Inorg. Chem. **2004**, *43*, 5183–5185

Inorganic Chemistry
Communication

Highly Selective Chromogenic Signaling of Hg^{2+} in Aqueous Media at Nanomolar Levels Employing a Squaraine-Based Reporter

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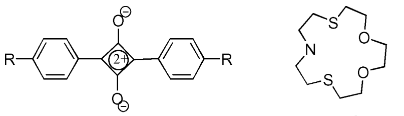
Received May 4, 2004

A chromogenic chemosensor for Hg^{2+} detection in aqueous media at the nanomolar level has been developed from squaraine scaffoldings.

A number of chemosensors for metal ions have been reported to be capable of correlating metal ion concentration with changes in spectroscopic characteristics such as hyper-, hypo-, hypso-, or bathochromic modulations of absorption or emission bands.[1] Many of these chemosensors are based on the interaction of a metal ion (bound to a receptor that is equipped with a suitable binding site) with the π electron system of a chromo- or fluorophore in the host−guest ensemble, resulting upon complexation. However, although many interesting results have been reported in recent years there are still abundant aspects that need to be conveniently addressed in this field. Especially, reports of highly selective and sensitive chemosensors for target metal ions are still limited. To reach this goal is particularly interesting for prominent and environmentally important cations such as Hg^{2+}. Hg^{2+} is one of the environmentally most important cations whose toxicity has long been recognized as a ubiquitous environmental problem, attracting a great deal of public attention still today.[2] Chemosensors for Hg^{2+} do not only have to give a differential response toward Hg^{2+} over other "thiophilic" cations such as Pb^{2+} or Ag^{+}, but also need to be applicable to sensing purposes in neat or mixed aqueous media at very low concentrations. In fact, the maximum level of mercury in drinking water permitted, e.g., by the United States Environmental Protection Agency (EPA), is 2 ppb. An ideally designed chemosensor should thus display an even lower detection limit but retain its selectivity toward Hg^{2+}.

There are several examples of the design of redox-active[3] and fluorogenic[4] chemosensors for Hg^{2+} in organic,[4a−e] aqueous,[4f−j] or mixed aqueous[4k−t] solution, the latter including various approaches relying, e.g., on extraction techniques. Less frequent are reports on chromogenic chemosensors for Hg^{2+}, operating mostly in organic solutions[5] and only in a very few cases in pure water[6] or water−organic solvent environments via simple color variations.[7] However, the development of new selective and sensitive colorimetric probes for Hg^{2+} is of interest as they require usually low-cost and widely used instrumentation.


AT$_2$15C5

1: R = AT$_2$15C5
2: R = N(C$_4$H$_9$)$_2$

We report here a rational design, relying on the "binding site-signaling unit" approach, toward the development of colorimetric probes for Hg^{2+} cation sensing in mixed aqueous media. As binding site, we selected the dithia-dioxa-aza crown AT$_2$15C5. This topological combination of sulfur, oxygen, and nitrogen donor atoms anchored by ethylenic chains has recently proven to be highly effective in forming Hg^{2+} complexes that guarantee efficient signal transduction in optical chemosensing ensembles.[5c,6d] As signaling unit, we chose the squaraine scaffold which displays very attractive photophysical properties for chromogenic sensing applications.[8] Squaraines are symmetric nonalternating cross-conjugated polymethine chromophores,[9] consisting of the central four-member squaric acid ring and, e.g., two substituted aniline moieties at the 1,3-positions of the cyclobutadienyl ring. Squaraines usually show a remarkably intense absorption ($\log \epsilon = 5.3-5.5$) in an analytically advantageous

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10.1021/ic049422q CCC: \$27.50

wavelength region,[10] at the lower end of the "spectral window" for many biological and environmental applications.[11] Furthermore, upon proceeding to aqueous media, squaraines tend to show aggregation phenomena,[12] a fact which we addressed here in view of the possibility to explore an alternative sensing concept in aqueous environments.[13] With these design principles in mind, we synthesized **1** and model compound **2** by reaction of the corresponding aniline derivatives and squaric acid in butanol−toluene 1:1 mixtures with azeotropic removal of water.[14]

Table 1. Spectroscopic Data of **1** and **2** in Acetonitrile at 298 K

dye	λ_{abs} (nm)	λ_{em} (nm)	ϕ_f	τ_f (ns)
1	640	661	0.129	0.67
2	642	661	0.087	0.46

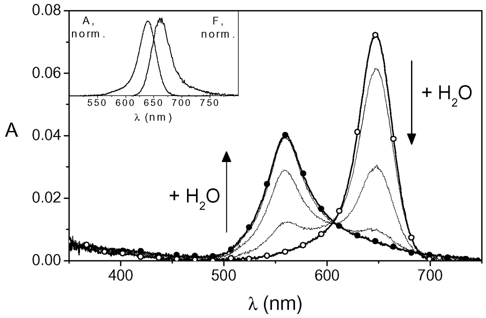

Figure 1. Spectroscopic behavior of **1** in MeCN−H_2O mixtures: 33:67 (○), 28:72, 22:78, 15:85, 6:94 v/v (●). Inset: Absorption and fluorescence spectra of **1** in MeCN ($\epsilon_{640\ nm}$[**1**] $= 307040 \pm 3460\ M^{-1}\ cm^{-1}$).

One spectroscopic endpoint of the studies reported here is the behavior of the compounds in pure acetonitrile, shown in Table 1. The corresponding spectra of **1** in MeCN shown in the inset of Figure 1 reveal the cyanine-related nature of the squaraine chromophore. The bands of mirror-image shape are narrow and weakly Stokes-shifted, and no hints for multiple emission bands are found,[15] with the monoexponential fluorescence lifetimes in Table 1 supporting this assignment. The low to moderate fluorescence quantum yields in the highly polar solvents most probably result from an intramolecular charge separation process in the excited state, involving large amplitude motions and the population of a species that decays radiationlessly to the ground state.[10d] Figure 1 reveals that despite minor spectral shifts (e.g., $\lambda_{abs} = 647$ nm for **1** in 33:67 MeCN−H_2O), the spectroscopic properties of **1**, as well as **2** (not shown here), are largely preserved in the presence of up to 67 vol % water. Further increasing the amount of water induces the reversible formation of hypsochromically shifted H-aggregates (e.g., $\lambda_{abs} = 560$ nm for **1**), that were found to be entirely nonfluorescent. These blue-shifted aggregates have also been found for other squaraine dyes and were associated with face-to-face π-stacking interactions between the monomers.[16]

Aiming at the development of Hg^{2+}-selective sensing protocols, we tested the response of **1** toward Hg^{2+} as well

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(14) Both compounds were prepared by condensation of the corresponding aniline derivate with squaric acid, in *n*-butanol−toluene 1:1 mixtures with azeotropic distillation of water. ^1H NMR ($CDCl_3$) of both compounds shows two characteristic doublets in the aromatic zone at 8.39 and 6.74 ppm (**1**) and 8.37 and 6.74 ppm (**2**). FAB high-resolution mass spectroscopy: (**1**) M + 1 733.2474, calculated 733.2474; (**2**) M + 1 489.3460, calculated 489.3481. Both **1** and **2** complexes are stable in the solid state but slowly decompose in water at basic pH.

(15) After correction of the fluorescence spectra (traceable to the primary standard blackbody radiator at PTB, Berlin), no hints on multiple emitting species (as described, e.g., in ref 10c) could be detected. Moreover, spectral analysis of the absorption and emission bands of **1** and **2** employing three Gaussian functions revealed virtually identical half-widths (340 and 350 cm^{-1}) and vibronic spacings (780 ± 20 cm^{-1}) for both bands, stressing the interpretation that squaraines can be regarded as polymethines rather than as donor−acceptor−donor substituted dyes, showing a typical intramolecular charge transfer.

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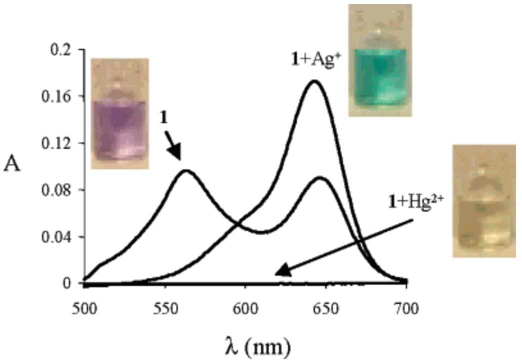

Figure 2. Absorption spectrum of **1** in 80:20 v/v $H_2O-MeCN$ and in the presence of 5 equiv of Hg^{2+} or Ag^{+}. The photographs show the color of the three corresponding solutions.

as various other metal ions in a $MeCN-H_2O$ 20:80 mixture. Figure 2 shows that Hg^{2+} binding leads to a complete bleaching of both the monomer and the aggregate bands that is fully reversible.[17] This decoloration of the solution upon formation of the complex gives further support to the fact that cyanine dyes are well-suited for long-wavelength chromogenic sensing with drastic signal changes. In accordance with previous studies on crowned hemi-cyanines,[18] fully engaging the lone electron pair of the nitrogen atom of the polymethine chain in complexation literally "switches off" the cyanine features, entailing gigantic hypo- and hypsochromic shifts: the lowest-energy absorption band of the complex is centered at 285 nm and has a more then 10-fold reduced molar absorptivity. Besides these advantageous sensitivity features, the selectivity of **1** is also remarkable. Cations such as Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} do not lead to any noticeable change in either the monomer or the aggregate band, and counterion effects were also not observed for Cl^{-}, Br^{-}, NO_3^{-}, PO_4^{3-}, or SO_4^{2-} salts. Moreover, this monomer-to-aggregate "two-band" system possesses another prominent advantage; i.e., it is able to discriminate between thiophilic cations. Whereas our target Hg^{2+} strongly diminishes both bands at 647 and 560 nm, Ag^{+} induced disaggregation and thus a shift toward the formation of the monomer (Figure 2), and Pb^{2+} remains entirely silent. Furthermore, model **2** does not respond to any of the ions tested under similar conditions.

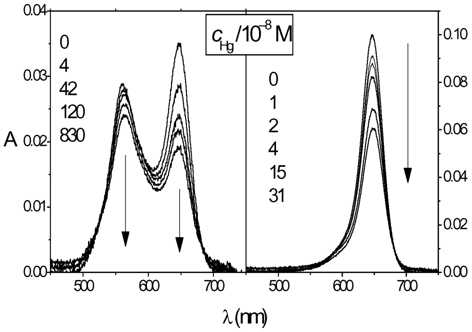

Figure 3. Variation in the bands of **1** in 22:78 (left) and 33:67 v/v (right) $MeCN-H_2O$ (HEPES, 0.08 M) mixtures in the presence of increasing amounts of Hg^{2+} as indicated in the plots.

To assess the potential of this new chromogenic Hg^{2+} chemosensor for selective colorimetric detection, we performed various titrations in 33:67, 22:78, and 6:94 v/v $MeCN-H_2O$ mixtures, employing a 0.08 M HEPES buffer (pH 6.90) instead of pure water. In all the cases, successive decoloration occurred with no detectable changes in the band ratios. Apparently, once Hg^{2+} binds to a dye molecule, the π-delocalization is switched off.[19]

Initial studies on the detection limit were also promising. By using different solvent mixtures, Hg^{2+} could be detected down to ca. 1×10^{-7} M with fully aggregated **1** (6:94 $MeCN-H_2O$), down to ca. 2×10^{-8} M in 22:78 mixtures and even down to less than 1×10^{-8} M in monomer-containing solutions (Figure 3). For a certain solvent combination, a Hg^{2+} response range between 1.5 and 3 orders of magnitude was observed.[20] The increase in complex stability constant and molar absorptivity, $\lambda_{mon} > \lambda_{agg}$, are most probably responsible for the highest sensitivity in the one-band case, meeting well the EPA requirements. Accordingly, this monomer/aggregate system covers a significantly larger dynamic sensing range than conventional molecular probes while enabling a straightforward adjustment of the system for the concentration range of interest by simple choice of the solvent ratio.

In conclusion, a new sensing protocol for aqueous Hg^{2+} operating at long wavelengths in the visible has been developed via the unique coordination properties of the dithia-dioxa-aza crown and the remarkable physicochemical and photophysical properties of the squaraine scaffold, especially the very high molar absorptivity and the two-band monomer-to-aggregate equilibrium sensing system. The probe allows discrimination of Hg^{2+} over other thiophilic cations such as Ag^{+} and Pb^{2+} and remarkably selective chromogenic double signaling (via the 647 and/or 560 nm bands) of Hg^{2+} in aqueous media at nanomolar levels.

Acknowledgment. This work was supported by Projects MAT2003-08568-C03, REN2002-04237-C02-01, and GRUPOS03/035. J.V.R.-L. thanks the Ministerio de Educacion Cultura y Deporte for a doctoral fellowship.

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(17) Colorless solutions of **1**$-Hg^{2+}$ changed back to blue upon addition of an excess of EDTA.

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(19) On the basis of the results obtained with Ag^{+}, it is most likely that attack of the aggregates by Hg^{2+} also leads to their disintegration with subsequent binding and decoloration.

(20) In the monomer/aggregate equilibria, the complex stoichiometries vary as a function of the solvent composition, and the optical response to Hg^{2+} would require the use of calibration procedures.