

Chemosensors displaying pH controlled multistage fluorescence emission

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Abstract

A large number of systems displaying pH controlled multistages of fluorescence emission intensity, have not been recognized from this point of view. In this work we present two paradigmatic cases: (i) polyamine receptors linked to a fluorophore unit, in which the signal is modulated by an intra-molecular pH dependent electron transfer process, (ii) phenols presenting adiabatic excited state proton transfer. In the first example the chemosensors are constituted by a receptor unit containing a polyaza moiety suitable for binding protons (but also metal ions or anions). The receptor is linked to a benzene or naphthalene unit that acts as a fluorophore. The fluorescence signal is dependent on the protonation state of the receptor. In the second example, addition of buffers are used to tune the intensity of the fluorescence emission. ©1999 Elsevier Science S.A. All rights reserved.

1. Introduction

Chemosensors constitute a class of molecules able to bind while at the same time signal the presence of other molecules, in particular ions. Taking advantage of this behavior, molecular devices capable of signalling the presence/absence of stimuli (for example, those constituted by addition of protons) have been designed. Systems displaying these type of responses open the possibility of multiple applications, namely, ion sensing in life sciences, detectors responding to particular chemicals in the environment, memories and logic gates [1–3]. Fluorescent chemosensors are particularly appealing due to the very high sensitivity of this technique. Most of these fluorescent systems are limited to a bimodal response ‘on’ and ‘off’ just like computer memory (base two), but multistage response would be desirable for many applications [4–6]. It is generally accepted that these multistage systems are less common [5]. Although this is probably the case, there are many examples of multistage fluorescence based on switching systems that have not been recognized from this point of view [7,8]. In this work we wish to report on two paradigmatic cases, one based on polyaza receptors and the other one on the excited state proton transfer, both of them working in aqueous solution.

2. Experimental

2.1. Materials

Polyazacyclophanes [9], *N,N'*-dibenzylated polyamines [10], naphthalenophanes [9,11], were synthesized as described in the references. All other materials were of analytical grade (Sigma, Aldrich).

2.2. pH measurements

The pH was measured with a Metrohm 713 pH meter. Adjustment to the desired pH values was accomplished by addition of HCl or NaOH.

2.3. Absorption spectroscopy and fluorescence emission measurements

Absorption spectra were recorded on a Perkin–Elmer lambda 6 spectrophotometer, and fluorescence emission spectra were measured using a SPEX F111 Fluorolog spectrofluorimeter.

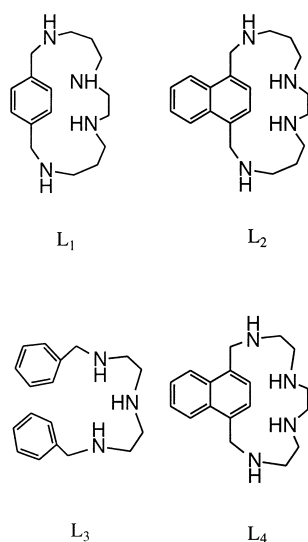
2.4. Temperature

All measurements were carried out at 25°C by using a Haake thermostated water bath.

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Scheme 1.

3. Results and discussion

3.1. Polyamine macrocyclic receptors

Recently we have reported on the fluorescence emission properties of two families of polyaza[n]paracyclophane and *N,N'*-dibenzylated polyamines, as examples see L_1 and L_3 , respectively in Scheme 1 [12,13]. The compounds are constituted by a polyamine chain of different dimensions (C_8N_4 in the case of L_1 shown in Scheme 1) with the function of binding and one (or two) benzene fluorophores with the role of signaling. Both these units are linked by benzylic carbon atoms that act as spacers.

In Fig. 1 are represented the fluorescence emission titration curves of one representative element of each one of these families, L_1 and L_3 .

In all the compounds, the fully protonated form exhibits the highest fluorescence emission intensity. The molar fraction distribution of the several species in solution obtained by potentiometric measurements fits quite well to the titration fluorescence emission curves, showing a large decrease of the emission intensity following the first deprotonation, and an almost disappearance of the fluorescence upon the second one. This quenching effect was attributed to an intramolecular electron transfer process involving the deprotonated amines and the excited aromatic unit. The formation of the plateaus in the emission intensity can be explained taking into account the deprotonation sequences obtained by NMR as reported in Scheme 2, for L_1 .

First deprotonation of L_1 occurring in one of the nitrogen atoms in the central part of the chain (N2 in Scheme 1) brings about a charge reorganization and one of the nitrogens N2 (and partially nitrogen N1) becomes available to the quenching process. The fluorescence emission intensity is at this stage reduced by a factor of ca. two. The quenching effect is not complete because the geometry of the molecule

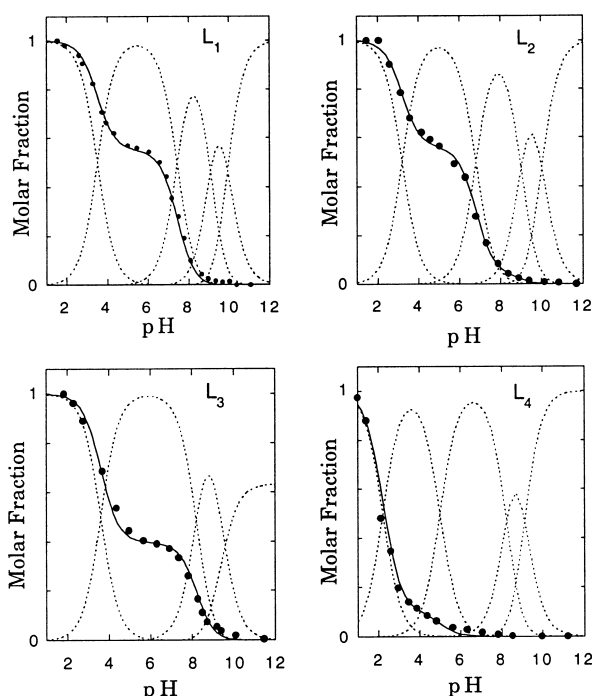
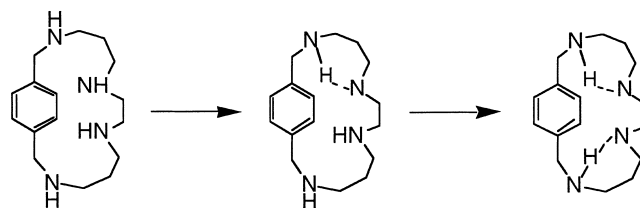


Fig. 1. Fluorescence emission titration curves of the compounds L_1 , L_2 , L_3 , and L_4 (●) fluorescence emission intensity upon normalization; dashed lines: molar fraction distribution of the several species according to the respective protonation state; solid lines: fitting curve achieved by a linear combination of the several molar fraction distributions.

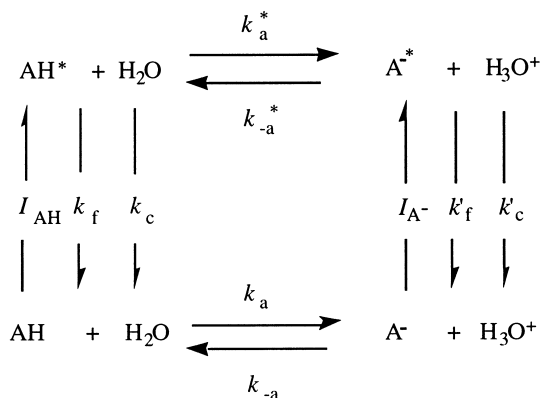


Scheme 2.

does not allow the deprotonated nitrogen to approach the excited fluorophore more than a certain distance. When second deprotonation occurs, the re-distribution of the protons is compatible with a C_{2v} symmetry and thus the quenching probability is roughly duplicated, explaining the disappearance of the remaining emission.

In the case of the *N,N'*-dibenzylated open-chain polyamine L_3 , the first deprotonation, according to the NMR data, occurs in the central nitrogen atom of the chain leading to a large quenching effect. As in the previous example, the emission is totally quenched upon removal of the second proton.

In order to improve the absolute quantum yield of the fluorescence emission the benzene unit was substituted by the more efficient naphthalene fluorophore, L_2 and L_4 . The results reported in Fig. 1, show that the behavior of L_2 is quite similar to its parent compound L_1 . Concerning L_4 , this derivative contains a smaller receptor unit (C_6N_4) [11]. As in the previous examples, the NMR data shows that first de-



Scheme 3. Ground and excited state acid–base equilibria in the absence of buffer effects.

protonation occurs in the central nitrogens of the molecule. However, when comparing the distances involved, it is clear that in L₄ the deprotonated nitrogen is closer to the fluorophore, explaining the dramatic quenching effect observed.

In conclusion, there is the possibility of preparing numerous compounds based on polyamine chains linked to fluorophore units capable of displaying three level (or even more) signals switched by pH.

3.2. Excited state proton transfer systems: buffer effects

The other multistage system we are reporting in this work, is based on the excited state proton transfer (ESPT) phenomenon. Although other authors already suggested this possibility [7], we would like to stress on the effect of buffers. There are many examples of ESPT like the cases of hydroxyquinolines [14,15], 2-naphthol studied by Weller [16–22], polyphenols such as synthetic flavylum salts [23,24], or simply phenol, see below.

In recent years we developed a simple treatment [25,26] of the ESPT phenomenon, on the framework of the Weller theory [16–22], see Scheme 3.

According to this approach the ESPT can be easily interpreted on the basis of two parameters K_{ap}^* and $\eta_{\text{A}^-}^*$ Eqs. (1) and (2)

$$K_{\text{ap}}^* = \frac{k_a^* \tau_{\text{AH}}}{k_{-a}^* \tau_{\text{A}^-}} \frac{1}{\eta_{\text{A}^-}^*} = \frac{1 + k_a^* \tau_{\text{AH}}}{k_{-a}^* \tau_{\text{A}^-}} \quad (1)$$

where τ_{A^-} ($\tau_{\text{A}^-} = 1/(k_c' + k_f')$) and τ_{AH} ($\tau_{\text{AH}} = 1/(k_c + k_f)$) being the lifetimes of the basic and acidic species and $\eta_{\text{A}^-}^*$ being the proton transfer efficiency in the excited state is defined by Eq. (2)

$$\eta_{\text{A}^-}^* = \frac{k_a^*}{k_a^* + k_c + k_f} \quad (2)$$

the other symbols being defined according to Scheme 3 are as follows: (i) for the acidic species k_f , k_c , and I_{AH} are, respectively, the fluorescence emission rate constant, the sum of the non-radiative rate constants and the number of photons

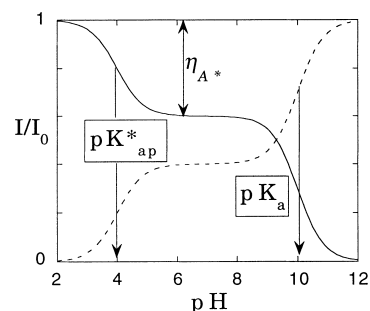


Fig. 2. Normalized fluorescence emission titration curves of the acidic (full line) and basic (traced line) forms of a compound exhibiting ESPT: $\eta_{\text{A}^-}^* = 0.4$; $pK_{\text{ap}}^* = 4$; $pK_a = 10$.

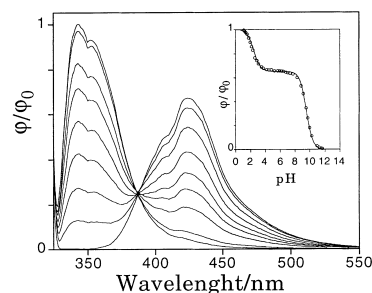


Fig. 3. Fluorescence emission spectra of 2-naphthol as a function of pH, as described in [25]. Excitation wavelength at the isosbestic point 345 nm. Inset: pH dependence of the fluorescence emission titration curve at the same excitation wavelength and emission of 424 nm.

absorbed per second, (ii) for the basic species k_f' , k_c' , I_{A^-} have an equivalent meaning.

In Fig. 2 the use of this methodology is summarized. The pH dependent fluorescence emission curves are usually obtained by excitation at the isosbestic point of the pH dependent absorption spectra. Whenever the emissions from the acidic and basic species overlap, they can be easily separated [25,26], and after division by the respective maximum, the final representation is that shown in Fig. 2. The parameter $\eta_{\text{A}^-}^*$ (proton transfer efficiency) represents the yield of formation of the excited base from the excited acid, and defines the plateau of the data represented in Fig. 2. The apparent pK_{ap}^* , Eq. (1), and the ground state pK_a are also represented in the figure.

Fig. 3 shows the pH dependence of the fluorescence emission spectra of 2-naphthol in water. As can be observed in the inset of this figure, the fluorescence emission intensity obtained by excitation at 345 nm (isosbestic point) monitored at 345 nm is a clear example of a three level signal. From this figure the parameters $\eta_{\text{A}^-}^* = 0.36$ and $pK_{\text{ap}}^* = 2.4$ can be calculated.

3.3. Buffer effects in ESPT

It is well known that [17,20] some buffers can compete with water for the adiabatic ESPT. In Fig. 4 the changes in the shape of the fluorescence emission titration curve

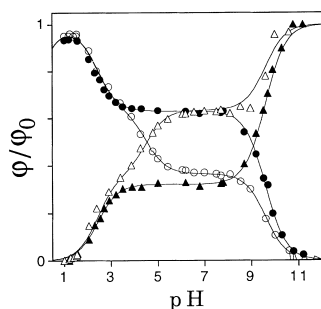


Fig. 4. Fluorescence emission titration curves of the normalized emission of 2-naphthol: (●) acidic form in the absence of buffer, (▲) basic form in the presence of buffer, (○) acidic form in the presence of 0.1 M acetate, and (△) basic form in the presence of 0.1 M acetate.

of 2-naphthol in the presence of 0.1 M sodium acetate are represented. As shown in Fig. 4, acetate is more efficient than water to carry out ESPT and for this reason the intermediate plateau of the acidic form decreases (and the plateau of the basic form increases by the same amount).

In order to quantify this new situation, with water and acetate competing for the proton, Eq. (3) has to be added to those reported in Scheme 3.



The model described above can be extended in order to also quantify buffer effects [25,26]. Without entering into this in detail, it must be emphasized that the position of the plateau in the presence of buffer effects is given by the parameter $\eta_{\text{A}^-}^{b*}$ Eq. (4), which like $\eta_{\text{A}^-}^*$ in the absence of buffer gives the position of the intermediate plateau.

$$\eta_{\text{A}^-}^{b*} = \frac{\eta_{\text{A}^-}^* + \varphi_{\text{B}}^*[\text{B}]}{1 + \varphi_{\text{B}}^*[\text{B}]} \quad (4)$$

where φ_{B}^* is given by Eq. (5)

$$\varphi_{\text{B}}^* = \frac{k_{\text{buf}}^*}{k_{\text{a}}^* + k_{\text{c}} + k_{\text{f}}} \quad (5)$$

In the case of 2-naphthol in the presence of 0.1 M acetate, $\varphi_{\text{B}}^* = 8$ and $\eta_{\text{A}^-}^{b*} = 0.64$. An interesting feature of this system is the possibility of tuning the relative intensity of this signal by simply controlling the concentration of the buffer. For example, in order to obtain a plateau with exactly half the intensity ($\eta_{\text{A}^-}^{b*} = 0.64$), a concentration of 0.035 M acetate has to be used.

Buffer effects are rather common and can be found for example in phenol. In this compound, the acidic form shows fluorescence emission but the basic species is not emissive. No ESPT can be detected in water in the absence of buffers. However, in the presence of 0.1 M acetate ESPT involving acetate occurs, and the shape of the fluorescence emission of the acidic species displays the typical three level signal, see Fig. 5. The shape of the curve can be fitted with the model for the parameters $\varphi_{\text{B}}^* = 4.2$ and $\eta_{\text{A}^-}^{b*} = 0.30$.

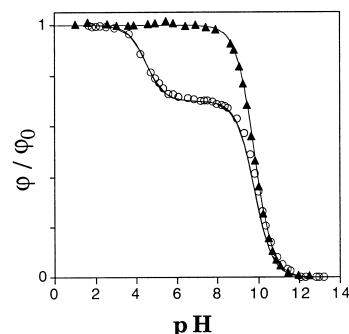


Fig. 5. Fluorescence emission titration curve of phenol in the absence, (▲), and presence, (○), of 0.1 M acetate buffer.

4. Conclusion

In the last years there is a huge interest on molecules displaying on/off (base two) or multilevel signals switched by an external stimulus [27,28]. It is not clear if these systems can find a real application, but the research in this field can introduce new ideas in the bottom-up approach to nanotechnology. Through this work we have shown that it is possible to find or design numerous molecules capable of displaying fluorescence signals in a three level stages, (base three) switched by pH.

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