Squaraines as Fluoro−Chromogenic Probes for Thiol-Containing Compounds and Their Application to the Detection of Biorelevant Thiols

Jose V. Ros-Lis,[†] Beatriz García,[†] Diego Jiménez,[†] Ramón Martínez-Máñez,*,[†] Félix Sancenón,[†] Juan Soto,[†] Fernando Gonzalvo,[‡] and M. Carmen Valldecabres[‡]

GDDS, Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, E-46071 Valencia, Spain, and Hospital de la Ribera, Ctra de Corbera, Km 1, E-46600 Valencia, Spain

Received December 29, 2003; E-mail: rmaez@qim.upv.es

Amino acids are very well-known compounds with an angular importance in biological systems as they join together to yield proteins, enzymes, structural elements, and many other molecules of biological activity. This role of building blocks in living systems, along with the discovery that a number of disorders are associated with changes in concentration of particular amino acids, has resulted in a rising interest in their detection in fields such as chemistry, biochemistry, and clinical chemistry.[1]

Natural α-amino acids show similar properties due to the special arrangement of their carboxyl and amino groups. Therefore, chemosensors relying on the coordination with these common moieties are not as selective as wanted although additional coordination with other groups in the amino acid's side chains has sometimes resulted in some preferential coordination. This is the case of two fluorogenic chemosensors for α-amino acids, one containing a Zn^{2+} complex as binding site for carboxylates[2a] (preferential sensing of amino acids bearing aromatic residues due to π-staking interaction with an anthracene group) and the other a heteroditopic system containing an aza-crown ether for ammonium binding and guanidinium units for carboxylate coordination[2b] (preferential response with lysine and glycine). Both contain anthracene as signaling unit and follow the general "binding site−signaling unit" approach. On the other hand, a terpyridine−Zn^{2+} complex also bearing guanidinium groups[2c] has been described that shows certain selectivity for aspartate via metal−ligand coordination and hydrogen bonding involving the side-chain carboxylate and guanidinium groups. This case follows a "displacement" approach in which color change was observed as the aspartate binding induces the displacement of the pyrocatechol violet dye previously bound to the receptor. As an attractive alternative to these preferential coordination systems, a higher selective amino acid signaling has been achieved when the interaction between the receptor and the amino acid does not rely primarily on the coordination of the carboxylate−ammonium groups but rather on the interaction with the side-chain residues of certain amino acids. Following this approximation, three recent examples have been described. The first one follows a "displacement" approach and is based on the interaction of the imidazolate residue of the histidine with a dimetallic Cu^{2+} complex of a bisdien macrocycle.[2d] The other two are so-called "chemodosimeters". One displays a highly selective fluorescence signal due to coordination of cysteine and cysteine derivatives (such as methionine, homocysteine, and glutathione) to a Pt(II) center, in a Ru(II)−Pt(II) trinuclear complex[2e] and the liberation of the highly fluorescent *cis*-[Ru(phen)-(CN)$_2$]. Another recent example displays visual detection of cysteine and homocysteine via thiazolidine formation with a xanthene dye.[2f] We are interested in further exploring the high potential of the "chemodosimeter" approach toward thiol-selective recognition and

Scheme 1. Molecular Structure of the Squaraine Derivatives L[1] and L[2]
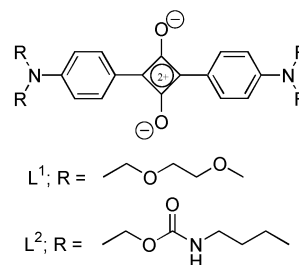
its potential application to the detection of biorelevant thiols. Here the target concept is specific reactivity instead of selective coordination.[3] Related with that "chemodosimeter" approach several thiol-reactive probes are also known and used for the determination of thiol-containing compounds. These are common alkylating agents such as iodoacetamides, maleimides, benzylic halides, etc. However, these and other reported thiol reagents show certain limitations, typically comprising reactivity with other nucleophiles such as amines including amino acids with amine residues (histidine or tyrosine), instability in light, hydrolysis of the linkage to the fluorophore, etc. Additionally, many of these reagents show signals near the UV zone, and relatively few (usually rather complex and high in cost) are designed to display transduction near the IR regions of the spectra.

We have focused our attention to water-soluble squaraine derivatives such as the compounds shown in Scheme 1, L[1] and L[2].

Squaraines are electrophiles because of their formally electron-deficient central four-membered ring. For instance, they have been proved to react with the nucleophilic anion cyanide.[4] Another interesting aspect with relation to squaraines is that they show a very intense band at long wavelengths and are usually highly fluorescent even in aqueous environments. Thus, both L[1] and L[2] show a band at ca. 640 nm (ϵ ca. 1×10^5 mol cm^{-1}) and have a quantum yield in acetonitrile/water, 20:80 v/v, of ca. 0.1.

The reactivity of L[1] and L[2] was studied with amino acids. Besides the presence of the amino group, natural α-amino acids also contain in the side chain some other potentially reactive nucleophilic groups such as alcohols (serine, threonine), amines (lysine, histidine), and thiols (cysteine). In a typical assay acetonitrile/water, 20:80 v/v, solutions of L[1] ([L[1]] = 1.21×10^{-5} mol dm^{-3}) were buffered at pH 6 (MES 0.01 mol dm^{-3}), and 10 equiv of different natural α-amino acids were added. The results are displayed in Figure 1 that shows a remarkable bleaching in the presence of cysteine. A concomitant complete fluorescence quenching was also observed. A very similar decoloration and fluorescence quenching was found for L[2].

These chromo- and fluorogenic effects are most likely due to the thiol residue of the cysteine amino acid. Additional studies (see
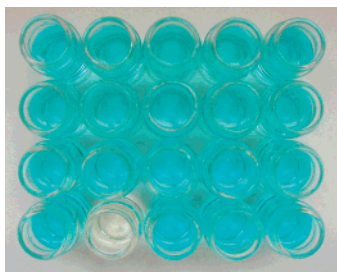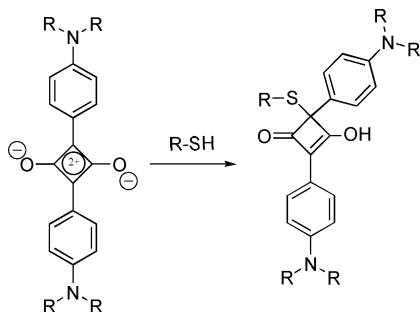
Figure 1. Photograph showing the color change in acetonitrile/water, 20:80 v/v, at pH 6 (MES 0.01 mol dm^{-3}) of the squaraine L^1 ([L^1] = 1.21 × 10^{-5} mol dm^{-3}) in the presence of 10 equiv of certain amino acids. From left to right and top to bottom: no amino acid, phenylalanine, threonine, arginine, histidine, asparagine, leucine, alanine, proline, valine, glycine, lysine, glutamine, methionine, isoleucin, serine, cysteine, tryptophan, glutamic acid, and aspartic acid.

Scheme 2. Proposed Reaction between Squaraine Derivatives and Thiol-Containing Compounds

Table 1. Determination of Total Aminothiols in Human Plasma Using L^1

sample	determined total aminothiols (mol dm^{-1} × 10^{-6})	added cysteine (mol dm^{-1} × 10^{-6})[a]	measured (mol dm^{-1} × 10^{-6})	recovery (%)
1	306	196	494	98.4
		390	710	102.0
2	372	196	535	94.2
		390	730	95.8

[a] Known amounts of cysteine added to the plasma.

Supporting Information) showed that L^1 and L^2 gave decoloration and fluorescence quenching in the presence of other thiol-containing compounds, whereas alcohols, phenols, and amines (primary, secondary, and tertiary) or sulfides produced no color changes. At this neutral pH even known nucleophiles such as cyanide gave no color variation. The bleaching process was tentatively assigned to the reaction shown in Scheme 2 (see Supporting Information for more details).

Additional studies were carried out to demonstrate the use of L^1 or L^2 as chromo−fluorogenic chemodosimeters. They could surely be used for the postcolumn HPLC detection of thiol-containing compounds. However, we took a further step and studied their potential applicability for the direct determination of biorelevant thiols (such as cysteine and cysteine derivatives, see below) in a complex matrix such as human blood.

In plasma, cysteine can be found free or linked to other amino acids, for instance, forming cysteine-glycine and glutathione. These together with homocysteine are known as low-molecular mass aminothiols. In blood, they are in a large percentage bound to proteins, but after reduction they are found free in the plasma. Thus, free low-molecular weight aminothiols in human plasma include cysteine as the main component (ca. 83% of the total aminothiols) and the cysteine derivatives, glutathione (ca. 3%), cysteine-glycine (ca. 11%), and homocysteine (ca. 3%).[5] L^1 and L^2 were found to react with these aminothiols but not with other sulfur-containing compounds such as sulfides or disulfides. Recently, the ability of cysteine and cysteine derivatives to act as biomarkers has begun to be explored, and their determination in biological fluids has gained importance in clinical chemistry. For instance, the determination of aminothiols is important for the diagnosis of certain diseases such as vascular disorders, arteriosclerosis, etc. Their determination is usually carried out using rather sophisticated and time-consuming chromatographic techniques.[6] In this respect, the

development of straightforward, low-cost, and undemanding probes for the determination of biomarkers such as total aminothiols in blood might be of general interest. Total cysteine and cysteine derivatives in human plasma have been determined using L^1 and a colorimetric addition standard method with fine results (see Table 1 and Supporting Information).

In summary, L^1 and L^2 are specific fluoro−chromo chemodosimeters for thiol-containing compounds in aqueous environments. The squaraine derivatives are quite simple, easy to synthesize, and show favorable optical properties with absorption and emission not far from the IR region. The squaraine derivatives have been successfully applied to the determination of low-molecular mass aminothiols in a complex multicomponent mixture such as human plasma. The design principle utters the high potential applicability of the chemodosimeter approach in the search for new or improved chromogenic selective or specific probes for target biorelevant molecules.

Supporting Information Available: Synthetic procedures, reactivity studies of L^1 and L^2, and its use in the determination of total aminothiols in human plasma. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Sulfur Amino Acids: Biochemical and Clinical Aspects*; Proceedings of an International Symposium and the 5th Annual Meeting of the Japanese Research Society on Sulfur Amino Acids (JRSSA), August 7−10, 1982, Tokyo; Kuriyama, K, Huxtable, R. J., Iwata, H., Eds.; A. R. Liss: New York, 1985.

(2) (a) Fabbrizzi, L.; Licchelli, M.; Perotti, A.; Poggi, A.; Rabaioli, G.; Sacchi, D.; Taglietti, A. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2108−2113. (b) Sasaki, S.; Hashizume, A.; Citterio, D.; Fujii, E.; Suzuki, K. *Tetrahedron. Lett.* **2002**, *43*, 7243−7245. (c) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am Chem. Soc.* **2001**, *123*, 11296−11297. (d) Hortalá, M. A.; Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *J. Am. Chem. Soc.* **2003**, *125*, 20−21. (e) Chow, C.; Chiu, B. K. W.; Lam, M. H. W.; Wong, W. *J. Am. Chem. Soc.* **2003**, *125*, 7802−7803. (f) Rusin, O.; St. Luce, N. N.; Agbaria, R. A.; Escobedo, J. O.; Jiang, S.; Warner, I. M.; Dawan, F. B.; Lian, K.; Strongin, R. M. *J. Am. Chem. Soc.* **2004**, *126*, 438−439.

(3) (a) Jiménez, D.; Martínez Máñez, R.; Sancenon, F.; Ros-Lis, J. V.; Benito, A.; Soto, J. *J. Am. Chem. Soc.* **2003**, *125*, 9000−9001. (b) Sancenón, F.; Descalzo, A. B.; Martínez-Máñez, R.; Miranda M. A.; Soto, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2640−2643. (c) Descalzo, A. B.; Jiménez, D.; El Haskouri, J.; Beltrán, D.; Amorós, P.; Marcos, M. D.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2002**, 562−563. (d) Mohr, G. J. *Chem. Commun.* **2002**, 2646−2647.

(4) Ros-Lis, J. V.; Martínez-Máñez, R.; Soto, J. *Chem. Commun.* **2002**, 2248−2249.

(5) Melnyk, S.; Pogribna, M.; Pogribny, I.; Hine, R. J.; James, S. J. *J. Nutr. Biochem.* **1999**, *10*, 490−497.

(6) Bald, E.; Kaniowska, E.; Chwatko, G.; Glowacki, R. *Talanta* **2000**, *50*, 1233−1243.

JA031987I