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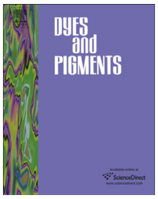

Tuning the limits of pH interference of a rhodamine ion sensor by introducing catechol and 3-hydroxy-4-pyridinone chelating units

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ABSTRACT

A previously reported fluorescein-based dye containing a catechol unit showed high sensitivity to Fe(III) at physiological pH, but also undesirable pH sensitivity, mainly due to the lower pK_a values of the phenolic hydroxyl groups of the fluorescein core. Aiming to synthesize compounds that interact with metal ions at physiological pH, exhibiting no pH sensitivity, we designed two novel fluorescent compounds by assembling, through amide linkage, a rhodamine fluorophore with two selected chelating moieties, catechol and 3-hydroxy-4-pyridinone, both possessing high affinity for Fe(III) but distinct pK_a values.

The fluorescent compounds were prepared using straightforward synthetic protocols and characterized by NMR, mass spectrometry and electronic spectroscopy (UV–Vis and fluorescence) and one of the compounds was also characterized by single crystal X-ray diffraction. The results reveal that, while the fluorescent 3-hydroxy-4-pyridinone derivative exhibits a significant dependence of fluorescence emission with increasing pH, due to the pK_a values arising from the 3-hydroxy-4-pyridinone residue, the fluorescent catechol ligand responds mainly to interactions with metal ions, preferentially to Fe(III), showing less sensitivity to pH than the related ligands over the pH range 3–8, fact that is relevant concerning the application of this compound as an ion sensor in biological media.

1. Introduction

Rhodamine derivatives have been widely used in the development of powerful fluorescence analytical methods that enable detection and quantification of environmental and biologically important species such as metal ions [1–3] anions [4], highly reactive oxygen species [5,6] and also pH values [7,8]. Among the many analytical methods employed for sensing purposes, fluorescence has attracted particular attention because it is sensitive and has numerous parameters that can serve as analytical information, including decay time, energy transfer and quenching efficiency in addition to the more conventional measurement of intensity or polarization [9].

Rhodamines and their analogues typically exhibit excellent photophysical properties [10]. These compounds have been particularly attractive for biological applications by virtue of their lipophilic character and long-wavelength emission maxima. When rhodamines are linked to a specific ligand (recognition site) *via* a suitable linker or spacer, the fluorescence properties of the resulting conjugate will be changed upon reaction with the target analyte which may lead to an attenuation of fluorescence (quenching) or an enhancement in proportion to the analyte concentration, thereby providing a mechanism of detection.

There is a wide versatility of synthetic pathways to model the peripheric positions of rhodamines (Fig. 1) thus allowing conception of a wide range of structures with desired spectroscopic and photophysical characteristics [10,11]. The most common synthetic transformations involve the derivatization reaction of the carboxylic group at position 2′ of the xanthene core, in order to prepare fluorescent compounds based on spirocyclic derivatives [12]. Further modifications can be performed namely at positions 3 and 6, or in the periphery of the phenyl ring at positions 4′ and/or 5′. Curiously, modifications at position 9, which can lead to the synthesis of dihydro derivatives have been less explored [13,14]. An alternative method is to perform the substitution of the xanthene

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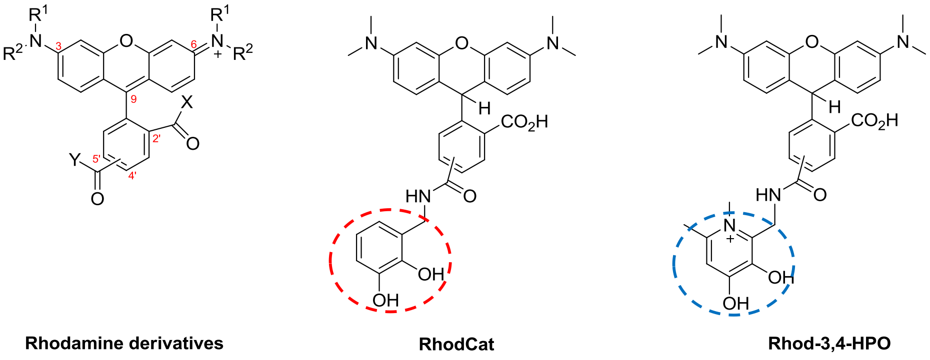

Fig. 1. Chemical structures of rhodamine derivatives, **RhodCat** and **Rhod-3,4-HPO**.

oxygen with other atoms, including Si and C, yielding dyes with red-shifted spectra [15,16].

The design of rhodamine-based pH probes has been generally focused on the introduction of pH-sensitive groups causing a structural change between spirocyclic and open-ring forms. From the recent examples reported in the literature, we highlight a rhodamine-based fluorescent sensor having a methylcarbitol group at position 2′, which has been successfully employed to quantitatively detect the chloroquine induced increase in lysosomal pH and monitor changes in the acidity of lysosomes during apoptosis in live cells [17]. Also a series of pH dependent rhodamine analogues possessing an anilino-methyl moiety at position 2′ was recently reported, showing a unique photophysical response to pH [18].

For metal ions detection, suitable ligands or cavities have been commonly attached to the various positions of the xanthene core. For instance, rhodol and rhodamine dyes carrying a carboxylate di(2-picolyl)amine ligand at positions 3 and/or 6 were prepared, showing enhance affinity for Zn(II) and a Zn-induced shift of emission wavelength [19]. In some cases, the ligand may be also attached to one or more positions of the phenyl ring, as exemplified by a probe that contains a hexathioether ligand bonded to the phenyl ring, allowing a reversible detection of Hg(II) with femtomolar binding affinity in aqueous solutions [20]. Dimeric rhodamine derivatives have also been prepared as metal ion probes through spirocyclic ring–opening reactions [21,22].

Despite these recent advances, the development of strategies to prepare fine-tuned fluorescent rhodamine derivatives having suitable solubility in physiological conditions is of great interest in this research field.

Our group has recently reported the preparation of a Fe(III) selective fluorescein dihydro derivative containing a catechol chelating unit, which showed quenching of the fluorescence at 518 nm (λ_{exc} = 493 nm) in MOPS buffer (pH = 7.4) [23]. Furthermore, this compound revealed to be highly sensitive to the pH, in which the phenolic hydroxyl groups of the fluorescein scaffold contributed with two pK_a values at 4.31 and 6.43, hindering the potential application of this compound as an ion sensor in biological media.

In order to achieve fluorescent compounds less sensitive to pH, we designed two ligands derived from rhodamine containing the catechol and the 3-hydroxy-4-pyridinone (3,4-HPO) unit – **RhodCat** and **Rhod-3,4-HPO** (Fig. 1). We anticipated that by using the rhodamine fluorophore, the spectroscopic and photophysical properties of these ligands could be fine-tuned, at physiological pH, by attaching peripherial chelating units at the appropriate positions of the xanthene core.

The photophysical characteristics of both fluorescent compounds, the evaluation of the fluorescence properties at variable pH and their response to metal ions were studied and are reported in this work. These studies will be determinant for a future analytical application of these compounds and to understand the influence of the chelating unit in the sensing properties of the compounds.

2. Experimental section

2.1. Materials and physical measurements

Reagents and solvents were purchased as reagent-grade products and used without further purification unless otherwise stated.

NMR spectra were recorded on a Bruker Avance III 400, operating at 400.15 MHz for ^{1}H and 100.62 MHz for ^{13}C atoms, equipped with pulse gradient units, capable of producing magnetic field pulsed gradients in the *z*-direction of 50.0 G/cm or on a Bruker Avance III HD 600, operating at 600.13 MHz for proton and 150.92 MHz for carbon atoms, equipped with pulse gradient units, capable of producing magnetic field pulsed gradients in the *z*-direction of 6.57 G/cmA. Two-dimensional ^{1}H/^{1}H correlation spectra (COSY), gradient selected ^{1}H/^{13}C heteronuclear single quantum coherence (HSQC) and ^{1}H/^{13}C heteronuclear multiple bond coherence (HMBC) spectra were acquired using the standard Bruker software.

Microwave-assisted reactions were carried out in a CEM Discovery Labmate circular single-mode cavity instrument (300 W max magnetron power output) from CEM Corporation.

Mass spectra were acquired by Unidade De Espectrometria De Masas of Santiago de Compostela and microanalyses were acquired by Unidad De Análisis Elemental of Santiago de Compostela. Flash chromatography was carried out using silica gel Merck (230–400 mesh).

Absorption spectra were acquired with a Varian Cary bio50 spectrophotometer equipped with a constant-temperature cell holder, at 25 °C, in 1 cm cuvettes. Fluorescence measurements were performed with a Varian Cary Elipse Spectrofluorometer equipped with a constant-temperature multicell cell holder, at 25 °C, with 5 mm slit width for excitation and emission in 1 cm cuvettes. All fluorescence spectra were recorded using the maximum absorption wavelength as the excitation wavelength (λ_{exc}). To minimize reabsorption effects, the absorbance's sample values were kept below 0.1.

2.2. Synthetic procedures

The synthetic procedure of **RhodCat** and **Rhod-3,4-HPO** is described in Scheme 1. The precursors 2,3-dibenzyloxy benzylamine **2** and 1,6-dimethyl-2-aminomethyl-3-benzyloxy-4-