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A dansylated peptide for the selective detection of copper ions†

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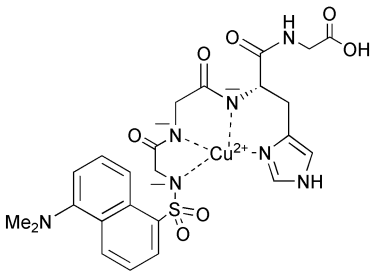
A new peptidyl fluorescent chemosensor for the selective detection of copper ions was studied.

There have been intense investigations on the fluorescent chemosensors for the detection of Cu^{2+} for the past few years.[1] A basic ionic sensor is composed of two intramolecularly linked functional units: a fluorophore and an ionophore.[2] The foremost work is to design a selective binding motif, and then a fluorophore is attached to the vicinity of the receptor for signal transduction. This methodology provides considerable flexibility of design. However, one disadvantage is that the recognition event is hard to be fully detected because the fluorophore does not directly contact the bound metal ion. In this aspect, an ideal fluorescent probe will be that whose fluorescent unit is directly involved in the interaction with metal ions.

Gly–gly–his is a peptide motif originated from the amino terminal Cu and Ni-binding (ATCUN) site.[3] Fluorescent chemosensor based on this motif has been studied by Imperiali and coworkers.[1d] Selectivity was obtained by modulating the backbone structure of the peptide. In this paper, we report our work in which a new fluorescent chemosensor (dansyl-gly–gly–his–gly, **1**) was developed such that the fluorophore itself is part of the recognition site. This model provides excellent selectivity of detection, even more, the involved difference is significant. First, it provides the advantage of synthetic simplicity. Previously reported fluorescent chemosensors for Cu^{2+} are usually designed such that a fluorophore was attached on the side branch,[1] therefore a side branch needs to be first added or branched organic residues are required. In our approach, only the simple essential amino acids were used and no branched amino acids are needed for the synthesis. Additionally, the synthesis can be conveniently achieved by using the well established Fmoc solid phase peptide protocol. The second difference lies in the unique structure in which a dansyl fluorophore directly participated in the binding with Cu^{2+} (Scheme 1). This is advantageous to the previously used method. In the side-branch labeling method, the fluorophore is on a side chain and does not participate in the binding, so it is hard to efficiently detect the binding event. In our method, since the dansyl sulfonamide group directly joins in the binding with Cu^{2+}, the molecular fluorescence is quenched to a maximum level and therefore a high molecular sensitivity is attained. Like the peptide gly–his, the free N-terminal amine of gly–gly–his is reported as an essential requirement for the binding of the peptide with Cu^{2+}.[3] Our results here have shown that this requirement is actually not absolute. We carefully considered the special acidity of the dansyl sulfonamide group (whose pK_a is 10 while that of the peptide amide nitrogen is 18) which may offer sufficient reactivity to assist the peptide to bind with Cu^{2+}. On the other hand, the requirement of deprotonation of the dansyl sulfonamide group for the binding will provide the improved selectivity indispensable to the resultant sensor.



Scheme 1 Binding model of **1** with Cu^{2+}.

In the absorption spectrum of the peptide, maximum bands were observed at 215, 247 and 329 nm. Upon the addition of Cu^{2+}, each absorption band shifted to lower wavelengths (207, 244 and 322 nm, respectively). Additionally, in the visible region, an absorption band was observed at 572 nm, which also supports the complex formation.

In the fluorescence spectra of peptide **1**, maximum excitation and emission wavelengths were observed at 343 and 558 nm, respectively. The emission intensity of **1** decreased with the addition of copper ions and a turning point was observed when one equivalent of Cu^{2+} was added indicating a 1 : 1 binding ratio (Fig. 1). The binding constant was calculated as $3.8 \times 10^6\ M^{-1}$ using the method reported by Connors.[4]

Peptide **1** exhibits an excellent selectivity toward Cu^{2+} (Fig. 2). An aqueous solution containing **1** (5.0 μM), several competitive transition metal ions including Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+} (each is of 5.0 μM) showed an emission almost overlapped with that of the free ligand **1**(98% of relative intensity at the maximum λ_{em}). On the other hand, when one equivalent of copper ions was added, the fluorescence intensity was considerably quenched and only 5% of the intensity remained.

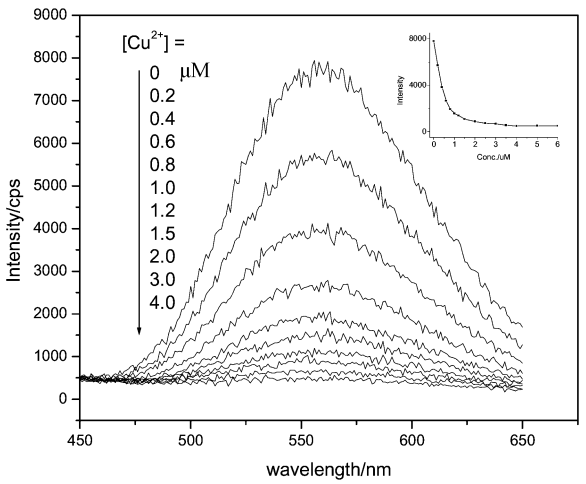


Fig. 1 Fluorescence emission spectra of **1** (1.0 μM) in the presence of increasing concentration of Cu^{2+}. Insert: fluorescence intensity at λ_{em} = 558 nm as a function of copper concentration. Excitation is selected at 340 nm. Measured at 20 °C in phosphate buffer (pH = 6.8).

† Electronic supplementary information (ESI) available: experimental procedures of organic synthesis and measurements of spectral data. See http://www.rsc.org/suppdata/cc/b2/b208012e/

DOI: 10.1039/b208012e

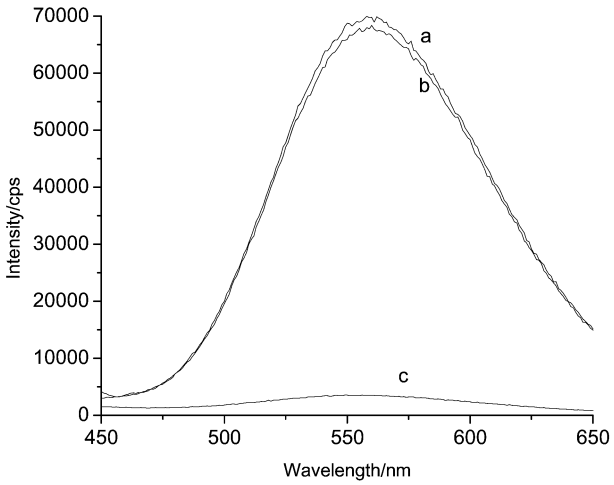


Fig. 2 Fluorescence selectivity of ligand **1** toward Cu^{2+} (a) free **1**, (b) **1** and miscellaneous ions, and (c) **1**, miscellaneous ions and Cu^{2+}. Concentration of **1** is kept constant at 5.0 μM. Concentration of Cu^{2+} is 5.0 μM. The miscellaneous ions contain Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, and each has a concentration of 5.0 μM. Excitation is selected at 343 nm. Phosphate buffer (pH = 7.2).

To investigate the range of pH in which ligand **1** can effectively detect Cu^{2+}, we measured the titration curve of fluorescence intensity *versus* pH of the aqueous solution (Fig. 3, curve a). The free **1** exhibited a strong fluorescence in the range of pH > 4. When the pH was lower than 4, the fluorescence intensity began to decrease and completely vanished when the pH reached 2.0. The quenching phenomenon under this acidic condition is caused by the protonation of the dimethylamino group of the dansyl fluorophore (pK_a ~ 4) which prevents the charge transfer between the amine and naphthyl ring.[5] Another feature on the pH titration curve is that, when the pH was higher than 8.6, the intensity increased substantially, which was related to the deprotonation of the dansyl sulfonamide group.

The effect of pH on the fluorescence of **1**-Cu^{2+} solution exhibited a quite different feature from that of the free peptide (Fig. 3, curve b). The pronounced difference was observed in the range of pH > 6.8 in which the fluorescence intensity was deeply quenched. Evidently the binding with Cu^{2+} caused this quenching. When the pH was lower than 6.8, the fluorescence appeared to increase, a process related to the dissociation of the **1**-Cu^{2+} complex. And when the pH was decreased to 5.6, the fluorescence intensity reached the same level as that of the free ligand indicating a complete dissociation; thus Cu^{2+} had no influence on the fluorescence of the ligand. Same as curve a, when the pH was lower than 4, the fluorescence began to disappear due to the protonation of the dimethylamino group of the dansyl fluorophore.

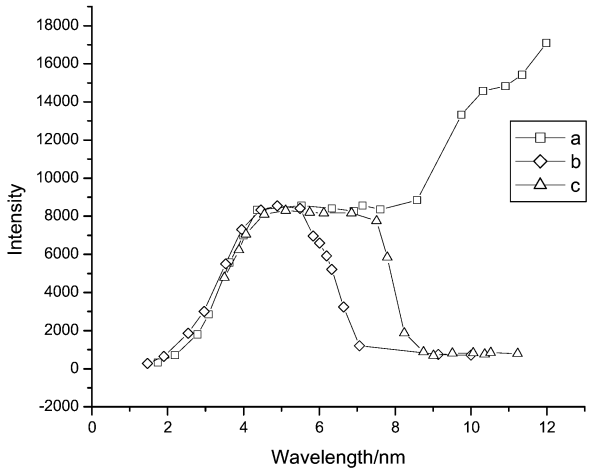


Fig. 3 Fluorescence intensity of **1** at different pH (a) free **1**, (b) **1** and Cu^{2+}, (c) **1** and miscellaneous ions. Concentration of **1** is kept constant at 1.0 μM. Concentration of Cu^{2+} is 1.0 μM. The miscellaneous ions contain Fe^{2+}, Fe^{3+} Zn^{2+} Co^{2+} Ni^{2+}, and each has a concentration of 1.0 μM. Excitation is selected at 340 nm.

To determine the detecting specificity of **1** toward Cu^{2+} at various pH, we tested the influence of several competitive miscellaneous transition metal ions (Fe^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}) on the fluorescence of **1** in the same pH range (Fig. 3, curve c). It is seen that these competitive ions exhibited a fluorescence quenching at a higher pH than Cu^{2+}. It is this thermodynamic difference causing the selective detection of Cu^{2+}. Clearly the ideal range for the detection of Cu^{2+} is pH 6.8–7.5 since during this region, Cu^{2+} causes a maximum quenching while the miscellaneous metal ions only have a marginal effect on the molecular fluorescence. It is meaningful to point out that this range is within the physiological pH region. The fluorescence intensity of the miscellaneous ions-added solution appeared to be lower than that of the free ligand when the pH was higher than 7.6 where the dansyl sulfonamide group began to deprotonate. This observation is understandable since the nitrogen atom of the sulfonamide group, after being deprotonated, carries a negative charge (electron donor), and therefore promotes complexation interaction with the miscellaneous ions. Obviously, the miscellaneous metal ions changed fluorescence of the peptide only after the dansyl sulfonamide group was deprotonated in the alkaline condition. But in the case of Cu^{2+}, the fluorescence was efficiently quenched from a pH of 6.8.

In conclusion, we have studied an N-terminally dansylated peptide which can detect Cu^{2+} with an excellent selectivity and molecular sensitivity. Our design provides a greatly improved and simplified model compared with previously investigated fluorescent probes for Cu^{2+}.

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Notes and references

1 (*a*) M. Beltramello, M. Gatos, F. Mancin, P. Tecilla and U. Tonellato, *Tetrahedron Lett.*, 2001, **42**, 9143; (*b*) A. Singh, Q. Yao, L. Tong, W. C. Still and D. Sames, *Tetrahedron Lett.*, 2000, **42**, 9601; (*c*) Y. Zheng, Q. Huo, P. Kele, F. M. Andreopoulos, S. M. Pham and R. M. Leblanc, *Org. Lett.*, 2001, **3**, 3277; (*d*) A. Torrado, G. K. Walkup and B. Imperiali, *J. Am. Chem. Soc.*, 1998, **120**, 609; (*e*) S. Bhattacharya and M. Thomas, *Tetrahedron Lett.*, 2000, **41**, 10313; (*f*) G. DeSantis, L. Fabbrizzi, M. Licchelli, C. Mangano, D. Sacchi and N. Sardone, *Inorg. Chim. Acta*, 1997, **257**, 69; (*g*) R. Corradini, A. Dossena, G. Galaverna, R. Marchelli, A. Panagia and G. Sartor, *J. Org. Chem.*, 1997, **62**, 6283; (*h*) T. Mayr, D. Wencel and T. Werner, *Fresenius J. Anal. Chem.*, 2001, **371**, 44; (*i*) J. Yoon, N. E. Ohler, D. H. Vance, W. D. Aumiller and A. W. Czarnik, *Tetrahedron Lett.*, 1997, **38**, 3845; (*j*) K. A. Mitchell, R. G. Brown, D. Yuan, S.-C. Chang, R. E. Utecht and D. E. Lewis, *J. Photochem. Photobiol. A: Chem.*, 1998, **115**, 157; (*k*) R. Kramer, *Angew. Chem., Int. Ed.*, 1998, **37**, 772.

2 (*a*) F. Pina, M. A. Bernardo and E. Garcia-Espana, *Eur. J. Inorg. Chem.*, 2000, 2143; (*b*) L. Fabbrizzi, M. Licchelli, P. Pallavicini, L. Parodi and A. Taglietti, in *Transition Metals in Supramolecular Chemistry, Perspective in Supramolecular Chemistry, Vol. 5*, ed. J.-P. Sauvage, John Wiley & Sons Ltd., New York, 1999, p. 93; (*c*) *Chemosensors of ion and molecule recognition*, ed. J. P. Desvergne and A. W. Czarnik, Kluwer Academic Publishers, Boston, 1997; (*d*) A. P. de Silva, H. O. Nimal Gunaratne, T. Gunnlaugusson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.

3 C. Harford and B. Sarkar, *Acc. Chem. Res.*, 1997, **30**, 123.

4 K. A. Connors, *Binding Constants, The Measurement of Molecular Complex Stability*, John Wiley & Sons, New York, 1987, p. 339.

5 (*a*) S. Aoki, H. Kawatani, T. Goto, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 2001, **123**, 1123; (*b*) E. Kimura and T. Koike, *Chem. Soc. Rev.*, 1998, **27**, 179; (*c*) T. Koike, T. Watanabe, S. Aoki, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1996, **118**, 12696.