Tetrahedron Letters 50 (2009) 7032–7034

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Metal ion detection using a fluorogenic 'click' reaction

Kris Varazo [a,*], Céline Le Droumaguet [b], Karmella Fullard [a], Qian Wang [b,*]

[a] Department of Chemistry, Francis Marion University, Florence, SC 29501, USA
[b] Department of Chemistry and Biochemistry and Nanocenter, University of South Carolina, Columbia, SC 29208, USA

ARTICLE INFO

Article history:
Received 16 June 2009
Revised 26 September 2009
Accepted 28 September 2009
Available online 2 October 2009

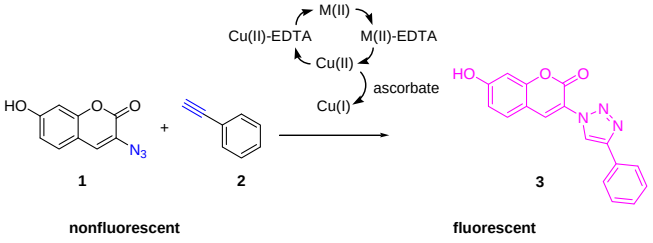
ABSTRACT

A fluorogenic Cu(I)-catalyzed azide–alkyne cycloaddition reaction (CuAAC) of 3-azido-7-hydroxycoumarin has been used to detect metal ions in solution. The formation of a highly fluorescent triazole product signals the presence of Cu(I) or Cu(II) ions at micromolar concentrations. CuAAC can be modified by using an exogenous ligand like EDTA to detect and quantify Zn(II), Ca(II), and Cd(II) ions at micromolar concentrations by an allosteric mechanism. The increase in the formation of the triazole product is regulated by the release of Cu(II) from the Cu(II)–EDTA complex by the addition of a second metal ion, the allosteric effector.

Published by Elsevier Ltd.

Recently, fluorogenic reactions have been employed as powerful tools in studying biological systems.[1] They have been used to label proteins for imaging bacterial cells,[2] label virus capsids,[3] monitor proteases in penicillin,[4] and for the fatty-acylation of proteins.[5] The Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) reaction,[6,7] a paradigm of 'click chemistry' as coined by Sharpless and coworkers,[6] affords superior regioselectivity and almost quantitative transformation under mild conditions. Alkyne and azide groups are very small in size, highly energetic, and have a particularly narrow distribution of reactivity; hence they are well suited to biological applications.[8] Recent work has shown the development and application of fluorogenic CuAAC reactions of azidoanthracenes and azidocoumarins.[1–3,9–15] Additionally, coumarin-based fluorogenic dyes have been used in Staudinger ligation[16] and *S*-nitrosothiol ligation reactions.[17] While many methods of metal ion detection employ fluorescent dyes that undergo off–on[18–20] and on–off[21–23] fluorescence changes during metal ion binding, metal-catalyzed reactions can be an alternative sensing method.[24–26] In this work, we used a 3-azido-7-hydroxycoumarin (**1**) based fluorogenic CuAAC reaction to detect and quantify copper and other biologically and environmentally important metal ions in solution.

For coumarin azide **1**, its fluorescence emission is essentially quenched due to the electron-rich azido group at the 3-position.[10] As shown in Scheme 1, **1** can react readily with phenylacetylene (**2**) catalyzed with Cu(I) to afford a highly fluorescent triazole product (**3**).[10] The formation of **3** can be followed by its fluorescence emission. Figure 1 shows a series of emission spectra (excitation at 350 nm) for a solution containing **1** (9 μM), **2** (9 μM), and sodium ascorbate (NaAsc, 1 mM) in 2.0 mL of DMSO:H_2O (1:1), as a function of added 0.10 mM $CuSO_4$ in 10 μL aliquots. The broad emission



Scheme 1.

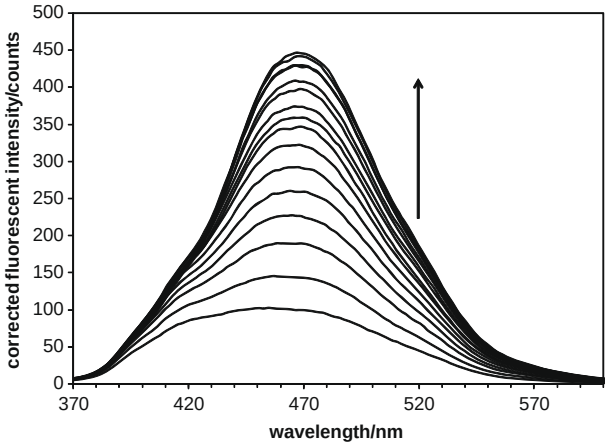


Figure 1. Fluorescence emission spectra of a 2.2 mL (1:1) DMSO:H_2O solution containing **1** (9 μM), **2** (9 μM), and NaAsc (1 mM) after adding 0.10 mM $CuSO_4$ in 10 μL aliquots (λ_{ex} = 350 nm).

* Corresponding authors. Tel.: +1 843 661 1450; fax: +1 843 661 4616 (K.V.); tel.: +1 803 777 8436; fax: +1 803 777 9521 (Q.W.).
E-mail addresses: kvarazo@fmarion.edu (K. Varazo), wang@mail.chem.sc.edu (Q. Wang).

0040-4039/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2009.09.166

band from approximately 380 nm to 550 nm, which is characteristic of coumarins,[10] was observed once the azide and alkyne were mixed, indicating a small amount of reaction without added Cu(II). As the Cu(II) concentration in the solution increases, it undergoes reduction by ascorbate to the catalytically active Cu(I) ion and the fluorescence emission increases strongly. The shoulder observed near 420 nm diminishes as the intensity at 470 nm increases. The increase in fluorescence begins with the first addition of Cu(II), and continues up to 5 μM Cu(II), reaching a plateau at slightly higher concentrations (Fig. 1). This fluorescence increase could be observed similarly in pure aqueous solution; however, the overall fluorescent signals were weaker than in a mixed solvent of DMSO/H_2O due to the poor solubility of compound **2** in pure water. Therefore, a 1:1 mixture of DMSO/H_2O was used in this study.

Generally, CuAAC reactions can be completed in a relatively short period of time.[3,6] For the data in Figure 1, measurements were made as soon as the reagents were mixed, and CuAAC reaction may not have been complete. Therefore, a study of the time dependence of the fluorescence increase of the reaction could lead to an improved sensitivity of Cu(II) detection. Figure 2 shows the relative fluorescence (F/F_0) emission at 470 nm for solutions containing 50 nM Cu(II) and 500 nM Cu(II) along with **1**, **2,** and NaAsc and a blank with no Cu(II). For each solution, there is an increase in the relative fluorescence that reaches a maximum in about 10 min, suggesting a relatively quick reaction. Using this method, Cu(II) at much lower concentrations than our previous experiments can be detected. For instance, 500 nM of Cu(II) produces an approximately fourfold higher signal than the blank, and 50 nM Cu(II) produces twofold higher signal than the background (Fig. 2). Hence, 50 nM can be taken as an estimate of the detection limit, where the signal-to-noise ratio is ~2.

Recent work in catalytic signal amplification using metal-catalyzed reactions has shown that an exogenous ligand can be used to detect metal ions that do not catalyze the reaction.[25,27] In this sensing protocol, a ligand like ethylenediaminetetraacetic acid (EDTA) is added to bind the metal catalyst, sequestering it. The addition of a second metal ion establishes a competitive equilibrium between the metal catalyst–ligand complex (1) (CuAAC as an example) and the added metal ion–ligand complex (2):

$$\mathrm{Cu(II)} + \mathrm{EDTA} \rightarrow \mathrm{Cu(II)}{-}\mathrm{EDTA} \quad (1)$$

$$\mathrm{M(II)} + \mathrm{Cu(II)}{-}\mathrm{EDTA} \rightarrow \mathrm{M(II)}{-}\mathrm{EDTA} + \mathrm{Cu(II)} \quad (2)$$

As more of the second metal ion is added, more of the metal catalyst is released, producing more fluorescent product, which signals the presence of the added metal ion. We chose to use (EDTA) as the ligand, which forms 1:1 complexes with most metal ions. Three important metal ions in biology and chemistry, that is, Zn(II), Ca(II), and Cd(II), were studied using our sensing cascade. To solutions containing 10 μM of **1** and **2**, a complex of 10 μM Cu(II)–EDTA was formed prior to the addition of NaAsc (80 μM), with a final volume of 2.1 mL. Then the second metal ion (0.1 mM) was added in 20 μL aliquots and the fluorescence emission of the solution was monitored.

As shown in Figure 3, compared to experiments without EDTA, the wavelength of maximum fluorescent intensity underwent a bathochromic shift of ~20 nm to 486 nm. As shown in Figure 4, the largest increase in relative fluorescence was observed when Cu(II) was used without EDTA, but all three metal ions were able to displace Cu(II) from the EDTA complex at micromolar concentration levels, liberating Cu(II) to subsequently undergo reduction with ascorbate and catalyze the reaction. The relative fluorescence ratios for Zn(II) and Ca(II) are nearly identical, indicating an equal ability to displace Cu(II) from the EDTA complex. Cd(II) produced a much weaker fluorescence increase, but it is significantly above the background intensity ($F/F_0 = 1$). Additionally, for Zn(II) and Ca(II),

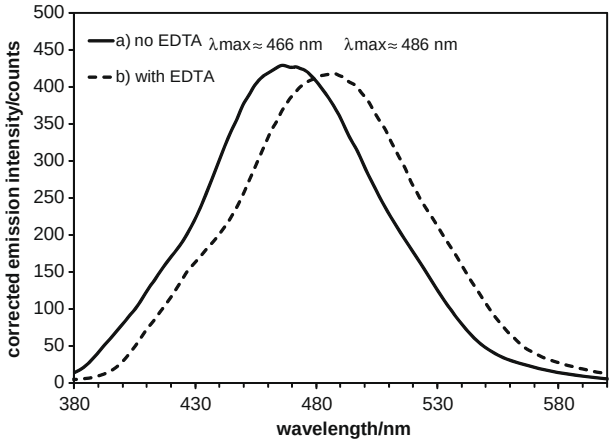


Figure 3. Fluorescence emission spectra ($\lambda_{ex} = 350$ nm) for (1:1) DMSO:H_2O solutions containing: (a) **1** (9 μM), **2** (9 μM), NaAsc (1 mM), and Cu(II) (4.76 μM); and (b) **1** (10 μM), **2** (10 μM), NaAsc (80 μM), Cu(II) (10 μM), EDTA (10 μM), and Zn(II) (7.2 μM).

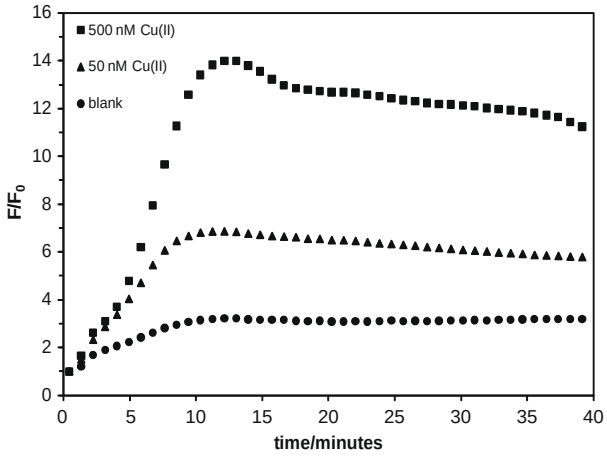


Figure 2. Relative fluorescence (F/F_0) at 470 nm versus time ($\lambda_{ex} = 350$ nm) for (1:1) DMSO:H_2O solutions containing **1** (10 μM), **2** (10 μM), and NaAsc (80 μM). The Cu(II) concentrations are • 0 nM, ▲ 50 nM, and ■ 500 nM, respectively.

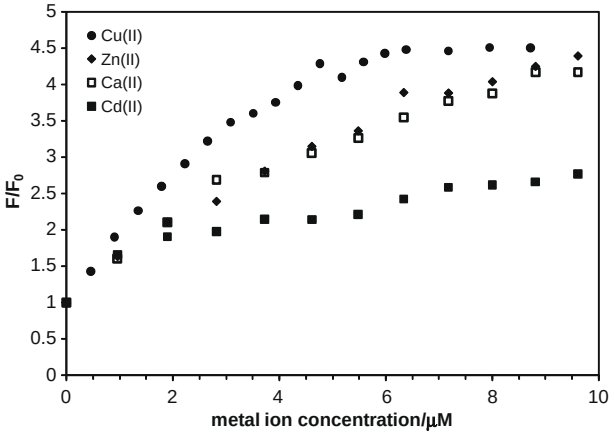


Figure 4. Relative fluorescence (F/F_0) at 486 nm of a (1:1) DMSO:H_2O solution containing **1** (10 μM), **2** (10 μM), NaAsc (80 μM), and Cu(II)–EDTA complex (10 μM) as a function of the concentration of added metal ion ($\lambda_{ex} = 350$ nm).

the maximum relative fluorescence was reached near 9 μM, which is enough for nearly complete displacement of Cu(II) from the EDTA complex. This is reflected in the similar F/F_0 values for Cu(II), Zn(II), and Ca(II).

In conclusion, the fluorogenic CuAAC reaction using 3-azido-7-hydroxycoumarin and phenylacetylene has been shown to be a sensitive method for detecting and quantifying Cu(I) and Cu(II) ions at micromolar and nanomolar concentrations. Fluorescence imaging experiments to detect intracellular zinc[28] and copper[19] ions typically range from minutes to hours, and the time needed for complete CuAAC reaction is compatible with this timescale, making fluorogenic CuAAC potentially useful. Additionally, the small size and bio-orthogonality of azide and alkyne make them suitable to intracellular studies where the probe must traverse cell membranes to detect intracellular levels of metal ions. Finally, by using EDTA as a regulatory element to form a Cu(II)–EDTA complex, the method can be extended to the detection of other biologically and environmentally important metals via allosteric control.

Acknowledgments

This work was supported by the South Carolina EPSCoR-CRP program, US NSF, the Alfred P. Sloan Foundation, the Camille Dreyfus Teacher-Scholarship, Merck AAAS USRP, and the W. M. Keck Foundation.

References and notes

1. Le Droumaguet, C.; Wang, C.; Wang, Q. *Chem. Soc. Rev*., in press, doi:10.1039/b901975h.
2. Beatty, K. E.; Xie, F.; Wang, Q.; Tirrell, D. A. *J. Am. Chem. Soc.* **2005**, *127*, 14150–14151.
3. Bruckman, M.; Kaur, G.; Lee, L. A.; Xie, F.; Sepulveda, J.; Breitenkamp, R.; Zhang, X.; Joralemon, M.; Russell, T. P.; Emrick, T.; Wang, Q. *ChemBioChem* **2008**, *9*, 519–523.
4. Meyer, Y.; Richard, J.-A.; Massonneau, M.; Renard, P.-Y.; Romieu, A. *Org. Lett.* **2008**, *10*, 1517–1520.
5. Charron, G.; Zhang, M. M.; Yount, J. S.; Wilson, J.; Raghavan, A. S.; Shamir, E.; Hang, H. C. *J. Am. Chem. Soc.* **2009**, *131*, 4967–4975.
6. (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (b) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
7. Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064.
8. (a) Prescher, J. A.; Bertozzi, C. R. *Nat. Chem. Biol.* **2005**, *1*, 13–21; (b) Wang, Q.; Chittaboina, S.; Barnhill, H. N. *Lett. Org. Chem.* **2005**, *2*, 293–301; (c) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192–3193.
9. Xie, F.; Sivakumar, K.; Zeng, Q.; Bruckman, M. A.; Hodges, B.; Wang, Q. *Tetrahedron* **2008**, *64*, 2906–2914.
10. Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603–4606.
11. Zeng, Q.; Li, T.; Cash, B.; Li, S.; Xie, F.; Wang, Q. *Chem. Commun.* **2007**, 1453–1455.
12. Beatty, K. E.; Liu, J. C.; Xie, F.; Dieterich, D. C.; Schuman, E. M.; Wang, Q.; Tirrell, D. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 7364–7367.
13. Seela, F.; Sirivolu, V. R. *Org. Biomol. Chem.* **2008**, *6*, 1674–1687.
14. O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. *Chem. Eur. J.* **2006**, *12*, 6776–6786.
15. Breed, D. R.; Thibault, R.; Xie, F.; Wang, Q.; Hawker, C. J.; Pine, D. J. *Langmuir* **2009**, *25*, 4370–4376.
16. Lemieux, G. A.; De Graffenried, C. L.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2003**, *125*, 4708–4709.
17. Pan, J.; Downing, J. A.; McHale, J. L.; Xian, M. *Mol. BioSyst.* **2009**, *5*, 918–920.
18. Cheng, T.; Xu, Y.; Zhang, S.; Zhu, W.; Qian, X.; Duan, L. *J. Am. Chem. Soc.* **2008**, *130*, 16160–16161.
19. Zeng, L.; Miller, E. W.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2006**, *128*, 10–11.
20. Xue, L.; Liu, C.; Jiang, H. *Org. Lett.* **2009**, *11*, 1655–1658.
21. Kim, S. H.; Kim, J. S.; Park, S. M.; Chang, S. K. *Org. Lett.* **2006**, *8*, 371–374.
22. Weng, Y. Q.; Yue, F.; Zhong, Y. R.; Ye, B. H. *Inorg. Chem.* **2007**, *46*, 7749–7755.
23. Varazo, K.; Xie, F.; Gulledge, D.; Wang, Q. *Tetrahedron Lett.* **2008**, *49*, 5293–5296.
24. Zhou, Z.; Fahrni, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 8862–8863.
25. Wu, Q.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 14682–14683.
26. Wu, Q.; Anslyn, E. V. *J. Mater. Chem.* **2005**, *15*, 2815–2819.
27. Zhu, L.; Lynch, V. M.; Anslyn, E. V. *Tetrahedron* **2004**, *60*, 7267–7275.
28. Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712–713.