

Amplified Analysis of Low-Molecular-Weight Substrates or Proteins by the Self-Assembly of DNAzyme–Aptamer Conjugates

Di Li, Bella Shlyahovsky, Johann Elbaz, and Itamar Willner*

Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

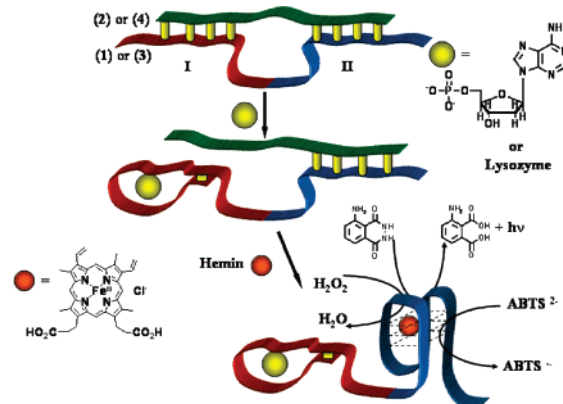
Received January 10, 2007; E-mail: willnea@vms.huji.ac.il

The SELEX (systematic evolution of ligand by exponential enrichment) procedure enables the selection and synthesis of specific binding nucleic acids (aptamer) or of catalytic nucleic acids.¹ Numerous nucleic acids that specifically bind to proteins, for example, thrombin, or to low-molecular-weight substrates, for example, cocaine or adenosine, were developed.² Similarly, catalytic nucleic acids such as DNAzymes that stimulate the activity of peroxidase³ or induce hydrolytic reactions⁴ were developed. The specific binding properties of aptamers were employed to develop sensors,⁵ while DNAzymes were used as labels that amplify biosensing events.⁶ Indeed, a variety of aptamer-based electrochemical,^{7,8} optical,^{9,10} and microgravimetric¹¹ sensors for low-molecular-weight substrates or proteins were developed in the past few years.

Among the DNAzymes acting as amplifying labels for bio-recognition events, the horseradish peroxidase-mimicking DNAzyme that consists of hemin intercalated in a G-quadruplex structure attracted special efforts. This DNAzyme was used to catalyze the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS²⁻, by H₂O₂ to the ABTS^{••} colored product⁶ or to stimulate the generation of chemiluminescence in the presence of H₂O₂/luminol.¹² Recently, the horseradish peroxidase-mimicking DNAzyme was used to amplify the analysis of nucleic acids by a DNA-based machine.¹³ Here, we wish to report on a new method to detect a low-molecular-weight substrate (adenosine 5'-monophosphate, AMP) or a protein (lysozyme) by a DNAzyme-aptamer/analyte hybrid complex. The DNAzyme provides the colorimetric or chemiluminescence readout signals for the formation of the aptamer–analyte complex.

Scheme 1 depicts the principles of the analytical process for analyzing AMP or lysozyme. The nucleic acids **1** or **3** include in their sequence the region I that consists of the aptamer for AMP or lysozyme, while the region II is composed of the base sequence that in the presence of hemin forms the horseradish peroxidase-mimicking DNAzyme. The nucleic acids **1** or **3** are hybridized with blocking nucleic acids **2** or **4** that are partially complementary to the DNAzyme sequence, and partially complementary to the aptamer regions. The “blocker” nucleic acid was designed to include two separate nine-base complementary domains to **1** or **3** that result in a cooperative binding. The separation of any hybridized domain through the formation of the aptamer–substrate complex results in the thermal melting of the remaining nine-base duplex. The hybridization of the blocker with the DNAzyme–aptamer domains minimizes the formation of any free hemin-containing active DNAzyme. Addition of the substrate for the aptamer region results in the dehybridization and the formation of the respective aptamer–substrate complex. This reaction depletes, however, the stabilization of the “blocker”–DNAzyme complex, and consequently, the DNAzyme sequence is separated from the “blocker”. The separated sequence self-assembles, in the presence of hemin, to the DNAzyme unit that amplifies the detection of the substrate through the

Scheme 1. An Amplified Aptasensor Based on the Generation of a DNAzyme upon the Formation of the Respective Aptamer–Substrate Complexes^a



^a The sequences of the respective nucleic acids are given as Supporting Information.

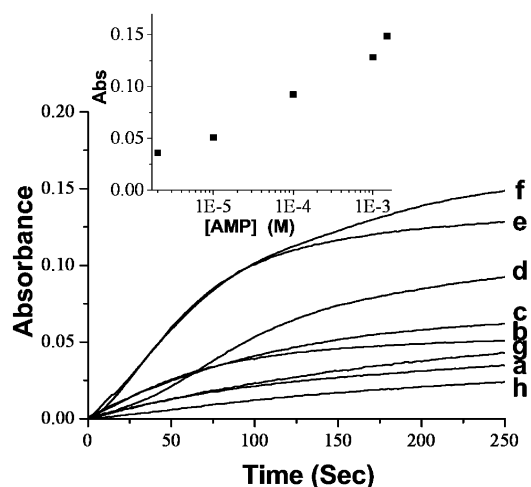


Figure 1. Time-dependent absorbance changes upon analyzing different concentrations of the AMP: (a) 0 M, (b) 2×10^{-6} M, (c) 1×10^{-5} M, (d) 1×10^{-4} M, (e) 1×10^{-3} M, (f) 1.5×10^{-3} M. In all systems, $[1] = 3 \times 10^{-7}$ M, $[2] = 6 \times 10^{-7}$ M, $[hemin] = 7 \times 10^{-7}$ M, $[ABTS^{2-}] = 2$ mM, $[H_2O_2] = 2$ mM, in 25 mM Tris acetate buffer that included 300 mM NaCl, pH = 8.2. Curve g corresponds to the response of the 1/2 system to cocaine, 1×10^{-3} M. Curve h shows the color developed by the hemin in the absence of 1/2 and AMP. The inset is the calibration curve.

biocatalyzed oxidation of ABTS²⁻ or the generation of chemiluminescence, in the presence of H₂O₂/luminol.

Figure 1 shows the time-dependent absorbance changes in the system upon analyzing different concentrations of AMP. As the concentration of AMP increased, the color generated by the system was intensified, implying enhanced dissociation of the “blocker”/1 complex and higher content of the DNAzyme. The response of the system in the absence of AMP is shown in Figure 1, curve a. The

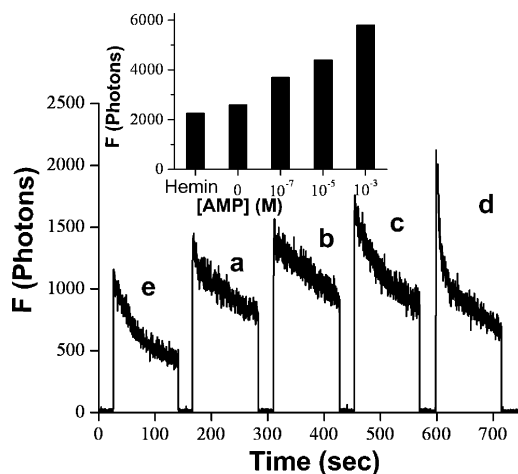


Figure 2. Time-dependent chemiluminescence generated upon the analysis of different concentrations of AMP by the 1/2 system: (a) 0 M, (b) 1×10^{-7} M, (c) 1×10^{-5} M, (d) 1×10^{-3} M, (e) light emitted by hemin only. The experimental conditions are identical to those in Figure 1, except that ABTS²⁻ was substituted by luminol, 2 mM. The inset shows the integrated light intensities (after 15 s) upon analyzing different concentrations of AMP.

resulting absorbance is almost similar to that generated by free hemin and may be considered as the background signal.

In further control experiments, the foreign substrates cocaine or cytidine monophosphate (CMP) were added to the system consisting of the 1/2. In these experiments, for example, cocaine resulted in the color changes observed in the absence of AMP, Figure 1, curve g, indicating that the dissociation of the “blocker”/1 complex is specific for AMP. Figure 1, inset, depicts the derived calibration curve. The detection limit for analyzing AMP corresponded to 4×10^{-6} M, a value that is improved as compared to other configurations of AMP aptasensors.¹⁴ At the highest concentration of AMP, we estimate that ca. 80% of the blocked aptamer is dissociated (see Supporting Information).

The analysis of AMP by the blocked DNAzyme–aptamer hybrid was also examined by the DNAzyme-generated chemiluminescence upon formation of the AMP–aptamer complex. Figure 2 shows the integrated light intensities generated upon the analysis of different concentrations of AMP by the 1/2 complex in the presence of luminol/H₂O₂ as cosubstrates for the DNAzyme. As the concentration of AMP increased, the emitted light intensities were intensified.

This approach was further applied to analyze lysozyme. The nucleic acid sequence 3 includes the domain that acts as an aptamer for lysozyme ($K_d = 31$ nM)¹⁵ and a region that assembles the horseradish peroxidase-mimicking DNAzyme in the presence of hemin. Figure 3 depicts the time-dependent spectral changes observed upon analyzing different concentrations of lysozyme. A control experiment revealed that the sensing of lysozyme is specific, and upon the analysis of the foreign protein, BSA, only the background color was observed (Figure 3, curve f). Figure 3, inset, shows the calibration curve for analyzing lysozyme. The detection limit for analyzing lysozyme is 1×10^{-13} M.

To conclude, the present study has introduced a new concept to analyze low-molecular-weight substrates or proteins by the design of nucleic acid composites consisting of blocked DNAzyme–

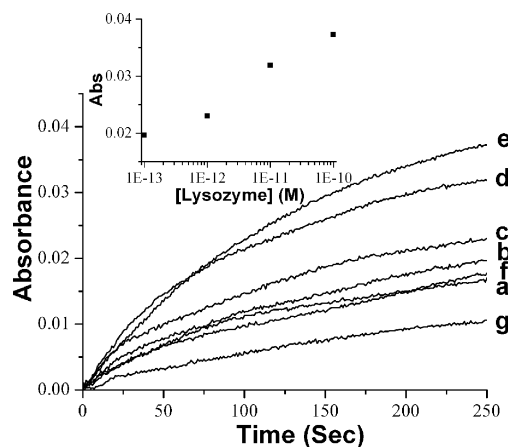


Figure 3. Time-dependent absorbance changes upon analyzing different concentrations of lysozyme: (a) 0 M, (b) 1×10^{-13} M, (c) 1×10^{-12} M, (d) 1×10^{-11} M, (e) 1×10^{-10} M. The experimental conditions are identical to those in Figure 1, except that 50 mM Tris buffer that included 100 mM NaCl, pH = 7.4, was used. Curve f corresponds to the analysis of BSA, 1×10^{-10} M, while curve g shows the color developed by the hemin in the absence of 3/4 and lysozyme.

aptamer chains that lead to the amplified biocatalytic readout of the formation of the aptamer–analyte complex. The detection limit for analyzing AMP is better than that reported for an optical aptasensor,¹⁴ while the detection limit for analyzing lysozyme is ca. 100-folds improved in the present system as compared to the reported electrochemical aptasensor.¹⁶

Acknowledgment. This research is supported by the Johnson & Johnson fund for innovative research.

Supporting Information Available: The sequences 1, 2, 3, and 4; calibration curve corresponding to the DNAzyme activity. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Osborn, S. E.; Ellington, A. D. *Chem. Rev.* **1997**, *97*, 349–370.
- (2) Klussmann, S. *The Aptamer Handbook: Functional Oligonucleotides and Their Applications*; Wiley-VCH: Weinheim, Germany, 2006.
- (3) (a) Travascio, P.; Li, Y. F.; Sen, D. *Chem. Biol.* **1998**, *5*, 505–517. (b) Travascio, P.; Bennet, A. J.; Wang, D. Y.; Sen, D. *Chem. Biol.* **1999**, *6*, 779–787.
- (4) Breaker, R. R. *Nature* **2004**, *432*, 838–845.
- (5) Tombelli, S.; Minunni, M.; Mascini, M. *Biosens. Bioelectron.* **2005**, *20*, 2424–2434.
- (6) Xiao, Y.; Pavlov, V.; Niazov, T.; Dishon, A.; Kotler, M.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 7430–7431.
- (7) Xiao, Y.; Piorek, B. D.; Plaxco, K. W.; Heeger, A. J. *J. Am. Chem. Soc.* **2005**, *127*, 17990–17991.
- (8) Radi, A. E.; Sanches, L. A.; Baldrich, E.; O’Sullivan, C. K. *J. Am. Chem. Soc.* **2006**, *128*, 117–124.
- (9) Ho, H. A.; Leclerc, M. *J. Am. Chem. Soc.* **2004**, *126*, 1384–1387.
- (10) Kirby, R.; Cho, E. J.; Gehrke, B.; Bayer, T.; Park, Y. S.; Neikirk, D. P.; McDevitt, J. T.; Ellington, A. D. *Anal. Chem.* **2004**, *76*, 4066–4075.
- (11) Pavlov, V.; Xiao, Y.; Shlyahovsky, B.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 11768–11769.
- (12) Pavlov, V.; Xiao, Y.; Gill, R.; Dishon, A.; Kotler, M.; Willner, I. *Anal. Chem.* **2004**, *76*, 2152–2156.
- (13) Weizmann, Y.; Beissenhirtz, M. K.; Cheglakov, Z.; Nowarski, R.; Kotler, M.; Willner, I. *Angew. Chem., Int. Ed.* **2006**, *45*, 7384–7388.
- (14) Liu, J. W.; Lu, Y. *Angew. Chem., Int. Ed.* **2006**, *45*, 90–94.
- (15) Cox, J. C.; Ellington, A. D. *Bioorg. Med. Chem.* **2001**, *9*, 2525–2531.
- (16) Hansen, J. A.; Wang, J.; Kawde, A.-N.; Xiang, Y.; Gothelf, K. V.; Collins, G. J. *Am. Chem. Soc.* **2006**, *128*, 2228–2229.

JA070180D