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Selective Sensor for Silver Ions Built From Polyfluorophores on a DNA Backbone

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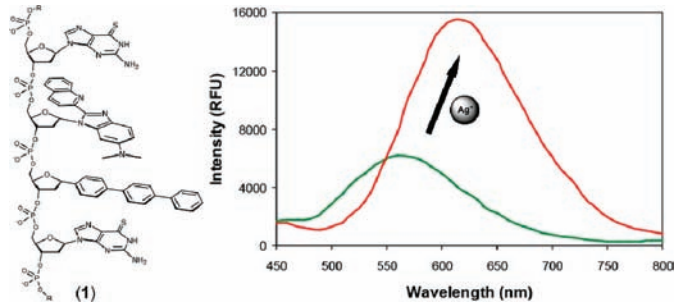
ABSTRACT



To explore a new modular metal ion sensor design strategy, fluorophores and ligands were incorporated into short DNA-like oligomers. Compound 1 was found to function as a selective sensor for Ag^+ in aqueous buffer, where low micromolar concentrations of Ag^+ induce a red-shifted, turn-on fluorescence signal. Experiments with HeLa cells show that 1 can penetrate cells and yield a signal for intracellular Ag^+. This suggests a broadly applicable approach to developing sensors for a wide variety of cations.

Fluorescent sensors for metal ions are widely useful tools in biology, chemistry, and environmental analysis. In cells and organisms, molecular imaging with such sensors is emerging as a powerful method to interrogate metal ion chemistry.[1] Selective sensors make available information on localization and bioavailability that is difficult or impossible to obtain using conventional analytical techniques such as atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, radioisotope labeling, and histochemical methods.

Currently, the fluorophore-spacer-receptor architecture remains one of the most popular designs for fluorescent metal ion sensors. The signals are typically transduced between fluorophore and receptor by a photoinduced electron transfer (PET) mechanism.[2,3] In this configuration, the fluorophore and receptor moieties of the system are carefully designed and optimized such that the interaction between the two moieties leads to quenching of the fluorescence. This communication is disrupted upon analyte binding to the receptor moiety, resulting in fluorescence recovery.

One of the problems faced in development of such sensors is that it can be difficult to design, synthesize, and optimize the molecules for each distinct metal ion of interest. In an effort to develop a new, general, and modular sensor design strategy for sensing a wide variety of metal ions, we have adopted a different

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approach, in which the fluorophores and ligands are incorporated into a DNA-like oligomer. This design brings the binding and reporting moieties into close proximity, allowing them to interact intimately. Unlike traditional sensor design, the signal transduction of this system is not limited to a single mechanism, such as PET. Moreover, the modular design allows for water solubility and for rapid synthesis and discovery from libraries. Herein we describe a selective sensor developed by this approach as a proof of concept: oligomer **1** is found to function as a selective sensor for silver ions in aqueous buffers and in human cells.

Because of the common and increasing human exposure to silver, convenient methods for monitoring the metal in water sources and in vivo are needed. The use of silver as an antimicrobial agent has increased of late, particularly in wound dressings, topical agents, and medical devices (catheters, endotracheal breathing tubes, bone prostheses, etc.). Silver is also used on surfaces and fabrics in order to reduce the spread of infections, particularly in hospitals. Colloidal silver is widely purchased by people who ingest the metal in expectation of health benefits. Chronic exposure to silver and silver compounds leads to argyria and argyrosis, and to other symptoms such as headaches, stomach distress, fatigue, skin irritation, or more serious neurological, renal, or hepatic complications.[4] Colloidal silver may also interfere with certain drugs, such as penacillamine, quinolones, tetracyclines, and thyroxine.[4d] Because silver is also used as an antibacterial agent in many home water treatment devices, the U.S. Environmental Protection Agency (EPA) has set a secondary maximum contaminant level (SMCL) for silver at 0.1 mg/L (0.93 μM). In toxic argyrosis, serum and urine concentrations of Ag^+ can be 2 μM.[4e] Study of the biological effects of this toxic exposure would be aided by a sensor that could detect this metal in aqueous media and in cells.

To date, fluorescent silver ion sensors for turn-on, -off, and ratiometric sensing have been developed based on the reactivity of Ag^+ or other signal transduction mechanisms, such as PET, metal-to-ligand charge transfer (MLCT), internal charge transfer (ICT), and excimer formation.[5,6] Notably, the majority of these studies have been carried out in nonaqueous or mixed solvents, due to limited solubility of the sensor molecule; and none has been shown to be effective in cells.

Compound **1**, identified in a screen of a library of fluorophore/ligand tetramers (see Supporting Information), contains fluorescent nucleoside metal ligands (**Q** and **I**), and a hydrocarbon fluorophore (**H**) (Figure 1).[7,8] Abasic spacers were

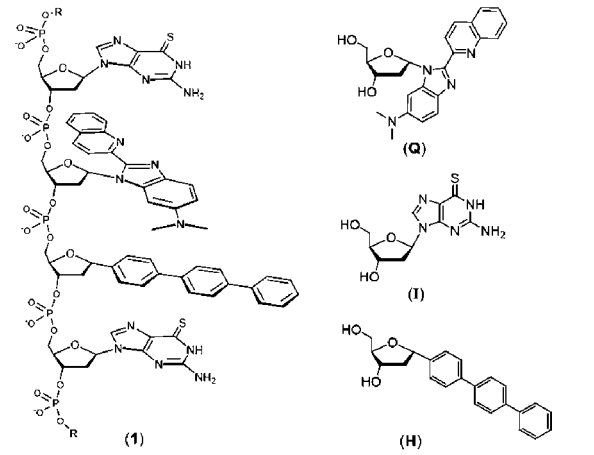

Figure 1. Structures of sensor **1** and its monomer components. R = two tetrahydrofuran abasic spacer monomers.

incorporated on both ends of the oligomer to increase aqueous solubility, prevent aggregation and facilitate purification. Unlike the deoxyriboside monomers (**H**, **Q**, and **I**), which are not appreciably soluble in water alone, compound **1** is functional in wholly aqueous media and in human cells (*vide infra*).

The addition of Ag^+ at micromolar concentrations to **1** in buffer results in a new emission band (λ_{max} = 620 nm) shifted 60 nm to the red from the original 560 nm fluorescence, and gives an 8-fold fluorescence enhancement at 710 nm (Figure 2). Titration studies revealed an apparent dissociation constant (K_d) of 1.67 ± 0.07 μM (Figure 2 inset). Time-dependent studies showed that the signal reaches near-saturation within 10 min (see Supporting Information, Figure S6). Sequential addition of a known chelator, dimercaptosuccinic acid (DMSA), to a mixture of **1** and Ag^+ resulted in a gradual reversion of the fluorescence spectrum from that of the **1**−Ag^+ complex to that of **1** (see Supporting Information, Figure S8), demonstrating the reversibility of the sensor.

One potential source of interference in sensing Ag^+ is the formation of $AgCl_{(s)}$ in the presence of Cl^- in the solution. This complicates sensing Ag^+ in intact mammalian cells,

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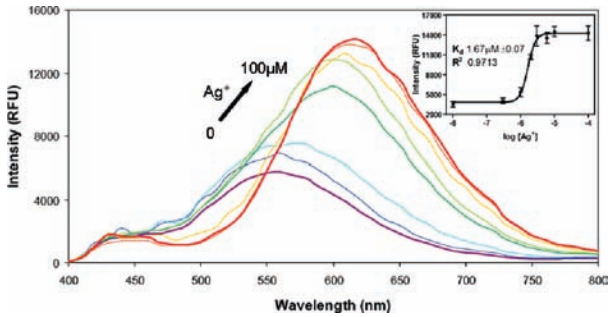

Figure 2. Fluorescence of **1** (2 μM) in 20 mM Tris-HCl, pH 7.3 with different concentrations of Ag^+. [Ag^+] was 0, 0.3, 1, 2, 3, 6, 10, and 100 μM. (Inset) Intensity at 620 nm vs log [Ag^+] ($\lambda_{ex.}$ = 350 nm).

which contain chloride. To circumvent this complication, previous studies of Ag^+ sensors avoided solutions containing Cl^-. However, for application in live cells and organisms, it is imperative that the sensor tolerate this counterion. Notably, we find that oligomer **1** can still function as a Ag^+ sensor despite the presence of Cl^- in the solution. Other counterions such as NO_3^-, ClO_4^-, OAc^-, and SO_4^{2-} have negligible effect on the signal (see Supporting Information, Figure S7). The data show that oligomer **1** can also respond to a suspension of $AgCl_{(s)}$ (Supporting Information).

Fluorescence measurements of **1** with various metals revealed good selectivity for Ag^+. High concentrations of alkali and alkaline-earth metal cations such as Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, and Ba^{2+} induced little to no interference; Mn^{2+} and Pb^{2+} also induced weak or no responses (Figure 3).

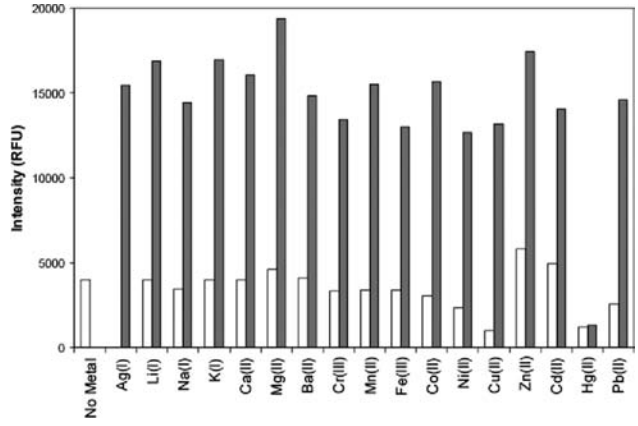

Figure 3. Fluorescence responses of **1** (2 μM) at 620 nm for various metal cations in 20 mM Tris-HCl, pH 7.3 ($\lambda_{ex.}$ = 350 nm). White bars represent the addition of the appropriate metal ion to the solution of **1**: 2 mM for Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}; 200 μM for Ba^{2+}, Mn^{2+}, Pb^{2+}; and 10 μM for Cr^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}. Dark bars represent the subsequent addition of 200 μM Ag^+ to the solutions.

Transition-metal ions that are known to be quenching metals such as Cr^{3+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Hg^{2+} quenched the fluorescence of **1** at high concentrations (see Supporting Information). However, the effects of the quenching metals and of Zn^{2+} and Cd^{2+} were mitigated at lower metal concentrations.

Competition experiments by subsequent addition of Ag^+ to each metal solution indicated that **1** can still function in the presence of the other potentially interfering metal ions (Figure 3). The data suggest that the quenching metals Co^{2+}, Ni^{2+}, and Cu^{2+} quench the fluorescence of **1** more effectively than they do the **1**−Ag^+ complex. Cu^{2+} is the strongest quencher of **1**; however, upon addition of Ag^+, the fluorescence from the **1**−Ag^+ complex can still be observed. The only metal that interfered with the fluorescence of both **1** and **1**−Ag^+ complex is Hg^{2+}, which caused quenching at high and low mercury concentrations. However, addition of the metal chelator TPEN to the solution resulted in the recovery of the fluorescence signal, presumably by preferentially binding the Hg^{2+} (see Supporting Information, Figure S9).[9] This suggests the possibility of using TPEN to mask the interference from Hg^{2+}.

Preliminary investigations of binding stoichiometry were carried out by performing titrations of ligand with metal and by measuring fluorescence at varied molar ratios (see Supporting Information, Figures S4a,b). The data are consistent with 1:1 complexes being primarily responsible for the observed signals; however, we cannot yet rule out higher-order complexes such as 2:2 and 2:3 ligand:metal.

Unlike most metal ion sensor designs, where the specificity of the ligand primarily determines the overall selectivity of the sensor, the ligands incorporated in **1** were not designed to be selective ligands for Ag^+. Neither of the nucleoside ligand monomers **Q**[8a] and **I** (see Supporting Information, Figure S10) are selective for Ag^+; both **Q** and **I** respond with quenching or blueshifts to a variety of metal cations. Thus, the response profile of tetramer **1** is very different from those of the ligand monomers (**Q** and **I**) which it is composed. For example, none of the components give a fluorescence increase for silver, while the oligomer displays a substantial enhancement. The data suggest that while oligomer **1** can likely bind multiple metal cations, the selective electronic interactions only yield the observed red-shifted, turn-on signal for Ag^+. This suggests the importance of cooperative electronic interactions among the ligands and the fluorophore in the sensing response of **1**, wherein the selectivity primarily stems from the signal specificity rather than from the selectivity of the ligands. This demonstrates a new approach to metal cation sensing that is distinct from traditional sensor compounds that rely heavily on the specificity of the metal ligand for metal selectivity.

Encouraged by the ability of **1** to sense Ag^+ in media containing chloride, we examined the possibility of employing oligomer **1** for silver sensing in live human cells. We know of no previous reports of cellular sensors for this metal. Intact HeLa cells incubated with 5 μM of **1** for 7 h in the absence of silver ions at 37 °C led to only very weak intracellular fluorescence as observed by widefield deconvolution microscopy (Figures 4a,c). However, after incuba-

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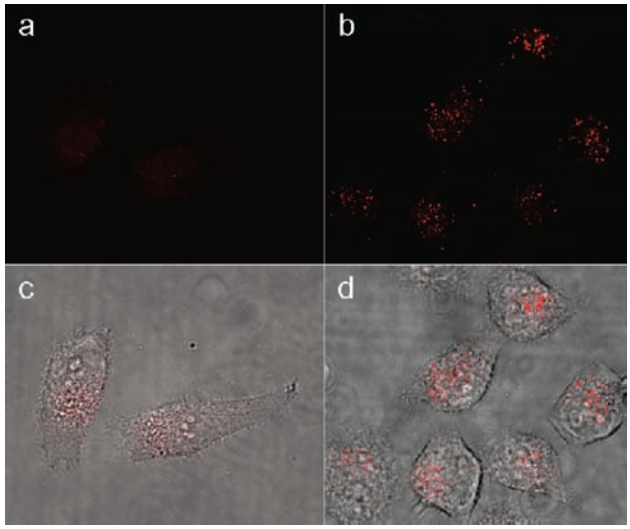

Figure 4. Widefield deconvolution microscopy images of HeLa cells. Fluorescence images (top) and the overlay of the fluorescence image on the DIC image (bottom). (a) and (c) Cells incubated with **1** (5 μM) for 7 h. (b) and (d) Cells incubated with **1** for 7 h, washed, and then incubated with $AgNO_3$ (20 μM) for 30 min.

tion with 20 μM $AgNO_3$ for 30 min at 37 °C, a significant increase in red fluorescence was observed in the cytoplasm of the cells (Figures 4b,d). These results indicate that oligomer **1** can penetrate cells and yield a signal for intracellular silver. The punctate nature of the observed signals likely indicates localization in intracellular vesicles, possibly late endosomes and lysosomes, as observed in previous studies with oligodeoxyfluorosides.[10] Work is ongoing to examine how the structure of fluorescent oligomers such as **1** affects cellular uptake and localization.

A significant advantage of the DNA scaffold of **1** is the aqueous solubility it confers. Our phosphate diester-based design enables more hydrophobic components (fluorophores, ligands, etc.) to be utilized as building blocks for designing water-soluble sensors. Notably, our control studies on the nucleoside ligand monomers (**Q** and **I**) could not be carried out in wholly aqueous media (methanolic solution and 1:1 $MeOH/H_2O$ were used respectively) due to the poor aqueous solubility of these nucleosides; this points out the value of the negatively charged phosphates in adding solubility to oligomer **1**.

Another advantage of this design strategy is that the sensors generated are not limited to a single sensing mechanism (e.g., PET). The design simply enables the fluorophores and ligands to interact on the DNA backbone, which can potentially generate sensors with different sensing mechanisms. Although the precise sensing mechanism of **1** remains under investigation, it is likely that the red-shifted, turn-on response of **1** upon binding Ag^+ may arise from changes in the oligomer structure that promote favorable alignment of the monomers, changes in the electronic properties of the ligand involved in binding, or a combination of both factors in a way that enhance delocalization in the ground and/or excited state. UV titration data shows a strong absorbance change at 343 nm, suggesting that monomer **I** (abs. $\lambda_{max} = 345$ nm) is directly involved in binding the metal (see Supporting Information, Figures S1 and S2); however, a small change at ca. 380 nm indicates that monomer **Q** (abs. $\lambda_{max} = 384$ nm) may also play a role directly or indirectly.[8a]

In conclusion, we have demonstrated a new molecular design for metal ion sensing that does not rely heavily on the selectivity of the ligands incorporated into the sensor. The strategy allows the ligands and fluorophores to interact among each other by incorporating them onto a DNA backbone, enabling the overall properties of the oligomer to be different from the monomers. Using this approach, **1** was identified and found to function as a selective sensor for Ag^+ in wholly aqueous media. In addition, fluorescence microscopy confirmed that **1** can also function as a Ag^+ sensor in live mammalian cells. The modular nature of this sensor design strategy suggests a broadly applicable approach to finding sensors for many different cations, simply by varying the sequence and composition of ligands and fluorophores using a DNA synthesizer.

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Supporting Information Available: Synthesis and characterization data; fluorescence data in vitro and in cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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