



## Highly sensitive and selective detection of Al(III) ions in aqueous buffered solution with fluorescent peptide-based sensor



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### ABSTRACT

A fluorescent sensor based on a tripeptide (SerGluGlu) with a dansyl fluorophore detected selectively Al(III) among 16 metal ions in aqueous buffered solutions without any organic cosolvent. The peptide-based sensor showed a highly sensitive turn on response to aluminium ion with high binding affinity ( $1.84 \times 10^4 \text{ M}^{-1}$ ) in aqueous buffered solutions. The detection limit (230 nM, 5.98 ppb) of the peptide-based sensor was much lower than the maximum allowable level (7.41  $\mu\text{M}$ ) of aluminium ions in drinking water demanded by EPA. The binding mode of the peptide sensor with aluminium ions was characterized using ESI mass spectrometry, NMR titration, and pH titration experiments.

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Aluminium is the most abundant metal and has regarded as one of useful metallic materials for industrial fields and medical fields.<sup>1–3</sup> However, high levels of Al(III) ions in human body have caused severe damages to the nervous system, inducing Alzheimer's disease or Parkinson's disease.<sup>4–8</sup> In addition, Al(III) ions with high concentrations induced several damages to the aquatic environment.<sup>9</sup> For example, Al(III) in water prevented the growth of plant due to the inhibition of phosphate intake.<sup>9</sup> Thus, Environmental Protection Agency (EPA) strictly regulated Al(III) ions in drinking water and surface waters. For these reasons, the detection of low concentrations of Al(III) ions in aqueous environments is of great interest. Even though conventional methods such as inductively coupled plasma-mass spectrometry (ICP-MS) and atomic absorption spectrometry (AAS) are currently used to monitor low levels of aluminium ions, they require expensive instruments, time-consuming procedure, and real time monitoring of Al(III) ions in water samples is not possible.<sup>10,11</sup>

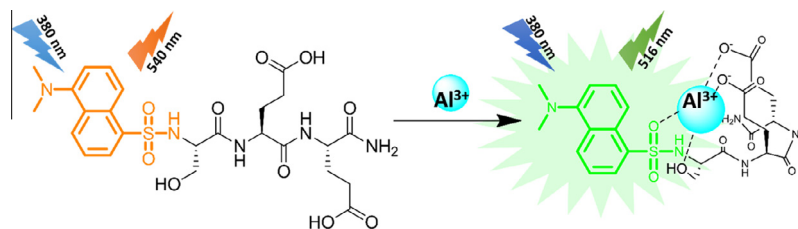
Fluorescent method is the most attractive approach to detect low concentrations of analytes due to their high sensitivity, simplicity, and inexpensive small instrumentation. Thus, the various types of fluorescent chemosensors have been reported for the detection of Al(III).<sup>12–19</sup> Even though there are considerable progresses for the detection of Al(III) ions using fluorescent chemosen-

sors, there are still several limitations to be solved. First of all, almost all fluorescent chemosensors for Al(III) operated in organic solution or in mixed aqueous/organic solution.<sup>12–19</sup> Even though some fluorescent chemosensors showed sensitive responses to Al(III) ions, most of them operated in mixed distilled water and organic solutions. However, the mixed distilled water and organic solution is not a suitable solvent system for sensing Al(III) ions as following reasons. As Al(III) ions act as Lewis acid in water, the addition of Al(III) ions into the mixed distilled water and organic solution, resulting in the change of pH of the sample solution,<sup>20</sup> which must affect the fluorescent emission of the chemosensors. Moreover, the solubility and soluble species of aluminium in water are highly dependent on pH. Al(III) ions dissolved well in water in acidic pH, whereas a series of OH<sup>-</sup> complexes of aluminium (Al(OH)<sup>2+</sup>, Al(OH)<sub>2</sub><sup>+</sup>, Al(OH)<sub>3</sub>, and Al(OH)<sub>4</sub><sup>-</sup>) were formed as pH increased.<sup>20</sup> Furthermore, most of the chemosensors for Al(III) commonly shared the Schiff base (imine) as a key ligand as receptors for Al(III).<sup>21–31</sup> It is necessary to develop new ligands for Al(III) for the detection of various concentrations of Al(III) ions in real sample solutions. Thus, it is highly challenging to develop new fluorescent chemosensors containing new receptors for Al(III) that operated well in aqueous buffered solution.

In recent years, peptide has received considerable attention as a receptor of the fluorescent sensors due to its structural diversity, biocompatibility, and potent binding affinities for metal ions.<sup>32–40</sup> Moreover, peptides dissolve well in aqueous buffered solutions with a wide pH range due to high solubility in water. However,

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**Scheme 1.** The proposed binding mode of the peptide-based sensor with Al(III).

there is no attempt for the peptide as the receptor of the fluorescent sensors for Al(III) ions. Using the advantages of peptides, we have first synthesized a new fluorescent sensors based on a peptide receptor for the detection of Al(III) ions in acidic aqueous buffered solution (Scheme 1).

The peptide-based sensor selectively detected Al(III) ions among various tested metal ions in aqueous buffered solution at acidic pH. Interestingly, the peptide-based sensor showed a highly sensitive turn-on response to Al(III) in 100% aqueous buffered solution without organic cosolvents.

## Material and methods

### Material

Fmoc-Ser(tbu)-OH, Fmoc-Glu(OtBu)-OH, Rink Amide MBHA resin (100–200 mesh, 0.43 mmol/g) and 1-hydroxybenzotriazole (HOBt) were purchased from Bead Tech. Trifluoroacetic acid (TFA), tri-isopropylsilane (TIS), *N,N'*-diisopropylcarbodiimide (DIC), triethyl amine (TEA), piperidine (pip), diethyl ether, acetonitrile (CH<sub>3</sub>CN) were purchased from Sigma Aldrich. Dansyl chloride was purchased from TCI. *N,N*-Dimethylformamide (DMF) were purchased from Acros Organics. All salts of metal ions were purchased from Sigma Aldrich and metal ion sample solution was prepared in de-ionized distilled water.

### Synthesis

#### Dansyl-SEE

(Dansyl-SerGluGlu) was synthesized in solid-phase synthesis using Fmoc chemistry (ESI, Scheme S1).<sup>41</sup> Activated Fmoc-Glu(OtBu)-OH (128 mg, 0.3 mmol) using DIC (47  $\mu$ l, 0.3 mmol) and HOBt (40 mg, 0.3 mmol) was added to swollen Rink Amide MBHA resin (235 mg, 0.1 mmol). After deprotection of the Fmoc group with 25% piperidine in DMF followed by washing, Fmoc-Glu(OtBu)-OH was coupled. After deprotection of Fmoc group, Fmoc-Ser(tbu)-OH (115 mg, 0.3 mmol) was assembled. To the resin bound tripeptide, dansyl chloride (80 mg, 0.3 mmol) in DMF and trimethylamine (80 l, 0.6 mmol) were added. Cleavage of the peptide from the resin was achieved by treatment with a cleavage solution of 3 ml TFA/TIS/H<sub>2</sub>O (95:2.5:2.5, v/v/v) at room temperature for 4 h. After filtration and washing of the resin by TFA, a gentle stream of nitrogen was used to remove the excess TFA. The crude was triturated with diethyl ether chilled at  $-20^{\circ}\text{C}$  and then centrifuged at 3000 rpm for 10 min at  $-10^{\circ}\text{C}$ . The crude product was purified by prep-HPLC with a Vydac C18 column using a water (0.1% TFA)–acetonitrile (0.1% TFA) gradient to give 80% yield.

#### Absorbance and fluorescence measurements

A stock solution of **Dansyl-SEE** at the concentration of 1 mM was prepared in distilled water and in a cold and dark place. This stock solution was used for all fluorescence and absorbance mea-

surements after appropriate dilution. UV/Vis absorbance spectrum (220–430 nm) of **Dansyl-SEE** in a 10 mm path length quartz cuvette was measured in 10 mM Hexamine buffer solution at pH 5.5 using a Perkin-Elmer UV-Vis spectrophotometer (model Lambda 45). And UV/Vis absorbance spectrum (300–800 nm) of **Dansyl-SEE** in a 10 mm path length quartz cuvette was measured in aqueous buffered solution (10 mM hexamine, pH 5.5) using a Perkin-Elmer UV-Vis spectrophotometer (model Lambda 45). The emission spectrum (400–700 nm) of the sample was measured with a Perkin-Elmer fluorescence spectrophotometer (model LS 55). The absorbance and emission spectra of **Dansyl-SEE** in the presence of metal ions (Hg(II), Cd(II), Ca(II), Co(II), Ag(I), Pb(II), Mg(II), Mn(II), Cu(II), Ni(II), Zn(II), Al(III) with perchlorate anion and Na(I), K(I) with chloride anion) were measured by excitation with 380 nm. The slit widths for excitation and emission were 12 and 8 nm, respectively.

#### Determination of dissociation constant ( $K_d$ )

The association constant ( $K_d$ ) was calculated based on the titration curve of **Dansyl-SEE** with metal ions. The dissociation constant was determined by a nonlinear least squares fitting of the data with the following equation, as referenced elsewhere.<sup>42</sup>

$$I = I_{\infty} \times \frac{([H] + [L] + K_d) - \sqrt{([H] + [L] + K_d)^2 - 4[H][L]}}{2[H]}$$

where  $I$  is fluorescence intensity and  $I_{\infty}$  is the final fluorescence intensity,  $[H]$  and  $[L]$  are the total concentration of the sensor ( $H$ ) and metal ion ( $L$ ).

#### Detection limit of Dansyl-SEE for Al(III)

The detection limit was calculated based on data of the fluorescence titration. To determine the  $S/N$  ratio, the fluorescence intensity of the **Dansyl-SEE** was measured 10 times and the standard deviation of the blank measurements was determined. Three separate measurements of the emission intensity were performed in the presence of aluminium ion and the mean intensity was plotted as a concentration of aluminium ion to determine the slope. The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/m$$

where  $\sigma$  is the standard deviation of the fluorescence intensity of the free sensor and  $m$  is the slope between the emission intensity vs the concentration of aluminium ion.<sup>43</sup>

## Results and discussion

As Al(III) is a typical hard metal ion, Al(III) ions prefer the coordination of the carboxylate, phenolate, and catecholate groups, respectively.<sup>44–46</sup> Glu, Asp, Ser, or Thr among amino acids has been frequently found in the Al(III) binding peptides because the negatively charged O donor groups preferred to forming the complex

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