Communications to the Editor

A Long-Wavelength Fluorescent Chemodosimeter Selective for Cu(II) Ion in Water

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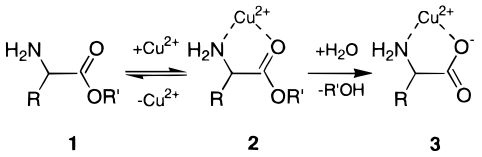
Chemodosimeters are devices, molecule-sized or larger, that utilize abiotic receptors to achieve analyte recognition with concomitant irreversible transduction of a human-observable signal. In fluorescent chemodosimeters, that signal is fluorescence. The properties of ideal fluorescent chemodosimeters are similar to those of fluorescent chemosensors,[1] except that while real-time response remains desirable, that response reflects a cumulative exposure to analyte and is therefore not reversible. In this paper, we report the design and synthesis of a new fluorescent chemodosimeter for Cu(II) ion in water that demonstrates usefully large selectivity and signal strength at a rhodamine-like emission wavelength.

Cu(II) ion displays very high affinities for various polyaza ligands (e.g., $K_{eq} = 6.3 \times 10^{24}$ M^{-1} toward cyclen at pH 7[2]), but unfortunately a variety of other transition metal ions also display affinities only somewhat lower. However, the Cu(II) ion has been known for almost 50 years to promote the hydrolysis of α-amino acid esters (**1**) at rates much greater than those of other metal ions (Scheme 1).[3] A key feature of this reaction is the intermediacy of chelate **2**. For most α-amino acid esters, hydrolysis is complete within seconds at room temperature and neutral pH under reasonable reactant concentrations, yielding the Cu(II)·α-amino acid chelate (**3**; often 1:2 stoichiometry) as product. Selectivity based on time, rather than on bound/free ratio, is ultimately easier to reduce to practice for dosimetry applications, as higher concentrations of the indicator (leading to larger signals) can be used without fear of saturation under conditions of competing analytes.

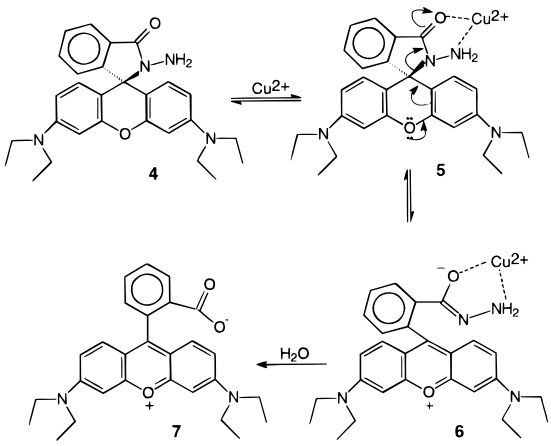
We envisioned that a similar molecular recognition/reactivity motif might be incorporated into a fluorophore derivative, such that Cu(II) complexation would lead to a fluorescence increase. Our approach is depicted in Scheme 2. Rhodamine B hydrazide (**4**), prepared in 80% yield by the reaction of rhodamine B with

Scheme 1



1 **2** **3**

Scheme 2



4 **5** **7** **6**

$POCl_3$ followed without purification by hydrazine,[4] is a colorless, nonfluorescent substance after crystallization from acetonitrile/water. We hypothesized that the hydrazide group of compound **4** would provide recognition for the Cu(II) by analogy to its behavior with α-amino esters. Hydrazides, hydroxamic acids, and *O*-acyl hydroxylamines are all known to bind Cu(II) thusly with resulting enhanced transacylation reactivity.[5] Upon addition of $Cu(OAc)_2$ to a colorless solution of hydrazide **4** in acetonitrile, both the pink color and fluorescence characteristic of rhodamine B appear instantly. Because both disappear upon addition of the chelating ligand cyclen (excess), we propose that Cu(II) in acetonitrile induces a **4** ⇄ **6** equilibrium in much the same way that the proton induces an analogous rhodamine B equilibrium in water.

The conditions of this reaction can be chosen to yield high selectivity for the Cu(II) ion. A solution of chemodosimeter **4** (0.5 μM) was prepared at pH 7 (0.01 M HEPES buffer), 100 equiv of various metal salts were then added, and the fluorescence intensity was monitored with time to produce the results shown. The salts tested were Ag(I), Al(III), Ca(II), Cd(II), Co(II), Cr(III), Cu(II), Eu(III), Fe(III), Ga(III), Gd(III), Hg(II), In(III), K(I), Li(I), Mg(II), Mn(II), Na(I), Ni(II), Pb(II), Rb(I), Sn(IV), Sr(II), U(IV), Yb(III), and Zn(II). After 1 h, only Cu(II) and Hg(II) showed a change in UV−visible absorption or fluorescence. Optimization of assay conditions requires 20% acetonitrile in pH 7 HEPES buffer. Under these conditions, the reaction of 0.5 μM **4** with 50 μM Cu(II) was complete within 1 min. The analogous reaction with Hg(II) requires 50 h to achieve completion. Both reactions afford increases in both fluorescence and absorbance, such that both fluorimetric and colorimetric analyses may be obtained.

The reaction with Cu(II) in water (as compared to acetonitrile as described above) predictably[5] effects a redox hydrolysis of

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(1) Czarnik, A. W. *Chem. Biol.* **1995**, *2*, 423. Available online at http://BioMedNet.com/fluoro. Fluorescent chemosensors for the Cu(II) ion have been described: *Chemosensors of Ion and Molecular Recognition*; Desvergne, J.-P., Czarnik, A. W., Eds.; NATO ASI Series, Series C: Vol. 492; Kluwer Academic Press: Dordrecht, 1997; chapters by L. Fabbrizzi, p 75; A. Czarnik, p 189; and F. Fages, p 221.

(2) Mutsuo, K. *J. Chem. Soc., Chem. Commun.* **1975**, 326.

(3) (a) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2034; *Ibid.* (b) 2036. (c) Bender, M. L.; Turnquist, B. W. *J. Am. Chem. Soc.* **1957**, *79*, 1889. The original discovery was made in the 1940s as part of a Department of Defense program, explaining why publication was delayed.

(4) Rhodamine B hydrazide (**4**): mp 190 °C dec; ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 12, NCH_2CH_3), 3.34 (q, 8, NCH_2CH_3), 3.61 (bs, 2, NH_2), 6.29 (dd, 2, xanthene-H), 6.42 (d, 2, xanthene-H), 6.48 (d, 2, xanthene-H), 7.11 (m, 1, Ar-H), 7.45 (m, 2, Ar-H), 7.94 (m, 1, Ar-H); ^{13}C NMR ($CDCl_3$) δ 12.5, 44.25, 65.75, 97.85, 104.45, 107.9, 122.8, 123.7, 127.90, 127.94, 129.9, 132.3, 148.7, 151.4, 153.7, 165.9; EI mass spectrometry, *m/e* 456.2526, 100% $(M)^+$; M^+, calcd 456.2525. Anal. Calcd for $C_{28}H_{32}N_4O_2$: C, 73.66; H, 7.06; N, 12.27; O, 7.01. Found: C, 73.61; H, 7.11; N, 12.34. Full experimental details can be found in the Supporting Information. Because this compound forms a stable hydrazone with acetone (fully characterized; mp 240−245 °C), the isomeric 6-membered ring alternative can be excluded.

(5) For lead references, see: Wathen, S. P.; Czarnik, A. W. *J. Org. Chem.* **1992**, *57*, 6129.

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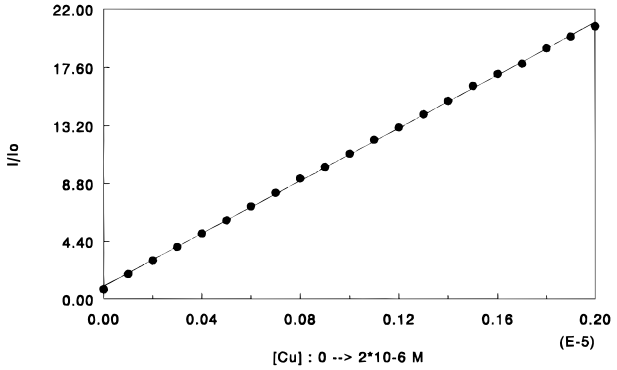

Figure 1. An assay for Cu^{2+}, 0−2 μM. Hydrazide **4** (10 μM) in 0.01 M HEPES buffer (pH 7) with 20% (v/v) added CH_3CN. Fluorescence was recorded every 2 min after adding Cu^{2+}: Excitation wavelength (λ_{ex}), 510 nm; slit width, 10 nm; emission wavelength (λ_{em}), 578 nm; slit width, 20 nm.

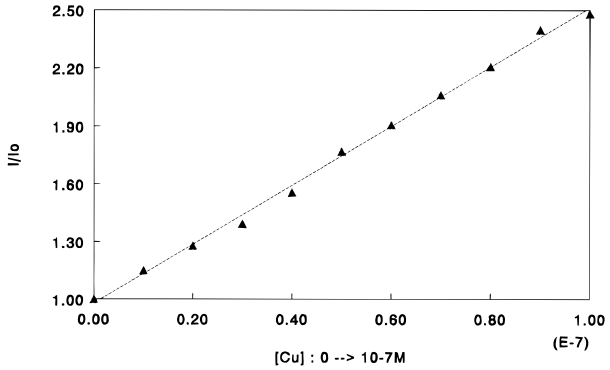

Figure 2. An assay for Cu^{2+}, 0−0.1 μM. Hydrazide **4** (10 μM) in 0.01 M HEPES buffer (pH 7) with 20% (v/v) added CH_3CN. Fluorescence was recorded every 2 min after adding Cu^{2+}: excitation wavelength (λ_{ex}), 510 nm; slit width, 10 nm; emission wavelength (λ_{em}), 578 nm; slit width, 20 nm.

6 leading to rhodamine B (**7**) itself in a stoichiometric[6] process. The formation of rhodamine B as product is confirmed by comparison of the product's TLC data (three-solvent systems), electrospray mass spectrometry data, and absorption and emission properties with those of authentic rhodamine B.[7] Addition of Cu(II) chelating agents, such as EDTA, does not decrease the intensity of the UV−visible absorption or fluorescence signals, confirming the irreversible character of this process. The resulting dosimetric analysis of Cu(II) can be used to measure Cu(II) ion concentrations in water. Figure 1 depicts such a titration, with linear response to 2 μM Cu(II) in water. Figure 2 shows the result of a titration to 0.1 μM Cu(II). Each reaction used to compose Figures 1 and 2 (including that for 10 nM Cu(II)) was effectively complete within 1 min of reagent mixing; 2 min were used.[8]

In summary, we have designed and created a compound with selectivity for Cu(II) in water based upon that ion's known reactivity toward carboxylate derivatives. While the unique propensity of Cu(II) to promote hydrolysis has been known for nearly 50 years, this information has not been used previously for Cu(II) ion quantitation in water. Because of the simplicity of the analysis, we believe the method may find application in a variety of settings requiring rapid and accurate Cu(II) ion analysis.

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Supporting Information Available: Experimental Details (3 pages). See any current masthead page for ordering and Internet access instructions.

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(6) While stoichiometric, the exact stoichiometry is nonintegral and dependent on hydrazide concentration. Experiments designed to understand the origin of this variable stoichiometry have not led to a defendable reaction mechanism; thus, neither mechanism nor stoichiometry are depicted or implied in Scheme 2. Furthermore, less than 1 equiv of rhodamine B is formed after complete reaction. The empirical observation is that at a given concentration of hydrazide, the fluorescence and UV intensity increases are proportional to Cu(II) concentration, permitting the creation of linear standard curves.

(7) For details of this and all other aspects of this study, see: Dujols, V. Ph.D. thesis, The Ohio State University, 1996.

(8) The solutions of Cu(II) were prepared using $CuSO_4$ in double-distilled water. Various Cu(II) solutions (10^{-5}, 10^{-4}, and 10^{-3} M) were prepared separately in volumetric flasks. Solutions of rhodamine B hydrazide were prepared in acetonitrile by serial dilution. Aqueous solutions (containing 20% acetonitrile) of chemodosimeter **4** were prepared by adding the acetonitrile solution with stirring to HEPES (0.01 M) at pH 7, with a final concentration of chemodosimeter **4** equal to 10 μM. Cu(II) assays using compound **4** were run in a polystyrene cuvette. Aliquots of Cu(II) were added every 2 min and the fluorescence was recorded.