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Detection of Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} ions through label-free poly-L-glutamic acid

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ABSTRACT

Detection of heavy metal ions in water is important for environmental sustainability and food safety. Current fluorescent sensors interact with metal ions directly through chelation or chemical reactions. Those sensors are expensive to produce and often can detect only one ion at a time. Here we report a fluorescent turn-on sensor that can detect three group IIB metal ions and Pb^{2+} ions through label-free polypeptides in water. In our sensor-polypeptide mixture, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} ions induce helix formation and inter-chain aggregation in poly-L-α-glutamic acid (PGA). The acridinium-based sensor molecules incorporate into the polypeptides and emit strongly with characteristic color for each group IIB ion under UV lamp. By adjusting the size of polypeptides or the length of the side chain carboxyl groups, we can selectively turn off or turn on the sensor emission for Hg^{2+} ions.

1. Introduction

Group IIB elements include the transition metals zinc, cadmium, and mercury. Zinc is the second most abundant trace element in the human body after iron. Zinc deficiency can cause severe neurological disorders including Alzheimer's and Parkinson's disease [1,2]. Cadmium exposure has been cited as a risk factor for several illnesses including kidney disease, early atherosclerosis, hypertension, low birth weight, and cardiovascular disease [3]. Mercury and lead ions are highly toxic and have been associated with numerous adverse effects on the lungs, kidneys, immune system, eyes, skin, and gastrointestinal tract [4,5]. Detection and quantification of group IIB and lead (II) ions are therefore important for environmental monitoring, food safety, and clinical diagnosis.

Fluorescent sensors have proven to be fast, noninvasive, cost-effective, and highly sensitive [6]. Most sensors for Zn^{2+}, Cd^{2+}, or Hg^{2+} are soluble in organic solvents and can be used only in partial aqueous media [7–9], although recently some water-soluble fluorophores have been reported to be effective in nearly 100% aqueous solutions [10–12]. Furthermore, some sensors can only detect one ion, while others cannot distinguish Cd^{2+} from Hg^{2+}. Here we report a turn-on fluorescent sensor that emits a characteristic color for each group IIB ion in aqueous solutions.

Fluorescent sensors often consist of two parts: one acts as a ligand to bind metal ions and another acts as a fluorophore to report the signals. The detection of target ions is based on changes of fluorescent properties, including the emission intensity and the wavelength of maximum emission in the presence of target metal ions. In most cases, there is a direct interaction between the sensor and the metal ions, often through chelating to change the electronic configuration or the geometry of sensor molecules. This interaction affects the energy transfer [13,14], or electron transfer pathway [15–17], and alters the fluorescence quantum yield. Our design reconceptualizes the fluorescent sensor design. Instead of using part of the sensor as a ligand to interact with metal ions, we use a polypeptide as a platform for metal ion binding. Sensing metal ions with peptides has been reported [18–20]. However, those peptides were tagged with fluorescent sensors. Our design uses commercially available, label-free polypeptides. Our fluorescent sensor does not interact directly with the metal ions. Instead, its fluorescent properties respond to the secondary and tertiary structure of polypeptides induced by the metal ions. Without the metal ions, it displays no emission in near neutral polypeptide solution. Upon binding with metal ions, our sensor emits strongly due to an induced conformational change in the polypeptides. Moreover, under UV light, it emits a characteristic color for each group IIB ions, enabling detection of three ions with the naked eye.

Previously we reported a donor-donor-acceptor triad, thiophene-phenylanilide-acridinium (TPA), which fluoresces strongly upon binding to poly-L-glutamic acid (PGA) in acidic conditions [21]. In the triad, acridinium is the fluorophore, while anilide and thiophenes are

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both electron donors. In aqueous solution and most organic solvents, the emission of acridinium is completely quenched due to photo-induced intramolecular electron transfer [21]. In acidic conditions (e.g., pH = 4.7), poly-L-glutamic acid formed a multi-helix tertiary structure with a hydrophobic interior capable of incorporating the TPA molecules [21]. This hydrophobic pocket restricted the rotation of TPA molecules, suppressed the second step photo-induced electron transfer (PET), and strongly enhanced the emission of charge-shift state (CSH) formed during the first step of PET [21]. In the present study, we use the triad to sense metal ions through poly-L-glutamic acid in near neutral pH. Recent studies showed that metal ions such as Ca^{2+}, Cu^{2+}, and Zn^{2+} can induce the helix formation of poly-L-glutamic acid at near neutral pH [22–24], and heavy metal ions such as Cd^{2+} and Hg^{2+} can bind strongly to glutamic acids [25–27]. We hypothesize that since metal ions can induce the helix formation, and the helix formation can enhance the CSH emission in the triad, then the presence of metal ions should enhance the CSH emission of the triad in poly-L-glutamic acid solutions.

It can be difficult to detect group IIB ions using spectroscopic methods because of their nd^{10} electron configuration. It is also difficult to differentiate zinc ions from cadmium and mercury ions because of their similarities. Herein we report a method that can differentiate those ions by size. We observed that at pH 6.5, the triad in PGA solution showed extremely weak emission without target ions. With 0.30 mM Zn^{2+}, it displayed strong CSH emission at 540 nm with 180-fold enhancement. With 0.30 mM Cd^{2+}, it emitted most strongly at 560 nm with 160-fold enhancement. With 0.30 mM Hg^{2+} or Pb^{2+}, it showed strong CSH emission at 575 nm with 170-fold enhancement for Hg^{2+} and 150-fold enhancement for Pb^{2+}. In the presence of Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, and Ag^{+}, there is no change in triad emission. We propose a model wherein metal ions induce helix formation and peptide aggregation to form hydrophobic interiors of various sizes which affect the CSH emission of the triad molecules.

2. Materials and methods

2.1. Materials

The preparation of thiophene-phenylanilide-acridinium triad (TPA, Scheme 1) has been reported previously [21]. Briefly, it was prepared by the coupling reaction between thiophene carbonyl chloride and the 4-aminophenyl-10-methylacridinium salt. Sodium salts of poly-L-α-glutamic acid were purchased from Sigma Aldrich with average molecular weights of 75,000 g/mol (MW: 50,000 to 100,000 g/mol), 32,500 g/mol (MW: 15,000–50,000 g/mol), and 3500 g/mol (MW: 1500 to 5500 g/mol), respectively. Sodium salts of poly-L-γ-glutamic acid (MW ≥ 750,000 g/mol), poly-acrylic acid (MW 15,000 g/mol), and poly-(αβ)-DL-aspartic acid (MW: 2000 to 11,000 g/mol) were purchased from Sigma Aldrich. The molar mass of one glutamic acid sodium salt residue ($C_5H_5NO_3Na$) is 151 g/mol. Thus, on average the α-PGA peptides contain 500, 215, and 23 residues, respectively. Metal salts, potassium nitrate, magnesium nitrate, calcium nitrate, strontium nitrate, barium nitrate, copper (II) nitrate, ammonium iron (II) sulfate, silver nitrate, mercury (II) chloride, zinc nitrate, lead (II) nitrate, cadmium nitrate, and 4-morpholineethanesulfonic acid hydrate (MES) were purchased from Sigma Aldrich and used without further purification.

2.2. Methods

The pH of all solutions was monitored with a VWR Symphony pH meter. UV–visible absorption spectra were recorded using a Cary 60 UV–Visible spectrophotometer. Steady-state emission spectra from 400 nm to 800 nm were recorded using a Shimadzu RF-6000 fluorophotometer with excitation wavelength of 360 nm. Fluorescent quantum yield was measured using Rhodamine B in water as the standard. Except where indicated, measurements were made at room temperature using 1-cm quartz cells in aqueous solutions. The slit size for excitation and emission are both 10 nm. The pH of all solutions was controlled by using 10 mM pH 6.50 MES buffer, except during the pH-effect experiment where small aliquot of dilute nitric acid and sodium hydroxide were used to adjust the pH. The TPA stock solution was prepared by dissolving 1.2 mg of triad in 3.00 ml of dry DMF. The metal ions stock solutions of about 40 mM were made by dissolving appropriate amount of metal salt in water. The final concentration of TPA in sample solution is 8.2 μM, and the R/D ratio (PGA residue/dye molecule) was kept at 100. Unless otherwise indicated, the PGA refers to the poly-L-α-glutamic acid with average molecular weight of 75,000 g/mol.

The detection limit was calculated based on fluorescent titration results. The signal of TPA-peptide mixture without metal ions was measured 10 times to determine the standard deviation (σ) of the blank sample. Three titrations of each Group IIB ions were carried out and the averaged fluorescent intensity was used to plot against the ion concentration to determine the slope (m). The detection limit was calculated using Eq. (1).

$$\text{Detection Limit} = \frac{3\sigma}{m} \quad (1)$$

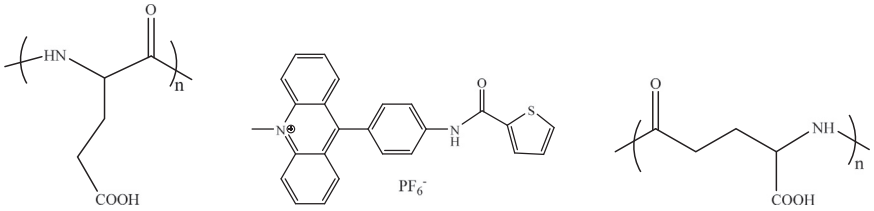
3. Results and discussion

3.1. UV–visible absorption spectra

TPA-PGA-metal ion mixtures displayed CSH absorption bands at various wavelengths. The CSH absorption band of TPA alone in pH 6.5 solution occurs at 428 nm. With PGA and 0.3 mM group IIB metal ions, the CSH absorption band maximum shifts to 433 nm for Zn^{2+}, 452 nm for Cd^{2+} and 454 nm for Hg^{2+} and Pb^{2+} (Fig. 1a). Addition of Hg^{2+} in small aliquots gradually shifted the CSH band from 428 nm to 454 nm (Fig. 1b). Based on the previously reported polarity effect, the red shift of CSH absorption band indicates the inclusion of dye molecules into the hydrophobic pockets inside the PGA aggregates [21].

3.2. Emission enhancement in the presence of Zn^{2+}, Cd^{2+}, Hg^{2+}, or Pb^{2+}

In aqueous solutions, the emission of acridinium chromophore at 505 nm is completely quenched due to ultrafast intramolecular electron



Scheme 1. Structure of α-PGA (left), TPA (middle) molecule, and γ-PGA (right).