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Quencher-free Oligonucleotide-based Fluorescent Probe for Pb^{2+} Ions

Min Sik Eom, Sudeok Kim, Seong Hyeok Seo, and Min Su Han*

*Department of Chemistry, Chung-Ang University, Seoul 156-756, Korea. *E-mail: mshan@cau.ac.kr*
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Lead is an extremely toxic metal ion and is harmful to human health because it interferes with nervous system development.[1] However, lead is used widely in products and industries such as lead-acid batteries, lead wire or pipes, paint, and metal recycling and foundries. The European Parliament regulates lead usage in electronics to prevent environmental contamination with lead ions and lead-related chemical waste, and the U.S. Environmental Protection Agency (EPA) has set the safe level of lead in drinking water at 15 μg/L.[2] Due to health concerns and legal restrictions, the detection of lead ions has been the focus of much research.

Atomic absorption spectroscopy, anodic stripping voltammetry, and inductively coupled plasma mass spectrometry are commonly used methods for detecting Pb^{2+} ions.[3] However, these methods are time consuming and require sophisticated equipment. As a result, probes have been developed based on organic dyes,[4] proteins,[5] polymers,[6] nanomaterials,[7] and oligonucleotides.[8,9] Oligonucleotides have been used intensively as scaffolds for developing Pb^{2+} probes due to their specific interaction with lead ions.

Previously reported oligonucleotide-based probes for Pb^{2+} can be divided into two types. The first type is based upon DNAzyme. RNA-cleaving DNAzyme triggered by Pb^{2+} is a very common platform for Pb^{2+} sensors, and a Pb^{2+}- induced allosteric G-quadruplex DNAzyme has also been utilized for Pb^{2+} detection.[8] These probes are quite sensitive and selective, but are limited in that they are quite sensitive to media conditions such as pH, temperature and composition because these systems rely on catalytic reactions. The second type is fluorescently labeled G-quadruplex.[9] Although these fluorescently labeled G-quadruplex probes are slightly less sensitive than those used in the DNAzyme based-method, they are not limited by reaction conditions. Generally, the probe is labeled with the donor and the quencher at its 5' and 3' termini, respectively, and the sensing mechanism is based on the change in the DNA strand's conformation from linear to a quadruplex upon binding with Pb^{2+} ions. This conformational change induces different degrees of FRET between the fluorophore and quencher at the termini of each DNA probe. Although fluorescent detection methods are sensitive and selective, they have some drawbacks. The fact that labeling is required at both ends of the oligonucleotide-based probe with specific dyes diminishes the overall yield and the labeling process is expensive and time-consuming.

Recently, single-labeled fluorescent oligonucleotides (SF-Oligo) have been developed as probes for silver ions and mercury ions using poly-deoxyguanosine in the probe as the quencher.[10] A conformational change is induced upon binding of metal ions to the probe, which causes the poly-deoxyguanosine to be oriented close to the fluorescent dye. The close proximity of the quencher to the dye results in a decrease in fluorescence due to the photo-induced transfer of electrons from the dye to the deoxyguanosine. In this paper, we describe a probe developed for the detection of Pb^{2+} in aqueous media using a single-labeled fluorescent G-quadruplex with a high binding affinity for Pb^{2+} ions. The sensing mechanism of this probe is based on a change in the DNA strand's conformation from linear to folded upon binding of lead ions.[11] These conformational changes affect the degree of FRET between the fluorophore and poly-deoxyguanosine in the SF-Oligo (Scheme 1).

The sensitivity of the SF-Oligo to Pb^{2+} ions was first determined by obtaining the fluorescence spectra of a solu-

Scheme 1. Schematic illustration of FRET changes of the SF-Oligo in the presence of Pb^{2+} ions.

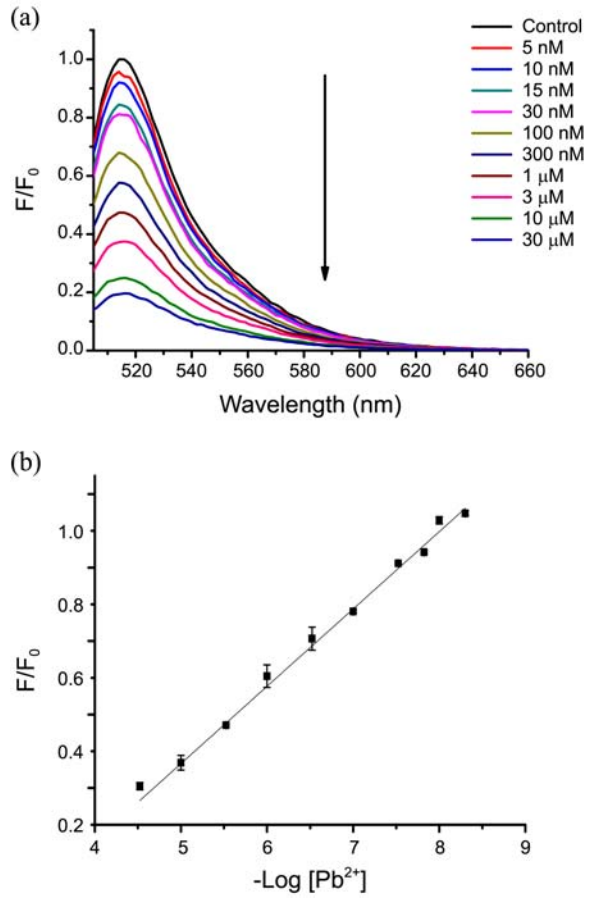

Figure 1. (a) Spectra obtained after the addition of Pb^{2+} (0-30 μM) to pH 7.4 buffer solution containing SF-Oligo (100 nM). (b) Plot of the normalized fluorescence intensities of the SF-Oligo at 515 nm as a function of Pb^{2+} concentration.

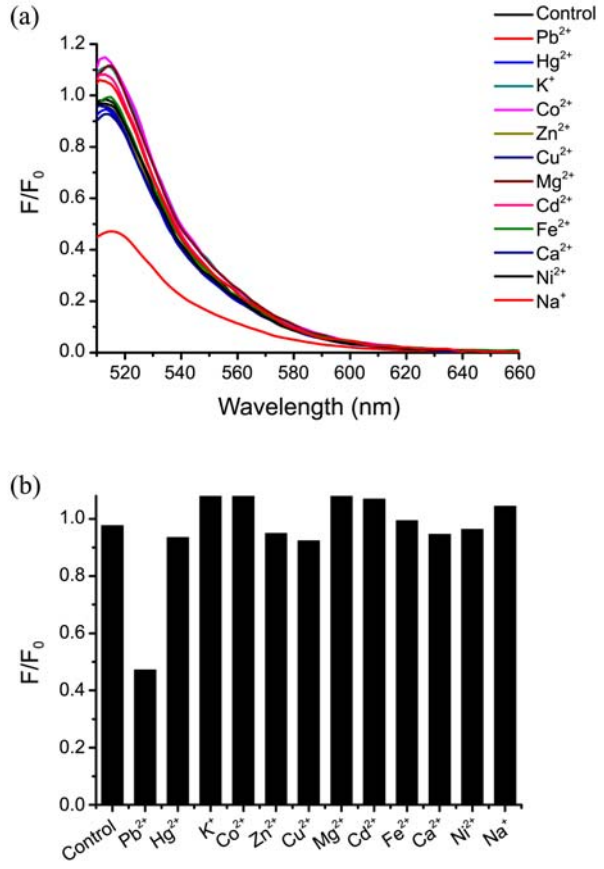

Figure 2. (a) Fluorescence emission spctra obtained after the addition of metal ions (1.0 μM) to a pH 7.4 buffer solution containing SF-Oligo (100 nM). (b) Plot of the normalized fluorescence intensities of the SF-Oligo at 515 nm in the presence of different metal ions.

tion containing SF-Oligo (100 nM) upon the addition of Pb^{2+} ions. Fluorescence emission spectra of the SF-Oligo in the presence of different concentrations of Pb^{2+} ions are shown in Figure 1(a). The addition of Pb^{2+} ions induced a decrease in fluorescence as shown in Figure 1(b). The observed fluorescence intensity was nearly proportional to the Pb^{2+} ion concentration. Additionally, based on titration results, the detection limit of the SF-Oligo for Pb^{2+} ions was estimated to be 3.0 nM. Although this probe is a turn-off type chemosensor and such decreased emission is undesirable for analytical purposes, the probe was highly sensitive to Pb^{2+} and the detection limit was sufficient for detecting Pb^{2+} ions in drinking water according to the limits stipulated by the US EPA (~72 nM).

We also evaluated the selectivity of the probe for Pb^{2+} detection by measuring changes in the fluorescence properties of the SF-Oligo induced by interaction with other metal ions. Fluorescence spectra of solutions of SF-Oligo (100 nM) were analyzed after the addition of 10 equiv of each metal ion (Figure 2(a)). The SF-Oligo was more highly selective for Pb^{2+} than for the other metal ions. A significant decrease in fluorescence intensity was not observed upon the addition of other metal ions, which implied that only Pb^{2+} ions were able to place deoxyguanosines in SF-oligo close to the fluorescent dye. Specifically, Hg^{2+} did not affect the fluorescence of the probe. This is important because previously reported double-labeled oligonucleotide sensors for Pb^{2+} had a higher affinity for Hg^{2+}.[9a] Hg^{2+} induced a conformational change in the oligonucleotide from a single strand to a hairpin-like structure, which induced degrees of FRET between the fluorophore and quencher at the termini of the probe.

The effect of coexisting metal ions on the detection of Pb^{2+} ions was also investigated by measuring the Pb^{2+}-induced fluorescence changes in the SF-Oligo in the presence of different metal ions. Fluorescence spectra of solutions of SF-Oligo were obtained after the addition of Pb^{2+} (1.0 μM) to a buffer solution (pH 7.4) containing SF-Oligo (100 nM) and other metal ions (1.0 μM). As shown in Figure 3, the fluorescence intensity changes caused by the addition of Pb^{2+} were not influenced by the presence of other metal ions.

In conclusion, we demonstrated that SF-Oligo is a highly effective fluorescent probe for detecting Pb^{2+} and polydeoxyguanosine in the probe is an effective quencher molecule similar to the dabsyl group used in traditional doubled-labeled fluorescent oligonucleotide probes. The probe was highly sensitive for detecting Pb^{2+}, and the detection limit of

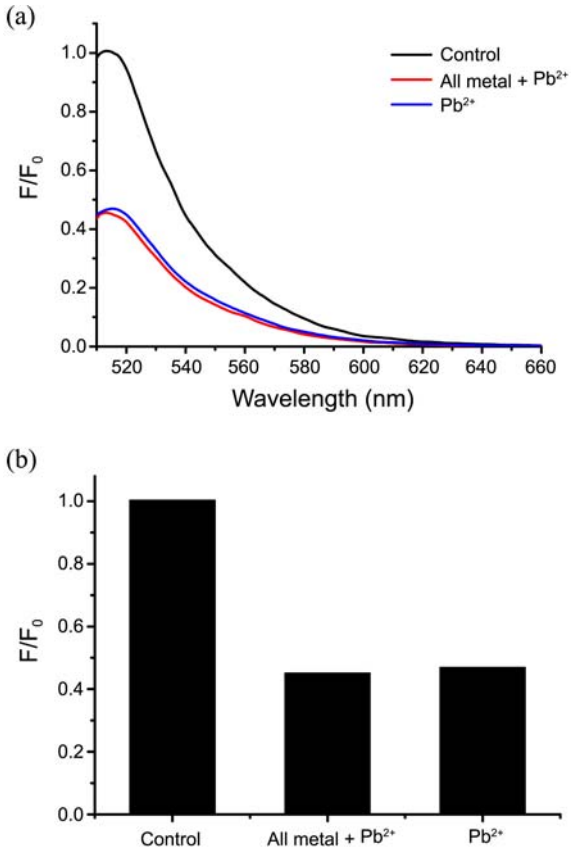

Figure 3. (a) Fluorescence emission spectra obtained after the addition of Pb^{2+} (1.0 μM) to a pH 7.2 buffer solution containing SF-Oligo (100 nM) and various metal ions (1.0 μM). (b) Fluorescence intensities of the SF-Oligo (100 nM) in the presence of Pb^{2+} (1.0 μM) and various metal ions (1.0 μM).

the SF-Oligo was estimated to be 3.0 nM. The detection limit is sufficient for detecting Pb^{2+} ions in drinking water according to the criteria stipulated by the US EPA (~72 nM). Additionally, poly-deoxyguanosine as quencher can be applied to develop other quencher free-fluorescent oligonucleotide probes.

Experimental Section

Chemicals and Equipment. All chemicals were of analytical grade or of the highest purity available. SF-Oligo was purchased from Bioneer (South Korea) and buffer and metal salts were purchased from Sigma Aldrich (USA). Milli-Q water was used to prepare all the solutions in this study. Fluorescence spectrometry was carried out with a FS-2 purchased from SCINCO.

Fluorometric Assay for Pb^{2+}. Solutions of SF-Oligo (100 nM) in Tris buffer (pH 7.2) were mixed with different concentrations of Pb^{2+} and the fluorometric spectra of the solutions were recorded after incubation for one hour.

Determination of Selectivity. Solutions of SF-Oligo (100 nM) in Tris buffer (pH 7.4) were mixed with various metal ions, such that the final concentration was 1.0 μM respectively. The fluorometric spectra of the solutions were recorded after incubation for one hour.

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