Development of Functional DNA-Based Sensors and Investigations into Their Mechanism

Nandini Nagraj Final Seminar January 21, 2010

Discoveries that nucleic acids can perform functional roles in addition to being carriers for genetic materials have opened doors to a new paradigm in nucleic acid chemistry.[1] Catalytic DNA molecules known as deoxyribozymes or DNAzymes were first isolated in 1994[2] through an *in vitro* selection procedure and have since been engineered and isolated to perform various functions.[3] The 8-17 DNAzyme is an RNA-cleaving DNAzyme that has been isolated under different selection conditions.[4-6] It exhibits activity in the presence of Pb^{2+} which is ~80-fold higher than its activity with other metal ions.[7]

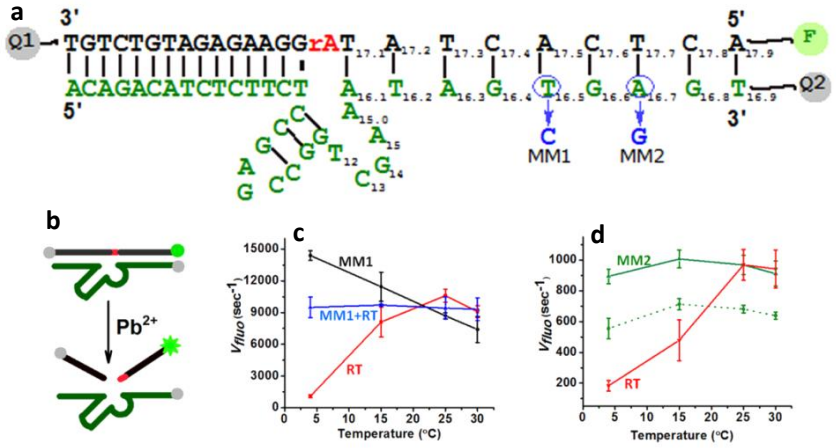

Figure 1: a) Predicted secondary structures of the fluorescent DNAzyme lead sensors. F represents the fluorophore, fluorescein or FAM, Q1 is the quencher BHQ-1® and Q2 is the DABCYL quencher. The single RNA base on the substrate arm is denoted by rA; b) Schematic representation of the fluorescent Pb^{2+} sensor; c) Temperature-dependent initial rate of substrate release of RT (sensor that contains no mismatch), MM1 (sensor that contains an A·C mismatch), and (4:1) MM1: RT upon addition of 2 µM Pb^{2+} and d) MM2 (sensor that contains a G·T mismatch) upon addition of 750 nM (solid line), 500 nM Pb^{2+} (dotted line) and RT upon addition of 750 nM Pb^{2+} in 50 mM HEPES with 100 mM NaCl at pH 7.2.

Fluorescent sensors based on the 8-17 DNAzyme for Pb^{2+}-detection have been previously developed.[8, 9] A critical barrier, however, of these sensors for practical applications, such as environmental monitoring, is their highly variable sensing performance with changing temperatures, due to the reliance of sensor design on temperature-dependent hybridization. This issue has been addressed through the use of the intrinsic lowered stability of mismatches on the DNA hybridization arms of the original 8-17 DNAzyme catalytic beacon sensor (referred to as RT).[10] In the process, it has been shown that the A·C mismatch (on MM1 sensor) and G·T wobble pair (on MM2 sensor) can both be utilized, albeit with different strategies, in tuning the

temperature response of the sensor. While a mixture of the RT and the MM1 sensors is required to show a relatively linear temperature response from 4-30 °C; MM2 alone can also exhibit this response as shown in Figure 1.

Currently, there is no crystal or NMR structure available for the 8-17 DNAzyme. Understanding the role of metal ions in its mechanism has, therefore, posed a challenge, particularly with regard to the high selectivity of Pb^{2+} for the catalytic activity of the DNAzyme. Hence, activity, folding and structural studies with both monovalent and divalent metal ions have been carried out which indicate that there is a clear trend that exists between the folding and activity of all the metal ions studied, the lower the activity, the lesser the folding and vice-versa. Structural studies based on CD and the folding studies based on FRET have both demonstrated that Pb^{2+} behaves in a manner that is different from other metal ions and hence it is hypothesized that the 8-17 DNAzyme might have evolved to have a stringent requirement for divalent metal ions and may have a binding pocket for Pb^{2+}.[11-13]

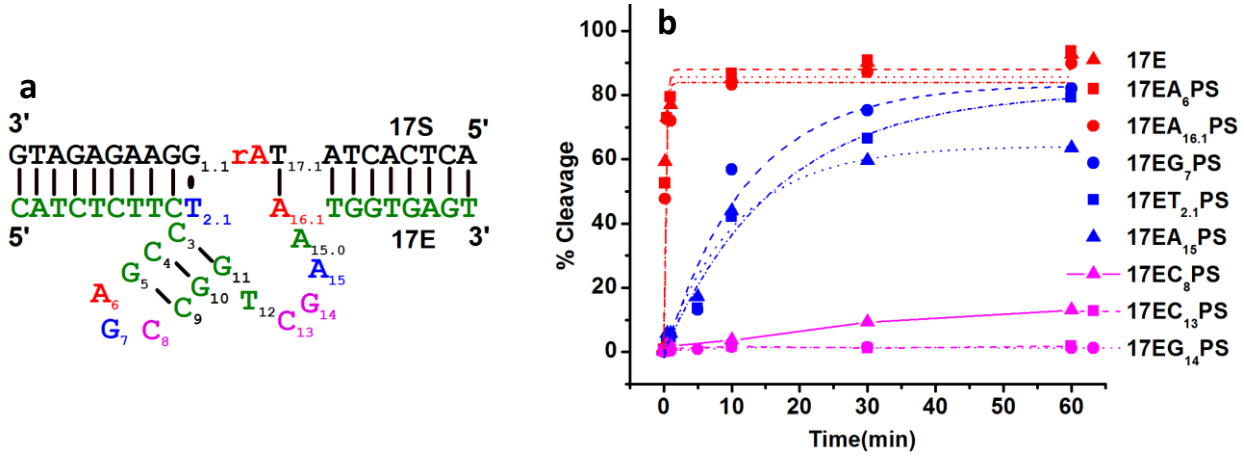

Figure 2: a) Secondary structure of the 8-17 DNAzyme showing positions of bases wherein individual phosphorothioate (or PS) modifications have been made; b) Effect of individual PS modifications on the substrate cleavage of the 8-17 DNAzyme, in the presence of 100 μM of Pb^{2+} in 50 mM MES,100 mM NaCl at pH 6.0.

Systematic phosphorothioate (PS) modifications on the backbone of the 8-17 DNAzyme have been carried out to investigate the role of backbone phosphates in the formation of a potential metal ion binding site for Pb^{2+}. Kinetic activity assays of these PS-modified enzyme-substrate complexes with Pb^{2+} have shown that specific bases on the enzyme strand are extremely perturbed upon the PS modification, and show no activity (magenta in Figure 2) while modifications on other bases show marginal perturbation (blue in Figure 2) and almost no perturbation (red on the enzyme strand in Figure 2) in comparison with the unmodified 17E. The activities of the identical PS modified enzymes are, however, not significantly altered in the presence of Mg^{2+} and Cd^{2+}. These results indicate that the phosphates of the highly perturbed bases maybe involved in metal binding, specifically towards Pb^{2+} in comparison with Cd^{2+} and Mg^{2+}. To complement these findings, ^{31}P NMR has also been used as an additional tool to directly visualize the backbone phosphates since significant shifts of the phosphate signal are obtained upon single PS modifications.

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