Fluorescent sensor of imidazole and histidine

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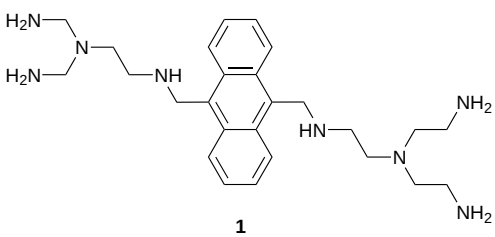
The dizinc(II) complex of an octamine containing the anthracene subunit binds both the imidazolate anion and the imidazolate moiety of L-histidine, and signals the binding through the fluorescence quenching of the fluorophore.

The design of multicomponent fluorescent systems able to detect the presence and monitor concentration changes of biologically active small molecules, in particular natural amino acids, is highly desirable. A fluorescent sensor of γ-amino-buyric acid has recently been reported.[1]

We have recently developed a receptor capable of recognising histidine in the presence of any other natural amino acid.[2] The receptor contains two Cu^{II} ions prepositioned within a poly-aza macrocycle. Recognition is based on the fact that the imidazole residue of histidine, in an aqueous solution adjusted to pH 9, deprotonates and bridges the metal centres. This situation is in some ways reminiscent of Cu-Zn superoxide dismutase (CuZn-SOD), in which a Cu^{II} and a Zn^{II} ion are bridged by the imidazolate residue of a histidine fragment.

We considered that the octamine **1**† could provide a convenient framework for the construction of a fluorosensor for histidine, as (i) it offers two quadridentate binding sites for Cu^{II} ions, leaving each metal centre coordinatively unsaturated and an open position for a further ligand (*i.e.* one of the two nitrogen atoms of an imidazolate subunit) and (ii) the anthracene fragment linking the tetraamine subunits gives an intense and characteristically structured fluorescent emission, suitable for signalling the occurrence of the receptor–substrate interaction.

The pH titration experiments were carried out on an aqueous solution containing **1** (1 equiv.) and Cu^{2+} (2 equiv.), in the absence and in the presence of 1 equiv. of imidazole (imH). Non-linear fitting of the titration curve in the absence of imH indicated the formation of the dimetallic species $[Cu^{II}_2L]^{4+}$ at pH 4. This is present as the major species in the 5–7 pH interval; at higher pH, hydroxide-containing species form. In the presence of imH, an imidazolate-containing dimetallic species $[Cu^{II}L(im)]^{3+}$ formed as a major species between pH 7–11. However, whereas the $[Cu^{II}_2L]^{4+}$ system appears to be an excellent receptor for imidazole, it cannot function as a fluorosensor since the Cu^{II} ions fully quench anthracene fluorescence in both the $[Cu^{II}_2L]^{4+}$ and the $[Cu^{II}L(im)]^{3+}$ complexes, and thus any monitoring of the recognition process through the variation of the fluorescent emission is prevented. Thus, we considered the use of a pair of Zn^{II} ions as binding sites for imidazole in the octamine receptor **1**. Zn^{II} is photophysically inactive and is expected to display some affinity towards a bridging imidazolate fragment, as it shows in the CuZn-SOD enzyme.



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Further pH titration experiments were carried out on an aqueous solution containing **1** (1 equiv.) and Zn^{2+} (2 equiv.), in the absence and in the presence of 1 equiv. of imH. Non-linear fitting of the titration curve in the absence of imH indicated the formation of stable dimetallic complexes at pH ⩾5. In particular, the complex of the neutral ligand $[Zn^{II}_2L]^{4+}$ forms at pH 5.5 and is present as the major species between pH 7–8.5. At pH ⩾9, hydroxide-containing complexes dominate: $[Zn^{II}_2L(OH)]^{3+}$ and $[Zn^{II}_2L(OH)_2]^{2+}$. Each hydroxide ion should be bound to the Zn^{II} centre and should result from the deprotonation of a coordinated water molecule.

Fig. 1 displays the distribution diagram of the species present at equilibrium in the system containing 1 equiv. of **1**, 2 equiv. of Zn^{II} and 1 equiv. of imH.‡ It is seen that at pH 7 the imidazolate-containing species $[Zn^{II}_2L(im)]^{3+}$ forms and exists as the dominant species in the pH 9–10 interval (60%), prevailing over the $[Zn^{ii}_2L(OH)]^{3+}$ complex (20%). A titration experiment was then carried out in a spectrofluorimetric cuvette, by adding standard NaOH to a solution containing 1 equiv. of **1**, 2 equiv. of Zn^{II}, 1 equiv. of imidazole and excess acid. The variation of the relative fluorescence intensity I_F (%) with the increasing pH profile is shown in Fig. 1 and can be explained as follows: in the pH 2–4 interval, **1** is present exclusively as LH_6^{6+} species. It is suggested that in each tetramine subunit of **1** the three peripheral amine groups (two primary and one secondary) are protonated and the central tertiary nitrogen atom is not. This situation would minimize electrostatic repulsions. In these circumstances, it seems possible that an electron transfer (eT) process from the lone pair of the tertiary nitrogen atom of each tren subunit to the excited anthracene moiety (An*) takes place, thus competing with radiative deactivation and partially quenching fluorescence. On addition of standard base (pH 4–5), the two secondary amine groups which are close to the anthracene subunit deprotonate, making the electron transfer to An* more effective and thus inducing a progressive I_F decrease. However, at pH 5, zinc-containing species begin to form (see the corresponding distribution curve in Fig. 1) and the Zn^{II}-bound

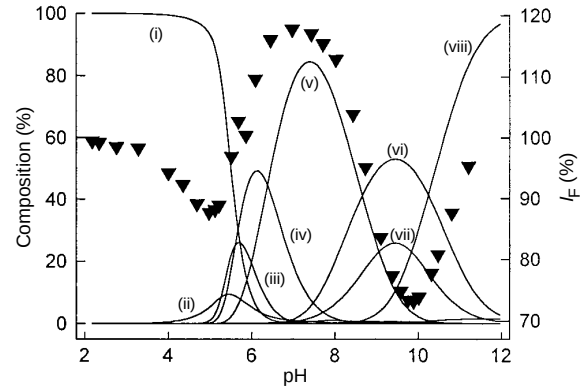
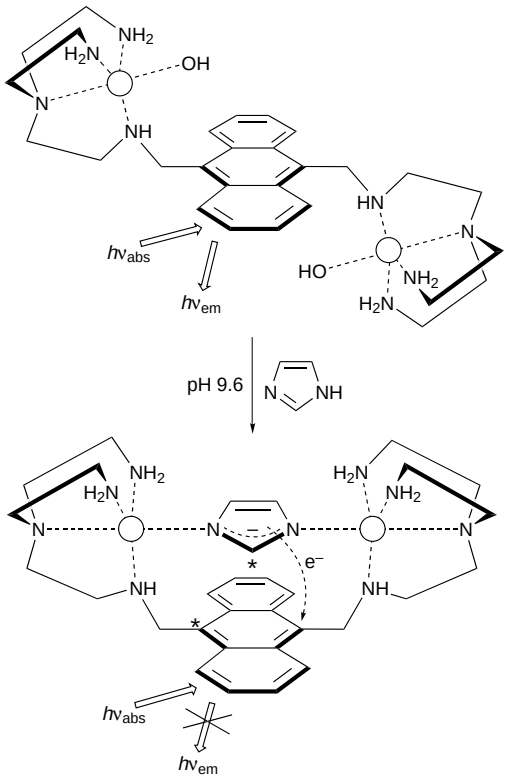


Fig. 1 Left vertical axis, pH dependence of the concentration of species present at equilibrium for the system **1** (L, 1 equiv.)–Zn^{II} (2 equiv.)–imidazole (imH) in aqueous 0.1 m $NaClO_4$ at 25 °C: (i) $[LH_6]^{6+}$; (ii) $[LH_5]^{5+}$; (iii) $[Zn^{II}_2(LH_2)]^{6+}$; (iv) $[Zn^{II}_2(LH)]^{5+}$; (v) $[Zn^{II}_2L]^{4+}$; (vi) $[Zn^{II}_2L(Im)]^{3+}$; (vii) $[Zn^{II}_2L(OH)]^{3+}$; (viii) $[Zn^{II}_2L(OH)_2]^{2+}$. Right vertical axis (full triangles), pH dependence of the fluorescence intensity of the solution (I_F).



Scheme 1

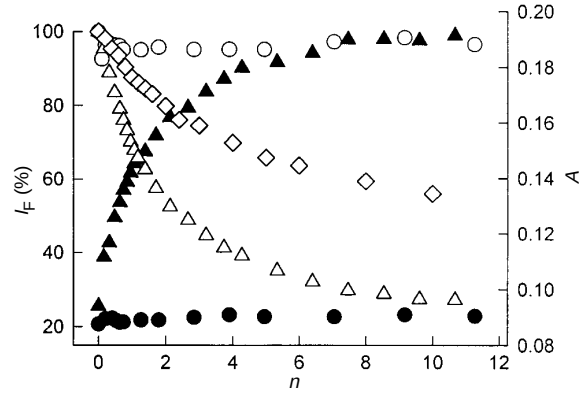


Fig. 2 Variation of the relative fluorescence intensity (I_F, left vertical axis) and of the absorbance at 300 nm (A, right vertical axis) during the titration of 9×10^{-6} m $[Zn^{II}_2L]^{2+}$, buffered at pH 9.6, with imidazole, histidine and acetate: (△) I_F (imidazole); (▲) A (imidazole); (○) I_F (acetate); (●) A (acetate); (◇) I_F (l-histidine). n = number of equivalents of the added substrate.

lone pairs of the anthrylamine groups (the secondary and the tertiary ones, in particular) are no longer available for the eT quenching mechanism, allowing fluorescence to revive. At pH 7.0–7.5, where $[Zn^{II}_2L]^{4+}$ is present as a major species, I_F reaches its maximum value. The formation of the imidazolate bridged complex $[Zn^{II}_2L(im)]^{3+}$ then induces a substantial fluorescence quenching. In particular, the minimum of the anthracene emission intensity corresponds to the maximum concentration of $[Zn^{II}_2L(im)]^{3+}$, at around pH 10. We ascribe this effect to the occurrence of an eT process from a π-orbital of the electron rich im^- moiety to a π-orbital of the photo-excited anthracene fragment within the $[Zn^{II}_2L(im)]^{3+}$ adduct. The photophysical effects associated with im^- binding by the $[Zn^{II}_2L]^{2+}$ receptor are outlined in Scheme 1.

Molecular modelling indicates that the imidazolate and anthracene fragments in the $[Zn^{II}_2L(im)]^{3+}$ adduct are quite close. In particular, a distance of 3.2 Å is calculated between the closest atoms of the two subunits; these are labelled with an asterisk in Scheme 1.

The fluorescence quenching that follows im^- binding opens the way to fluorescent sensing of imH and of any molecule bearing an imidazole residue. To demonstrate this, an aqueous solution containing 1 equiv. of **1** and 2 equiv. of Zn^{II} was adjusted to pH 9.6 with CHES buffer and was titrated with a solution of imH. Anthracene fluorescence was observed to decrease according to the profile displayed in Fig. 2. Non-linear fitting of the I_F *vs.* equiv. substrate curve indicates the formation of a 1 : 1 receptor–substrate adduct, *i.e.* $[Zn^{II}_2L(im)]^{3+}$, with a conditional constant of 3.65 ± 0.04 log units. The formation of the $[Zn^{II}_2L(im)]^{3+}$ adduct could also be followed spectrophotometrically, by monitoring an increase of the absorption band at 300 nm. The absorbance *vs.* equiv. substrate profile, shown in Fig. 2, is specularly symmetric to the I_F *vs.* equiv. substrate profile and gives the same log$K$ value (3.65 ± 0.09). It should be noted that titration with 1-methylimidazole, which *cannot* undergo deprotonation, did not induce any modification of the anthracene fluorescent emission, confirming that signalling is promoted by Zn–Zn bridging of the im^- fragment.

Spectrofluorimetric titration of $[Zn^{II}_2L]^{2+}$ with l-histidine induced a fluorescence quenching similar to that produced by imidazole (see Fig. 2): the shallower I_F *vs.* equiv. substrate profile corresponds to a lower value for the binding constant (log$K$ = 2.92 ± 0.01), which may reflect the existence of some steric repulsions between by the imidazole-appended amino acid fragment and the receptor framework. Interestingly, the titration profile is not modified when the solution contains an excess of any other amino acid. As an example, addition of l-histidine to a solution buffered at pH 9.6 and containing the receptor $[Zn^{II}_2L]^{2+}$ plus 10 equiv. of l-glycine produced an I_F *vs.* equiv. substrate profile superimposable onto that given in Fig. 2. The lack of interference may be due to the fact that the only anionic group any amino acid other than histidine can offer, *i.e.* the carboxylate fragment, does not display bridging tendencies towards the dimetallic core of $[Zn^{II}_2L]^{2+}$. Indeed, titration of a solution of $[Zn^{II}_2L]^{2+}$ with acetate did not induce any decrease of the fluorescent emission and did not cause any change of the absorbance at 300 nm nor at any other wavelength in the UV–VIS region. Thus, $[Zn^{II}_2L]^{2+}$ recognizes and senses l-histidine in the presence of any other natural amino acid.

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Footnotes

† The octamine **1** was prepared as follows: 9,10-dichloromethylanthracene[3] (1.5 g, 5.4 mmol) and tris(2-aminoethyl)amine (tren, 4.73 g, 32.4 mmol) were dissolved in toluene (50 ml) and the solution was refluxed under magnetic stirring for 6 h. After cooling to room temperature, the resulting yellow–orange solution was filtered, then the solvent and the excess tren were distilled off at reduced pressure, giving **1** as an orange oil (72%); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ 1.85 (br s, NH and NH_2), 2.3–2.9 (m, 24 H, CH_2N), 4.67 (s, 4 H, benzylic), 7.2–7.5 (m, 4 H, aromatic), 8.1–8.4 (m, 4 H, aromatic).

‡ The equilibrium constants were obtained by processing data of pH titration experiments with the non-linear least-squares program HYPERQUAD[4] and were used to calculate the distribution curves shown in Fig. 1.

References

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