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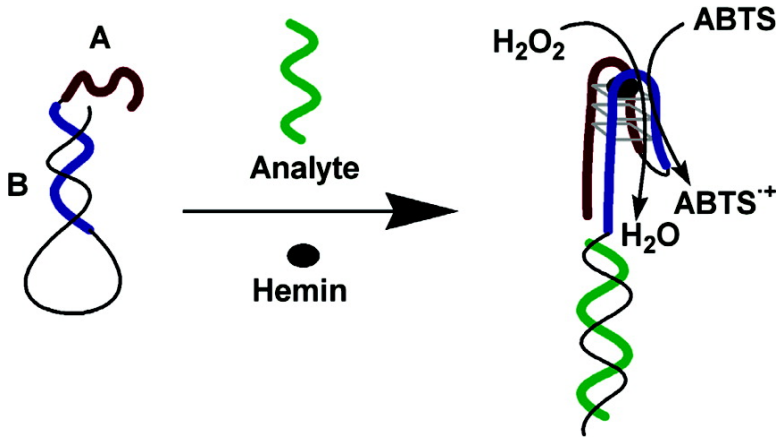

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Catalytic Beacons for the Detection of DNA and Telomerase Activity

Yi Xiao,[†] Valeri Pavlov,[†] Tamara Niazov,[†] Arnon Dishon,[‡] Moshe Kotler,[‡] and Itamar Willner*[,†]

The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, and Department of Pathology, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

Received December 21, 2003; E-mail: willnea@vms.huji.ac.il

The discovery of catalytic RNAs (ribozymes) sparked scientific interest directed to the preparation of new biocatalysts.[1,2] Analogous deoxyribozymes (catalytic DNAzymes) are not available in nature, but have been demonstrated synthetically.[3,4] An interesting DNAzyme that revealed peroxidase-like activities is a complex between hemin and a single-stranded guanine-rich nucleic acid (aptamer).[5] This complex catalyzed the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid, ABTS, by H_2O_2. It was suggested[6] that the intercalation of hemin into the complex results in the formation of the biocatalyst. We have shown that the hemin/G-quadruplex also mimics peroxidase by the generation of chemiluminescence in the presence of H_2O_2 and luminol.[7] The use of DNAzymes as catalytic labels for biosensing is attractive since nonspecific adsorption processes associated with protein-based labels are eliminated.

Nucleic acid beacons are extensively used as specific DNA sensing matrixes. The specific linkage of photoactive chromophores/quenchers to the hairpin termini results in the chromophore luminescence quenching. The subsequent lighting-up of the chromophore luminescence by the hybridization of DNA with the hairpin was used as a general motif for the photonic detection of DNA.[8] The quenching of dyes by molecular or nanoparticle quenchers[9] or the fluorescence resonance energy transfer (FRET) between dyes was used for the optical detection of the hybridization of the DNA to the beacon.[8] Recently, the labeling of the beacon termini with redox-active units led to the electrochemical detection of hybridization to the hairpins.[10] The development of catalytic beacons may provide a major advance in DNA sensing, and recently, efforts to apply beacon structures for the catalyzed sensing of the hybridization were reported.[11] Also, catalytic DNA coupled to gold nanoparticles was reported as a colorimetric sensor for lead ions.[12] Here we report on the tailoring of catalytic beacons for the sensing of DNA and telomerase activity originating from HeLa cancer cells. We design hairpin structures that upon opening yield, in the presence of hemin, DNAzymes that allow the biocatalytic detection of the hybridization process.

Scheme 1A depicts the method for applying the beacon (**1**) as a catalytic unit for the sensing of DNA (**2**).[13] The hairpin structure of (**1**) includes the sequence consisting of segments A and B that in an open configuration form the G-quadruplex with hemin that exhibits peroxidase-like activity. Since segment B is hybridized in the hairpin structure, the formation of the catalytic DNAzyme is prohibited. Hybridization of DNA (**2**) with the hairpin opens the beacon, and the released sequence (components A and B) self-assembles with hemin to form the catalytic DNAzyme that oxidizes ABTS (**3**) to the colored product (**4**) by H_2O_2. The hybridization and hairpin opening is detected spectroscopically by following the accumulation of (**4**) at $\lambda = 414$ nm ($\epsilon = 3.6 \times 10^4$ M^{-1} cm^{-1}). Figure 1, curve a, shows the time-dependent color evolution upon the analysis of DNA (**2**) 4.3 μM. Knowing the activity of the pure DNAzyme, we estimate that 85% of the beacon was opened. The control experiment, curve c, follows the spectral changes of the hairpin (**1**) in the presence of hemin, H_2O_2, and ABTS and does not lead to any development of a color. Also, the hybridization of (**2**) with a hairpin structure that lacks the B segment in the "hairpin

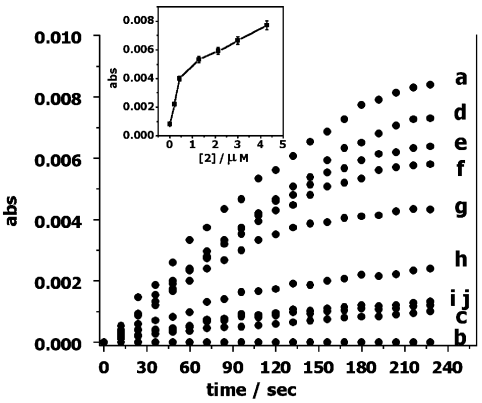
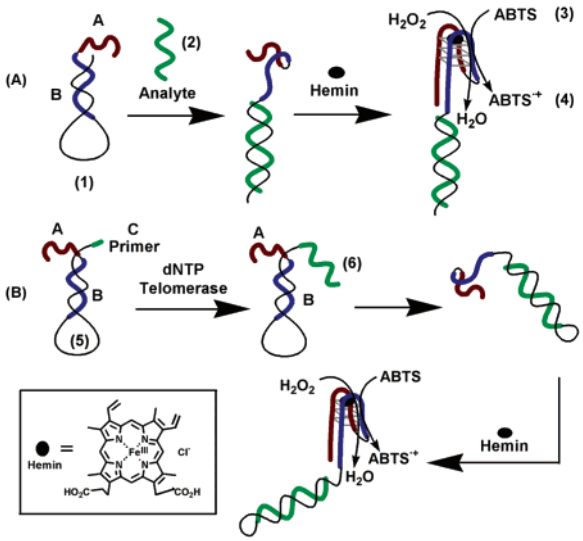


Figure 1. Absorbance changes originating from the formation of (**4**) upon analysis of: (a) (**2**), 4.3 μM. (b) Absorbance generated by hemin and (**2**), 4.3 μM, in the absence of (**1**). (c) Color formed by hemin and (**1**) without (**2**). (d)−(h) Analysis of variable concentrations of (**2**) corresponding to 3.0, 2.15, 1.30, 0.40, and 0.2 μM, respectively. (i) and (j) The analysis of the SNP mutations (**2a**) or (**2b**), 4.3 μM. Experiments were performed in the presence of (**1**), 0.43 μM, hemin, 0.43 μM, ABTS, 3.2 mM and H_2O_2, 3.2 mM in a 0.1 M Tris buffer solution, pH = 8.1, that included $MgCl_2$, 20 mM. Inset: Calibration curve corresponding to absorbance upon analyzing variable concentrations of (**2**) after a fixed time interval of 3 min.

Scheme 1. (A) Analysis of DNA by Opening of a Beacon Nucleic Acid and the Generation of a DNAzyme. (B) Analyzing Telomerase Activity by a Functional DNA Beacon that Self-Generates a DNAzyme



[†] The Institute of Chemistry.
[‡] Department of Pathology.

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stem" does not lead to an active DNAzyme. These results indicate that it is only upon the hybridization of (**2**) with the beacon (**1**) and its opening that the DNAzyme that oxidizes ABTS is generated. The extent of opening of the sensing beacons, and thus, the quantity of the generated DNAzyme, is controlled by the concentration of (**2**). Curves d−h of Figure 1 show the time-dependent evolution of the oxidized product (**4**), at variable concentrations of the analyzed DNA. As the concentration of (**2**) increases, the absorbance of (**4**) is higher. Figure 1, inset, shows the calibration curve obtained upon analyzing variable concentrations of (**2**) and monitoring the color accumulated. As expected, the biocatalytic process is enhanced as the concentration of (**2**) increases. The catalytic beacon reveals specificity, and single base mismatches may be discerned. For example, curves i and j of Figure 1 show the time-dependent accumulation of (**4**) upon analyzing the mutants (**2a**) and (**2b**), that include a single-base mismatch relative to the fully complementary analyzed DNA (**2**). Clearly, the signal for analyzing (**2**) is 8-fold higher than the signal for the mutants. The sensitivity limit is 0.2 μM (see Supporting Information).

Telomeres are nucleic acids consisting of constant repeat units at the ends of human chromosomes.[14] The telomeres protect the chromosomes, and their erosion during cell proliferation provides a cellular signal for the termination of the cell life cycle. Telomerase is a ribonucleoprotein that replicates the 3′-ends of the linear chromosomes with the telomere repeat units.[15] The accumulation of telomerase in cells results in the constant elongation of the telomeres, turning the cells into immortal units. In most cancer and malignant cells, increased levels of telomerase were detected, and telomerase is considered as an important biomarker for cancer.[16] Several analytical procedures for the determination of telomerase activity were developed, including the telomeric repeat amplification protocol (TRAP) that involves PCR amplification[17] or the functionalization of the telomeres with fluorescent labels.[18] We have applied a catalytic beacon as an active component for the analysis of telomerase activity (Scheme 1B).[19] The beacon (**5**) is designed to include at its two termini two functional nucleic acid components. One end of the hairpin structure ends with a nucleic acid that includes the base sequence that is a part of the DNAzyme in the presence of hemin (part A). The second part of the DNAzyme base sequence (part B) is "hidden" in the hybridized hairpin configuration. At the other end of the hairpin, a nucleic acid segment that is a primer for telomerase is tethered to the beacon (part C of the beacon). The single-stranded loop of the beacon is complementary to the telomere repeat units. Treatment of the beacons with HeLa cancer cell extract in the presence of the dNTP nucleotide mixture results in the telomerization of the hairpin end. The telomerization was confirmed by gel electrophoresis experiments that showed the extension of the primer attached to the beacon and the formation of telomeres of variable length. The elongated telomere self-generates the sequence for its hybridization with the complementary hairpin loop and leads to the beacon opening and to the generation of the DNAzyme. Thus, the telomerase activity is monitored by following ABTS oxidation by H_2O_2 upon opening of the hairpin structure.

Figure 2A, curve a, shows the time-dependent accumulation of the colored product (**4**) upon analyzing telomerase originating from 10 000 cells. Figure 2A, curve b, shows the results of the control experiment where the accumulation of (**4**) from a system that included a heat-treated deactivated telomerase (10 000 cells). Clearly, only residual formation of (**4**) is observed, presumably due to nonspecific binding of hemin to reaction components. The rate of the telomeres formation is controlled by the amount of telomerase

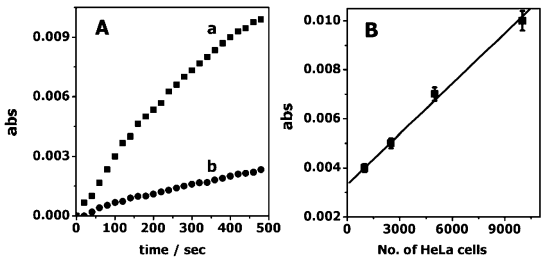

Figure 2. (A) Absorbance changes upon analyzing telomerase activity originating from: (a) 10 000 HeLa cells and (b) 10 000 heat-treated HeLa cells (95 °C, 10 min). In all experiments, the systems consisted of the catalytic beacon (**5**), 0.04 μM, hemin, 0.04 μM, ABTS, 3.2 mM and H_2O_2, 3.2 mM in 0.1 M Tris buffer solution, pH = 8.1, that included $MgCl_2$, 20 mM. (B) Calibration curve corresponding to absorbance changes of the system upon analyzing variable numbers of HeLa cells.

in the sample, and thus the accumulation of (**4**) is regulated by the number of analyzed HeLa cells. Figure 2B shows the absorbance of (**4**), obtained upon analyzing telomerase activity originating from different numbers of HeLa cells. (The absorbance of (**4**) is determined after a time interval of 8 min.) The detection limit of the HeLa cells is ca. 500 cells.

The present study has introduced a new concept of catalytic beacons for analyzing DNA and telomerase activity. The extension of the concept to include the DNAzyme linked to aptamers[20] for the detection of proteins is underway.

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Supporting Information Available: Sequences and results for analyzing the mutants (**2c**) and (**2d**). This material is available free of charge via the Internet at http://pubs.acs.org.

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