Dithioamide metal ion receptors on fluorescent lipid bilayers for the selective optical detection of mercuric ion

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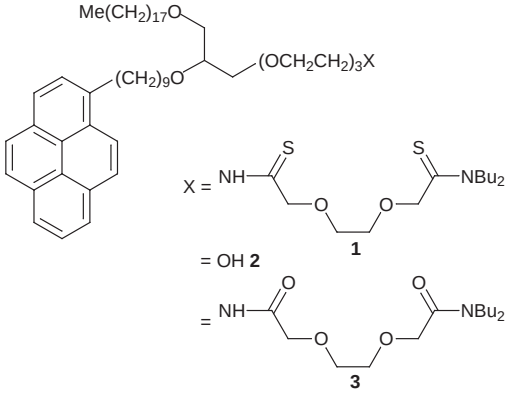
Functionalized lipid bilayers with dithioamide receptors at the surface and pyrene labels in the hydrophobic interior showed a selective fluorescence response to mercuric ion with sensitivity limits at the ppb level.

The release of mercury into the environment originates from a variety of man-made and natural sources, including fossil fuel combustion and the electronics industry.[1] As many mercurial compounds are highly toxic, the monitoring of mercurials in the environment and in industrial waste streams is highly desirable. The development of optically responsive sensor materials for the detection of heavy metal ions has received much attention in recent years offering an inexpensive and robust sensor platform with remote detection capability. Often the sensor material employs the use of ion recognition sites to selectively bind specific analytes from solution. In particular, sensors that can detect mercuric ion have been prepared using porphyrins,[2] bioreceptors,[3] borates[4] and polyamines[5] as the ion recognition ligands. Typically the recognition group operates in conjunction with a neighboring chromophore that produces a change in spectral properties upon binding. We describe here a novel sensor material that uses a molecular assembly as a platform for ion recognition as well as a reversible and sensitive optical transducer to the binding event.

Previously[6] we showed that a distearylphosphatidylcholine (DSPC) bilayer doped with a synthetic lipid having an iminodiacetic acid headgroup and a pyrene label in the tail could detect sub-ppb levels of Cu^{2+} selectively. The selectivity was determined to be a function of the ion binding headgroup, however, the mechanism of the fluorescence response and generality of the detection approach was uncertain. Herein, we describe a new sensor tailored for the binding and detection of 'soft' metal ions previously not recognized by the iminodiacetic acid group. As the recognition ligand we chose the thioamide functional group for selective detection of mercuric ion.[7,8] Lipid **1**,‡ which contains the dithioamide headgroup, was prepared by a series of steps starting from compound **2**.[9] The alcohol headgroup of **2** was converted to the primary amine by mesylation of the alcohol, followed by azide substitution with sodium azide in DMF, and then LAH reduction. The reaction of this amine with 8-(4-nitrophenyl)-3,6-dioxaoctanoate-1-dibutylamide, prepared through two steps from 3,6-dioxaoctanedioic acid, yielded the diamide product **3** in 83% yield. The dithioamide **1** was obtained as a light yellow–green viscous liquid in 75% yield through the reaction of **3** with P_2S_5 in pyridine at elevated temperature for 24 h followed by flash column chromatography.



Lipid bilayer preparation is briefly described below; a more detailed description can be found elsewhere.[10] Films of 5% **1** in DSPC were prepared by evaporation of a chloroform solution of the lipids on the inside of a conical tube. The films were then hydrated in a MOPS buffer solution (pH 7.4, 0.02 M morpholinopropanesulfonic acid–0.10 M NaCl) at 65 °C with vortex mixing. The solution temperature was maintained near room temperature while being probe sonicated for 12 min using a 2 mm tip at a power of 25 W under a nitrogen atmosphere. Following sonication the solution was centrifuged and the supernatant passed through a 0.2 μm filter. Total lipid incorporated into the bilayers was 50% as determined by phosphate analysis.[11] The **1**/DSPC aggregates imaged with transmission electron microscopy (not shown here), using a 1% ammonium molybdate stain, formed disc-like structures with no apparent inner volume unlike the typical spherical structures found with DSPC liposomes. Although the structural geometry and headgroup polarity of the lipid components determine the packing geometry of the bilayer,[11] it is interesting to find that incorporation of small amounts of **1** can significantly alter the DSPC bilayer geometry. Details of these and related results will be described elsewhere.

The fluorescence spectrum of the 5% **1**/DSPC lipid bilayers in buffered solution shows emission maxima from both the pyrene monomer at 376 nm and the excimer at near 470 nm [Fig. 1(*a*)]. With a total lipid concentration of 0.05 mM the observed excimer to monomer intensity ratio (*E*/*M*) is 2.2. Addition of certain metal ions (chloride salts) into solution causes a decrease in the fluorescence intensity of the pyrene excimer with a concomitant increase in the monomer emission. A highly selective response was found for mercuric ion with a limit of detection at 100 nM (20 μg l^{-1}). Fig. 1(*b*) shows an example of the fluorescence response from the 5% **1**/DSPC bilayers to the presence of 1.0 mM Hg^{2+}. The *E*/*M* response for Hg^{2+} was linear from a concentration of 10^{-7} to 10^{-4} M on a semilogarithmic plot shown in Fig. 2. Against the heavy metal

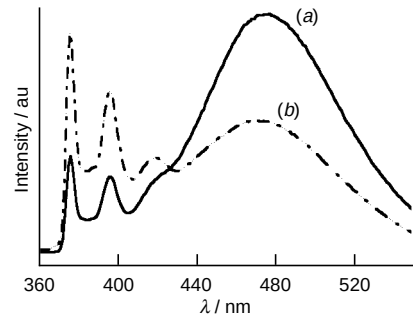


Fig. 1 Fluorescence spectra of 5% **1**/DSPC lipid bilayers in MOPS buffer solution (pH 7.4) in the absence (*a*) and presence of 1.0 mM $HgCl_2$ (*b*)

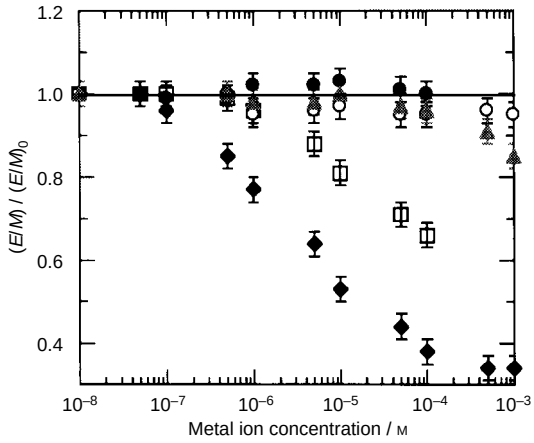


Fig. 2 Plot of the normalized ratio of fluorescence intensities from pyrene excimer to monomer (*E/M*) *vs.* metal ion concentration, showing metal ion selectivity of the 5% **1**/DSPC bilayers. Selectivity was in the order Hg^{2+} (◆) > Cu^{2+} (□) ≫ Cd^{2+} (▲) > Pb^{2+} (●) and Mn^{2+} (○). Equilibration of response took only moments following addition of metal ion and slight stirring.

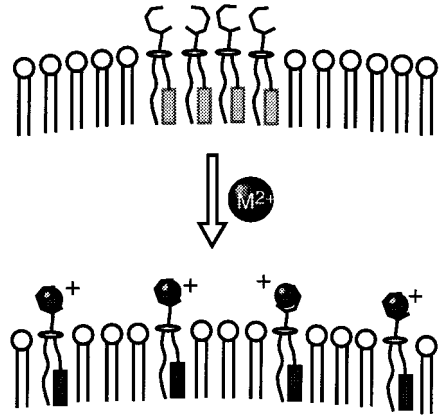


Fig. 3 Illustration of lipid molecule aggregational changes upon metal ion binding to receptor sites. Initially the receptor **1** is phase separated from the DSPC matrix. Metal ion complexation with the receptor and formation of charged headgroups causes static repulsion of the pyrene labeled molecules and loss of local aggregation.

ions Mn^{2+}, Ni^{2+}, Ca^{2+}, Cr^{3+}, Co^{2+} and Pb^{2+}, the bilayers were unresponsive up to mM concentrations. Of the metals evaluated only Cu^{2+} and Cd^{2+} gave responses with 10- and 1000-fold lower sensitivity, respectively, compared to Hg^{2+}. The material was also insensitive to high concentrations (100 mM) of monovalent alkali metals (*i.e.* Na^+, Li^+, K^+). Response times were rapid, limited only by the rate of mixing of the solution. To regenerate the material an excess of EDTA was added to solution to remove metal ions bound to the recognition sites. Complete fluorescence recovery was realized in a few minutes and the material could be reused. As a control, compound **3**, which has a poor metal chelating headgroup, when prepared as a bilayer with DSPC and tested for metal sensitivity showed no response to any of the above mentioned metal ions.

The observed fluorescence response to selected metal ions is due to a change in aggregational properties of the pyrene labeled lipid in the DSPC matrix. In the absence of metal ion, molecules of lipid **1** tend to aggregate into pools within the bilayer due to differences between the physical state of the two membrane components. At room temperature, DSPC (T_c = 55 °C) forms crystalline domains causing a partitioning from the liquid phase of lipid **1** (T_c < room temperature). This creates high local concentrations of pyrene which increases the collision rate of the fluorophores subsequently producing a large population of pyrene excimers compared to monomers.[12] Metal ion binding to the dithioamide headgroup induces a reorganization of molecules in the molecular assembly. Aggregates of **1** are dispersed into the DSPC matrix reducing the local concentration of pyrene resulting in a population shift in the excited state species and the observed loss in intensity of excimer emission and gain in monomer emission. Of the changes that could result from metal ion complexation, formation of a charged headgroup on **1** producing static repulsion between **1**–M^{2+} chelates would lead to the observed changes in molecular organization in the bilayer. Fig. 3 illustrates the molecular aggregate state before and after metal complexation. Initially, when **1** is neutrally charged, lipid organization is controlled by differences in the crystalline and liquid behavior of the components. Following metal ion binding and formation of charged headgroups on **1** electrostatic repulsion overcomes the separation of physical phases resulting in a dispersal of **1** in the matrix.

Although dithioamides have been used as ionophores for 'soft' metals, such as Hg^{2+}, Pb^{2+} and Cd^{2+}, the present sensor shows almost exclusive selectivity for Hg^{2+}. Some slight structural differences that exist between those in literature[7,8] and that on **1**, and the use of a lipid bilayer as the site of recognition, may account for the differences in selectivity. We are continuing to pursue new metal chelating headgroups to increase the range of metal ion selectivities for eventual configuration into a sensor array system for evaluation of mixed wastes.

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Notes and References

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‡ *Selected data* for **1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (br s, 1 H, NH), 8.28 (d, 1 H, *J* 9.3 Hz, Py-H), 8.10 (m, 7 H, Py-H), 7.87 (d, 1 H, *J* 7.8 Hz, Py-H), 4.54 [s, 2 H, $OCH_2C(S)NBu_2$], 4.39 [s, 2 H, $OCH_2C(S)NH$], 3.92 [dt, *J* 5.4, 5.4 Hz, 2 H, $CH_2NHC(S)$], 3.85 (t, *J* 7.5 Hz, 2 H, CH_2O), 3.76–3.40 (m, 25 H, CH_2O, CH_2N), 3.33 (t, *J* 7.7 Hz, 2 H, $PyCH_2$), 1.85 (tt, *J* 7.7, 7.7 Hz, 2 H, $PyCH_2CH_2$), 1.72–1.24 (m, 57 H, aliphatic CH_2), 0.95 [t, *J* 7.4 Hz, 6 H, $N(CH_2)_3CH_3$], 0.87 [t, *J* 6.9 Hz, 3 H, $(CH_2)_{17}CH_3$]. Anal. Calc. for $C_{66}H_{108}N_2O_7S_2$: C, 71.69; H, 9.84; N, 2.53; S, 5.80. Found: C, 71.51; H, 9.96; N, 2.50; S, 6.11%.

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