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Regular Article

On the role of a coumarin derivative for sensing applications: Nucleotide identification using a micellar system

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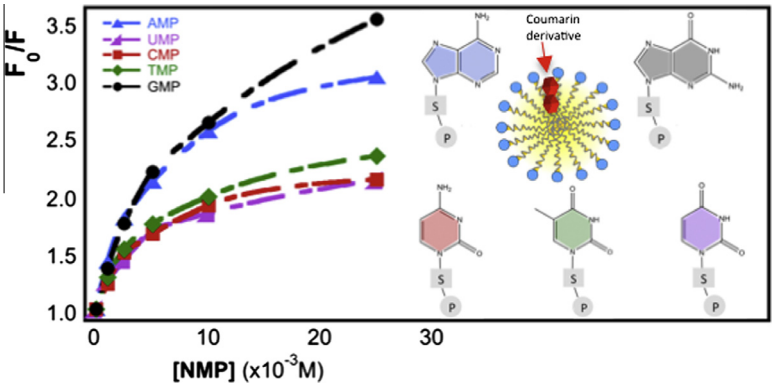
GRAPHICAL ABSTRACT



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ABSTRACT

The recognition of nucleotides is of crucial importance because they are the basic constituents of nucleic acids. The present study is focused on the selective interaction between a novel amphiphilic fluorophore containing coumarin and imidazole, CI (1-methyl-3-(12-((2-oxo-2H-chromen-7-yl)oxy)dodecyl)-1H-imidazol-3-ium bromide), and different nucleotide-monophosphates (NMPs). It was supposed that the solubilization of the low water soluble CI in a micelle system of hexadecyltrimethylammonium chloride (CTAC) would make the coumarin moiety of CI available to the interaction with the water-soluble NMPs. Changes in CTAC critical micelle concentration suggested that CI strongly interacted with the host cationic surfactant, thus forming a positively charged interface enriched with coumarin able to interact with the anionic NMPs. Steady-state fluorescence quenching revealed that CI/CTAC system was capable of distinguish between purine- and pyrimidine-based nucleotides. A modified Stern-Volmer equation permitted the use of a quenching model that accounted for the possible interactions between the micelles and the nucleotides. The data analysis allowed calculating selective parameters that differentiated according to the type of nucleotide either at 25 or 50 °C. Our results established the utility of the novel coumarin derivative fluorophore, supported by the simple and suitable micellar systems, as a tool for DNA sensing applications.

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1. Introduction

In the last few years, great research efforts have been devoted to the development of novel fluorescent chemosensors to selectively detect heavy metal ions [1–3], anions [4,5], enzymes [6], membranes [7] and proteins [8] with large impact in a variety of fields including medicinal science, environmental and analytical chemistry.

In particular, fluorescence chemosensors for nucleotides are appealing [9,10] since nucleotides are ubiquitously present in biological systems and play crucial roles in many cellular functions, such as transport across membranes, DNA synthesis, cell signaling, and energy- or electron-transfer processes. When designing a fluorescent chemosensor the choice of the reporting unit (i.e. the fluorophore) is fundamental and properties such as molar absorptivity, fluorescence quantum yield, chemical stability, low cost and toxicity should be taken into account. Among the most serious drawbacks of several commonly used fluorophores there are susceptibility to pH sensitivity and photobleaching [11,12].

Coumarins (or benzopyrones) are an important class of heterocycles that find applications in various fields spanning from lasers to biomedicine. They are characterized by good photostability, high fluorescence quantum yields (although unsubstituted coumarin, namely 1-benzopyran-2-one, has a quantum yield of just 0.03% in cyclohexane) [13], large Stokes shift, and low toxicity [14,15]. Due to their emission properties and good stability, substituted coumarins have been widely investigated for sensing applications. In particular, fluorescent chemosensors for anions [16–19], metal ions [20–23], pH [24], detection of nitric oxide [25], and hydrogen peroxide [26] have been recently studied.

The availability of coumarin derivatives having different size, shape, and hydrophobicity make them especially useful as fluorescent probes of heterogeneous environments [27–29]. In this regard, the use of colloidal systems such as micelles, vesicles and liposomes, which are able to create compartmentalized environments, has led to important results in a large number of fields, ranging from fundamental research focused on the physical-chemical properties of the lipid bilayer to suitable technology, interested in the development of new tools for sensory applications [30–34]. Schematically, micelles are composed of a hydrocarbon-like core and a headgroup region, containing charged surfactant headgroups, counterions, and water. Micelles made by the single-tailed cationic surfactants like cetyl trimethylammonium bromide (CTAB), cetyl trimethylammonium chloride (CTAC) belong to the archetype of cationic micelles. One of the principal properties of aqueous micellar solutions is their ability to solubilize a wide variety of organic solutes with quite distinct polarities and degrees of hydrophobicity. Cuomo and co-workers have recently demonstrated that diverse nucleotide-monophosphates (NMP) differently influence the fluorescence of pyrene solubilized in CTAB micelles [35,36]. Lately, the interest toward the recognition and sensing of nucleotides has been proven by the publication of several papers. Amendola and co-workers [37] studied a dicopper (II) cryptate complex that selectively recognized guanosine 5′-monophosphate (GMP) among the other nucleotides monophosphate in methanol/water solutions. Incl`an et al. [38] investigated on the ability of a scorpiand-based polyamine receptor to selectively distinguish guanosine-5′-triphosphate (GTP) from adenosine-5′-triphosphate (ATP). Kong et al. [39] proposed a Rhodamine modified fluorescent probe able to recognize adenine and cytidine based nucleotides. Moreover, Dong and co-workers have described the self-aggregation behavior of a family of carbazole-tailed imidazolium ionic liquids (ILs) able to form micelles in aqueous solution [40], and we have shown that stable ILs can be used as fluorescent chemosensors for metal ions recognition [41].

Herein, we report a study on the properties and use of an ad hoc synthesized amphipilic coumarin imidazole fluorophore, 1-methyl-3-(12-((2-oxo-2H-chromen-7-yl)oxy)dodecyl)-1H-imidazol-3-ium bromide (**CI**), suitable for inclusion in CTAC micelles. **CI** containing CTAC micellar solution was used for steady state fluorescence response to the presence of nucleotide-monophosphates (NMPs). Specifically, the sensing ability of the obtained system was analyzed toward purine-based monophosphates, i.e. adenosine 5′-monophosphate (AMP) guanosine 5′-monophosphate (GMP), and pyrimidine-based monophosphates, i.e. uridine 5′-monophosphate (UMP), cytidine 5′-monophosphate (CMP) and thymidine 5′-monophosphate (TMP).

2. Materials and methods

2.1. Materials

7-Hydroxy coumarin, 1-methyl imidazole, K_2CO_3, 1,12-dibromododecane, hexadecyltrimethylammonium chloride (CTAC), AMP (99%, disodium salt), UMP (>99%, disodium salt), CMP (>99%, disodium salt) GMP (>99%, disodium salt), TMP ($\geqslant$99%, disodium salt) were from Sigma-Aldrich and used without further treatment.

2.2. General procedures

All reactions were performed in oven-dried glassware under a slight positive pressure of nitrogen. ^{1}H NMR (400 MHz, 500 MHz) and spectra were determined on a Varian INOVA-400 spectrometer, and Varian INOVA-500 spectrometer. Chemical shifts for ^{1}H NMR are reported in parts per million (ppm), calibrated to the residual solvent peak set, with coupling constants reported in Hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet. Microanalytical data were obtained using a Fisons EA CHNS-O instrument (T = 1000 °C).

The critical micelle concentration was determined through conductivity measurements using a CDM230 conductivity meter (Radiometer Analytical) equipped with a two-pole conductivity cell tailored for small volumes (CDC749) calibrated with a standard solution of KCl 1×10^{-2} M. The conductivity was measured at 25 °C.

2.3. CI synthesis

2.3.1. Synthesis of 7-((12-bromododecyl)oxy)-2H-chromen-2-one (*1*)

K_2CO_3 (1.279 g, 9.254×10^{-3} mol) was added to a stirred solution of 7-hydroxy coumarin (0.500 g, 3.084×10^{-3} mol) dissolved in acetone (3.0×10^{-2} L). A solution of 1,12-dibromododecane (3.036 g, 9.252×10^{-3} mol) in acetone (2.0×10^{-2} L) was added dropwise, and the reaction mixture was refluxed under stirring for 24 h. The resulting mixture was cooled down, filtered off and evaporated under reduced pressure. The resulting solid was then recrystallized in hot EtOH (7.5×10^{-2} L), to obtain (**1**) as a white solid (0.961 g, 2.348×10^{-3} mol). Yield = 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.29 (m, 12H, 6xCH_2), 1.44 (m, 4H, 2xCH_2), 1.56 (H_2O), 1.83 (m, 4H, 2xCH_2), 3.40 (t, J = 8 Hz 2H, CH_2-Br), 4.01 (t, J = 8 Hz, 2H, CH_2-O), 6.24 (d, J = 4 Hz 1H, H_{Arom}), 6.80 (s, 1H, H_{Arom}), 6.83 (d, J = 4 Hz, 1H, H_{Arom}), 7.35 (d, J = 8 Hz, 1H, H_{Arom}), 7.62 (d, J = 8 Hz, 1H, H_{Arom}).

2.3.2. Synthesis of 1-methyl-3-(12-((2-oxo-2H-chromen-7-yl)oxy)dodecyl)-1H-imidazol-3-ium bromide *CI* (*2*)

1-Methyl imidazole (0.395 g, 4.811×10^{-3} mol) dissolved in MeCN (1.0×10^{-2} L) was added to a stirred solution of (1)