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Molecular Probe for Selective Detection of Thiols in Water of Neutral pH

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Abstract—The ternary complex, $[Zn(cyclen)(lumazine)]^+$ that can be readily obtained by mixing cyclen, zinc perchlorate, and lumzine in water in a 1:1:1 molar ratio serves as a molecular probe that detects selectively thiols in an aqueous solution of neutral pH. Thiols successfully displace the lumazine in the molecular probe, which is accompanied by a decrease of the fluorescence. The molecular probe is useful for identification of cysteine among essential amino acids and detection of glutathione in aqueous solution of neutral pH.

Thiols are widely distributed in the biological system and play important roles. Thiol group in cysteine residues of proteins is involved in determining three dimensional structures of the biomacromolecules through disulfide formation.[1] The thiol group in glutathione (L-γ-glutamyl-L-cysteinylglycine), a physiologically important tripeptide is responsible for its important roles in diverse biological process.[2] Chemically, thiol group differs uniquely from other functional groups by being readily oxidized to form disulfides especially in the presence of a trace amount of metal ions such as Fe^{2+}, Co^{2+}, and Mn^{2+}. Besides, thiol group readily undergoes electrophilic reactions by virtue of its strong nucleophilic property.

Molecular probes that can detect thiols have received much attention in recent years and numerous thiol probes have been reported. Most of these sensors take advantage of the strong tendency of thiols to form disulfides under oxidation conditions, and numerous electrochemical thiol detection devices that make use of various redox electrodes have been reported.[3–5] Other thiol probes utilize the nucleophilic property of thiol group to form a covalent bond upon reaction with molecules bearing an electrophilic moiety such as iodoacetamdes and maleimides.[6] In these probes, the irreversible bond formation is coupled to a fluorescence signaling unit.[6] We wish to report herein a structurally simple and easy to construct molecular probe that detects thiols with excellent selectivity in an aqueous solution of neutral pH. The present probe takes advantage of the unique property of thiol group to form a coordinative bond to zinc ion in an aqueous medium.

Chemosensors are, in general, composed of a receptor and a signaling unit that is being appended covalently to the receptor by a linker of alkyl chain.[7] The binding of analyte to receptor causes environmental perturbation of the receptor molecule, which is conveyed through the linker to the signaling unit, manifesting a signal of various forms. In the present sensor, the receptor is noncovalently coupled to a signaling unit and the target analyte is allowed to compete for the receptor with the signaling unit.[8] The successful competition of the analyte would displace the signaling unit from the receptor, and this displacement is communicated by the change of the fluorescence intensity. We chose $[Zn(cyclen)]^{2+}$ as the receptor.[9] The zinc ion in the receptor complex is highly acidic and is known to interact with lumazine (signaling unit) in an aqueous solution of pH 7.0 (HEPES = 10 mM) to form $[Zn(cyclen)(lumazine)]^+$, whereby the fluorescence of lumazine at 467 nm increases by 2.4-fold when excited at 355 nm.[10] It is well known that a thiol group has a strong affinity towards

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the zinc ion of enhanced Lewis acidity, and this property has been utilized for the design of inhibitors for zinc-containing proteolytic enzymes.[11] We have envisioned that thiol would compete successfully for $[Zn(cyclen)]^{2+}$ with the lumazine to form a coordinative bond with the receptor zinc ion, and thus it replaces the receptor-bound lumazine upon exposing to $[Zn(cyclen)(lumazine)]^{+}$, whereby attenuation of the fluorescence at 467 nm occurs (Fig. 1).

As expected, additions of structurally varied thiols to the neutral degassed aqueous solution of $[Zn(cyclen)(lumazine)]^{+}$ that was prepared by dissolving cyclen, zinc perchlorate, and lumazine in a 1:1:1 molar ratio resulted in a decrease of the fluorescence intensity, and such decrease was found to be extremely selective toward thiols (Fig. 2). No significant decrease in fluorescence intensity was observed upon additions of compounds having other functional groups than thiol such as amino acids (alanine, histidine, proline, methionine and serine) and *S*-benzylcysteine. Cysteine brought about a larger decrease of fluorescence than simple thiols such as glutathione, cysteine methyl ester, and *N*-acethylcysteine methyl ester, suggesting that the carboxylate of cysteine undergoes additional binding interactions with the receptor possibly forming a hydrogen bond to the amino nitrogen of the cyclen. Although the addition of aspartic or glutamic acid also caused a decrease of the fluorescence intensity, the extent of the decrease is much less compared with that caused by a thiol compound, corresponding to only 12% of that caused by cysteine. The probe showed negative responses to anions found widely in the biological systems such as phosphate, carbonate, acetate, and chloride as well as cations such as sodium, potassium, and calcium ions (data not shown). The present probe may thus be useful for identification of cysteine among essential amino acids.

Glutathione is the most abundant thiol in the biological system, amounting to the level of μM in human plasma and about 500 μM in red blood cells,[12] and plays major roles in cellular defenses against oxidative and nitrosative stresses as well as against reactive electrophiles.[13] Accordingly, we have evaluate $[Zn(cyclen)(lumazine)]^{+}$ as a probe for glutathione.[14] The pH dependence of fluorescence change of $[Zn(cyclen)(lumazine)]^{+}$ caused by glutathione was studied to find that the maximum fluorescence change occurs at pH 6.5–7.0 (Fig. 3). We have then examined the changes of fluorescence caused by incremental additions of glutathione. As can be seen in Figure 4, the fluorescence decreases linearly as glutathione is added until its concentration reaches about 2 molar equivalence of the probe. The plateau shown

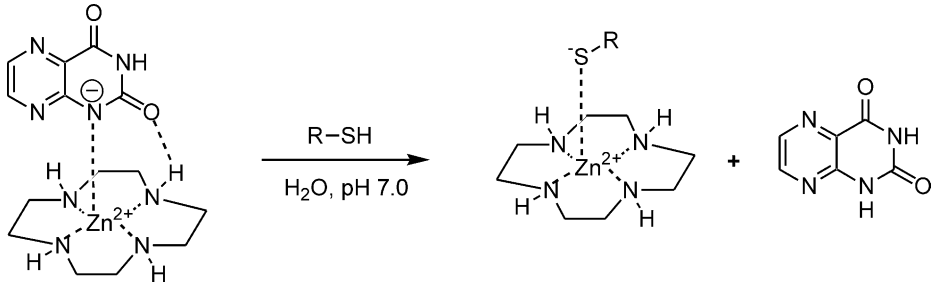

Figure 1. A schematic representation for the rationale used for designing $[Zn(cyclen)(lumazine)]^{+}$ as a probe for thiols.

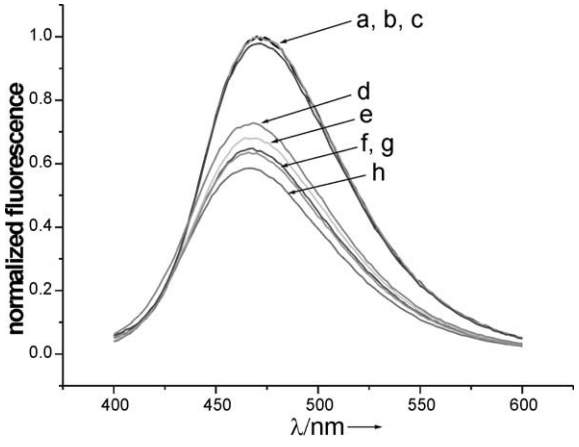

Figure 2. Fluorescence emission spectra obtained by the addition of various thiols (final concentration: 200 μM) to a pH 7.0 (HEPES 10 mM, KNO_3 100 mM) buffer solution of the sensing ensemble (final concentration: 100 μM): (a) no analyte, (b) Met, (c) GSSG, (d) *N*-Ac-Cys-OMe, (e) Cys-OMe, (f) *N*-Ac-Cys, (g) GSH, and (h) Cys. Excitation was made at 355 nm.

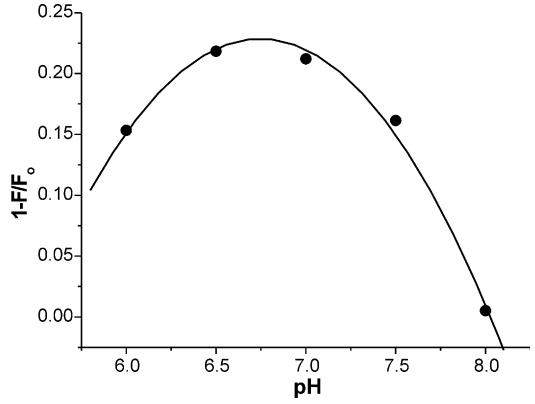

Figure 3. Plot of $1-F/F_0$ as a function of the pH value for the sensor (100 μM). F and F_0 represent fluorescence intensity at λ_{max} at the specific pH in the presence and absence of GSH (100 μM), respectively. Excitation was made at 355 nm.

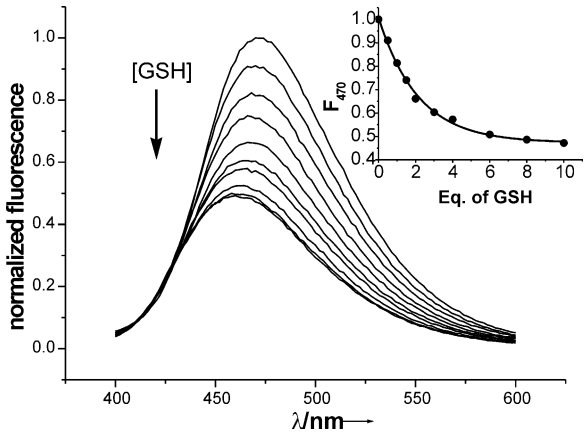

Figure 4. Fluorescence emission spectra obtained by the addition of glutathione (final concentration: 50, 100, 200, 300, 400, 600, 800, and 1000 μM) to the pH 7.0 (HEPES 10 mM, KNO_3 100 mM) buffer solution containing $[Zn(cyclen)(lumazine)]^+$ (final concentration: 100 μM). Excitation was made at 355 nm. Inset: Plot of normalized fluorescence (F_{470}) against concentration of glutathione expressed by number of equivalency to the concentration of the ensemble.

when the concentration of the analyte is higher than about 5 molar equivalence of the concentration of the probe indicates that more than one equivalent amount of glutathione is required to displace the receptor-bound lumazine completely.

The thermodynamic parameters and association constant for the binding of glutathione to the receptor were determined by the isothermal titration calorimetry (ITC).[15] Figure 5 depicts the ITC plot for the titration of the receptor with glutathione at 30 °C in a buffered (HEPES 10 μM) aqueous medium of pH 7.0, from which the molar enthalpy (ΔH^O) for the binding interactions was calculated to be −6.76 kcal mol^{-1}. The entropy change (ΔS^O) of −2.57 eu was obtained from the Gibbs–Helmholtz equation and the K_{ass} value[16] of $(2.06\pm0.06)\times10^4$ M^{-1} was calculated using the equation, $\Delta G^O = -RT \ln K_{ass}$, in which R and T represent the gas constant and absolute temperature, respectively. The stoichiometry parameter of 0.8 obtained from the curve fitting (Fig. 5B) suggests that the binding of glutathione to the receptor occurs with a 1:1 stoichiometry. These thermodynamic parameters suggest that the binding of glutathione to the receptor is essentially enthalpy driven, originating most likely from the strong binding interactions between the zinc ion in the receptor and the deprotonated thiol in glutathione. The K_{ass} value for the binding of glutathione to the receptor is only slightly larger than the K_{ass} value of $(0.66\pm0.013)\times10^4$ M^{-1} reported for the binding of lumazine to the receptor,[10] which is compatible with the observation that an excess amounts of glutathione is needed to completely displace the indicator from the ensemble (Fig. 4).

In conclusion, we have demonstrated that $[Zn(cyclen)(lumazine)]^+$ serves as an efficient molecular probe useful for real-time detection of thiols including biologically important tripeptide, glutathione in the aqueous medium of physiological pH. The probe is readily prepared by dissolving commercially available cyclen, zinc perchlorate, and lumazine in buffered water of pH 7.0 in a 1:1:1 molar ratio.

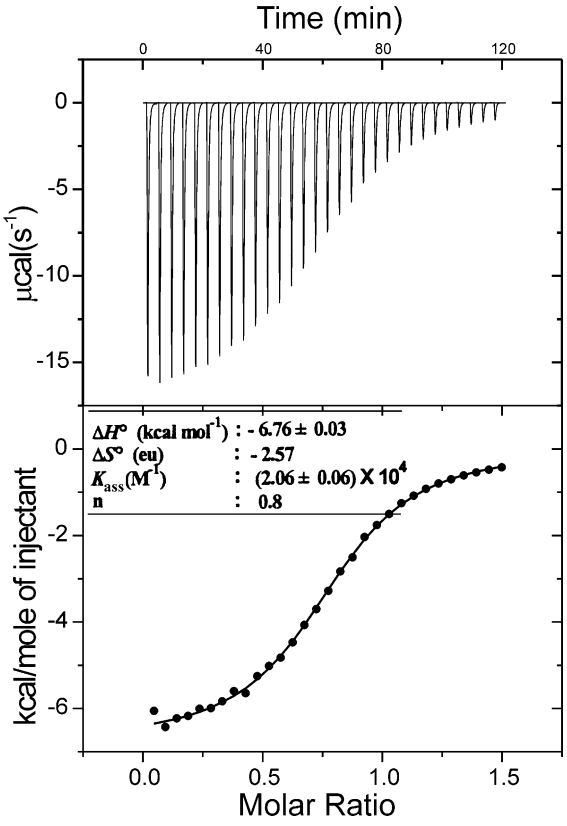

Figure 5. ITC plot for the titration of $[Zn(cyclen)]^{2+}$ with glutathione in an aqueous buffer pH 7.0 at 30 °C. An aqueous solution (1.5 mL, pH 7.0, HEPES 10 mM, KNO_3 100 mM) of $[Zn(cyclen)]^{2+}$ (1.0 mM) was added to the calorimeter cell. To this solution was injected a 7 μL portion of the aqueous glutathione solution (10 mM) 30 times. The mixture was continuously stirred and was kept at an operating temperature of 30 °C. The data were analyzed and fitted using the software Origin. Inset: Thermodynamic parameters (ΔH^O, ΔG^O and ΔS^O), association constant (K_{ass}), and stoichiometry (n) for the binding of glutathione to $[Zn(cyclen)]^{2+}$ in aqueous solution of pH 7.0 (HEPES).

References and Notes

1. (a) Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; Freeman & Co: New York, 1984; pp 2–4. (b) Zubay, G. L., Parson, W. W., Vance, D. E., *Principles in Biochemistry*, p 179.
2. (a) Black, M. *Ann. Rev. Med.* **1984**, *35*, 577. (b) Curello, S.; Ceconi, C.; Cargnoni, A.; Cornacchiari, A.; Ferrari, R.; Albertini, A. *Clin. Chem.* **1987**, *33*, 1448.
3. (a) Spätaru, N.; Sarada, B. V.; Popa, E.; Tryk, D. A.; Fujishima, A. *Anal. Chem.* **2001**, *73*, 514. (b) Chen, X.; Xia, B.; He, P. *J. Electroanal. Chem.* **1990**, *281*, 185. (c) Fawcett, W. R.; Fedurco, M.; Kovacova, Z. *J. Electroanal. Chem.* **1994**, *368*, 265. (d) Zagal, J. H.; Aguirre, M. J.; Parodi, C. G. *J. Electroanal. Chem.* **1994**, *374*, 215. (e) Li, H.; Li, T.; Wang, E. *Talanta* **1995**, *42*, 885. (f) Halbert, M. K.; Baldwin, R. P. *Anal. Chem.* **1985**, *57*, 591. (g) Chen, S. M. *Electrochim. Acta* **1997**, *42*, 1663. (h) Perez, E. F.; Kubota, L. T.; Tanaka, A. A.; De Oliveria Neto, G. *Electrochim. Acta* **1998**, *43*, 1665. (i) Inoue, T.; Kirchhoff, J. R. *Anal. Chem.* **2000**, *72*, 5755.

4. Zen, J.-M.; Kumar, A. S.; Chen, J.-C. *Anal. Chem.* **2001**, *73*, 1169.
5. (a) Shahrokhian, S. *Anal. Chem.* **2001**, *73*, 5972. (b) Sugawara, K.; Tanake, S.; Toga, M. *J. Electroanal. Chem.* **1991**, *316*, 305. (c) Mafatle, T. J.; Nyokong, T. *J. Electroanal. Chem.* **1996**, *408*, 213.
6. (a) Haugland, R. P., *Molecular Probes. Handbook of Fluorescent Probes and Research Chemicals*, 6th ed.; Molecular Probes, Eugene, OR, pp 47–62. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T. *Tetrahedron Lett.* **1998**, *39*, 5077.
7. de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515.
8. (a) This design protocol has bee exploited by Anslyn and coworkers: Wishur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963. (b) Snowden, T. S.; Anslyn, E. V. *Curr. Opin. Chem. Biol.* **1999**, *3*, 740.
9. (a) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805. (b) Kimura, E.; Koike, T. *Comments Inorg. Chem.* **1991**, *11*, 285.
10. Han, M. S.; Kim, D. H. *Supramol. Chem.* **2003**, *15*, 59.
11. (a) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441. (b) Chong, C. R.; Auld, D. S. *Biochemistry* **2000**, *39*, 7580. (c) Kim, D. H.; Guinosso, C. J.; Buzby, G. C.; Herbst, D. R.; McCaully, R. J. *J. Med. Chem.* **1983**, *26*, 394.
12. Jones, D. P.; Carlson, J. L.; Samiec, P. S.; Sternberg, P., Jr.; Mody, V. C., Jr.; Reed, R. L.; Brown, L. A. S. *Clinica Chim. Acta* **1998**, *275*, 175.
13. (a) Sies, H. *Free Radical Biol. Med.* **1999**, *27*, 916. (b) Griffith, O. W. *Free Radical Biol. Med.* **1999**, *27*, 922.
14. Numerous assay methods for glutathione in serum have been reported.[12] In these methods, glutathione is analyzed by HPLC after being converted into fluometrically detectable derivatives by treating the sample with a chromogenic agent such as dansyl chloride.
15. Jelesarov, I.; Bosshard, H. R. *J. Mol. Recog.* **1999**, *12*, 3.
16. We have also determined the K_{ass} value for the binding of glutathione to $[Zn(cyclen)]^{2+}$ by a non-linear regression analysis of the titration curve in Figure 4 to obtain 1.6×10^4 M^{-1}.