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Synthesis of a novel thiazole based dipeptide chemosensor for Cu(II) in water†

Santanu Bhattacharya* and Mini Thomas

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India

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Abstract

A thiazole-based novel dipeptide module has been synthesized, which exhibited selective Cu(II) recognition in water in the presence of other competing divalent transition metal ions at micromolar concentrations. This is the first example of an entirely synthetic peptide based chemosensor for Cu(II).

Chemosensors are molecular-sized or larger devices that signal interactions with analytes reversibly and in real time.[1] Fluorescence proffers high sensitivity among the many signal types available. Therefore this mode of signal transduction has been used widely in the detection of a number of transition metal ions.[2,3] Among these, the sensors targeted toward the detection and estimation of divalent copper ions are currently receiving a lot of attention.[3] Detection of individual metal ions with high specificity under physiologically relevant conditions is an important aspect in the design of fluorescent chemosensors for biological and environmental applications. In this regard peptide based sensors offer several advantages over other systems as they are biocompatible and also could be conveniently conjugated to appropriate systems of biological interest such as proteins or nucleic acids.

Herein we report the convenient synthesis of a Cu(II)-binding peptide module **1**, which is based on thiazole derivatives. This represents the first example of a Cu(II)-sensing peptide of entirely synthetic origin, which is also the smallest peptide motif that acts as a specific fluorescence chemosensor for a metal ion. Notably, the sensing of Cu(II) using **1** has been achieved in water under physiologically relevant pH and salt conditions, in the presence of other competing divalent transition metal ions.

To synthesize the thiazole containing peptide module, we took advantage of the commercially available 2-amino-4-thiazole acetic acid, **3a** as the key starting material. This was first converted to the corresponding ethyl ester using EtOH and $SOCl_2$ as described in Scheme 1. Removal of

* Corresponding author: Swarnajayanti Fellow. E-mail: sb@orgchem.iisc.ernet.in

† This paper is dedicated to Professsor Robert A. Moss on the occasion of his 60th birthday.

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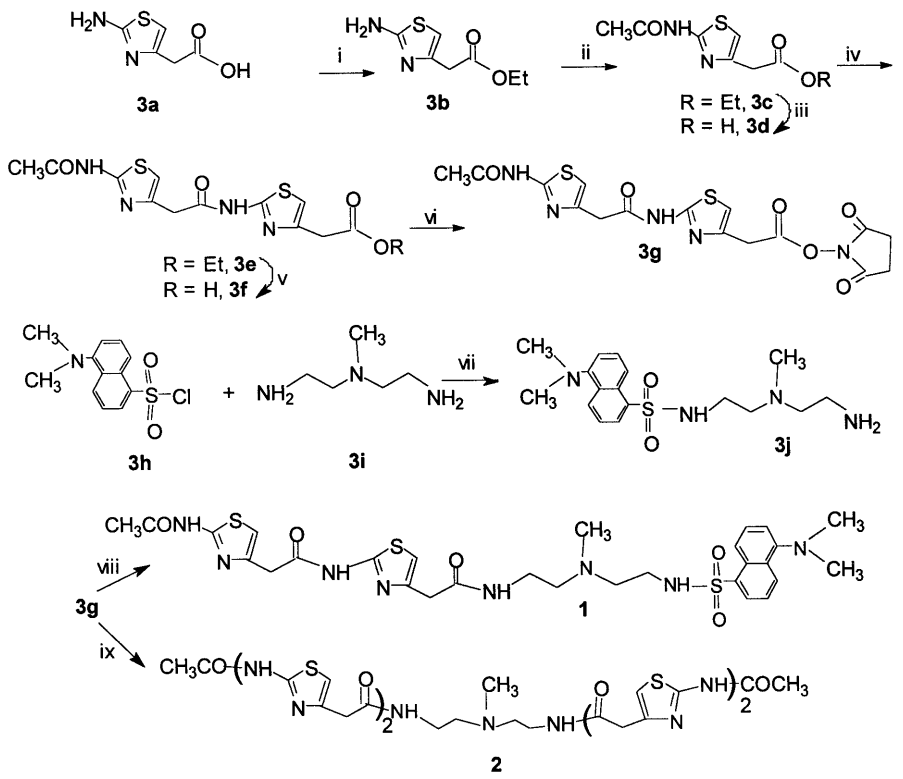

Scheme 1. *Reagents, conditions and yields*: (i) $SOCl_2$/EtOH, −5°C, 1 h→rt, 12 h; (ii) 1:1 v/v HOAc/Ac_2O, −5°C, 1 h→rt, 6 h, 95%; (iii) 0.5 M NaOH, rt, 30 min→0.5 M HCl, 0°C, 97%; (iv) **3b**, DCC, HOBT, DMF, 0°C, 1 h→rt, 6 h (85%); (v) 0.5 M NaOH, rt, 30 min→0.5 N HCl, 0°C, 98%; (vi) DCC, HOSU, DMF, 1 h→rt, 6 h; (vii) Et_3N, dry $CHCl_3$, 0°C, 1 h, 70%; (viii) **3j**, dry $CHCl_3$, rt, 2 h (80%); (ix) **3i**, dry DMF, rt, 4 h (85%)

excess reagents followed by aqueous work up furnished the ester, **3b** in quantitative yield. This was then reacted with Ac_2O/HOAc. Removal of excess reagents and recrystallization from EtOAc yielded an analytically pure sample of the *N*-acetylated product, **3c** in 95% yield. Saponification of this key intermediate and subsequent acidification provided the acid, **3d** in 97% yield. The coupling of **3d** with the amine, **3b** was effected by dropwise addition of a solution of dicyclohexyl carbodiimide (DCC) in DMF to a solution of **3b** in DMF in the presence of 1-hydroxybenzotriazole (HOBT). To ensure satisfactory amide coupling slow addition of DCC was important as bulk introduction of DCC resulted in the formation of the *N*-acyl isourea derivative of the corresponding acid. This could be due to low reactivity of the aromatic amine, **3b**. DCU was removed by filtration and the product was precipitated by the addition of water. The amide **3e** was obtained in pure form (85%) by successive washing of this solid with EtOAc and 5% $NaHCO_3$ solution, to remove the unreacted starting materials. Saponification and subsequent acidification of **3e** afforded the corresponding acid, **3f** in analytically pure form (98%) which was subsequently converted to the corresponding *N*-hydroxysuccinimide ester, **3g** employing DCC and *N*-hydroxysuccinimide (HOSU). DCU was removed by filtration and the product was precipitated by the addition of water, and was used as such for further reactions.

In a separate set of reactions, commercially available 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride), **3h** and *N*-methyldiethylenetriamine, **3i** (2 equiv.) were reacted in

$CHCl_3$ in presence of Et_3N as described in Scheme 1. The monodansylated amine, **3j** was isolated in 70% yield after column chromatography on neutral alumina. Aminolysis of the activated ester, **3g** with **3j** in dry $CHCl_3$ furnished the dipeptide chemosensor, **1** in 80% yield after column chromatography on neutral alumina. Similarly aminolysis of **3g** with **3i** (0.5 equiv.) furnished the tetrapeptide, **2** in 85% isolated yield after column chromatography on neutral alumina. All numbered intermediates and final compounds were characterized by their IR, NMR, mass spectra and elemental analysis.[4]

Fluorescence emission spectra of **1** were recorded upon excitation at 330 nm. A linear decrease in the fluorescence intensity was observed until one equiv. of Cu(II) was added (Fig. 1A). Further addition of Cu(II) produced only a nominal decrease in fluorescence intensity. The fluorescence quenching of **1** by Cu(II) was tested over a range of concentrations (2–20 μM of **1**) and was found to be linear with the added metal ion concentration. When 1 equiv. of each of Mn(II), Fe(II), Co(II), Ni(II) and Zn(II) were first added to a solution of the peptide, **1** in tris buffer, only a marginal quenching of the sensor fluorescence was observed (Fig. 1B, curve 'b'). Addition of 1 equiv. of Cu(II) to the above solution however, produced a drastic decrease in the

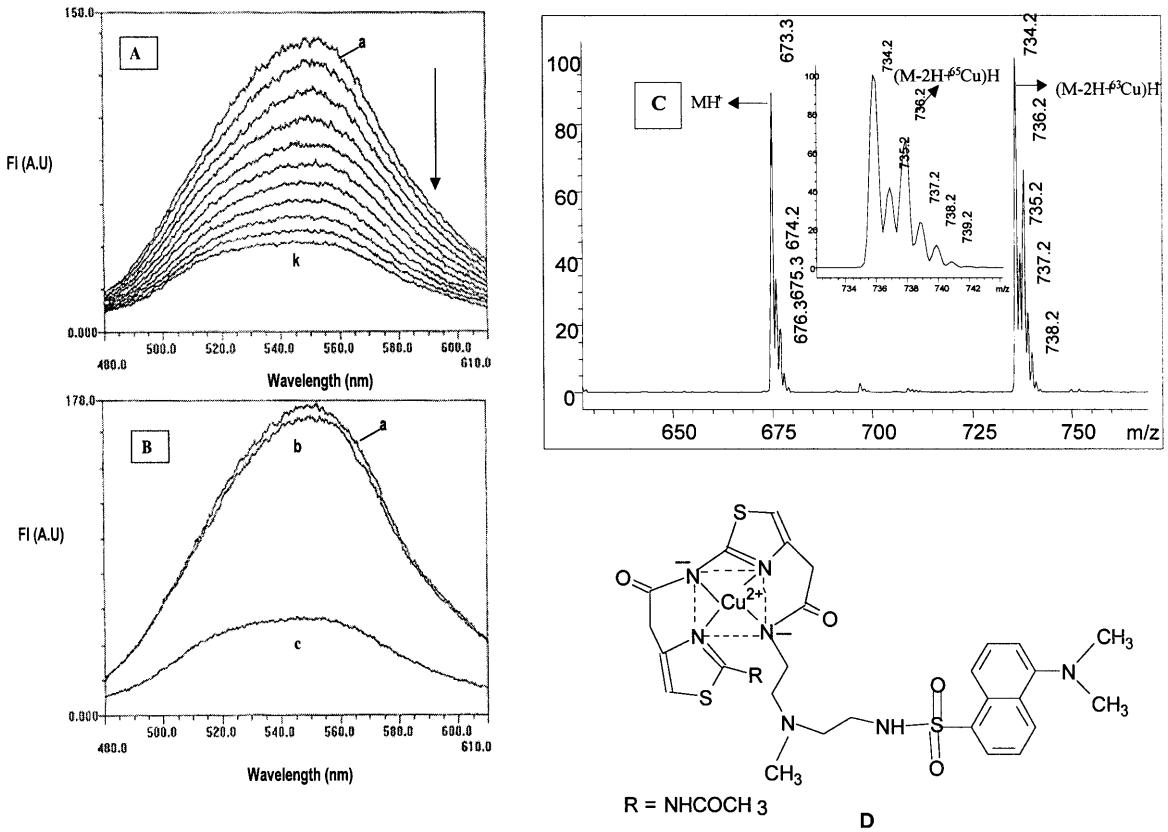

Figure 1. (A) Effect of addition of Cu(II) on the emission spectra (λ_{ex} = 330nm) of **1** in 20 mM tris buffer containing 150 mM NaCl (pH 7.4). Curve 'a', free probe (4.4 μM). 4.4 μM of Cu(II) was added in ten equal aliquots. Curve 'k' corresponds to 4.4 μM of Cu(II). (B) Effect of addition of Cu(II) in presence of other divalent transition metal ions. Curve 'a' is due to the free probe (5.4 μM), curve 'b' shows the effect of addition of 5.4 μM each of Mn(II), Fe(II), Co(II), Ni(II), Zn(II); curve 'c' shows the effect of addition of 5.4 μM of Cu(II). (C) ESI-MS of **1**/Cu(II) complex. (D) Possible coordination scheme for the **1**/Cu(II) complex

fluorescence intensity (Fig. 1B, curve 'c'). This experiment confirms that the peptide, **1** can act as a specific sensor for Cu(II) even in the presence of elevated levels of other competing divalent transition metal ions. The Cu(II) induced quenching was found to be reversible with the addition of excess EDTA.

To discern the nature of the complex formed between **1** and Cu(II) in solution, we examined the system by electrospray ionization mass spectrometry (ESI-MS) (Fig. 1C). At 1:1 metal/ligand ratio, the signal obtained at a mass/charge (m/z) unit of 734.2 could be assigned to a species corresponding to $[M-2H+Cu]\cdot H^+$. The presence of the peak at m/z of 673.2 is likely to be due to the free peptide formed by decomplexation under ESI-MS conditions as further addition of Cu(II) did not increase the intensity of the bound species. The corresponding tetrapeptide, **2** exhibited a signal corresponding to $[M-4H+2Cu]H^+$ in ESI-MS, suggesting that two independent co-ordination centers of similar nature as in **1** are present. Direct involvement of the central tertiary amino group and the dansyl group in metal-complexation is less likely. We propose a co-ordination scheme (Fig. 1D) that explains the ESI-MS results. Work is underway to obtain crystals of the Cu(II) complexes of **1** and **2** to establish firmly the structure of the metal ligation site.

The present system **1**, which could be easily synthesized in solution phase, represents a simple, yet unexplored class of peptide sensors for Cu(II) with high selectivity. As this motif selectively targets Cu(II) ions, intramolecular quenching of the covalently tethered fluorophore represents a convenient mechanism for signaling of Cu(II) binding. Functional devices based on **1** could be designed for environmental monitoring of Cu(II) ions. We are now exploring the incorporation of this peptide module into other peptides/proteins[5a,b] and minor groove binders[6a,b] for generation of affinity cleavage agents and chemical nucleases.[6c–e]

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4. Selected spectroscopic data **1**: ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 2.25 (s, 3H); 2.30 (br s, 2H); 2.37 (br s, 2H); 2.89 (s, 6H); 3.01 (br s, 2H); 3.13 (br s, 2H); 3.59 (s, 2H); 3.87 (s, 2H); 6.59 (s, 1H); 6.77 (s, 2H); 6.80 (s, 2H); 7.16 (d, 1H, $J=7.5$ Hz); 7.41 (t, 1H, $J=8.1$ Hz); 7.53 (t, 1H, $J=8.1$ Hz); 8.26 (d, 1H, $J=7.2$ Hz); 8.34 (d, 1H, $J=8.7$ Hz); 8.56 (d, 1H, $J=8.4$ Hz). ESI-MS: calcd for $C_{29}H_{37}N_8O_5S_3$ (MH^+) 673.3, found: 673.3. **2**: ^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6): δ (ppm) 2.09 (s, 6H); 2.16 (s, 3H); 2.41 (t, 4H, $J=7.6$ Hz); 3.15–3.23 (m, 4H); 3.49 (s, 4H); 3.74 (s, 4H); 6.69 (s, 2H); 6.76 (s, 2H); 7.72 (br s, 2H); 11.96 (br s, 2H). ESI-MS: calcd for $C_{29}H_{36}N_{11}O_6S_4$ (MH^+) 762.3, found: 762.2.
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