the monosubstituted-{Pd–porphyrin}-12-N_4. Introduction of the three chiral pendant arms onto the remaining ring nitrogens was carried out by reaction with either (*S*)- or (*R*)-2-chloro-*N*-(methylbenzyl)ethanamide to afford (*SSS-S*)-L^1 and the (*RRR-R*) isomer, respectively. Alkylation also occurred at the rather acidic amide NH position on the porphyrin periphery. Reaction of L^1 with lanthanide trifluromethanesulfonates (Yb, Nd, Gd) afforded cationic complexes which were purified by alumina preparative plate chromatography. Complexes gave ESMS, microanalyses and ^{1}H NMR spectra in accord with the proposed structures. For example, for $[\text{YbL}^1]^{3+}$, ESMS analysis revealed peaks at *m/z* 738 $[\text{M}]^{3+}$ and 1181 $[\text{M} + \text{CF}_3\text{SO}_3]^{2+}$ and the ^{1}H NMR spectrum in CD_3OD(293 K, 200 MHz) showed a single set of resonances ($>87\%$) for the most shifted ring axial proton for both stereoisomers at δ +100.8, 95.5, 78.2 and 72.9. Such chemical shift values are in a similar range to those observed for related tetraamide Yb complexes with a capping water molecule, in which the complex adopts a square antiprismatic coordination environment.[11]



Ar = $\text{C}_6\text{H}_3\text{Bu}^t{}_2$-3,5

1 R = H
2 R = COCH_2I

The circular dichroism spectra for the (*RRR*)-$[\text{NdL}^1]^{3+}$, (*RRR*)-$[\text{YbL}^1]^{3+}$ and (*SSS*)-$[\text{YbL}^1]^{3+}$ complexes, in the visible range, are shown in Fig. 1. The CD spectra are dominated by the porphyrin chromophore, with the characteristic Soret band at *ca.* 422 nm (log ε = 5.36 $\text{M}^{-1}\ \text{cm}^{-1}$), and the α and β bands at 520 (log ε 4.37) and 560 nm (log ε 3.57), respectively (295 K, MeOH). The weak induced CD obtained for the (*RRR*)-Yb

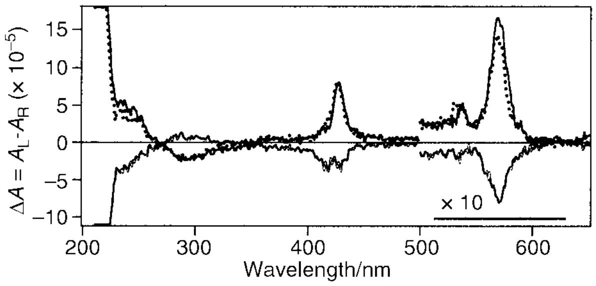


Fig. 1 CD spectra of (*RRR-R*)-YbL[1] (upper bold), (*RRR-R*)-NdL[1] (dashed) and (*SSS-S*)-YbL[1] (lower) (295 K, MeOH).

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† Electronic supplementary information (ESI) available: emission spectra. See http://www.rsc.org/suppdata/cc/b0/b002452j/

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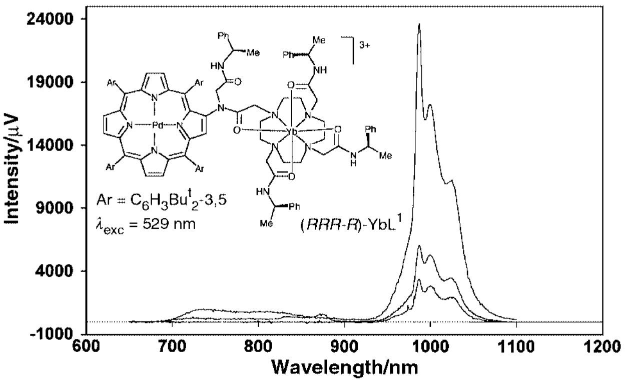

Fig. 2 Emission spectra for (*RRR*-*R*)-YbL^1 (295 K, 20% D_2O–CD_3OD, 5 μM) in degassed solution (top), in aerated solution (bottom) and in the presence of 0.1 mM $[(CG)_6]_2$.

complex resembles that of the Nd complex, with g_{abs} values of $+1.7 \times 10^{-4}$ (560 nm) and $+0.8 \times 10^{-4}$ (423 nm). Intriguingly the CD spectrum of the (*SSS*) stereoisomer showed a split Soret band (perhaps related to the change in the tertiary amide configuration in the two complexes, giving a different orientation of the porphyrin chromophore with respect to the Yb ion) and a reduced ICD at 420 and 566 nm. The chirality of the excited state of the lanthanide centre is conveniently probed using circularly polarised luminescence spectroscopy (CPL);[12] the first examples of CPL in the near-IR, following sensitised excitation in the near-UV, have only recently been reported.[13] With (*RRR*)-$[YbL^1]^{3+}$, weak CPL was observed following excitation at 529 nm, with $g_{em} = -0.04$ (985 nm) and $+0.05$ (963 nm) and a very similar spectrum, with the opposite polarisation was observed for the (*SSS*) complex. CPL spectra were also measured for the (*RRR*)-Nd complex and, for the first time, small, but measurable, CPL was observed for the $^4F_{3/2}$ – $^4I_{9/2}$ transitions [*e.g.* $g_{em} = +0.015$ (884 nm)] and for the $^4F_{3/2}$–$^4I_{11/2}$ transition [$g_{em} = -0.006$ (1055 nm)].

The total emission spectra for the Yb and Nd porphyrin conjugates were measured in aerated and degassed MeOH and CD_3OD (Fig. 2), following excitation at 529 nm. Enhanced emission from the Ln ion was observed in the deuteriated solvent (a factor of 4 for Yb and 2.5 for Nd), reflecting the known sensitivity of the excited ion to quenching by OH oscillators.[6] In the absence of oxygen, the phosphorescence from the Pd–porphyrin appeared as a broad emission with maxima at *ca.* 735 and 800 nm, while that due to the lanthanide was also enhanced (a factor of 7 for Yb and 2 for Nd). The excitation spectra of degassed samples, observing the emission from Yb at 980 nm and Nd at 1064 or 870 nm, revealed the same spectrum which matched the porphyrin absorption spectrum of the separate complexes. Taken together such behaviour is consistent with competitive rates of energy transfer and triplet quenching by oxygen. The energy transfer to Nd is considerably more efficient than to Yb, as a consequence of the better spectral overlap integral (Nd: absorption maxima at 740, 794 and 865 nm (with ε values of 6, 9 and 3 $dm^3\ mol^{-1}\ cm^{-1}$, respectively) overlap well with the porphyrin emission band, but poorly with Yb at 980 nm (2 $M^{-1}\ cm^{-1}$). The absence of the porphyrin emission in aerated solution reflects the efficiency of the bimolecular quenching process (values of $k_q[O_2]$ of *ca.* $1 \times 10^9\ s^{-1}$ have been reported for palladium porphyrins).[8] In addition, the very presence of Ln emission is an indication that the rate of intramolecular energy transfer is relatively fast. The Yb emission from the stereoisomeric (*SSS*)-YbL^1 complex was identical in form but was 40% lower in intensity.

Preliminary time resolved studies have allowed the decay of the porphyrin triplet and the grow-in and decay of the Nd or Yb emission to be monitored. The rate constant for oxygen quenching was calculated to be $5 \times 10^8\ M^{-1}\ s^{-1}$ for the Nd complex. Complex decay profiles were observed consistent with the similar rates of decay of the Yb excited state and the rate of dissociative solvent exchange.[11] However, qualitative agreement between the rate of triplet decay and the rate of Ln 'grow-in' was observed, consistent with the conventional mechanism of triplet-mediated lanthanide sensitisation.[2,6]

In the presence of a 20 mol excess of $[(CG)_6]_2$ oligonucleotide or calf-thymus DNA, in aerated solution {20% D_2O–CD_3OD, 296 K, 5 μM (*RRR*)-$[YbL^1]^{3+}$, λ_{exc} 529 nm}, the porphyrin emission re-appeared to 30% of its value in deoxygenated media; the Yb emission also increased by a factor of 2, *i.e.* also to *ca.* 30% of the value in deoxygenated solution. Very similar behaviour was observed with the (*SSS*) stereoisomer. Evidently binding to the nucleic acid inhibits quenching of the porphyrin triplet by oxygen. That the porphyrin moiety might be interacting with the CG base pairs was suggested by the observation of a red-shift (9 nm) and a pronounced hypochromicity of the Soret band (40%) on binding.

The behaviour reported here is of interest with respect to time-resolved near-IR imaging studies. The sensitivity of the Nd and especially Yb emission to pO_2 may also be of interest in defining regions of low oxygen tension.

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Notes and references

1 A. Casnati, C. Fischer, M. Guardigli, A. Isernia, I. Manet, N. Sabbatini and R. Ungaro, *J. Chem. Soc., Perkin Trans. 2*, 1996, 395; M. P. Oude-Wolbers, F. C. J. M. Van Veggel, B. H. M. Snellink-Ruel, J. W. Hofstraat, F. A. J. Guerts and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 1997, **119**, 138.

2 N. Sabbatini, M. Guardigli and J.-M. Lehn, *Coord. Chem. Rev.*, 1993, **123**, 201.

3 D. Parker, K. Senanayake and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2129.

4 M. I. Gaiduk, V. V. Grigoryants, A. F. Mironov, V. D. Rumyantseva, V. I. Chissov and G. M. Sukhin, *J. Photochem. Photobiol. B*, 1990, **7**, 15.

5 T. F. Kachura, A. N. Sevchenko, K. N. Solov'ev and M. P. Tsvirko, *Dokl. Akad. Nauk SSSR*, 1974, **217**, 1121; M. Gauterman, C. D. Schumaker, T. S. Srivastava and T. Yaneta, *Chem. Phys. Lett.*, 1976, **40**, 456.

6 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, 493; M. P. Oude-Wolbers, F. C. J. M. van Veggel, B. H. M. Snellink-Ruel, J. W. Hofstraat, F. A. J. Guerts and D. N. Reinhoudt, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2141; M. H. V. Werts, J. W. Verhoeven and J. W. Hofstraat, *J. Chem. Soc., Perkin Trans. 2*, 2000, 433.

7 P. M. Gewehr and D. T. Delpy, *Med. Biol. Eng. Comput.*, 1993, **31**, 2; P. M. Gewehr and D. T. Delpy, *Med. Biol. Eng. Comput.*, 1993, **31**, 11; D. B. Papkovsky, *Anal. Chem.*, 1995, **67**, 4112; P. Hartmann and W. Trettnak, *Anal. Chem.*, 1996, **68**, 2615; A. Beeby and S. Faulkner, *Chem. Phys. Lett.*, 1997, **266**, 116 and references therein.

8 A. M. Brun and A. Harriman, *J. Am. Chem. Soc.*, 1994, **116**, 10 383.

9 L. J. Govenlock, C. E. Mathieu, C. L. Maupin, D. Parker, J. P. Riehl, G. Siligardi and J. A. G. Williams, *Chem. Commun.*, 1999, 1699.

10 M. J. Crossley and L. J. King, *J. Chem. Soc., Chem. Commun.*, 1984, 920.

11 A. S. Batsanov, A. Beeby, J. I. Bruce, J. A. K. Howard, A. M. Kenwright and D. Parker, *Chem. Commun.*, 1999, 1011.

12 J. P. Riehl and F. S. Richardson, *Chem. Rev.*, 1986, **86**, 1; J. P. Riehl and F. S. Richardson, *Methods Enzymol.*, 1993, **226**, 539.

13 C. L. Maupin, D. Parker, J. P. Riehl and J. A. G. Williams, *J. Am. Chem. Soc.*, 1998, **120**, 10 563.