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A Lead(II)-Driven DNA Molecular Device for Turn-On Fluorescence Detection of Lead(II) Ion with High Selectivity and Sensitivity

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Abstract: A Pb^{2+}-driven DNA molecular device which is constructed based on a DNA duplex−quadruplex exchange is utilized for the highly selective and sensitive detection of Pb^{2+}. The power of this DNA device originates from the excellent efficiency of Pb^{2+} for stabilizing G-quadruplexes, which makes the DNA duplex unwind thereby driving the device. This device can be reset to the original state by addition of a strong Pb^{2+} chelator DOTA, endowing the device with good reusability. In the whole process, the signal readout is modulated via a fluorescent probe binding to and being released from the G-quadruplex. Such a DNA device can serve as a novel turn-on fluorescent sensor for Pb^{2+} detection with high selectivity and sensitivity.

Since ionic lead is a major environmental pollutant, the sensitive and selective detection of aqueous Pb^{2+} is of particular interest. In the past few years, functional nucleic acids became a powerful and extensively used tool for Pb^{2+} analysis.[1] In particular, a Pb^{2+}-dependent RNA-cleaving DNAzyme called "8−17" is a very common sensing element for Pb^{2+} sensors.[1a−g,i] In addition, a Pb^{2+}-induced allosteric G-quadruplex (G4) DNAzyme is also utilized for Pb^{2+} detection.[1h] These previous Pb^{2+} sensors all exhibit high sensitivity and selectivity for Pb^{2+} analysis. Herein, we report a Pb^{2+}-driven DNA molecular device as a conceptually new fluorescent Pb^{2+} sensor, which integrates high sensitivity and selectivity with good reusability. This Pb^{2+}-driven DNA device is mainly constructed based on a duplex−quadruplex exchange, a common operating principle for DNA nanodevices.[2] Different from previous counterparts powered by DNA strands[2a,b,d,e] or protons,[2c,f] our DNA device is driven by the binding of Pb^{2+} to the G4 structure. Pb^{2+} proves to have excellent efficiency for stabilizing G4 DNA.[3] This unique feature enables Pb^{2+} to disrupt a duplex consisting of a G4 DNA and its complementary strand. Most importantly, the ultrahigh G4-stabilizing efficiency of Pb^{2+} endows the DNA device with high sensitivity and selectivity for sensing Pb^{2+}.

Figure 1 depicts a typical Pb^{2+}-driven DNA molecular device consisting of T30695, $(GGGT)_4$, and its partly complementary strand (**X**, CAC_3TC_3AC). In the absence of coordination cations, T30695 and **X** are hybridized to form a DNA duplex. Upon addition of Pb^{2+}, the duplex is induced to unwind while T30695 folds into the G4 structure stabilized by Pb^{2+}. We previously demonstrated that the Pb^{2+}-stabilized T30695 can interact with zinc protoporphyrin IX (ZnPPIX) and sharply enhance its fluorescence intensity.[4] In contrast, the DNA duplex does not. This allows us to utilize ZnPPIX as a fluorescent probe to indicate the formation of the G4 structure and monitor the operation of the DNA device, unlike previous counterparts[2] which required labeling oligonucleotides with fluorophores. In a reverse process, a strong Pb^{2+} chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) is introduced into the system. Since DOTA has an extremely high capacity of complexing with Pb^{2+} ($\log K \approx 23$),[5] it is able to competitively bind Pb^{2+} and remove this coordination cation from the G4-Pb^{2+} complex ($\log K \approx 7$).[3a,6] As a result, T30695 and **X** tend to form the duplex again; namely, the DNA device is reset to the original state. To achieve the best performance of this DNA device, the DNA sequences are optimized (see Figure S1a in the Supporting Information). The well-chosen DNA device has a quite appropriate stability (see Figure S1b), which keeps it stable at room temperature whereas a minimum amount of Pb^{2+} is sufficient to start it up.

As the fluorescence intensity of ZnPPIX is tuned by its binding to and release from the G4 structure, the structural changes of DNA device can be directly reflected by an increase or decrease in fluorescence signal. Figure 2a shows that, without Pb^{2+}, the DNA device has a low signal (curve 1). Upon addition of Pb^{2+}, there is a sharp increase in the fluorescence intensity (curve 2), indicating the Pb^{2+}-induced formation of the G4 structure. Subsequently, the

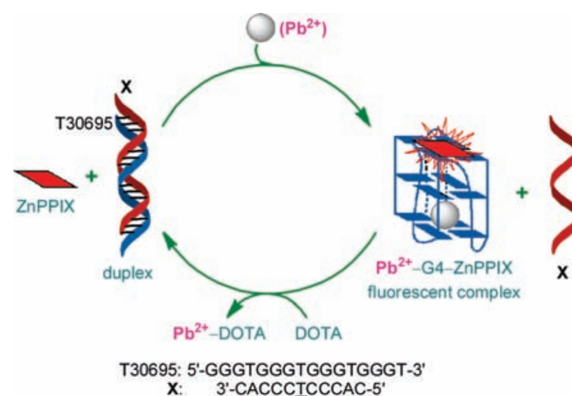

Figure 1. Oligonucleotide sequences and working cycle of the Pb^{2+}-driven DNA device consisting of T30695 and **X**. The position of a mismatch in the duplex is underlined.

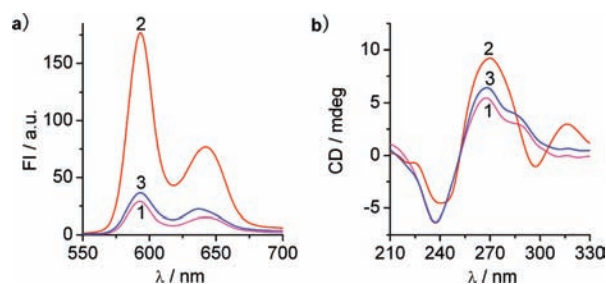

Figure 2. Fluorescence (a) and CD (b) spectra for characterizing the DNA structural conversion of the Pb^{2+}-driven DNA device: (1) no cation; (2) 3 μM Pb^{2+}; (3) 3 μM Pb^{2+} plus 10 μM DOTA. Experimental conditions: 10 μM ZnPPIX, 6 μM duplex (total concentration of DNA strands) in pH 6.5, 50 mM MOPS-NH_2OH buffer.

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fluorescence intensity decreases when DOTA is added (curve 3). It indicates the disruption of the G4 structure and meanwhile the reformation of DNA duplex. These observations are further confirmed by circular dichroism (CD), as shown in Figure 2b. In the absence of Pb^{2+}, there is a positive band near 265 nm with a shoulder at 285 nm in the CD spectrum, indicating the existence of a B-form duplex.[7] The addition of Pb^{2+} causes an increase in the positive band and the disappearance of the shoulder. Meanwhile, a small positive peak appears near 310 nm, which is the typical CD characteristic of the Pb^{2+}-stabilized antiparallel G4 structure.[1h,3a,4,6a] This CD spectrum suggests the coexistence of the parallel G4 structure with a small amount of the antiparallel one. After incubation with DOTA, the CD spectrum becomes quite similar to that of the original state, indicating that the duplex is formed again.

Since our DNA device responds to external stimulus Pb^{2+}, it can serve as a novel fluorescent Pb^{2+} sensor. Unlike the previous counterparts,[1] this DNA device is thought able to be reused for Pb^{2+} detection, owing to its ability to operate repeatedly.

Figure 3 depicts the fluorescent analysis of different concentrations of Pb^{2+} using the DNA device as a Pb^{2+} sensor. As the concentration of Pb^{2+} increases, the fluorescence intensity is enhanced (Figure 3a). This indicates that the duplex gradually converts into the G4 structure, since the fluorescence increase originates from the promotion of the G4 structure on ZnPPIX. It is found that there is an observable change in the fluorescence spectra upon addition of 20 nM Pb^{2+}. Figure 3b outlines the relationship between the fluorescence intensity at 594 nm (FI_{594}) and the concentration of Pb^{2+}. A linear range is observed from 20 nM to 1 μM (Figure 3b, the inset), and a limit of detection (20 nM) for Pb^{2+} analysis is achieved.

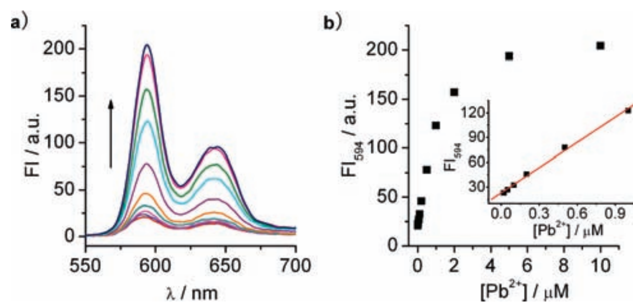

Figure 3. Pb^{2+}-driven DNA device as a fluorescent sensor for Pb^{2+} detection. (a) Fluorescence spectra for analyzing different concentrations of Pb^{2+} (from bottom to top): 0, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 μM. (b) Dependence of FI_{594} on the concentration of Pb^{2+}. The inset shows a linear relationship ($R = 0.998$) in the concentration range from 20 nM to 1 μM.

Because the DNA device can be reset from the open to closed state via addition of DOTA, it is able to be reused as a turn-on Pb^{2+} sensor. A limit of detection (5 nM) and good linear range for Pb^{2+} analysis are achieved in the second cycle (see Figure S2 in the Supporting Information).

To test the selectivity of our DNA device for Pb^{2+} analysis, other common metal ions are adopted in place of Pb^{2+}. Figure 4 shows a high selectivity of the DNA device for Pb^{2+} against other metal ions. As we know, K^+ is highly able to stabilize the G4 structure.[8] However, DNA melting experiments reveal the stability of K^+-T30695 is much lower than that of Pb^{2+}-T30695 under the same conditions (see Figure S3 in the Supporting Information). So, K^+ cannot drive the DNA device to work, thereby not interfering with Pb^{2+} detection. These data demonstrate that the Pb^{2+}-driven DNA device can serve as a novel fluorescent sensor for sensitive and selective Pb^{2+} detection.

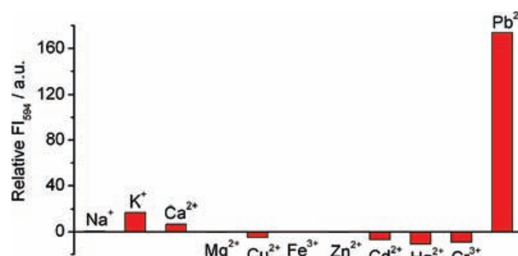

Figure 4. Selectivity of Pb^{2+} analysis using the DNA device. Pb^{2+} was used at 3 μM, and other tested metal ions were used at 10 μM.

In conclusion, we have reported a Pb^{2+}-driven DNA molecular device and utilized it as a turn-on fluorescent Pb^{2+} sensor. This DNA device exhibits a high selectivity for Pb^{2+}, with a high sensitivity comparable to (or better than) those of DNAzyme-based Pb^{2+} sensors.[1] In addition, This DNA device has some unique features: (1) It can be reused for Pb^{2+} detection; (2) It does not require labeling or modification of oligonucleotides, with ZnPPIX as a specific structural probe for signal readout. These important features endow our DNA device with great promise in environmental monitoring.

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Supporting Information Available: Experimental details and supplementary figures and discussion. This material is available free of charge via the Internet at http://pubs.acs.org.

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