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A new peptidyl fluorescent chemosensors for the selective detection of mercury ions based on tetrapeptide

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ABSTRACT

A novel peptidyl chemosensor ($PySO_2$-His-Gly-Gly-Lys($PySO_2$)-NH_2, **1**) was synthesized by incorporation of two pyrene (Py) fluorophores into the tetrapeptide using sulfonamide group. Compound **1** exhibited selective fluorescence response towards Hg(II) over the other metal ions in aqueous buffered solutions. Furthermore, **1** with the potent binding affinity (K_d = 120 nM) for Hg(II) detected Hg(II) without interference of other metal ions such as Ag(I), Cu(II), Cd(II), and Pb(II). The binding mode of **1** with Hg(II) was investigated by UV absorbance spectroscopy, ^{1}H NMR titration experiment, and pH titration experiment. The addition of Hg(II) induced a significant decrease in both excimer and monomer emissions of the pyrene fluorescence. Hg(II) interacted with the sulfonamide groups and the imidazole group of His in the peptidyl chemosensor and then two pyrene fluorophores were close to each other in the peptide. The decrease of both excimer and monomer emission was mainly due to the excimer/monomer emission change by dimerization of two pyrene fluorophores and a quenching effect of Hg(II).

1. Introduction

The design and synthesis of fluorescent chemosensors for the detection and quantification of low level contamination of heavy and transition metal (HTM) ions have received considerable attention because these types of metal ions are toxic for humans and other living organisms.[1,2] Among the various HTM ions, mercury is most toxic and hazardous. The contamination of mercury occurs through many ways and it became a worldwide environmental problem.[3] In general, the oxidized form of mercury, Hg(II) and methyl mercury enters the food chain through the contaminated water and accumulates in higher organisms.[3c,4,5] Even accumulation of low concentration of mercury in human body causes a variety of diseases such as prenatal brain damage, serious cognitive, and motion disorders.[6,7] Hence, it is highly recommended to develop selective and sensitive ways for Hg(II) in aqueous solutions.

Among the various analytical detection techniques for Hg(II), fluorescence has received attention because of its inexpensive instrument, high sensitivity, convenient, rapid and accurate detection of small amounts of analytes in the sample.[3c,8] Thus, in recent years, various types of fluorescent chemosensors for Hg(II) have been reported. In general, the fluorescent chemosensor consists of a ligand-binding site (receptor), responsible for recognizing analytes and a signal transduction site (fluorophore), converting the recognition events into fluorescent signals.[9] Various type of scaffolds such as calixarene,[10a,b] hydroxyquinolines,[10c,d] azines,[10e] azadiene,[10f] dioxaoctane-diamide,[10g] cyclams,[10h] and crown ethers,[10i–l] have been reported as a receptor part for the detection of Hg(II) in fluorescent chemosensors. However, most of them suffer from the cross-sensitivity or interference from other metal ions, for example, Cu(II), Ag(I), Cd(II) and Pb(II) due to the similar size or softness of these metal ions to Hg(II).[10] Therefore, it is highly desirable to develop fluorescent chemosensors based on new scaffolds as a receptor for the selective and sensitive detection of heavy and transitional metal (HTM) ions in aqueous solutions.

In recent years, the peptide scaffolds have been exploited as the basic molecular framework for fluorescent chemosensors in the construction of the binding site due to its structural diversity, good solubility in water, and biological and environmental compatibility.[11,12] We and other research groups have successfully demonstrated that the fluorescent chemosensors based on the peptide scaffolds showed sensitive responses to heavy metal ions in aqueous solutions.[11,12] However, almost all peptidyl chemosensors except the peptide sensors based on copper-binding motif originating from the amino terminal Cu and Ni binding (ATCUN) site showed cross-sensitivity to other heavy metal ions or suffered from the interference of other heavy metal ions.[12] Alternatively, the fluorescent peptidyl chemosensors based on copper-binding motif and similar sequences of ATCUN site such as GGH, GHK, GGHG, and HGGG showed highly selective response only to Cu(II).[11a,c–e,h] Thus, it is highly challenging and important to synthesize a highly selective peptidyl chemosensor for a specific metal ion except Cu(II) and to investigate the binding mode of the peptidyl chemosensor for the specific metal ion.

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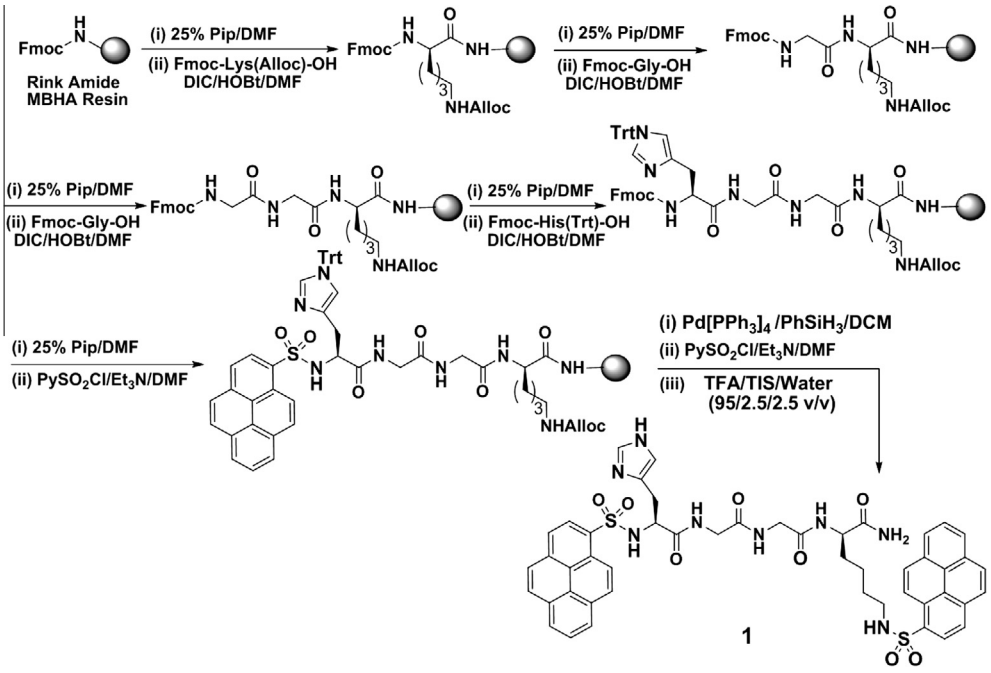
Considering the binding mode of the reported chemosensors based on amino acids and peptides,[12,13] we designed and synthesized a new fluorescent chemosensor based on the tetrapeptide scaffold and investigated the fluorescent response to metal ions. Two pyrene fluorophores were incorporated into the tetrapeptide ($PySO_2$-His-Gly-Gly-Lys($PySO_2$)-NH_2, **1**) using sulfonamide group because the pyrene fluorophore has an interesting photophysical properties such as high fluorescence quantum yield, chemical stability, and dual fluorescence emissions (monomer and excimer)[14] and the sulfonamide group in several chemosensors based on amino acids acted as a ligand for specific metal ions.[13a–g] A His residue was included in the peptidyl chemosensor because His as a metal chelating amino acid played a critical role in the metal recognition in several metalloenzymes.[12a,d,e,15] As shown in Scheme 2, we assumed that when the peptidyl chemosensor might interact with metal ions, it might fold and His and two pyrene fluorophores with sulfonamide group might be closer or far to each other, resulting in the change of the pyrene monomer as well as the excimer emissions. Gly residue was introduced in the sequence of the peptidyl chemosensor **1** for increasing flexibility of the peptide between two pyrene flourophores.[12c] Peptidyl chemosensor **1** exhibited a selective and sensitive response to Hg(II) over the other metal ions in aqueous solution. Furthermore, we investigated the binding mode of **1** for Hg(II) by using ^{1}H NMR titration, fluorescent titration, and pH titration experiment to understand the potent binding affinity for Hg(II) and to explain the decrease of monomer emission intensity as well as the decrease of excimer emission intensity.

2. Results and discussion

Two pyrene labeled peptidyl chemosensor **1** was easily synthesized in solid phase synthesis with a high yield (89.4%) using Fmoc chemistry (Scheme 1).[16] Details on synthesis and characterization of **1** are described in the Section 4 (Figs. S1–S7).

We measured the fluorescent spectrum of **1** in aqueous solution (10 mM HEPES at pH 7.4) containing DMSO (Fig. 1). Interestingly, the fluorescence behavior of **1** was dependent on the amount of DMSO in aqueous solutions. The spectrum of **1** measured in aqueous solution containing 0.1% (v/v) DMSO displays a strong excimer emission at 494 nm, originated from the π–π-stacking between two pyrene moieties, and weak monomer emissions at 381 and 400 nm. Upon increasing the amount of DMSO in aqueous solution (10 mM HEPES at pH 7.4), the decrease of excimer emission and the considerable increases of monomer emissions were observed, respectively. The higher amount of DMSO in aqueous solution (10 mM HEPES at pH 7.4) induced a strong monomer emission intensity and the weak excimer emission intensity. The π–π interactions between the two pyrene moieties of the peptide sensor may depend on the hydrophobicity of solvent system. Small amounts of DMSO in aqueous solutions provided more hydrophilic environments for the peptide and the π–π interactions between the two pyrene moieties increased. Thus, two pyrene moieties of the peptide sensor **1** might come closer to each other due to strong π–π stacking between the two pyrene moieties, which leaded to the strong excimer emission at 494 nm. Large amounts of DMSO in aqueous solutions provided more hydrophobic environments and the π–π interactions between the two pyrene moieties relatively decreased. As the decrease of the π–π stacking interactions between the two pyrene moiety, the decrease of excimer emission and the increase of monomer emission intensity were observed. Among the various solvent conditions, aqueous solution containing 30% DMSO (H_2O/DMSO = 7:3, v/v, 10 mM HEPES at pH 7.4) was chosen for further studies because the peptide sensor **1** showed a significant changes in fluorescence emission intensities in the presence of Hg(II) in this solvent condition (Fig. 1).

As shown in Figure 2a, peptidyl chemosensor **1** displayed both typical monomer emission and excimer emission. Upon addition of Hg(II), the intensities of monomer and excimer emissions significantly decreased whereas both monomer and excimer emissions of **1** were not changed upon addition of other tested metal ions including Na(I), K(I), Mg(II), and Al(III) as chloride anion and Ca(II), Co(II), Cr(III), Fe(III), Mn(II), Ni(II), Pb(II) and Zn(II) as perchlorate anion. Figure 2b shows the gradual emission intensity change of peptidyl chemosensor **1** upon addition of Hg(II). The intensities of monomer emission at 381 and 400 nm and excimer emission at 494 nm decreased significantly. The monomer emission



Scheme 1. Solid phase synthesis of **1**.