Coumarin-derivative-based off–on catalytic chemodosimeter for Cu^{2+} ions†

Mi Hee Kim, Hyun Hye Jang, Sujung Yi, Suk-Kyu Chang and Min Su Han*

Received (in Cambridge, UK) 30th April 2009, Accepted 11th June 2009
First published as an Advance Article on the web 3rd July 2009
DOI: 10.1039/b908638b

In this study, a new coumarin derivative is shown to be a highly effective turn-on fluorescent sensor that is catalytically hydrolyzed by Cu^{2+}, and the catalytic process induces a large increase in the fluorescence intensity, due to amplification of the fluorescence signal.

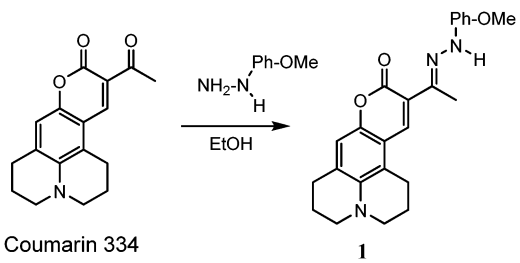
Many transition-metal ions function as essential trace elements in biological systems. Among such metal ions, Cu^{2+} is a particularly important divalent cation that plays a vital role in many cellular processes such as those occurring in the human nervous system, gene expression, and the functioning and structural enhancement of proteins; further, it functions as a cofactor of many enzymes such as nuclease.[1] However, if the concentration level of copper exceeds that required in cellular processes, copper can be toxic and can cause oxidative stress and disorders associated with neurodegenerative diseases such as Alzheimer's disease.[2] As a result, Cu^{2+} has attracted considerable attention in recent years, and numerous fluorescent Cu^{2+} sensors have been developed.[3,5]

Conventional Cu^{2+} sensors developed in the past are fluorescent sensors that are based on chelating moieties.[3] However, most of these sensors show decreased emission upon Cu^{2+} binding, due to quenching of fluorescence by mechanisms inherent to paramagnetic species; such decreased emission is undesirable for analytical purposes.[3,4] Therefore, many efforts have been made to develop various fluorescent sensors specifically for Cu^{2+} ion detection with fluorescence enhancement.[5] Only a few sensors among such "off–on" Cu^{2+} sensors have nanomolar sensitivity and high selectivity.[5] These sensors detect Cu^{2+} ions on the basis of highly selective catalytic reactions induced by these ions.[5a–c] This approach is very attractive for achieving high selectivity and sensitivity of sensors.[6] For example, Lu *et al.* developed a catalytic chemosensor for Cu^{2+} by using DNAzyme;[5a] Czarnik prepared a catalytic hydrolyzable rhodamine B hydrazide by using Cu^{2+} ions;[5b] finally, Anslyn detected Cu^{2+} by carrying out signal amplification on the basis of the Heck reaction.[5c] These sensors had both nanomolar sensitivity and high selectivity in the detection of Cu^{2+} ions; their fluorescence was enhanced due to the detection. Moreover, these sensors can possibly be used to amplify the fluorescence signal induced by a catalytic reaction.

In this communication, we present a new turn-on type chemodosimeter that has nanomolar sensitivity and high selectivity because of the catalytic hydrolysis of a hydrazone derivative by Cu^{2+}. Hydrazone derivatives have been used as protecting groups for carbonyl compounds, and carbonyl compounds are readily regenerated from hydrazones by undergoing catalytic hydrolysis with Cu^{2+} ions.[7] To develop a selective chemodosimeter for Cu^{2+}, we carried out the catalytic regeneration of carbonyl compounds from hydrazone derivatives by using Cu^{2+} ions. For this purpose, we selected coumarin 334, which is a fluorophore with a high quantum yield (θ = 0.83), as a signaling moiety.[8] When the catalytic hydrolysis of the hydrazone occurs in the presence of Cu^{2+} ions, the regeneration of coumarin from the hydrazone derivative induces a fluorescence change. The hydrazone derivative used in this study (**1**) was synthesized from coumarin 334 and a hydrazine, as shown in Scheme 1.

Fluorescence spectra of solutions of **1** were recorded 5 min after the addition of Cu^{2+}. Fluorescence emission spectra of **1** in the presence of Cu^{2+} in various concentrations are shown in Fig. 1. The addition of Cu^{2+} ions induced the enhancement of the fluorescence, and, as can be found from the inset of Fig. 1, the observed fluorescence intensity was nearly proportional to the Cu^{2+} concentration. From the titration results, the detection limit of **1** for Cu^{2+} was estimated to be 8.7×10^{-8} M (see ESI†). This detection limit is acceptable within the US EPA limit (~20 μM) for the detection of Cu^{2+} in drinking water.

Another important property of the chemodosimeter is its high selectivity for Cu^{2+} over other metal ions. To evaluate the Cu^{2+} selectivity of **1**, changes in the fluorescence properties of **1** caused by other metal ions were also measured. Fluorescence spectra of solutions of **1** (1 μM) recorded 5 min after the addition of 10 equiv. of each of these metal ions are shown in Fig. 2. Compound **1** showed remarkably high selectivity for



Scheme 1 Synthetic route to **1**.

Department of Chemistry, Chung-Ang University, Seoul 156-756, Republic of Korea. E-mail: mshan@cau.ac.kr; Fax: +82 2 825 4736; Tel: +82 2 820 5198

† Electronic supplementary information (ESI) available: Synthesis of **1**, determination of the detection limit of **1** for Cu^{2+}, the method for quantification of Cu^{2+}, UV-Vis and fluorescence spectra of both **1** and **1** in the presence of Cu^{2+}, ^{1}H-NMR spectrum of **1** in the presence of Cu^{2+} and ^{1}H-NMR spectrum of a mixture of coumarin 334 and 4-methoxyphenylhydrazine. See DOI: 10.1039/b908638b

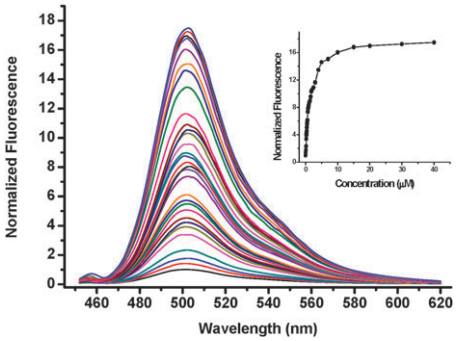


Fig. 1 Fluorescence emission spectra obtained by addition of Cu^{2+} (0–40 μM) to pH 5.0 buffer solution (acetonitrile : 0.01 M acetate buffer = 5 : 5) containing **1** (1 μM). Inset: plot of normalized fluorescence intensities of **1** at 502 nm *versus* Cu^{2+} concentration.

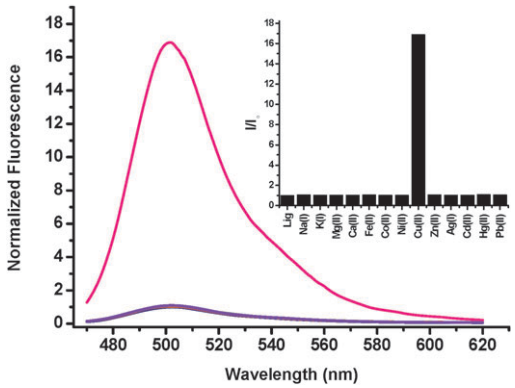
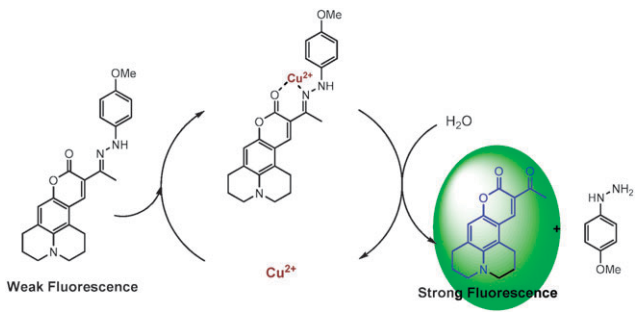


Fig. 2 Fluorescence emission spectra obtained by addition of various metal ions to pH 5.0 buffer solution (acetonitrile : 0.01 M acetate buffer = 5 : 5) containing **1** (1 μM). Inset: plot of normalized fluorescence intensities of **1** at 502 nm *versus* metal ions.

Cu^{2+} over the other metal ions. No significant increase in the fluorescence intensity was observed upon the addition of any other metal ions.

To gain a better understanding of the properties of **1**, having high sensitivity and selectivity, we postulate its sensing mechanism as follows: because Cu^{2+} ions show high affinity for various amine ligands, Cu^{2+} is bound to **1**, which in turn promotes the hydrolysis of the hydrazone bond in **1**. The hydrolyzed products and the Cu^{2+} ion separate, and the Cu^{2+} ion binds to another **1** and subsequently again promotes the hydrolysis of the hydrazone bond *via* the



Scheme 2 Cu^{2+}-induced catalytic sensing cycle of **1**.

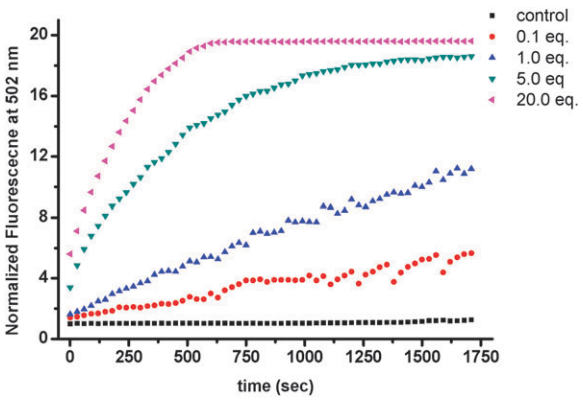


Fig. 3 Kinetics of fluorescence over background at varying Cu^{2+} levels. Conditions: [**1**] = 1.0×10^{-6} M, λ_{ex} = 450 nm, buffer pH = 5.0.

catalytic sensing cycle. Finally, the catalytic cycle induces and amplifies the fluorescence signal (Scheme 2).

To confirm the sensing mechanism of **1** for Cu^{2+}, products of **1** catalytically hydrolyzed by Cu^{2+} were investigated. UV-Vis and fluorescence spectra of **1** incubated with 10 equiv. of Cu^{2+} for 10 min were compared with those of coumarin 334, and the spectra of these two compounds were found to be almost identical (see ESI†). Further, **1** was reacted with Cu^{2+} in deuterated acetonitrile-d_3–D_2O (1 : 1, v/v) for 10 min, and the NMR spectrum of the solution was measured after eliminating the paramagnetic metal ion (Cu^{2+}) using Chelex resin.[9] The ^{1}H-NMR spectrum of the resulting solution was consistent with that of a 1 : 1 mixture of coumarin 334 and 4-methoxyphenylhydrazine (see ESI†). These data clearly confirmed that the hydrolyzed product of **1** was coumarin 334. Further, the time-dependent fluorescence change in **1** was evaluated in the presence of Cu^{2+} ions. In a typical experiment, Cu^{2+} ions at fixed concentrations were added to a solution of **1**. Upon the hydrolysis of the hydrazone group in **1** by Cu^{2+}, the fluorescence of the solution gradually increased and the hydrolysis process was traced by measuring the fluorescence at 502 nm (Fig. 3). The entire quantity of **1** was hydrolyzed within 10 min in the presence of 20 μM Cu^{2+} (20 equiv. to **1**), and the fluorescence was enhanced and became ~18 times (100% hydrolysis of **1**) the initial fluorescence. However, no changes in fluorescence were detected in the absence of Cu^{2+}. In particular, in the presence of 0.1 μM Cu^{2+} (0.1 equiv. to **1**), the fluorescence became more than four times (>20% hydrolysis of **1**) the initial fluorescence at 1500 s. This observation implies that **1** was catalytically hydrolyzed by Cu^{2+} and could successfully detect a very low concentration of Cu^{2+} by signal amplification, as shown in Scheme 2. Therefore, because of signal amplification *via* the catalytic reaction, the developed chemodosimeter is advantageous in that it has better sensitivity than traditional chemodosimeters without any catalytic properties.

The applicability of the developed chemodosimeter to the analysis of Cu^{2+} ions in a practical sample was also investigated. Possible interferences by other metal ions were assessed by measuring Cu^{2+}-induced fluorescence changes in **1** in the presence of background metal ions. Fluorescence spectra of solutions of **1** (1 μM) recorded 5 min after the addition of

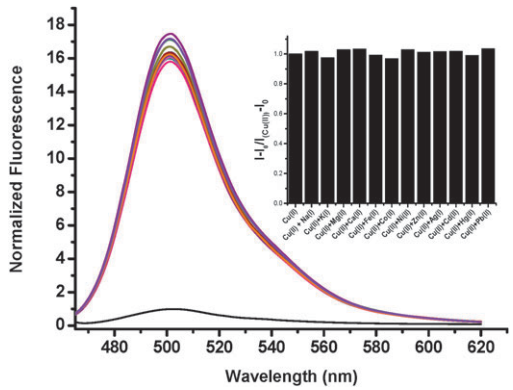


Fig. 4 Fluorescence emission spectra obtained by addition of Cu^{2+} (10 μM) to pH 5.0 buffer solution (acetonitrile : 0.01 M acetate buffer = 5 : 5) containing **1** (1 μM) and metal ions (100 μM). Inset: fluorescence intensities of **1** (1 μM) in the presence of Cu^{2+} (10 μM) and metal ions (100 μM).

Cu^{2+} (10 μM) to a buffer solution (pH 5.0) containing **1** and 100 equiv. of the other metal ions are shown in Fig. 4. The fluorescence intensity changes caused by the addition of Cu^{2+} are not influenced by the presence of the other metal ions, which may be attributed to the following reasons: (a) Cu^{2+} ions show higher affinities for various amine ligands than other metal ions and (b) the hydrazone moiety was not cleaved by other metal ions.[10] It is worth mentioning that a fluorescence enhancement similar to that in the presence of just Cu^{2+} ions was achieved in the same incubation time in spite of the presence of an excess of other metal ions.

In conclusion, we have proposed a new hydrazone derivative that is easily prepared in a single step from coumarin 334, and we have demonstrated that the hydrazone derivative is a highly effective fluorescent sensor with strong fluorescence enhancement in the presence of paramagnetic Cu^{2+} ions. This sensor is catalytically hydrolyzed by Cu^{2+}, and the catalytic process induces a large increase in the fluorescence intensity, due to signal amplification. Importantly, this compound has high selectivity for Cu^{2+} over other anions; moreover, this selectivity is retained even in the presence of an excess of other metal ions. In addition, the detection limit for Cu^{2+} is below an aqueous Cu^{2+} concentration of 100 nM and this detection limit is acceptable within the US EPA limit. Consequently, the chemodosimeter **1** can be employed in a practical system for monitoring Cu^{2+} concentrations in aqueous samples.

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2007-331-C00162).

Notes and references

1 (*a*) H. Tapiero, D. M. Townsend and K. D. Tew, *Biomed. Pharmacother.*, 2003, **57**, 386; (*b*) R. Uauy, M. Olivares and M. Gonzalez, *Am. J. Clin. Nutr.*, 1998, **67**, 952S.
2 D. Strausak, J. F. B. Mercer, H. H. Dieter, W. Stremmel and G. Multhaup, *Brain Res. Bull.*, 2001, **55**, 175.
3 (*a*) Y. Zheng, J. Orbulescu, X. Ji, F. M. Andreopoulos, S. M. Pham and R. M. Leblanc, *J. Am. Chem. Soc.*, 2003, **125**, 2680; (*b*) A. Torrado, G. K. Walkup and B. Imperiali, *J. Am. Chem. Soc.*, 1998, **120**, 609; (*c*) K. C. Chang, L. Y. Luo, E. W. G. Diau and W. S. Chung, *Tetrahedron Lett.*, 2008, **49**, 5013; (*d*) S. H. Kim, J. S. Kim, S. M. Park and S. K. Chang, *Org. Lett.*, 2006, **8**, 371; (*e*) M. Boiocchi, L. Fabbrizzi, M. Licchelli, D. Sacchi, M. Vazquez and C. Zampa, *Chem. Commun.*, 2003, 1812; (*f*) N. Shao, Y. Zhang, S. Cheung, R. Yang, W. Chan, T. Mo, K. Li and F. Liu, *Anal. Chem.*, 2005, **77**, 7294; (*g*) G. Klein, D. Kaufmann, S. Schurch and J. L. Reymond, *Chem. Commun.*, 2001, 561; (*h*) Y. Xiang, Z. Li, X. Chen and A. Tong, *Talanta*, 2008, **74**, 1148; (*i*) H. H. Zeng, R. B. Thompson, B. P. Maliwal, G. R. Fones, J. W. Moffett and C. A. Fierke, *Anal. Chem.*, 2003, **75**, 6807; (*j*) H. S. Jung, P. S. Kwon, J. W. Lee, J. I. Kim, C. S. Hong, J. W. Kim, S. Yan, J. Y. Lee, J. H. Lee, T. Joo and J. S. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 2008.
4 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.
5 (*a*) J. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 9838; (*b*) V. Dujols, F. Ford and A. W. Czarnik, *J. Am. Chem. Soc.*, 1997, **119**, 7386; (*c*) Q. Wu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 14682; (*d*) M. Royzen, Z. Dai and J. W. Canary, *J. Am. Chem. Soc.*, 2005, **127**, 1612; (*e*) L. Mei, Y. Xiang, N. Li and A. Tong, *Talanta*, 2007, **72**, 1717; (*f*) Z. C. Wen, R. Yang, H. He and Y. B. Jiang, *Chem. Commun.*, 2006, 106; (*g*) Z. Liu, L. R. Lin, R. B. Huang and L. S. Zheng, *Spectrochim. Acta, Part A*, 2008, **71**, 1212.
6 (*a*) R. Martínez-Máñez and F. Sancenón, *Coord. Chem. Rev.*, 2006, **250**, 3081; (*b*) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008, **37**, 1465; (*c*) A. Chatterjee, M. Santra, N. Won, S. Kim, J. K. Kim, S. B. Kim and K. H. Ahn, *J. Am. Chem. Soc.*, 2009, **131**, 2040; (*d*) Y. K. Yang, K. J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760; (*e*) B. Liu and H. Tian, *Chem. Commun.*, 2005, 3156; (*f*) W. Lin, L. Yuan and X. Cao, *Tetrahedron Lett.*, 2008, **49**, 6585; (*g*) R. J. T. Houk, K. J. Wallace, H. S. Hewage and E. V. Anslyn, *Tetrahedron*, 2008, **64**, 8271; (*h*) K. C. Song, J. S. Kim, S. M. Park, K. C. Chung, S. Ahn and S. K. Chang, *Org. Lett.*, 2006, **8**, 3413; (*i*) J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoon, *J. Am. Chem. Soc.*, 2005, **127**, 10107.
7 E. J. Corey and S. Knapp, *Tetrahedron Lett.*, 1976, **17**, 3667.
8 E. Arici, A. Greiner, F. Raubacher and J. H. Wendorff, *Macromol. Chem. Phys.*, 2000, **201**, 1679.
9 D. Y. Lee and H. C. Zheng, *Plant Soil*, 1994, **164**, 19.
10 R. D. Hancock and A. E. Martell, *Chem. Rev.*, 1989, **89**, 1875.

