

# Investigation of metal-dependence in DNazymes and applications of DNazymes and aptamers for diagnostics

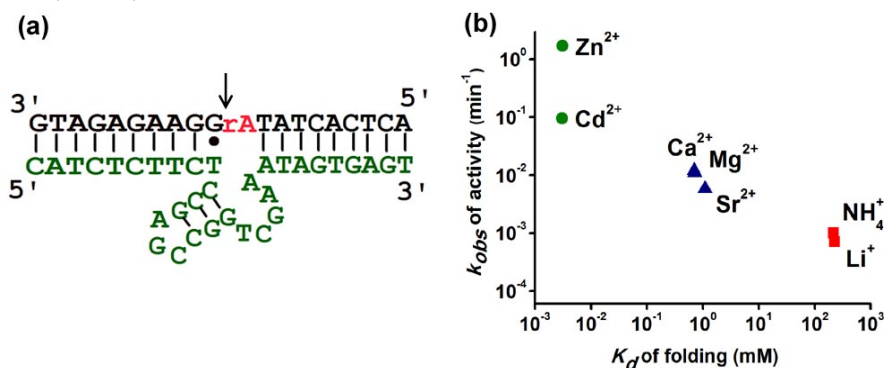
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DNazymes and aptamers are functional DNA molecules which can catalyze a reaction or can bind specifically to a chemical or biological target, respectively. They have not been found in nature and were first isolated by a combinatorial biology process called *in vitro* selection in the 1990's.<sup>1-3</sup> A large random pool of nucleic acid sequences ( $\sim 10^{14}$  sequences) is subjected to iterative rounds of selection and amplification. In each round, 'winner' sequences with the desired activity or binding affinity are separated and then amplified by polymerase chain reaction to be used for the next round of selection.

Most DNazymes are metalloenzymes which require divalent metal ion cofactors for optimum functionality; however, knowledge about the structural and functional roles of these metal ions as DNzyme cofactors is limited. *In vitro* selection has been utilized previously in our laboratory to obtain  $\text{Pb}^{2+}$ -,  $\text{Zn}^{2+}$ -, and even  $\text{UO}_2^{2+}$ -dependent RNA-cleaving DNazymes, which have been converted into practical biosensors for their respective metal ions.<sup>4-7</sup> These DNazymes can catalyze the cleavage of a DNA substrate with a single embedded RNA base that acts as the cleavage site. In order to test if *in vitro* selection can be used to impart metal specificity among a group of transition metal ions that share similar physical and chemical properties, selection for a  $\text{Cd}^{2+}$ -dependent DNzyme was performed. We found that the choice of the dinucleotide junction, which is engineered as the cleavage site, can have an impact on the metal ion specificity. A  $\text{Cd}^{2+}$ -specific DNzyme was obtained with 5'-rC↓T-3' at the cleavage site; however, when 5'-rA↓G-3' was employed as the cleavage site, the selection resulted in a DNzyme that was not only active with  $\text{Cd}^{2+}$ , but also had similar activity with other metal ions such as  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Ni}^{2+}$ .

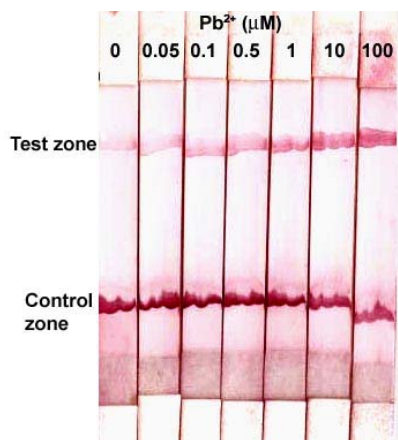


**Figure 1:** (a) Predicted secondary structure of the 8-17 DNzyme. The enzyme strand is shown in green and the substrate is in black with a single riboadenosine (in red) at the cleavage site (indicated by the arrow). (b) Correlation between activity and folding with different metal ions.

Although divalent metal-dependent catalysis is the most common mode of activation in nucleic acid enzymes, monovalent metal ion-dependent catalysis has been observed in some naturally occurring catalytic RNA, called ribozymes.<sup>8</sup> Biochemical assays of the *in vitro* selected

8-17 DNAzyme (Figure 1a),<sup>4,9</sup> which utilizes  $\text{Pb}^{2+}$  as its most efficient cofactor for catalysis, revealed detectable activity in the presence of 4 M  $\text{Li}^+$  and  $\text{NH}_4^+$  (observed rate constant,  $k_{\text{obs}} \sim 10^{-3} \text{ min}^{-1}$ ), which is  $\sim 1000$ -fold lower than the  $k_{\text{obs}}$  with  $\text{Mg}^{2+}$  and  $\sim 200,000$ -fold lower than the estimated  $k_{\text{obs}}$  with  $\text{Pb}^{2+}$  at the same pH. This result is different from some naturally occurring RNA catalysts such as the hammerhead ribozyme, where the activity in the presence of monovalent ions can approach those observed for divalent metal ion-dependent activity. In addition to activity, metal ion-dependent global folding and structural changes were monitored using fluorescence resonance energy transfer (FRET) and circular dichroism (CD) respectively, and the results were compared to divalent metal ion cofactors. We found that monovalent ions which have the lowest charge density also have the weakest  $K_d$ , obtained from both FRET and CD ( $\sim 100 \text{ mM}$ ), as well as the lowest activity. In contrast, the divalent transition metal ions that have higher Z/r ratio show the strongest  $K_d$  ( $\sim 10^{-3} \text{ mM}$ ) and the highest activity (Figure 1b), suggesting that the observed folding and structural changes are at least partially due to electrostatic interactions between the metal ions and the DNAzyme. Surprisingly, the DNAzyme did not show any folding<sup>10</sup> or structural changes with  $\text{Pb}^{2+}$ , implying that  $\text{Pb}^{2+}$ -dependent activity follows a different mechanism as compared to other metal ions.

Practical applications of DNA in diagnostics and sensing were examined using DNAzymes and aptamers. By labeling DNAzymes and aptamers with colored gold nanoparticles, our laboratory had previously constructed colorimetric sensors for analytes recognized by these nucleic acids.<sup>11,12</sup> In order to make these sensors more user-friendly, dipstick tests similar to the home pregnancy tests, were developed using lateral devices. Aptamers specific for adenosine and cocaine were used to develop diagnostic tests for these molecules, which were capable of working in human serum.<sup>13</sup> The 8-17 DNAzyme was used to develop a dipstick test for  $\text{Pb}^{2+}$ , in which the substrate was labeled with gold nanoparticles. The test for  $\text{Pb}^{2+}$  was based on selective capture of the uncleaved substrate at a control zone and capture of the cleaved substrate at a test zone on the device, leading to accumulation of gold nanoparticles at those areas, producing red lines (Figure 2). The applicability of this test in the detection of  $\text{Pb}^{2+}$  in paint in accordance with the federally defined threshold for paint classified as ‘lead-based’ was demonstrated. Apart from the ease-of-use and storage, the dipstick tests were  $\sim 10$  times more sensitive than solution-based colorimetric tests using gold nanoparticles for the detection of analytes with the naked eye.



**Figure 2:** ‘Dipstick’ tests for  $\text{Pb}^{2+}$ . A single red line at the control zone is observed in the absence of  $\text{Pb}^{2+}$ . As the concentration of  $\text{Pb}^{2+}$  is increased, a second red line of increasing intensity appears at the test zone.

## References

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