A new reagent for duplex DNA detection

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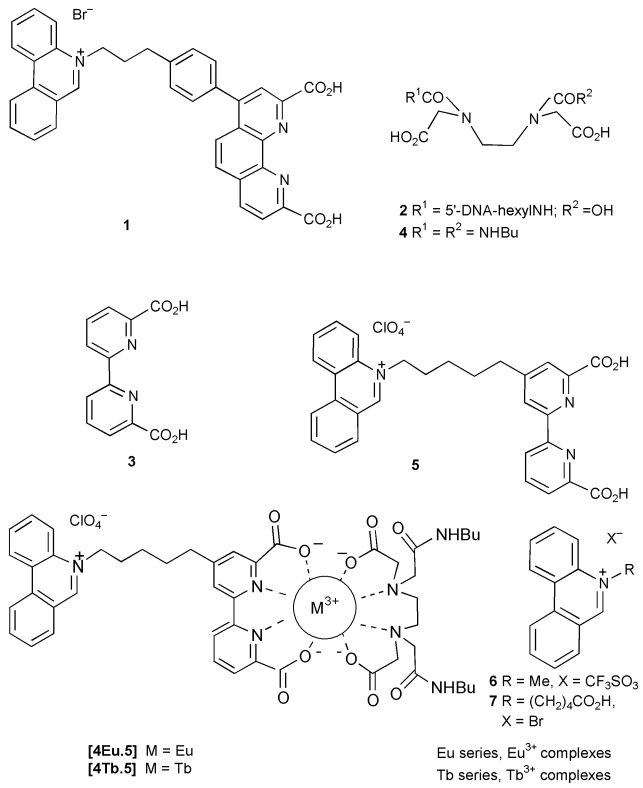
The phenanthridinium-linked terbium complex [4Tb.5] serves as a novel probe for DNA duplexes.

In recent work[1] we have demonstrated a cooperative procedure for the detection of DNA sequences. In this procedure, the phenanthridinium group of the sensitiser **1** intercalates in the locality of the duplex DNA formed between a probe DNA strand, to which is attached an EDTA complexing group **2**, for a europium ion, and a target sequence of DNA. Under appropriate conditions, the sensitiser **1** can also bind to the europium complex of **2** to form a 1 : 1 : 1 complex [**2Eu.1**][2] that,



when irradiated, exhibits a strong luminescence signal from the europium ion. The system thus signals the presence of a target strand of DNA since, in its absence, the complex dissociates and only a very weak signal is observed.

In attempting to develop a two-colour signalling system suitable for discriminating between normal and point mutated target DNA strands, a sensitiser system was required that would work for both europium and a second luminescent lanthanide ion, such as terbium. Thus, for example, a 'normal' DNA sequence could be matched by a europium probe (red emission) and a mutated sequence by the corresponding terbium probe (green emission). The phenanthroline sensitiser, such as **1**, was not suitable for use with terbium, presumably because terbium requires a higher energy triplet sensitiser than europium.[3]

Examination of several alternative systems revealed that the 2,2′-dipyridyl-6,6′-dicarboxylic acid system, **3** is suitable, giving excitation of both europium and terbium chelated with EDTA bisbutylamide **4**, as complexes [**4Eu.3**] and [**4Tb.3**] respectively, although the optimal excitation wavelength with this system was shorter (λ_{ex} = *ca.* 320 nm) compared to that possible with the phenanthroline sensitiser **1** (λ_{ex} = 340 nm). In addition, luminescence intensities were temperature dependent and measurements were therefore conducted within a narrow ambient range (around 23 °C).

The dipyridyl dicarboxylic acid unit was then incorporated into the probe **5**.† Examination of the properties of this probe revealed that the complex with the Eu^{3+}·EDTA bisbutylamide, chelate [**4Eu.5**] exhibited a strong luminescence signal on irradiation at 318 nm (Table 1). However, on standing, the intensity of the luminescence dropped with time. After exploring several possible explanations for this fading of the signal it was discovered that the effect could be countered by the addition of a surfactant, such as the cationic CTAB (cetyl trimethylammonium bromide), whereupon a stable signal was observed. Independent experiments showed that the complex [**4Eu.5**] has a strong tendency to adhere to the surface of the cuvette, thus removing it from solution.

The corresponding Tb^{3+} complex, [**4Tb.5**], only exhibited a very weak signal (Table 1), which was not enhanced by the addition of CTAB. This inefficiency with the terbium complex is in contrast with the behaviour of the simpler complexes described above, *e.g.* [**4Tb.3**], and is ascribed to an intramolecular quenching process from the attached phenanthridinium group. Since only the terbium complex was affected but not the europium complex, this quenching process was not of the sensitising dipyridyl species, common to both systems, but of the excited lanthanide ion.

It is known[4] that the triplet level of the phenanthridinium group (*ca.* 21 300 cm^{-1}) is near in energy to that of the excited $^5D_4$ state of the terbium ion (*ca.* 20 500 cm^{-1}) and is capable of quenching the latter. In order to confirm this triplet quenching mechanism an experiment involving the intermolecular quenching of the terbium complex [**4Tb.3**] with the phenanthridinium salts **6** and **7** was carried out. The results followed Stern–Volmer plots (Fig. 1). Of note was the observation that the carboxylic acid derivative **7** was a less efficient quencher than the *N*-methyl salt **6**. Presumably, electrostatic effects play a part

Table 1 Luminescence signals for various chelates

Ln^{3+}	Complex	I_{max}[a]	Notes
Eu^{3+}	[**4Eu.3**]	500	
Tb^{3+}	[**4Tb.3**]	350	
Eu^{3+}	[**4Eu.5**]	170[b]	
Eu^{3+}	[**4Eu.5**]	150	calf thymus DNA added[c]
Tb^{3+}	[**4Tb.5**]	1	
Tb^{3+}	[**4Tb.5**]	300	calf thymus DNA added[c]
Tb^{3+}	[**4Tb.5**]	210	SDS added[d]

[a] I_{max} in arbitrary units. Measurements all with same instrument settings using a PE LS50B spectrofluorimeter; slit widths 10 nm; delay time 0.1 ms; gate time 1.0 s. Measurements at 23 °C, pH 7.5 in buffer; complex concentrations at 1×10^{-6} mol dm^{-3}. For Eu^{3+} I_{max} for the band at *ca.* 614 nm was measured; for Tb^{3+} I_{max} for the emission at *ca.* 545 nm was measured. [b] Initial intensity in the absence of CTAB. [c] Intensity measured at a ratio of DNA base pair : complex of > 10 : 1. [d] Intensity measured at a concentration of SDS of 2×10^{-4} mol dm^{-3}.

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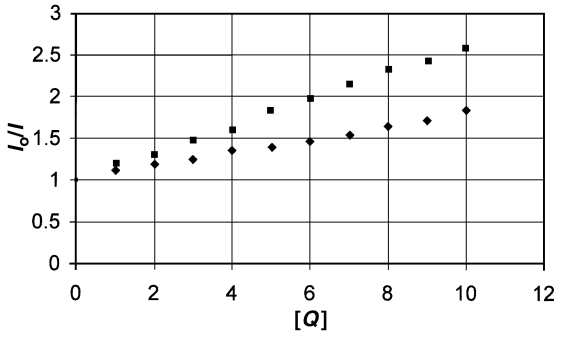

Fig. 1 Stern–Volmer plots: Tb^{3+} signal at λ_{em} = 545 nm, delay time 0.1 ms; [**4Tb.3**] at 1×10^{-6} mol dm^{-3}, buffered at pH 7.5 at 23 °C; ◆ addition of **6** as quencher; ■ addition of **7** as quencher; no degassing. [Q] in molar equivalents of quencher to complex.

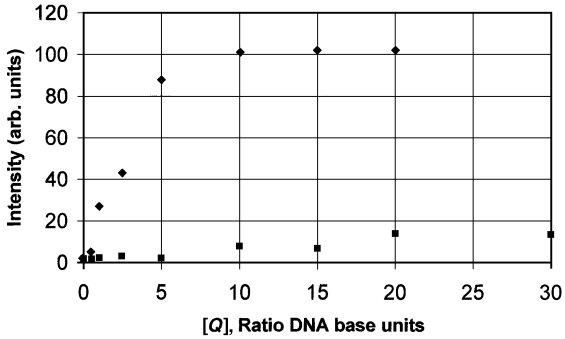

Fig. 2 Enhancement of Tb^{3+} signal at λ_{em} = 545 nm, delay time 0.1 ms; [**4Tb.5**] at 1×10^{-6} mol dm^{-3}, buffered at pH 7.5 at 23 °C; ◆ addition of calf thymus duplex DNA; ■ addition of synthetic 15-mer DNA single strand oligomer. [Q] in molar DNA base equivalents to the probe.

and the latter, net positively charged species, can approach the net negatively charged complex [**4Tb.3**] more easily than the former, which is net neutral under the quenching conditions utilised.

The possible involvement of oxygen, as a known quenching agent for the excited phenanthridinium species[5] was also examined but deoxygenation of the system by a number of freeze–thaw cycles had little effect on the overall levels of emission from the europium [**4Eu.5**] complex, whilst, as anticipated there was a small increase for the terbium [**4Tb.5**] complex. This indicates that, although the *N*-substituted phenanthridinium species can act as a sensitiser for the terbium ions, under our conditions the dominant sensitiser is the dipyridyl chromophore, the phenanthridinium ion acting as a net triplet energy sink.

Triplet quenching requires close contact of the interacting species in order to operate. Quenching would not be expected to occur if the terbium ion could be held apart from the phenanthridinium unit. Since the phenanthridinium ion has a propensity to intercalate with duplex DNA,[6] it was of interest to test whether or not quenching was observed in the presence of such DNA. Thus to a solution of the terbium complex [**4Tb.5**] in HEPES buffer, was added a solution of duplex DNA (calf thymus); the onset of the terbium signal appeared within seconds and approached a maximum at DNA base to complex concentration ratios > 10 : 1 (Fig. 2). Thus intercalation of the phenanthridinium group is sufficient to prevent its intramolecular approach to the complexed terbium ion. In contrast, the luminescent properties of the corresponding europium complex, [**4Eu.5**] were only slightly altered by the addition of calf thymus DNA, a slight reduction in the europium emission being observed. In both cases no substantial changes in the patterns of lanthanide emissions were observed upon the addition of the DNA.

Further experiments have shown that these effects could be reproduced using synthetic DNA hybrids. Addition of the complex [**4Tb.5**] to single stranded DNA showed only a small increase in the terbium signal (Fig. 2).

The triggering of the terbium signal thus serves as a useful 'switch' for the detection of duplex DNA in solution and complements the behaviour of the dipyridophenazine–ruthenium complexes described by Barton *et al.*[7]

Since we believe the onset of the terbium signal was caused by a physical barrier to approach of the phenanthridinium ion to the chelated terbium species and hence quenching, we subsequently tried use of the anionic surfactant, SDS (sodium dodecyl sulfate). No effect was observed at low concentrations but at molar equivalents > 10 : 1 SDS: [**4Tb.5**] a typical terbium emission signal appeared, reaching a maximum at molar concentrations > 100 : 1. We ascribe this to a micellar effect, the phenanthridinium group being surrounded by SDS molecules as micellation occurs thus effectively restricting approach by these groups to the hydrophilic and net anionic, dipyridyl lanthanide segment of the complex. Thus the complex [**4Tb.5**] can also act as a probe for anionic micellation.

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Notes and references

† All new compounds were characterised with supporting microanalytical and/or accurate mass measurements.

1 J. Coates, P. G. Sammes, G. Yahioglu, R. M. West and A. J. Garman, *J. Chem. Soc., Chem. Commun.*, 1994, 2311; J. Coates, P. G. Sammes and R. M. West, *J. Chem. Soc., Chem. Commun.*, 1995, 1107.
2 J. Coates, P. G. Sammes and R. M. West, *J. Chem. Soc., Perkin Trans. 2*, 1996, 1277; J. Coates, P. G. Sammes and R. M. West, *J. Chem. Soc., Perkin Trans. 2*, 1996, 1283.
3 E. Soini and T. Lövgren, *CRC Crit. Rev. Anal. Chem.*, 1987, **18**, 105; *Handbook on the Physics and Chemistry of Rare Earths*, ed. K. Schneider and L. Eyring, North-Holland, Amsterdam, 1979.
4 D. Parker, P. K. Senanayake and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2*, 1998, 2129.
5 D. Parker and J. A. G. Williams, *Chem. Commun.*, 1998, 245.
6 H. W. Zimmerman, *Angew. Chem., Int. Ed. Engl.*, 1986, **25**, 115.
7 J. K. Barton and Y. Jenkins, *J. Am. Chem. Soc.*, 1992, **114**, 8736; J. K. Barton and R. M. Hartshorn, *J. Am. Chem. Soc.*,1992, **114**, 5919.