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The effect of absorbance of Fe^{3+} on the detection of Fe^{3+} by fluorescent chemical sensors

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ABSTRACT

We investigated the effect of ferric ion absorbance on the detection of Fe^{3+} by fluorescent sensors. The presence of Fe^{3+} ion resulted in a decrease of emission intensity of fluorophores (naphthalene, dansyl chloride, and anthracene) excited with UV light. Absorbance spectrum and reversibility studies revealed that the decrease in emission intensity of the fluorophores without a receptor moiety was mainly due to the absorbance of ferric ion. We synthesized fluorescent chemical sensors consisting of the receptor for Fe^{3+} and naphthyl fluorophore or dansyl fluorophore, respectively. The sensors detected Fe^{3+} by a fluorescent quenching effect of Fe^{3+}, as well as by the absorbance effect of Fe^{3+}. The absorbance of Fe^{3+} has a considerable interference effect on the excitation of the fluorophore with a short excitation wavelength range in UV light. Hence, fluorophores with longer excitation wavelength are highly recommended in the synthesis of fluorescent sensors for Fe^{3+}.

1. Introduction

During the past couple of decades, research on fluorescent chemical sensors for the detection of metal ions has received increasing interest due to their inherent simplicity, high sensitivity, and an easy measurement [1–6]. Fluorescent chemosensors consist of receptor and fluorophore. The receptor is responsible for the recognition of analytes and the fluorophore converts the recognition events into fluorescent signals. Several types of fluorescent chemical sensors have been synthesized by incorporating various fluorophores into receptors for environmental or biological purposes of detection. The ideal fluorophore in chemosensors has high quantum yields of fluorescence, high extinction coefficients, long excitation and emission wavelengths, a long lifetime, and photostability. Therefore, the choice of fluorophore is the most important factor in the design of new fluorescent chemosensors.

Recently, fluorescent chemosensors for Fe^{3+} have received increasing attention. Iron is a highly important metal ion for most organisms, playing a significant role in many biochemical processes [7,8]. Specifically, Fe^{3+} acts as a cofactor in many enzymatic reactions involved in the mitochondrial respiratory chain, and both its deficiency and excess can induce a variety of diseases [8–10]. Therefore, methods of ferric ion detection have received a great deal of study. However, in spite of the important role of Fe^{3+} in chemical and biological processes, selective and sensitive fluorescent chemosensors for Fe^{3+} are still rare.

Various research groups have independently synthesized Fe^{3+} chemosensors containing fluorophores such as naphthyl, dansyl, anthracene, and coumarin compounds that have excitation wavelengths in the range of UV and near-visible light [13–24]. Very recently, we successfully synthesized fluorescent sensors containing the dansyl fluorophore for heavy and transition metal ions such as Zn^{2+}, Cu^{2+}, Hg^{2+}, and Cd^{2+} [11,12]. In this process, we found that the ferric ion, unlike other metal ions, exhibits substantial UV and near-visible absorbance. Absorbance by the ferric ion had a considerable interfering effect on the excitation of dansyl fluorophore. In the present study, we investigated the effect of ferric ion's absorbance on the detection of fluorescent chemosensors for Fe^{3+}. For this, we chose naphthalene, dansyl chloride, and anthracene among common fluorophores that have excitation wavelengths in the UV range, and investigated their fluorescent spectra in the presence of ferric ion. Even though these fluorophores did not have the receptor for Fe^{3+} incorporated in them, increasing concentration of ferric ion resulted in a decrease of emission intensity of the fluorophores. Absorbance spectrum and reversibility studies revealed that the decrease of emission intensity of the fluorophores was due to the absorbance of ferric ion rather than the quenching effect of ferric ion. Furthermore, we synthesized fluorescent chemical sensors consisting of the receptor for Fe^{3+} and naphthyl or dansyl fluorophores and investigated the effect of the absorbance of ferric

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ion on the detection of the fluorescent sensors for Fe^{3+}. The overall results indicated that if the sensors monitored ferric ion by turn-off detection, and the excitation wavelength of their fluorophores was shorter than that of visible light, the absorbance of ferric ion caused oversensitivity in the Fe^{3+} sensors. Thus, it is desirable that the fluorophore of Fe^{3+} sensors operates with an excitation wavelength longer than UV, which calls for sensitivity studies of fluorescent sensors of Fe^{3+}. To the best of our knowledge, this is the first paper to describe the effect of absorbance of ferric ion on the detection of fluorescence sensors for Fe^{3+} ion.

2. Experimental

2.1. Materials and instruments

Naphthalene, 2-naphthoic acid, dansyl chloride, anthracene, trifluoroacetic acid (TFA), tri-isopropylsilane (TIS), dansyl chloride, triethylamine, diethyl ether, dimethyl sulfoxide (DMSO), N,N-dimethylformamide (DMF), and piperidine were purchased from Aldrich.

Fmoc-Asp (Otbu)-OH, N,N-diisopropylcarbodiimide, 2-Cl trityl chloride resin from Novabiochem. All UV–visible spectra were measured by UV/visible spectrometer (Lambda 40, PerkinElmer, UK), fluorescence spectra by Luminescence spectrometer (LS 55, PerkinElmer, UK).

2.2. Synthesis of **10**

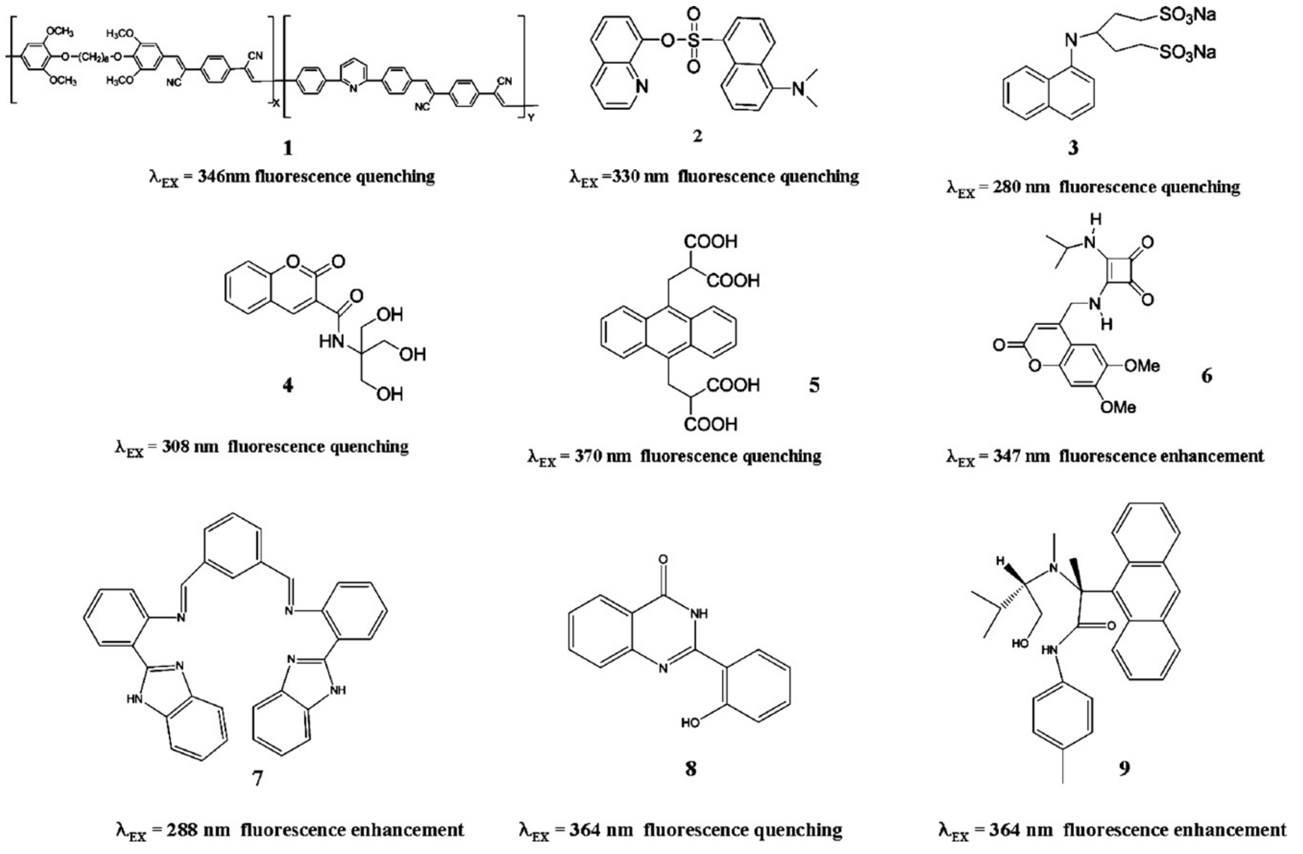
Compound **10** was synthesized in solid phase synthesis in a 2-Cl trityl chloride resin. After Fmoc-Asp(Otbu)-OH was introduced into the resin, the coupling of 2-naphthoic acid was performed. To the resin-bound amino acid (0.05 mmol), DMF solution (3 mL) containing 2-naphtholic acid (0.15 mmol, 3 equiv.), DIPC (0.15 mmol, 3 equiv.) and HOBt (0.15 mmol, 3 equiv.) were added and then shaken for 24 h at room temperature. Deprotection and cleavage were achieved by treatment with a mixture of TFA/TIS/H_2O (9.5:0.25:0.25, v/v/v) at room temperature for 4 h. After precipitation of the crude product in ether solution, the crude product was purified by semi-HPLC with a C_{18} column using water (0.1% TFA)/acetonitrile (0.1% TFA) gradient. Successful synthesis (**10** $[M+H]^+$ calcd. 287.10; obsd. 287.90) and purity (>95%) were confirmed by ESI mass spectrometer (Platform II, Micromass, Manchester, UK) and analytical HPLC with C_{18} column.

2.3. Synthesis of **11**

Compound **11** was synthesized by solid phase synthesis in 2-Cl trityl chloride resin. Fmoc-Asp(Otbu)-OH was introduced into the resin. To the resin-bound amino acid (0.05 mmol), dansyl chloride (0.15 mmol, 3 equiv.) in DMF (3 mL) containing triethylamine (0.15 mmol, 3 equiv.) were added and kept for 2 h at room temperature. Deprotection and cleavage were achieved by treatment with a mixture of trifluoroacetic acid (TFA)/TIS/H_2O (9.5:0.25:0.25, v/v/v) at room temperature for 3–4 h. After precipitation of the crude product in ether solution, the crude product was purified by semi-HPLC with a C_{18} column using water (0.1% TFA)/acetonitrile (0.1% TFA) gradient. Successful synthesis (**11** $[M+H]^+$ calcd. 366.40; obsd. 366.98) and purity (>95%) were confirmed by an ESI mass spectrometer (Platform II, Micromass, Manchester, UK) and analytical HPLC with C_{18} column.

2.4. Fluorescence measurements

Fluorescence emission spectrum of a compound in a 10 mm path length quartz cuvette was measured using a PerkinElmer luminescence spectrophotometer (model LS 55). Emission spectra of



1 λ_{EX} = 346nm fluorescence quenching

2 λ_{EX} =330 nm fluorescence quenching

3 λ_{EX} = 280 nm fluorescence quenching

4 λ_{EX} = 308 nm fluorescence quenching

5 λ_{EX} = 370 nm fluorescence quenching

6 λ_{EX} = 347 nm fluorescence enhancement

7 λ_{EX} = 288 nm fluorescence enhancement

8 λ_{EX} = 364 nm fluorescence quenching

9 λ_{EX} = 364 nm fluorescence enhancement

Fig. 1. Structures and fluorescent properties of reported fluorescent sensors for Fe^{3+}.