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Detection of Amines and Unprotected Amino Acids in Aqueous Conditions by Formation of Highly Fluorescent Iminium Ions

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Fluorescent probes have become a common means of investigating cellular events in real time via fluorescence microscopy. For such applications, chemical sensors have proven to be invaluable, particularly for tracking certain metal ions.[1] We have been interested in the development of practically useful chemical sensors for metal ions and small organic compounds. We have reported progress toward this goal using a structurally novel pinwheel receptor motif which operates by cooperative interactions.[2] As a potential sensor substrate, amino acids are an attractive target, especially the excitatory amino acids glutamate and aspartate.[3] To adapt our pinwheel receptors for recognition of amino acids, a good recognition element for the amine portion of the amino acid was required. Aqueous phase recognition of amines by groups such as crown ethers rely on the charged nature of the protonated form.[4] Given that amino acids are zwitterionic and overall neutral, these functionalities are less useful for recognition of the amino group of α-amino acids. Furthermore, ionic interactions are particularly weakened in the high salt conditions necessary for cellular work.[5] Thus, metal chelation has been employed to recognize unprotected amino acids in aqueous conditions.[6] Particularly noteworthy is Anslyn's aspartate sensor which uses dye displacement to produce a chromophoric response.[7]

Taking a cue from the success of boronic acids in "binding" diols via the formation of covalent boronate esters,[8] we chose to explore aldehyde containing chromophores to detect amines and amino acids via the reversible formation of imines.[9] The major questions to be addressed in this study are: can imine formation be used for amine recognition under neutral, high ionic strength aqueous conditions, and can a system be designed which will generate an appropriate fluorescent response upon imine formation?

The formation of imines of amino acids under aqueous conditions has already been examined in detail in the context of understanding the pyridoxal phosphate (PLP) dependent transamination.[10] In fact, the formation of PLP and related imines has been studied by fluorescence.[11,12] Our initial work focused on the fluorescent behavior of several aldehyde substituted coumarins because of their ready accessibility and desirable spectral properties. Imines of amino acids have pK_a's above 7 and are largely protonated at neutral pH.[13] Thus, coumarins such as **1** (Scheme 1) were particularly attractive in that the iminium ion formed by reaction with an amine could hydrogen bond to the carbonyl of the coumarin, thus effectively modulating its fluorescence. Such fluorescence changes have been observed upon metal chelation to properly designed coumarin systems.[14]

The coumarin aldehyde **1a**[15] and its 4-butyl derivative **1b**[16] were examined for their interactions with amino acids using UV/vis spectroscopy under high salt aqueous conditions similar to those found in minimal cell culture media (100 mM NaCl, 50 mM HEPES, pH = 7.4). In this media, the two aldehydes displayed remarkably distinct spectroscopic behavior upon the addition of glycine (Figure 1). The equilibrium formation of the iminium ion was poor for **1a** (K_{eq} < 1) as evidenced by a small red shift in the absorption spectrum. In contrast, formation of the iminium ion was substantially more favorable for **1b** (K_{eq} = 4.0) and was accompanied by a much larger (34 nm) red shift in absorption. The red shifted absorption that both compounds exhibited is consistent with the proposed mechanism of hydrogen bonding of the iminium ion to the chromophore carbonyl. Indeed, basifying a solution of **1b** and saturating glycine to pH = 10 shifted the visible band from 479 to 420 nm, consistent with a shift from iminium ion to imine.

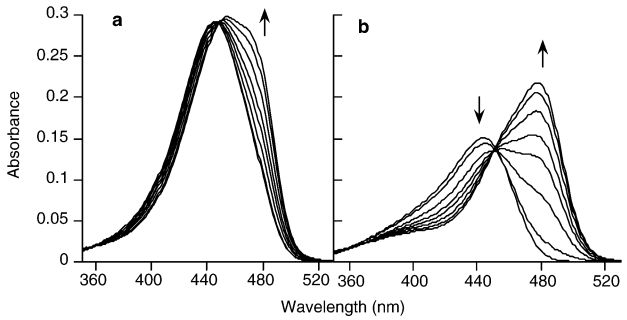
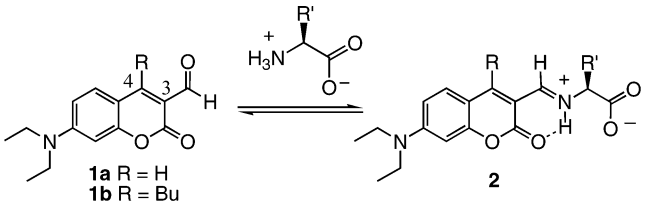


Figure 1. UV/visible absorption spectra for (a) compound **1a** and (b) compound **1b** upon addition of glycine (10 μM in sensor with 100 mM NaCl, 50 mM HEPES, pH = 7.4, 37 °C).

Scheme 1



NMR studies were then undertaken to elucidate the differences between the two aldehydes. The coumarin aldehydes were not sufficiently water soluble to study by NMR in pure D_2O. Therefore, the analogous water soluble compounds **3** and **4** were prepared.[16] The spectral properties of compounds **3** and **4** were similar to the corresponding coumarins (**1a** and **1b**, respectively). Neither compound exhibited evidence of hydrate in D_2O, and both converted cleanly to an equilibrium mixture of aldehyde and the corresponding iminium upon addition of d_7-glycine. Given the similarities in NMR behavior, we attribute the dramatic difference in spectroscopic properties of **1a** and **1b** to steric effects. Apparently, the electronic effect of substituting an iminium ion for an aldehyde carbonyl has a minimal effect on the absorption spectrum in both cases. In the case of **1b**, steric hindrance between the C4 substituent and the iminium ion enforces the *s-trans* conformation which is favorable for hydrogen bonding interactions with the chromophore carbonyl, thereby producing a large change in absorbance. In water, hydrogen

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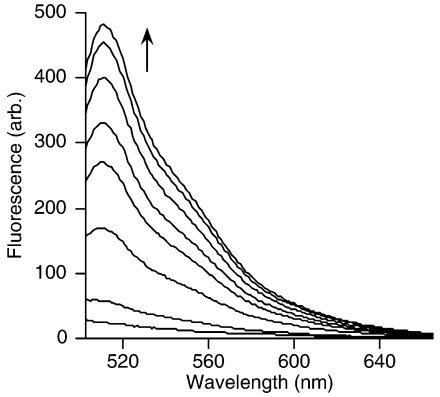


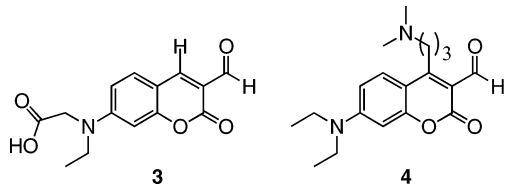
Figure 2. Fluorescence spectra for compound **1b** as a function of added glycine (λ_{ex} = 495 nm, 10 μM in sensor with 100 mM NaCl, 50 mM HEPES, pH = 7.4, 37 °C).

Table 1. Equilibrium Constants and Maximum Fluorescence Enhancements at 513 nm of Compounds **1a** and **1b** with Amines[a]

entry	sensor	analyte	K_{eq} (M^{-1})	I_{max}/I_0[b]
1	**1b**	glycine	4.0	26
2	**1b**	aspartate	2.3	40
3	**1b**	glutamate	2.4	45
4	**1b**	lysine	6.5	29
5	**1b**	serine	5.2	23
6	**1b**	β-alanine	2.5	23
7	**1b**	alanine	1.4	29
8	**1b**	ethanolamine	6.7	15
9	**1b**	1,3-diaminopropane	12.5	22
10	**1b**	lactic acid	–	–
11	**1b**	diethylamine	–	–
12	**1a**	glycine	<1	1.5[c]
13	**1a**	glutamate	<1	2.5[c]

[a] Measured by fluorescent titration of **1** with amines at 37 °C; λ_{ex} = 495 nm; 100 mM NaCl; 50 mM HEPES; pH = 7.4. [b] I_{max} is the maximum intensity at saturation taken from the fit of the titration data. [c] Estimated from the titration curve as the data could not be fit accurately.

bonds are not expected to be very strong, and thus the iminium ion derived from compound **1a** can adopt conformations in which the chromophore carbonyl is not hydrogen bonded, giving a very small response.



Fluorescence studies of compound **1b** were then performed using a variety of small molecule amines. By exciting in a region where the iminium ion preferentially absorbs (495 nm), the sensor produced a strong increase in fluorescence upon addition of an amine. For example, upon titration with glycine, the fluorescence intensity at 513 nm increased 26-fold (Figure 2). Results from a number of amines and amino acids (Table 1) indicate that all primary amines give strong fluorescence responses with compound **1b**. The acidic amino acids (entries 2 and 3) had smaller equilibrium constants and larger fluorescence increases than the neutral amino acids. Both of these observations are likely rooted in the higher pK_a of the ammonium groups and the resultant iminium ions derived from these amino acids. Secondary amines and hydroxy acids did not interact with the sensor (entries 10 and 11). Finally, in agreement with the absorption data, compound **1a** did not give significant fluorescence changes upon addition of amines or amino acids (entries 12 and 13).

These results suggest that properly substituted coumarin aldehydes are an excellent fluorogenic substrate for amines, operating efficiently in high salt, neutral solution with excitation and emission profiles similar to commercial fluorophores (e.g., BODIPY). Furthermore, intramolecular hydrogen bonding in water can be used to induce strong fluorescent responses to the binding of organic compounds. Because of the ready accessibility of 4-substituted coumarins, these chromophores should function as the fluorescent read-out for amine containing compounds within a larger receptor architecture. Work toward this goal is currently underway in our laboratory.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) *Chemosensors of Ion and Molecule Recognition*; Desvergne, J. P., Czarnik, A. W., Eds.; NATO ASI Series C: 492; Kluwer Academic Press: Dordrecht, 1997. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Radermacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.
(2) (a) Raker, J.; Glass, T. E. *J. Org. Chem.* **2002**, *67*, 6113−6116. (b) Raker, J.; Glass, T. E. *J. Org. Chem.* **2001**, *66*, 6505−6512.
(3) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423.
(4) (a) Tsubacki, K.; Kusumoto, T.; Hayashi, N.; Nuruzzaman, M.; Fuji, K. *Org. Lett.* **2002**, *4*, 2313−2316. (b) de Silva, A. P.; Sandanayake, K. R. A. S. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1173−1175.
(5) (a) Rekharsky, M.; Inoue, Y.; Tobey, S.; Metzger, A.; Anslyn, E. *J. Am. Chem. Soc.* **2002**, *124*, 14959−14967. (b) Hosaain, M. A.; Schneider, H.-J. *Chem.-Eur. J.* **1999**, *5*, 1284−1290. (c) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208−9.
(6) (a) Imai, H.; Misawa, K.; Munakata, H.; Uemori, Y. *Chem. Lett.* **2001**, 688−689. (b) Mizutani, T.; Wada, K.; Kitagawa, S. *J. Am. Chem. Soc.* **1999**, *121*, 11425−11431.
(7) Aït-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296−11297.
(8) (a) James, T. D.; Shinkai, S. *Top. Curr. Chem.* **2002**, *218*, 159−200. (b) James, T. D.; Sandanayake, S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910−1922.
(9) (a) Mertz, E.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2003**, *125*, 3424−3425. (b) Mertz, E.; Bell, J. B.; Zimmerman, S. C. *Org. Lett.* **2003**, *5*, 3127−3130.
(10) (a) French, T. C.; Bruice, T. C. *Biochemistry* **1964**, *3*, 1589−1596. (b) French, T. C.; Auld, D. S.; Bruice, T. C. *Biochemistry* **1965**, *4*, 77−84.
(11) (a) Cambrón, G.; Sevilla, J. M.; Pineda, T.; Blázquez, M. *J. Flouresc.* **1996**, *6*, 1. (b) Blázqez, M.; Ineda, T.; Sevilla, J. M.; Domímguez, M.; García-Blanco, F. *J. Phys. Org. Chem.* **1991**, *4*, 372. (c) See also: Vargas, V.; Amigo, L. *J. Chem. Soc., Perkin Trans. 2* **2001**, 1124.
(12) Fluorescent imines of amino acids have been studied under nonaqueous conditions: Hwang, T. K.; Miller, J. N.; Burns, D. T. *Anal. Chim. Acta* **1978**, *99*, 305−315.
(13) Kayser, R. H.; Pollack, R. M. *J. Am. Chem. Soc.* **1977**, *99*, 3379−3387.
(14) (a) Chen, C.-T.; Huang, W.-P. *J. Am. Chem. Soc.* **2002**, *124*, 6246−6248. (b) Leray, I.; Lefevre, J.-P.; Delouis, J.-F.; Delaire, J.; Valeur, B. *Chem.-Eur. J.* **2001**, *7*, 4590−4598 and references therein.
(15) Takechi, H.; Oda, Y.; Nishizono, N.; Oda, K.; Machida, M. *Chem. Pharm. Bull.* **2000**, *48*, 1702−1710.
(16) See Supporting Information for synthesis and characterization.

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