

## IN VITRO SELECTION OF HIGHLY EFFICIENT DNA ENZYMES AS RNA NUCLEASES AND METAL BIOSENSORS

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Nucleic acid enzymes are RNA and DNA molecules that can catalyze chemical reactions. Since the discovery of RNA enzymes in the early 1980's,<sup>1,2</sup> tremendous work has been carried out to elucidate the chemical and structural basis of their catalytic power. Most nucleic acid enzymes require divalent metal ions, especially  $Mg^{2+}$  and  $Ca^{2+}$ , for structure and catalysis.<sup>3</sup> A better understanding of the role of metal ions in their folding and catalysis will assist in the design of nucleic acid enzymes with higher efficiency or for special needs. So far, our knowledge of the metal binding sites in nucleic acid enzymes is limited by the low metal binding affinity and the lack of spectroscopic properties of  $Mg^{2+}$  and  $Ca^{2+}$  ions.

The ability of some RNA enzymes to catalyze sequence-specific cleavage of phosphodiester bonds allows for potential application as ribonucleases for RNA manipulation and as therapeutic agents against cancer, or viral and genetic diseases.<sup>4</sup> However, these applications have been hindered by their low activity, poor stability and high cost. To overcome these limitations, we applied the *in vitro* selection technique to isolate DNA enzymes that use transition metal ions (such as  $Zn^{2+}$ ) as cofactors for RNA substrate cleavage. Isolating DNA enzymes that are specific for a certain metal ion also permits the generation of DNA-based metal ion sensors.

*In vitro* selection is a combinatorial approach that allows RNA or DNA molecules with certain functions to be isolated from a large number ( $10^{13}$ - $10^{16}$ ) of sequence variants through multiple cycles of selection and amplification.<sup>5-7</sup> After 12 rounds of selection, we obtained a DNA enzyme, named 17E (Figure 1).<sup>8</sup> 17E consists of a catalytic core and two substrate-binding regions. It has a broad choice of cleavage targets and can catalyze the cleavage of any RNA or DNA/RNA chimeric substrates containing a guanosine (G) following a ribonucleotide (N).

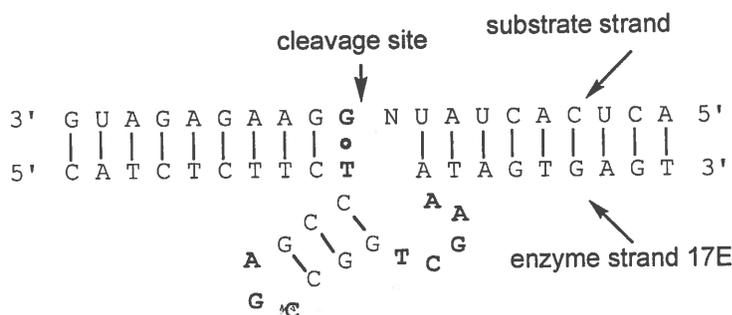
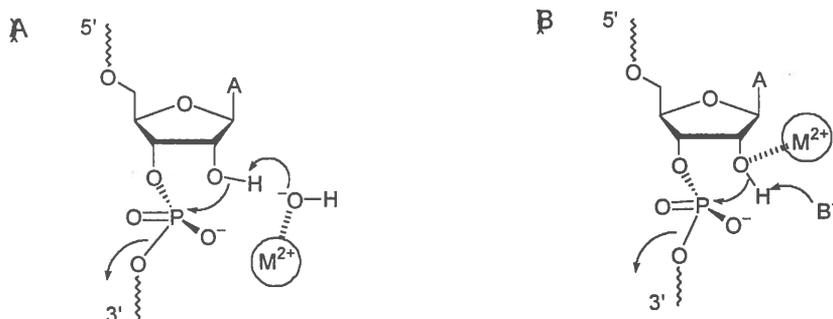


Figure 1

The catalytic rate of 17E surpasses that of most nucleic acid enzymes under similar conditions.<sup>8</sup> It undergoes a reaction pathway similar to that of small RNA enzymes, in which a 2'-OH group in the substrate nucleophilically attacks the scissile phosphate.<sup>9</sup> 17E is much more active in the presence of transition metal ions (especially  $Zn^{2+}$ ) than with alkaline-earth

metal ions. The higher reactivity of 17E with transition metal ions compared to alkaline-earth metal ions is probably due to the more efficient deprotonation of the 2'-OH group with the metal ion acting either as a general base or as a Lewis acid (Figure 2).<sup>10</sup>



**Figure 2**

Fluorescence resonance energy transfer (FRET) spectroscopy was carried out to study the structural role of divalent metal ions on 17E and to monitor the kinetics of substrate cleavage. The efficiency of resonance energy transfer increased with the concentration of divalent metal ions, revealing a metal-ion-induced conformational change, probably due to the two enzyme-substrate binding regions moving closer together upon the binding of divalent metal ions.

Although 17E was selected using  $Zn^{2+}$  as the metal ion cofactor, it has higher activity and higher affinity with  $Pb^{2+}$  ions than with other divalent metal ions. Taking advantage of the high selectivity of 17E towards  $Pb^{2+}$  and the high sensitivity of fluorescence detection, we have developed a DNA enzyme-based fluorescent chemosensor for  $Pb^{2+}$  ions (Figure 3).  $Pb^{2+}$  ion is toxic even at concentrations as low as 100 ppm ( $\sim 500$  nM) in the blood.<sup>11</sup> Laboratory techniques routinely used for lead analysis, such as atomic absorption spectrometry, inductively coupled plasma mass spectrometry and anodic stripping voltametry, require sophisticated equipment, sample pretreatment and skilled operators. A simple, highly selective and sensitive method that permits real time detection of  $Pb^{2+}$  is important in the fields of environmental monitoring, clinical toxicology, wastewater treatment, and industrial process monitoring.

The DNA enzyme-based  $Pb^{2+}$ -sensor we developed is highly specific and able to measure the presence of  $Pb^{2+}$  ions over a concentration range of three orders of magnitude, with a detection limit as low as 10 nM. The presence of other divalent metal ions does not inhibit the  $Pb^{2+}$ -sensing ability. This system represents the first example of a DNA-based biosensor for metal ion detection. Similar approaches can be applied to develop DNA enzyme-based biosensors for probing other toxic metal ions and at various concentration ranges.

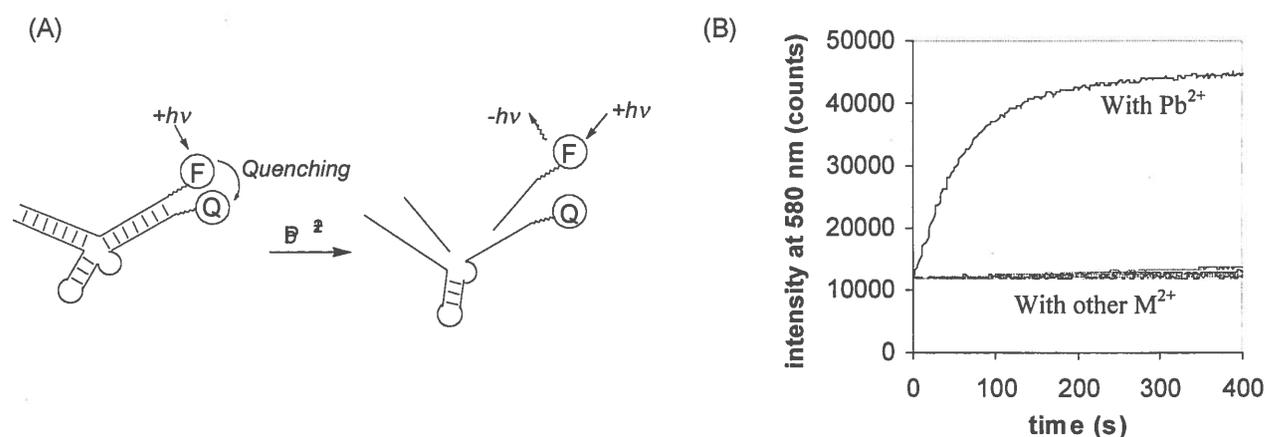


Figure 3

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