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Pyrene fluorescence quenching in supramolecular systems based on dimethylaminomethylated resorcinarene

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ABSTRACT

The aim of this work is the development of stable fluorophore-quencher pairs with a controlled response for bioassay in the laboratory. The widely used in bioanalysis pyrene and its water-soluble derivative were selected as organic fluorophores. Water-soluble aminomethylated calixarene with sulfonatoethyl groups at the "lower rim" was chosen as a stabilizer. We have established a strong fluorescence quenching in the solution of resorcinarene even at low premicellar concentrations which indicates the pyrene transition into a non-polar microenvironment. The data on the influence of pH, buffer composition, and the macrocyclic platform on the fluorescence quenching of pyrene in the presence of resorcinarene were obtained. The temperature dependence of the fluorescence quenching of pyrene was obtained with the aim of establishing the mechanism of the formation of complex fluorophore-resorcinarene (static versus dynamic quenching).

1. Introduction

The pH monitoring is of primary importance for various fields of science, in particular for cell biology [1]. Intracellular pH changes play an important part in many biological processes occurring in a cell, i.e. in drug resistance and in ion transport [2,3]. The pH of the medium affects the biological activity of proteins and nucleic acids [4,5]. The abnormal pH related to the functions of cells, their growth and division has been observed in some of the common types of diseases such as cancer [6] and the Alzheimer's disease [7]. The most common method of detecting changes in the pH is the use of fluorescent indicators, in which on/off luminescence in a certain pH range has been observed. However, many intracellular factors have a significant influence on the properties of indicators and can lead to errors when measuring their pH. Currently used fluorescent probes (fluorescein and pyrene derivatives) have several limitations, such as, stability, cell membrane permeability and sensitivity toward the cell constituents [1–3]. Currently many research groups are involved in the problem of creating new fluorescent indicators for biological applications. Uchiyama and Makino have developed new digital fluorescent pH sensors based on the copolymers of acrylic acid amide. These sensors might change the output signals in a narrow range of pH (within one unit). With various derivatives of acrylic acid it is possible to obtain indicators switched in a tunable pH window (4–5, 6–7, 8–9, 9–10) [8]. The scientists from Hong Kong have obtained the pH sensor–tetraaminosilikat (IV) phthalocyanine, for which there is a significant increase in the fluorescence and the generation of reactive oxygen species in the 5–7 pH range, which makes it a promising pH-controlled fluorescent antitumor probe as well as a photosensitizer in photodynamic therapy [9]. Based on the nanocrystalline quantum dots CdSe/CdZnS the fluorescent pH detectors in the biological ranges (pH 6–8) were developed. The detectors have a good resolution of the response correlation at the pH values between 6 and 8 with a linear or two-photon excitation, and in the presence of the 4% bovine serum albumin solution [10]. (See Table 1.)

Aminomethylated resorcinarenes are a known class of pH-receptors [11]. The presence of amino and hydroxyl groups at the upper rim allows to modify their charge from −1 to +4 depending on the pH [12]. This class of pH-switchable receptors binds volume cations [13–16], anions [17], and neutral molecules [18–20]. Water-soluble aminomethylated derivatives of tetrasulfanatoethyl resorcinarene [21] are effective chiral NMR solvating agents for compounds with aromatic indole rings [22,23]. In the presence of surfactants [24,25] they can be used as pH-switchable ditopic receptors [26], as well as supramolecular cationic amphiphiles.

In this paper we present the results of the investigations of the interaction between dimethylaminomethylated resorcinarene (1) and pyrene (Py) and its water-soluble derivative — 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt (PTS) (Scheme 1). The data on the influence of the pH, buffer composition, and the macrocyclic platform on the fluorescence quenching of pyrene in the presence of resorcinarene were obtained. The comparison of the fluorescence of pyrene-resorcine 1 pair makes it possible to elucidate the contribution of electron-donating amino group of 1 to the quenching mechanism, while the replace of 1 by

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Table 1
The self-diffusion coefficients of 1 and PTS in the mixed and individual solutions, D_2O, 296 K.

System, concentration	D_1, $\times 10^{-10}$ m^2/s	D_{PTS}, $\times 10^{-10}$ m^2/s
1, 1 mM	2.53	–
1, 20 mM	1.94	–
PTS, 1 mM	–	4.01
PTS, 20 mM	–	3.91
1 + PTS, 20:20 mM	1.88	3.63

aliphatic amine may reveal the role of macrocyclic scaffold. The temperature dependence of the fluorescence quenching of pyrene was obtained with the aim of establishing the mechanism of the formation of complex fluorophore-resorcinarene (static versus dynamic quenching).

2. Materials and methods

2.1. Materials

Resorcinarenes 1 and 2 were synthesized as described in [27]. Pyrene, 1,3,6,8-pyrenetetrasulfonic acid tetrasodium salt, and triethylamine (Sigma-Aldrich) were used as received. Doubly-distilled deionized water (18.2 MΩ cm resistivity at 25 °C) from the Direct-Q 5 UV equipment was used for all the solution preparations. Samples of lower concentration were prepared daily and used immediately after preparation.

2.2. Methods

2.2.1. Fluorescence spectroscopy

Stock solutions of the fluorescence probes Py and PTS (6×10^{-4} M) were prepared in ethanol and water accordingly. The Py stock solution of 5 μL was added to the investigated solutions (3 ml) to make the Py concentration equal 1×10^{-6} M. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorometer with the excited wavelengths for Py at 335 nm using 1 cm path length quartz cuvettes. The emission spectra were recorded in the range of 350–500 nm. The data obtained were analyzed using the Microsoft Excel and Origin Pro 8.5 software.

2.2.2. pH metry

The pH values of solutions were measured on the HANNA instrument (pH 2–11 at 25 °C with an accuracy of 0.05 pH units).

2.2.3. NMR-experiments

All NMR experiments were performed on a Bruker AVANCE-600 spectrometer operating at 600.1 MHz for the ^{1}H. The spectrometer was equipped with a Bruker multinuclear z-gradient inverse probe head capable of producing gradients with strength of 50 G cm^{-1}. All experiments were carried out at 30 ± 0.2 °C. Chemical shifts were reported relative to HDO (4.70 ppm) as an internal standard.

3. Results and discussion

3.1. The quenching of Py and PTS fluorescence in the individual solution of resorcinarene 1

It has been previously observed that due to the formation of an inclusion complex between resorcinarene and Py [28] morpholinomethylated tetraethylresorcinarene quenches pyrene fluorescence in alkaline solutions. This type of quenching is interpreted in terms of the "sphere-of-action" model. The amino groups located at the upper rim of resorcinarene and phenolate groups act as quenchers. Morpholinomethylated tetraethylresorcinarene is soluble only in a highly alkaline medium (1 M NaOH). Resorcinarene 1 can be soluble in water at the concentration of up to 30 mM [24] due to the presence of sulphonate groups at the lower rim. Due to acid–base interactions of amino groups at the upper rim with water spontaneous solution pH was around 9.0 ± 0.2. Unlike the acidifying solutions, under these unbuffered conditions, nitrogen atom should exhibit its electron-donating (and hence quenching) ability. Resorcinarene 1 like morpholinomethylated tetraethylresorcinarene effectively quenches the fluorescence of Py in the aqueous medium, even without alkalizing it (Fig. 1(a)). The addition of the Py ($C = 1 \times 10^{-6}$ M) to the solution of 1 in the concentration of 0.5 mM results in a 72-fold decrease in the fluorescence intensity of fluorophore (Fig. 2(a)).

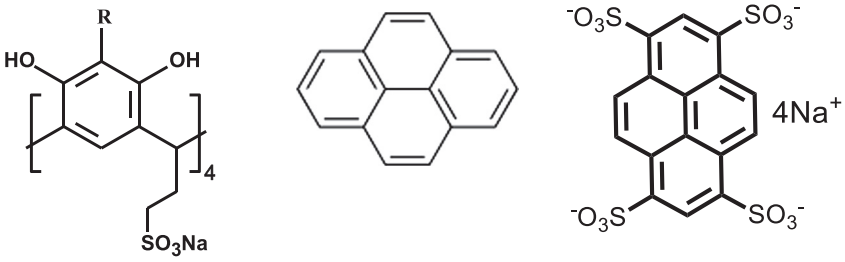
Resorcinarene 2 with no amino groups at the upper rim has essentially no effect on the fluorescence of pyrene. A similar phenomenon has been observed for triethylamine, i.e. the increase in its concentration in the aqueous solution does not affect the fluorescence intensity (Fig. 2(a)). From the results obtained it can be concluded that the quenching of Py occurs due to its interaction with amino groups at the upper rim of 1 when the Py is encapsulated into the cavity of resorcinarene 1.

The change in the microenvironment polarity of the Py in the solution of 1, which is reflected in the change in the ratio of the fluorescence intensity of the Py (I_{395}/I_{375}) at 395 and 375 nm (Fig. 3(a)) confirms the complex formation between 1 and the Py [29] (Fig. 3(a)).

Similar to morpholinomethylated tetraethylresorcinarene [28] the quenching of the Py fluorescence in the presence of 1 may be treated in terms of the "sphere-of-action" model and described by Eq. (1):

$$I_0/I = \left(1 + K_D \times C_q\right) \exp\left(\upsilon \times N \times C_q/1000\right) \quad (1)$$

in which K_D is the quenching constant, Cq is the quencher concentration, υ is the volume of the sphere, and N is the Avogadro's number. The mathematical processing of the data obtained has shown that the value of the quenching constant K_D is 7656 ± 350 M^{-1} (R^2 = 0.99987), and the diameter of the sphere is approximately 30 Å, with



1 R = $CH_2N(CH_3)_2$
2 R = H

Py

PTS

Scheme 1. Formulas of the compound used.

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