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Aluminium fluorescence detection with a FRET amplified chemosensor†

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A selective Al^{3+} fluorescence chemosensor able to detect concentrations of metal ion in the nanomolar range has been realized. The remarkable sensitivity is the result of the FRET amplification of the fluorescence emission of the ligand subunit.

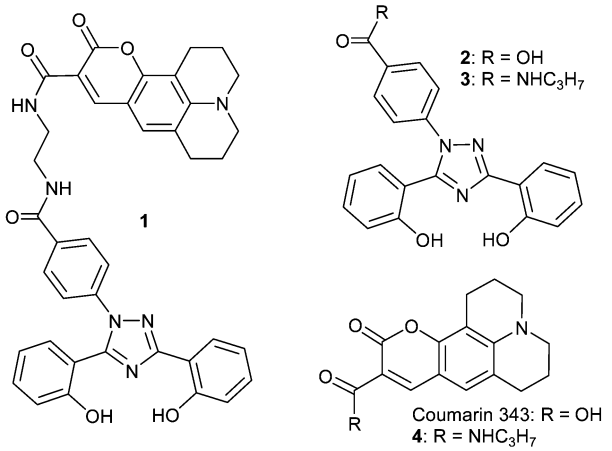
There is an increasing interest in the realization of fluorescent chemosensors, *i.e.*, structurally simple supramolecular systems that can effectively signal the complexation of a proper guest and provide an extremely sensitive and selective method to recognize and evaluate the concentration of different substrates.[1] The design of such chemosensors faces a key problem related to the implementation of an efficient transduction mechanism, which converts the binding of the substrate into a modification of the fluorescence emission of the sensor.[1b] Several mechanisms for signal transduction have been exploited and, among them, one of the most reliable implies a direct interaction between the bound substrate and the conjugated electronic system of the fluorophore.[2] This strategy, although successfully employed in the realization of several efficient chemosensors,[1c] is far from straightforward. In fact, on the one hand, it implies the not so trivial transformation of a fluorescent dye into a ligand selective for the desired substrate and, on the other hand, the desired photophysical properties cannot easily be predicted so as to encourage the needed synthetic effort.

Here we describe the fluorescent molecular species **1** not only as a very effective chemosensor for Al^{3+} but as an example of a novel and promising strategy for the realization of an intrinsic chemosensor. In the present case, in fact, a selective ligand with poor sensing properties has been converted into an efficient chemosensor by the introduction of a fluorescence resonance energy transfer (FRET) amplification of the produced signal.[3]

The metal complexing properties of 3,5-bis(*o*-hydroxyphenyl)-1,2,4-triazole substituted with benzoic acid (**2**) in solution have been previously investigated by Hegetschweiler and coworkers. Their studies[4] revealed that **2** shows an unexpectedly high affinity for the Al^{3+} ion, probably due to the ideal preorientation of the ligand donor set.

The detection of Al^{3+} is of great interest because of the potential toxicity and the widespread presence of this ion.[5] We reasoned that, in analogy with related biarylpyridines,[6] ligands **2** and **3** could display an enhanced fluorescence emission as a consequence of the binding of the metal ion, due both to the system rigidification and/or the formation of an emissive charge transfer emission state.[6] In fact, we found that **3** alone, in a 1 : 1 water–ethanol solution buffered at pH 5.0, does not show any significant fluorescence emission. On the other hand, upon addition of Al^{3+} ions, we observed the appearance of an emission band centred at 445 nm (Fig. 1)[7] which is, however, too weak to allow the detection of the metal ion with high sensitivity.

Based on these preliminary results, we designed sensor **1**, in which a fluorophore, Coumarin 343, is connected to the benzoic acid moiety of ligand **2** *via* an ethylene spacer.[8] The absorbance of Coumarin 343 overlaps very well with the weak emission of the **3**–Al^{3+} complex which can hence act as a donor in a FRET process (Fig. 1). As the FRET process is usually more efficient than non-radiative decay processes,[9] we anticipated that the added fluorophore would strongly amplify the fluorescence signal emitted by the ligand upon binding of Al^{3+}.



This turned out as expected. As shown in Fig. 2, the spectrofluorimetric titration of a 3.1 μM solution of receptor **1** in water–ethanol 1 : 1, buffered at pH 5.0, with $Al(NO_3)_3$ shows an increase of the intensity of the coumarin fluorescence emission band at 489 nm (excitation at 350 nm) up to 700%. As a result, Al^{3+} concentrations as low as 50 nM can be detected (5% increment of the sensor fluorescence emission). Interpolation of the emission intensity *versus* Al^{3+} concentration data, assuming a 1 : 1 binding model, gives a good fit and allows estimation of a log K_{app} value of 5.8 ± 0.1. The kinetics of complex formation were measured spectrophotometrically at pH 5 and the second order rate constant, $k_2 = 1.3 \times 10^3\ s^{-1}\ M^{-1}$,[10] was determined.

In contrast, addition of Al^{3+} ions has no effect on the emission spectra of **1** when the sensor is irradiated at 445 nm which is the

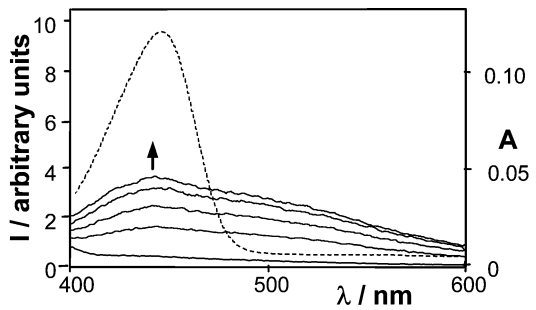


Fig. 1 Fluorescence emission spectra (λ_{exc} = 350 nm) of ligand **3** in the presence of increasing amounts of $Al(NO_3)_3$ in EtOH–H_2O (1 : 1) at pH = 5.0. [**1**] = 8.7×10^{-6} M, [acetate buffer] = 0.01 M. The dotted line is the absorption spectrum of Coumarin 343.

† Electronic supplementary information (ESI) available: experimental details and spectra. See http://www.rsc.org/suppdata/cc/b3/b303195k/

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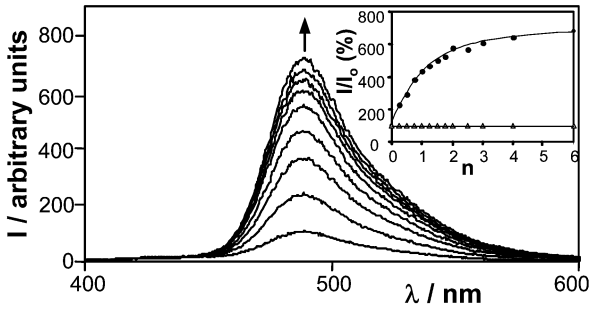

Fig. 2 Fluorescence emission spectra (λ_{exc} = 350 nm) of sensor **1** in the presence of increasing amounts of $Al(NO_3)_3$ in EtOH–H_2O (1 : 1) at pH = 5.0. [**1**] = 3.1×10^{-6} M, [acetate buffer] = 0.01 M. Inset: spectrofluorimetric titration curve at λ_{exc} = 350 nm (●) and λ_{exc} = 445 nm (△). *n* = number of added equivalents of Al^{3+}.

typical excitation wavelength for the Coumarin 343 dye (Fig. 2, inset). This clearly indicates that the fluorescence enhancement observed is due to the FRET process between the ligand subunit and the dye and not to a direct interaction of the metal ion with the latter. Also a mechanism involving the suppression of a pre-existing quenching process of the coumarin emission by the phenolic moiety of the ligand subunit can be excluded on the basis of this observation.[11]

To test the sensor selectivity, compound **1** was titrated with different metal ions (Fig. 3). The addition of Mg^{2+}, Ca^{2+}, Ni^{2+}, and Zn^{2+} did not produce any effect on the emission intensity of the system in the concentration range explored (up to 2×10^{-5} M). Titration of **1** with Al^{3+} in the presence of 10 equiv. of these ions gives the same fluorescence increase as in the absence of these ions (see Supplementary Information†). The selectivity is hence to be ascribed to the very low affinity of the above metal ions for the ligand. However, as typically observed for Al^{3+} ligands, interference was observed with Cu^{2+} and Fe^{3+} although to a different extent. Addition of these ions to a solution of receptor **1** leads to a significant quenching of the fluorescence intensity (respectively 70% and 75% of the initial value). In the case of Cu^{2+}, a log K_{app} of 6.9 for the 1 : 1 complex was determined, while in the case of Fe^{3+} the formation of 1 : 1 and 2 : 1 (ligand to metal) complexes was detected with log K_{app} values of 5.6 and 9.5 respectively.[12] As expected, in the presence of 10 equiv. of the interfering metal ions, no fluorescence increase is observed after Al^{3+} addition in the case of Cu^{2+}, while in that of Fe^{3+} the fluorescence increase is about one third of that observed in the absence of the metal ion (see Supplementary Information†). However, appropriate procedures have been reported to eliminate or mask these ions from Al^{3+} containing samples.[4a]

In conclusion, we have presented the fluorescent chemosensor **1** that operates with an innovative FRET-based amplification mechanism and is able to recognise Al^{3+} with high affinity and selectivity. Chelation enhanced fluorescence (CHEF) is a further valuable property of the system.[1c] The proposed transduction mechanism is based on the binding induced fluorescence activation of the ligand subunit followed by energy transfer between the ligand and the attached fluorophore which results in signal amplification. This innovative design is of general application in the realization of other intrinsic chemosensors, because amplification of the fluorescent signal by a FRET mechanism allows the use of ligand subunits which are characterized by a strong and selective binding of the substrate but by poor fluorescent properties.

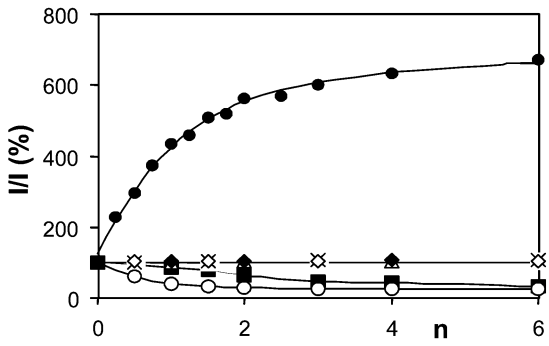

Fig. 3 Spectrofluorimetric titration of **1** at pH 5.0 with different metal ions: Al^{3+} (●), Mg^{2+} (◆), Ca^{2+} (x), Ni^{2+} (◇), Zn^{2+} (△), Fe^{3+} (■) and Cu^{2+} (○). *n* = number of added equivalents of metal ion. [Acetate buffer] = 0.01 M, [**1**] = 3.3×10^{-6} M (λ_{exc} = 350 nm).

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Notes and references

1 (*a*) Special Issue on "Luminescent Sensors", *Coord. Chem. Rev.*, 2000, **205**; (*b*) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515–1566; (*c*) *Fluorescent Chemosensors for Ion and Molecule Recognition*, ed. A. W. Czarnik, ACS Symposium Series 538, American Chemical Society, Washington DC, 1993.

2 These types of fluorescent chemosensors are usually classified as "intrinsic chemosensors" to distinguish them from the "conjugate chemosensors" in which the binding site and the fluorescent dye are two separate subunits of the sensor, see ref. 1*c*.

3 This phenomenon is referred to by other authors as EET (electronic energy transfer) or RET (resonance energy transfer). Examples of FRET-based chemosensors have been reported. In these cases, however, a different transduction mechanism is used and the FRET originates from a variation of the distance between two fluorophores. See: (*a*) S. E. Schneider, S. N. O'Neil and E. V. Anslyn, *J. Am. Chem. Soc.*, 2000, **122**, 542–543; (*b*) M. Di Casa, L. Fabbrizzi, M. Lichelli, A. Poggi, A. Russo and A. Taglietti, *Chem. Commun.*, 2001, 825–826; (*c*) A. Arimori, M. L. Bell, C. S. Oh and T. D. James, *Org. Lett.*, 2002, **4**, 4249–4251; (*d*) H. Ueyama, M. Takagi and S. Takenala, *J. Am. Chem. Soc.*, 2002, **124**, 14286–14287.

4 U. Heinz, K. Hegetschweiler, P. Acklin, B. Faller, R. Lattmann and H. P. Schnebli, *Angew. Chem., Int. Ed.*, 1999, **38**, 2568–2570. The ligand binds the metal ion upon deprotonation of the two phenolic hydroxyl groups. Formation of the 1 : 1 **1** (− $2H^+$)–Al^{3+} complex has a log *K* value of 19.8 in H_2O–DMSO (4 : 1). **1** has also a strong affinity for Fe^{3+} (log *K* = 23.3) and Cu^{2+} (log *K* = 18.8) ions.

5 For some examples of fluorimetric detection of Al^{3+} see: (*a*) J. L. Ren, J. Zang, J. Q. Luo, X. K. Pei and Z. X. Jiang, *Analyst*, 2001, **126**, 698–702; (*b*) C. Jiang, B. Tang, R. Wang and J. Yen, *Talanta*, 1997, **44**, 197–202; (*c*) M. P. Mànuel-Vez and M. Garcìa-Vargas, *Talanta*, 1994, **41**, 1553–1559; (*d*) F. Will, *Anal. Chem.*, 1961, **33**, 1360–1362.

6 J. V. Mello and N. S. Finney, *Angew. Chem., Int. Ed.*, 2001, **40**, 1536–1538.

7 The origin of such fluorescence switching is under investigation. The addition of Al^{3+} also leads to a modification of the absorbance spectrum of **3** with the decrease of the ligand absorption band centred at 300 nm and the appearance of a new band at 325 nm. Similar spectral changes can also be observed on deprotonation of the phenolic groups at pH > 13 but no fluorescence emission is observed in this case. Addition of Al^{3+} to solutions of **1** also leads to a modification of the absorption spectra with an increase of the absorbance at 350 nm (see Supplementary Information†).

8 The synthesis of the compounds **1–4** is described in the Supplementary Information†.

9 A. Adronov, S. L. Gilat, J. M. J. Fréchet, K. Ohta, F. V. R. Neuwahl and G. R. Fleming, *J. Am. Chem. Soc.*, 2000, **122**, 1175–1185.

10 Due to the relatively slow complexation, all measurements were made 12 h after the preparation of the samples. A slow complexation is observed also in the case of the reagents currently used for the fluorimetric detection of aluminium[5] and does not hamper their practical application.

11 If a PET-based quenching mechanism from the excited coumarin to the ligand subunit is present it will be operative also at the typical excitation wavelength of the coumarin dye. Moreover, the quantum yields of the coumarin amide **4** and **1** are almost the same in the operative conditions. See Supplementary Information†.

12 The formation of the Cu^{2+} complexes is immediate after mixing, while a second order rate constant k_2 of 1.7×10^3 s^{-1} M^{-1} was measured for the formation of the 1 : 1 Fe^{3+} complex.