Visual Discrimination of Homocysteine from Cysteine through Selective Fluorescent Gel Formation

Donghak Jang, Kyung-Sik Lee, and Jong-In Hong*

*Department of Chemistry, Seoul National University, Seoul 08826, Korea. *E-mail: jihong@snu.ac.kr*
Received March 21, 2017, Accepted April 7, 2017, Published online May 25, 2017

Keywords: Chemodosimetric gelator, Gelation, Homocysteine, Cysteine, 1,3-Thiazinane

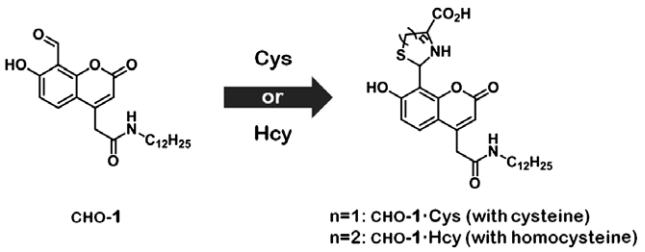
Biothiols, such as cysteine (Cys) and homocysteine (Hcy), are intriguing biologically active small molecules that are closely associated with several metabolic pathways.[1–3] Abnormal levels of Cys and Hcy induce changes in redox states, resulting in the development of various diseases. At high concentrations, products of Hcy autoxidation may damage endothelial cells by forming reactive oxygen intermediates.[4–7] The deficiency of Cys decreases the level of glutathione (GSH), which acts as a scavenger for reactive oxygen species, and results in liver damage.[8–10] Because Hcy and Cys have a wide range of effects on metabolic pathways, extensive research efforts are focused on the detection and discrimination of these two biothiols.

The majority of studies that sought to detect Hcy and Cys have failed in distinguishing these molecules because of their similar spatial arrangement and chemical reactivity.[11–18] Recently, several studies detected Cys by using chemodosimetric probes through Michael-type reaction.[19–21] In contrast to Cys, a few methods enabled the selective discrimination of Hcy. They were based on chemical reactions[22–26] and aggregation of gold nanoparticles.[27–29] It is still necessary to develop a reliable strategy for distinguishing Hcy from Cys. This reports presents a method for the selective detection of Hcy by using a chemodosimetric gelator that self-assembles into a fluorescent gel upon the addition of Hcy.

Low molecular weight gelators are known to construct superstructures through the self-assembly of components in gelation systems.[30–32] In particular, target-responsive gelators have received much attention, because they have wide application and their structural changes can be easily detected. A self-assembling process can be controlled by external stimuli, such as introduction of additives,[33,34] induction of monomer structural changes,[35–37] and environmental changes.[38,39] The smallest changes in the molecular structure of gelators may result in massive changes in the self-assembling structures. We hypothesized that Hcy could be potentially distinguished from Cys if such strong and specific changes in the self-assembling process could be created. Herein, we report a novel strategy for discriminating Hcy based on chemodosimetric gelation system.

We recently synthesized a fluorescent probe for Hcy and Cys by using the condensation reaction between aldehyde and thiol.[40] The formyl moiety in the probe was changed into five- and six-membered cyclic thiaza-adducts as a result of the reaction with Cys and Hcy, respectively. Both adducts induced similar fluorescence changes, although they showed differences in interactions with themselves and solvent molecules, which suggested that they influenced the phase transition. Based on these differences, we designed CHO-**1**, a chemodosimetric gelator that contained the formyl and *N*-dodecylacetamido moieties in the 7-hydroxycoumarin backbone (Scheme 1). The *N*-dodecylacetamido moiety in CHO-**1** adducts of Hcy and Cys initiated a self-assembling process through van der Waals and hydrogen bonding interactions. This self-assembling process was controlled by different hydrophilic head groups in the CHO-**1** adducts. Through the phase transition that resulted from the controlled self-assembling process, we were able to differentiate between Hcy and Cys.

The chemodosimetric gelator CHO-**1** was synthesized from 7-hydroxycoumarin-4-acetic acid according to a previously described method.[41] First, we examined the self-assembling phenomena with and without Hcy and Cys. CHO-**1** itself did not dissolve in water even after heating to boiling temperature and adding a polar organic cosolvent such as ethanol. However, the addition of Cys (CHO-**1**·Cys) and Hcy (CHO-**1**·Hcy) induced changes in the solubility and self-assembling behaviors. Heating a mixture of CHO-**1** and 2.0 equiv Hcy in 1.0 M HEPES buffer and ethanol (1:2, vol/vol) produced a clear solution. Interestingly, while the clear solution was allowed to cool to ambient temperature, it turned cloudy and, finally, was transformed



Scheme 1. Molecular structures of CHO-**1** and its adducts obtained as a result of condensation reaction with Hcy and Cys.

 © 2017 Korean Chemical Society, Seoul & Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

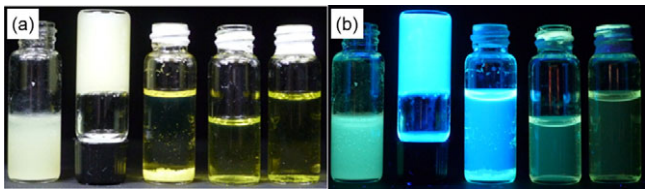


Figure 1. (a) Optical and (b) fluorescence images of the self-assembling behaviors of CHO-**1** (5 mg/mL, 12 mM) with biothiols in HEPES buffer and ethanol (1:2 vol/vol). CHO-**1**, CHO-**1**·Hcy, CHO-**1**·Cys, CHO-**1**·GSH, and CHO-**1**·Met are indicated from left to right.

into a physical gel showing fluorescence (Figure 1). The sol-to-gel transition of CHO-**1**·Hcy occurred when the concentration of CHO-**1** was reduced to 2 mg/mL (5 mM) (Figure S3 in Appendix S1, Supporting Information). As in the case with CHO-**1**·Hcy, we also observed the self-assembling behavior in the presence of Cys. However, the addition of Cys did not result in gelation, but only induced fluorescent precipitation (Figure 1), Thus, the chemodosimetric gelator (CHO-**1**) successfully discriminated Hcy from Cys through selective gelation of CHO-**1**·Hcy.

Fluorescence observed in the CHO-**1**·Hcy gel and CHO-**1**·Cys precipitate indicates that CHO-**1** formed 1,3-thiazinane and thiazolidine adducts with Hcy and Cys, respectively. Their ^{1}H NMR spectra also confirmed the formation of thiaza cyclic adducts (Figure 2). Although these biothiols had similar chemical structures, the self-assembling process of CHO-**1**·Hcy was distinct from that of CHO-**1**·Cys. Scanning electron microscopy (SEM) images of xerogels and dry precipitates were analyzed to gain a better insight into differences between self-assembling behaviors of CHO-**1**·Hcy and CHO-**1**·Cys. SEM images of CHO-**1**·Hcy and CHO-**1**·Cys revealed that both adducts self-assembled into fibrous microstructures (Figure 3(a) and (b)). It is noteworthy that the self-assembling fibers of CHO-**1**·Cys were thinner and thus, more tightly intertwined than those of CHO-**1**·Hcy, which resulted in the exclusion of solvent molecules from the networks of CHO-**1**·Cys fibers. This is why CHO-**1**·Cys precipitated. However, the self-assembling fibers of CHO-**1**·Hcy were thicker than those of CHO-**1**·Cys. In addition, CHO-**1**·Hcy fibers were intertwined, forming networks with relatively large cavities between fibers. Solvent molecules were stored within those cavities, possibly facilitating the transition of CHO-**1**·Hcy from sol to gel.

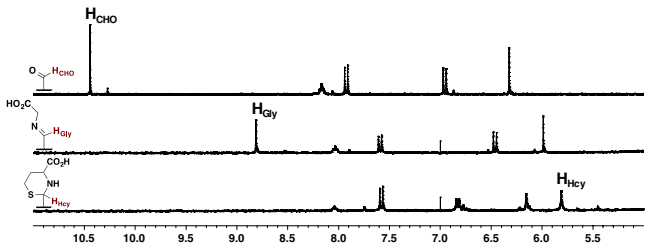


Figure 2. ^{1}H NMR spectra of (top to bottom) CHO-**1**, CHO-**1**·Gly precipitate, and CHO-**1**·Hcy xerogel in DMSO-d_6, respectively.

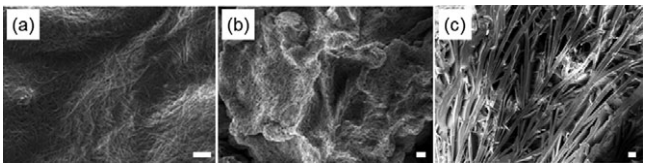


Figure 3. SEM images of (a) xerogels of CHO-**1**·Hcy (×1000, scale bar = 20 μm), (b) dried precipitates of CHO-**1**·Cys (×5000, scale bar = 2 μm), and (c) CHO-**1**·Gly (scale bar = 20 μm).

Because biothiols and amino acids could react with the formyl moiety of CHO-**1**, we examined the effect of addition of biothiols and amino acids to CHO-**1** on the morphology of the resulting adducts. A mixture of GSH or methionine (Met) and CHO-**1** was dissolved in a mixture of 1 M HEPES buffer and ethanol (1:2, vol/vol) (Figure 1(a)) to give a yellow solution and a small amount of precipitate upon heating, which were not fluorescent (Figure 1(b)). Similar results were also observed after adding other amino acids. In particular, aspartic acid (Asp), glycine (Gly), lysine (Lys), serine (Ser), and glutamine (Gln) were selected as acidic, neutral, basic, nucleophilic, and amide species, respectively. The addition of these amino acids did not result in gelation or fluorescence emission. However, precipitation occurred upon the addition of Gly, Ser, or Lys (Figure 4).

Variations in the self-assembling behavior could be possibly attributed to the differences in adduct molecular structures. The complete reaction of other biothiols and amino acids with CHO-**1** resulted in the formation of imine intermediates, which was confirmed by the absence of fluorescence (Figure 4(b)) and characteristic ^{1}H NMR spectra (Figure S4). In contrast to the self-assembling behavior of CHO-**1**·Hcy cyclic thiaza adduct, the flexible noncyclic imine adducts with other biothiols and amino acids are likely to self-assemble into well-stacked one-dimensional aggregates, and this was verified using SEM and wide-angle X-ray scattering (WAXS) analysis. SEM analysis of CHO-**1**·Gly revealed that the precipitates consisted of thick plate-like microstructures (Figure 3(c)), while the WAXS spectrum showed that CHO-**1**·Gly underwent self-assembly according to a lamellar pattern, which was different from that observed in CHO-**1**·Hcy (Figure S5). Therefore, unlike

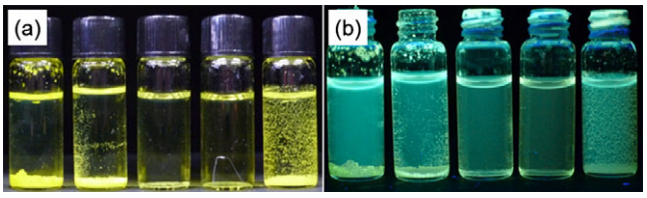


Figure 4. (a) Optical and (b) fluorescence images of the self-assembling behaviors of CHO-**1** (5 mg/mL, 12 mM) with amino acids (each 2.0 equiv to gelator) in 1 M HEPES buffer and ethanol (1:2 vol/vol). CHO-**1**·Gly, CHO-**1**·Ser, CHO-**1**·Asp, CHO-**1**·Gln, and CHO-**1**·Lys are indicated from left to right.

 © 2017 Korean Chemical Society, Seoul & Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

CHO-**1**·Hcy, the imine-containing adducts were unable to self-assemble into physical gels.

In summary, we developed CHO-**1**, a chemodosimetric gelator, which selectively responded to Hcy through the formation of a thiaza cyclic adduct followed by self-assembly into a fluorescent gel. The use of other biothiols and amino acids did not result in gelation. Selective gelation feature of CHO-**1** was able to distinguish between Hcy and Cys, the molecules, which have a difference in only one carbon. This chemodosimetric gelation system could be thus used for differentiating a target analyte from other chemically and physically similar compounds.

Experimental

7-Acetoxycoumarin-4-dodecylacetamide (Ac-1): $BF_3{\cdot}OEt_2$ (0.1 mL, catalytic amount) was added to a suspension of 7-hydroxycoumarin-4-dodceylacetamide (210 mg, 0.5 mmol)[16] in acetic anhydride (5 mL). The resulting clear solution was stirred at room temperature for 3 h. Distilled water (10 mL) was carefully added, and then, the reaction mixture turned into suspension. The organic phase was washed successively with water and brine, dried with Na_2SO_4, and concentrated *in vacuo*. The residue was purified using SiO_2 column chromatography, eluting with CH_2Cl_2 and then 1% methanol in CH_2Cl_2. Ac-**1** was further purified via recrystallization from methanol to give a white solid (210 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (3H, *J* = 6.8 Hz, t), 1.21–1.23 (18H, m), 2.32 (3H, s), 3.21 (2H, *J* = 6.4 Hz, q), 3.65 (2H, s), 5.53 (1H, bs), 6.34 (1H, s), 7.05 (1H, *J* = 2.4, 6.4 Hz, dd), 7.12 (1H, s), 7.68 (1H, *J* = 8.8 Hz, d). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 21.1, 22.7, 26.8, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 40.1, 40.9, 110.7, 116.1, 116.6, 118.5, 125.9, 149.0, 153.5, 154.4, 160.0, 166.9, 168.6. HRMS (FAB+) *m/z*: calcd. for $C_{25}H_{36}NO_5$, 430.2593; found *m/z*, 430.2597.

8-Formyl-7-hydroxycoumarin-4-dodecylacetamide (CHO-1): Ac-**1** (210 mg, 0.5 mmol) and hexamethylenetetramine (300 mg, 2.2 mmol) were dissolved in trifluoroacetic acid (10 mL). The mixture was heated at reflux with stirring for 6 h and then cooled to room temperature. All volatiles were removed *in vacuo*. Distilled water (10 mL) was added, and the resulting yellow suspension was stirred at room temperature overnight. The yellow precipitate was filtered and washed with water. The crude product was recrystallized from methanol and then washed with diethyl ether to give CHO-**1** (70 mg, 34% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (3H, *J* = 6.8 Hz, t), 1.21–1.27 (18H, m), 1.45 (2H, m), 3.22 (2H, *J* = 6.4 Hz, q), 3.62 (2H, s), 5.54 (1H, bs), 6.25 (1H, s), 6.89 (1H, *J* = 8.8 Hz, d), 7.81 (1H, *J* = 9.2 Hz, d), 10.59 (1H, s), 12.24 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1, 22.7, 26.8, 29.2, 29.3, 29.4, 29.5, 29.6, 30.9, 31.9, 40.1, 40.9, 108.7, 110.9, 113.5, 114.7, 158.8, 165.7, 166.6, 193.2. HRMS (FAB+) *m/z*: calcd. for $C_{24}H_{34}NO_5$, 416.2437, found *m/z*, 416.2433.

Gelation Method. To a suspension of CHO-**1** in ethanol, 2.0 equiv biothiols or amino acids in 1 M HEPES buffer (pH 7.4) was added, while maintaining the 2:1 volume ratio of ethanol and HEPES buffer. The mixture was heated until it turned into a clear solution and then cooled to room temperature. It turned into gel or precipitate in a several minutes, depending on the nature of the added substance.

Acknowledgments. This work was supported by the NRF grant (No. 2015R1A2A1A15055347) funded by the MSIP.

Supporting Information. Additional supporting information is available in the online version of this article.

Synthesis of CHO-1, its self-assembling behaviors with Cys and Hcy, NMR spectra of mixtures of CHO-1 and various amino acids, and wide angle X-ray scattering analysis.

References

1. Z. A. Wood, E. Schröder, J. R. Harris, L. B. Poole, *Trends Biochem. Sci.* **2003**, *28*, 32.
2. J. B. Schulz, J. Lindenau, J. Sevfried, J. Dichgans, *Eur. J. Biochem.* **2000**, *267*, 4904.
3. R. O. Ball, G. Courtney-Martin, P. B. Pencharz, *J. Nutr.* **2006**, *136*, 1682S.
4. B. Beije, E. Arrhenius, *Chem.-Biol. Interact.* **1978**, *21*, 167.
5. J. Hidalgo, J. S. Garvey, A. Armario, *J. Pharmacol. Exp. Ther.* **1990**, *225*, 554.
6. S. Shahrokhians, *Anal. Chem.* **2001**, *73*, 5972.
7. M. H. Stipanuk, J. E. Dominy Jr.. , J.-I. Lee, R. M. Coloso, *J. Nutr.* **2006**, *136*, 16525.
8. K. S. McCully, *Ann. Clin. Lab. Sci.* **2009**, *39*, 219.
9. C. Zhang, Y. Cai, M. T. Adachi, S. Oshiro, T. Aso, R. J. Kanufman, S. Kitajima, *J. Biol. Chem.* **2001**, *276*, 35867.
10. J. A. McMahon, T. J. Green, C. M. Skeaff, R. G. Knight, J. I. Mann, S. M. N. Williams, *N. Eng. J. Med.* **2006**, *354*, 2746.
11. O. Rusin, N. N. St. Luce, R. A. Agbaria, J. O. Escobedo, S. Jiang, I. M. Warner, F. B. Dawan, K. Lian, R. M. Strongin, *J. Am. Chem. Soc.* **2004**, *126*, 438.
12. X. Chen, Y. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.* **2010**, *39*, 2120.
13. X. Yang, Y. Guo, R. M. Strongin, *Angew. Chem. Int. Ed.* **2011**, *50*, 10690.
14. S.-Y. Lim, D.-H. Yoon, D. Y. Ha, J. Ahn, D. I. Kim, H. Kwon, H.-J. Ha, H.-J. Kim, *Sens. Actuat. B Chem.* **2013**, *188*, 111.
15. H. S. Jung, T. Pradhan, J. H. Han, K. J. Heo, J. H. Lee, C. Kang, J. S. Kim, *Biomaterials* **2012**, *33*, 8495.
16. D. P. Murale, H. Kim, W. S. Choi, D. G. Churchill, *Org. Lett.* **2013**, *15*, 3630.
17. Y.-K. Yang, S. Shim, J. Tae, *Chem. Commun.* **2010**, *46*, 7766.
18. D. H. Ma, D. Kim, E. Seo, S.-J. Lee, K. H. Ahn, *Analyst* **2015**, *140*, 422.
19. L. Yuan, W. Lin, Y. Yang, *Chem. Commun.* **2011**, *47*, 6275.
20. X. Zhou, X. Jin, G. Sun, X. Wu, *Chem. Eur. J.* **2013**, *19*, 7817.

 © 2017 Korean Chemical Society, Seoul & Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

21. J. Wang, B. Li, W. Zhao, X. Zhang, X. Luo, M. E. Corkins, S. L. Cole, C. Wang, Y. Xiao, X. Bi, Y. Pang, C. A. McElory, A. J. Bird, Y. Dong, *ACS Sens.* **2016**, *1*, 882.
22. F. Wang, Z. Guo, X. Li, C. Zhao, *Chem. Eur. J* **2014**, *20*, 11471.
23. Y.-W. Wang, S.-B. Liu, W.-J. Ling, Y. Peng, *Chem. Commun.* **2016**, *52*, 827.
24. F. Guo, M. Tian, F. Miao, W. Zhang, G. Song, Y. Liu, X. Yu, J. Z. Sun, W.-Y. Wong, *Org. Biomol. Chem.* **2013**, *11*, 7721.
25. Y. Yue, F. Huo, X. Li, Y. Wen, T. Yi, J. Salamanca, J. O. Escobedo, R. M. Strongin, C. Yin, *Org. Lett.* **2017**, *19*, 82.
26. W. Wang, J. O. Escobedo, C. M. Lawrence, R. M. Strongin, *J. Am. Chem. Soc.* **2004**, *126*, 3400.
27. C. Lu, Y. Zu, *Chem. Commun.* **2007**, *43*, 3871.
28. J.-H. Lin, C.-W. Chang, W.-L. Tseng, *Analyst* **2010**, *135*, 104.
29. K.-S. Lee, T.-K. Kim, J.-H. Lee, J.-I. Hong, *Chem. Commun.* **2008**, *44*, 6173.
30. L. A. Estroff, A. D. Hamilton, *Chem. Rev.* **2004**, *104*, 1201.
31. N. M. Sangeetha, U. Maitra, *Chem. Soc. Rev.* **2005**, *34*, 821.
32. D. Jang, H. Y. Lee, M. Park, S. R. Nam, J.-I. Hong, *Chem. Eur. J.* **2010**, *16*, 4836.
33. M.-O. M. Piepenbrock, G. O. Lloyd, N. Clarke, J. W. Steed, *Chem. Rev.* **2010**, *110*, 1960.
34. H. Maeda, *Chem. Eur. J.* **2008**, *14*, 11274.
35. H. Y. Lee, S. R. Nam, J.-I. Hong, *J. Am. Chem. Soc.* **2007**, *129*, 1040.
36. P. Duan, H. Cao, M. Liu, *Soft Matter* **2014**, *10*, 5428.
37. C. Wang, Q. Chen, F. Sun, D. Zhang, G. Zhang, Y. Huang, R. Zhao, D. Zhu, *J. Am. Chem. Soc.* **2010**, *132*, 3092.
38. U. K. Das, S. Banerjee, P. Dastidar, *Chem. Asian J.* **2014**, *9*, 2475.
39. S. Bai, S. Debnath, N. Javid, P. W. J. M. Frederix, S. Fleming, C. Pappas, R. V. Uljn, *Langmuir* **2014**, *30*, 7576.
40. K.-S. Lee, T.-K. Kim, J. H. Lee, J.-I. Hong, *Chem. Commun.* **2008**, 6173.
41. M. Park, D. Jang, S. Y. Kim, J.-I. Hong, *New J. Chem.* **2012**, *36*, 11451.

 © 2017 Korean Chemical Society, Seoul & Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim