A highly selective lead sensor based on a classic lead DNAzyme†

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A catalytic beacon sensor for Pb2+ has been developed based on the first DNAzyme discovered in the field, and such a sensor has shown a much higher metal ion selectivity (40 000 times) than the previously reported Pb2+ sensor based on 8-17 DNAzyme and thus is suitable for a wider range of practical applications.

Wide-spread Pb2+ contamination has posed adverse effects on human health, especially on children.1 To reverse the negative effects, accurate, on-site and real-time detection and quantification of Pb2+ in the environment or in vivo is an important first step. Toward this goal, a number of Pb2+ sensors have been developed.2–5 Among them, DNAzymes have emerged recently as a promising class of molecules to build sensors. DNAzymes that are selective for a number of metal ions have been obtained from a large DNA library through in vitro selection.6–8 More importantly, these DNAzymes exhibit high catalytic turnovers that allow signal amplification and employ rate-based measurement that is much less vulnerable to the sample background interferences that have hampered other intensity-based measurement methods. Because of these features, DNAzymes have been converted into fluorescent, colorimetric and electrochemical sensors for a wide range of metal ions, including Pb2+.3–6

Currently, all DNAzyme-based Pb2+ sensors are built on the 8–17 DNAzyme, which is capable of catalyzing a phosphodiester bond cleavage reaction in the presence of Pb2+. This DNAzyme has been obtained through in vitro selection by several groups under different conditions.9 Even though the same 8–17 DNAzyme was selected against different metal ions such as Mg2+, Zn2+ and Ca2+, a survey of the metal-ion-dependent activities showed that the 8–17 DNAzyme displays substantially higher activity in the presence of Pb2+ than any other metal ion,4,10 and thus this DNAzyme has been converted into sensors for Pb2+.3–5 Although being selective for Pb2+, the 8–17 DNAzyme is still active in the presence of other metal ions, such as Mg2+, Zn2+, Mn2+, Co2+ and Ca2+. For example, the activity of the 8–17 DNAzyme at millimolar concentrations of Zn2+ has been shown to be equivalent to nanomolar concentrations of Pb2+ and Zn2+.9,10 As a result, the 8–17 DNAzyme would be a good Pb2+ sensor in the presence of equal concentrations of Pb2+ and Zn2+, but it would become ineffective if Zn2+ concentration is much higher. Therefore, a more selective DNAzyme sensor is required in such a situation.

The first DNAzyme was selected using Pb2+ as the cofactor more than a decade ago.5 Similar to the 8–17 DNAzyme, this classic DNAzyme (to avoid confusion, we name this DNAzyme GR-5 DNAzyme) can also catalyze the cleavage of an RNA base embedded in the DNA substrate in the presence of Pb2+. To find out if this DNAzyme has a higher selectivity for Pb2+ over other competing metal ions, we carried out metal-ion-dependent activity assays with this DNAzyme and found that it has excellent selectivity for Pb2+ (Fig. S1f). For example, under single turnover conditions, the kobs (observed rate constant) was obtained by fitting the equation y = y0 + Ae−x/t) was measured to be 0.64 min−1 for 100 μM Pb2+ in HEPES buffer (pH 7.0 plus 500 mM NaCl, 500 mM KCl, 50 mM MgCl2). In contrast, under the same conditions, the kobs was measured to be 0.014 min−1 for 20 mM Zn2+, and no other metal ion surveyed has a kobs higher than 0.0034 min−1 (Table S1f). Encouraged by this high selectivity, we report here a new catalytic beacon sensor for Pb2+ based on the GR-5 DNAzyme and compare its performance with that of the 8–17 DNAzyme.

The new sensor is designed similarly to a temperature independent 8–17 DNAzyme sensor variant called +5_17E reported previously (Fig. 1a).4d It consists of a substrate strand (in black) labelled with a fluorophore (F) at the 5′ end and a quencher (Q1) at the 3′ end, and an enzyme strand (in green) labelled with a second quencher (Q2) at the 3′ end. Previous studies have shown that while the intermolecular quenching between Q2 in the enzyme strand and F in the substrate accounts for the majority of the quenching because of their close proximity to one another,4d a small percentage of dehybridization of the substrate from the enzyme at room temperature resulted in F being away from Q2 and thus high fluorescent background.4e To improve the quenching efficiency, Q1 was added to the 3′ end of the substrate to promote intramolecular quenching when the substrate strand was dehybridized from the enzyme strand, lowering the fluorescent background by ~75%.4e Fluorescein (FAM) was used as the fluorophore in both sensors in Fig. 1. For the 8–17 DNAzyme sensor, a black hole quencher (BHQ-1′) was used at 3′ end of the substrate strand while Dabcyl was used at the 3′ end of the enzyme strand, as reported previously.4a,b,d For the new sensor design, we chose to use BHQ-1′ at the 3′ ends of both the substrate and the enzyme strands to simplify the design even further (Fig. 1b). Previous studies indicate that both Dabcyl and BHQ-1 quench FAM efficiently at such short a distance.12 In the absence of Pb2+, the arms of the enzyme-substrate complex maintain a double helical structure, placing
the FAM molecule close to a BHQ-1\(^\text{\textsuperscript{1}}\) quencher, resulting in the fluorescent signal being quenched. When Pb\(^{2+}\) is present in the solution, it facilitates the cleavage of the phosphodiester bond of the internal RNA base (rA) by the enzyme strand (Fig. 1c). After cleavage, the base pairing between the enzyme and the substrate is destabilized and the cleaved substrate dissociates from the complex. As a result, the fluorophore is no longer quenched by BHQ-1\(^\text{\textsuperscript{1}}\), resulting in an increased fluorescent signal.

Prior to testing the sensor, the buffer conditions were optimized for the best sensor performance (see Fig. S2\(^\text{\textsuperscript{6}}\)). Under these optimized conditions (50 mM NaHEPES, 50 mM NaCl and 5 mM MgCl\(_2\) at pH 7.26), the GR-5 DNAzyme’s observed rate was higher than the observed rate obtained under the selection conditions.

In consistent with the original study that showed Mg\(^{2+}\) played no role in the catalysis\(^\text{\textsuperscript{8}}\), Mg\(^{2+}\) alone did not generate an enhanced fluorescent signal in the study here. Since a low concentration of Mg\(^{2+}\) (<5 mM) has no significant effect on the rate of the sensor, while maintaining a more stable enzyme-substrate complex over the course of the experiment, we chose the buffer conditions for the GR-5 DNAzyme sensor in the subsequent experiments to be 50 mM NaHEPES, 50 mM NaCl and 5 mM MgCl\(_2\) at pH 7.26. Interestingly, pH ~7.2 has been used in previous investigations of the 8–17 DNAzyme catalytic beacon sensors.\(^\text{\textsuperscript{6}}\) Therefore the performance of the two generations of the sensors can be fairly compared at the same pH.

Although the 8–17 DNAzyme sensor was shown to be selective for Pb\(^{2+}\), the interference from other metal ions at very high concentration has not been thoroughly investigated. In 50 mM NaHEPES, 50 mM NaCl, at pH 7.2, the 8–17 DNAzyme catalytic beacon exhibited a high fluorescent enhancement over the background (\(F/F_0\)) in the presence of 25 nM–2 \(\mu\)M of Pb\(^{2+}\) (Fig. 2a), similar to those reported previously.\(^\text{\textsuperscript{4}}\) When the competing metal concentration was \(\geq 400 \mu\)M, both Co\(^{2+}\) and Zn\(^{2+}\) gave a fluorescent enhancement that was comparable to 2 \(\mu\)M Pb\(^{2+}\). Although to a lesser degree, Mn\(^{2+}\) and Cd\(^{2+}\) also gave a strong signal with concentration \(\geq 40 \mu\)M and their signal saturated at \(\sim 400 \mu\)M. The 8–17 DNAzyme sensor was less active with Ni\(^{2+}\), Cu\(^{2+}\) and Ca\(^{2+}\) (note that Cu\(^{2+}\) had a quenching effect on the fluorophore used, Fig. S3\(^\text{\textsuperscript{6}}\)). Ba\(^{2+}\), Sr\(^{2+}\) and Hg\(^{2+}\) were the only metal ions that the 8–17 DNAzyme sensor did not respond to. Based on these measurements, the sensor offers no more than 160-fold selectivity against the most active interfering ion, Zn\(^{2+}\).

In contrast, the fluorescent enhancement (\(F/F_0\)) for the GR-5 Pb\(^{2+}\) DNAzyme sensor, shown in Fig. 1h, was much more selective for Pb\(^{2+}\) under the same conditions. First the \(F/F_0\) for the GR-5 sensor in the presence of 25 nM–2 \(\mu\)M Pb\(^{2+}\) were higher than those of the 8–17 DNAzyme sensor, reaching \(\sim 24\) (Fig. 2b) instead of \(\sim 8\) (Fig. 2a). Remarkably, other competing metal ions displayed little \(F/F_0\) for the new sensor based on the GR-5 Pb\(^{2+}\) DNAzyme. Even in the presence of 1 mM of the competing metal ions, only a very small \(F/F_0\) (~2) was observed with Zn\(^{2+}\) and Cu\(^{2+}\). Other metal ions did not give any fluorescent signal increase with concentrations up to 1 mM. This GR-5 DNAzyme sensor is about 40000 times more selective for Pb\(^{2+}\) against Zn\(^{2+}\), the most active interfering metal ions.

To investigate the selectivity further, the fluorescent response to Pb\(^{2+}\) in the presence of a “metal soup” containing 100 \(\mu\)M of Zn\(^{2+}\), Mn\(^{2+}\), Cd\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Sr\(^{2+}\) and Ba\(^{2+}\) was tested for both sensors. For the 8–17 DNAzyme sensor (Fig. 2b inset), a high fluorescent enhancement was observed with the metal soup in the absence of Pb\(^{2+}\). Addition of 0.1 \(\mu\)M Pb\(^{2+}\) to the metal soup resulted in a similar fluorescent response, suggesting poor selectivity. Remarkably, for the GR-5 DNAzyme sensor, no fluorescent enhancement was observed with the metal soup without Pb\(^{2+}\), and \(\sim 3\)-fold fluorescent enhancement was observed with 0.1 \(\mu\)M of Pb\(^{2+}\). Therefore the new sensor based on the GR-5 DNAzyme has demonstrated an excellent selectivity for Pb\(^{2+}\) when tested against the metal ions individually or as a mixture of many metal ions.
In addition to selectivity, the sensitivity of the two sensors was also investigated and compared under the same condition. The kinetics of the 8–17 DNAzyme sensor shown in Fig. S4 is similar to those reported previously.\(^4\) In contrast, the GR-5 sensor displayed faster kinetics, as its signal reached saturation in about 100 s with 2 \(\mu\)M \(\text{Pb}^{2+}\) (Fig. S4b) while the 8–17 sensor took almost 200 s (Fig. S4a). The faster kinetics are advantageous for practical applications because it allows rapid detection. The initial rates were clearly faster for the GR-5 DNAzyme, as predicted by mfold. While the “rA” is base-paired to a T in the 8–17 DNAzyme, it is in a bulge region without any base pairing partner in the 8–17 DNAzyme. This difference may have contributed to its superior selectivity and sensitivity. Further investigations are under way to elucidate the origin of such high selectivity.

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### Notes and references