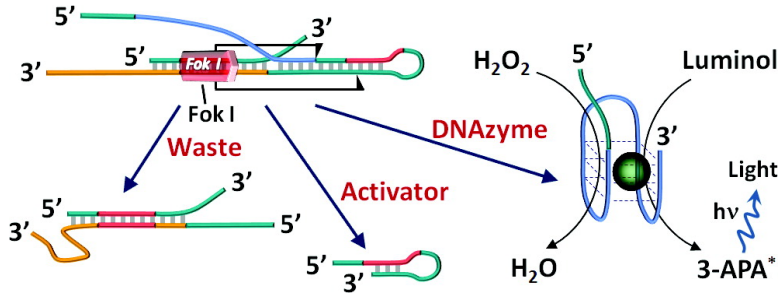
Communication

A Fok I/DNA Machine that Duplicates its Analyte Gene Sequence

Yossi Weizmann, Zoya Cheglakov, and Itamar Willner

J. Am. Chem. Soc., **2008**, 130 (51), 17224-17225 • DOI: 10.1021/ja806222e • Publication Date (Web): 02 December 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 12/02/2008

A Fok I/DNA Machine that Duplicates its Analyte Gene Sequence

Yossi Weizmann, Zoya Cheglakov, and Itamar Willner*

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

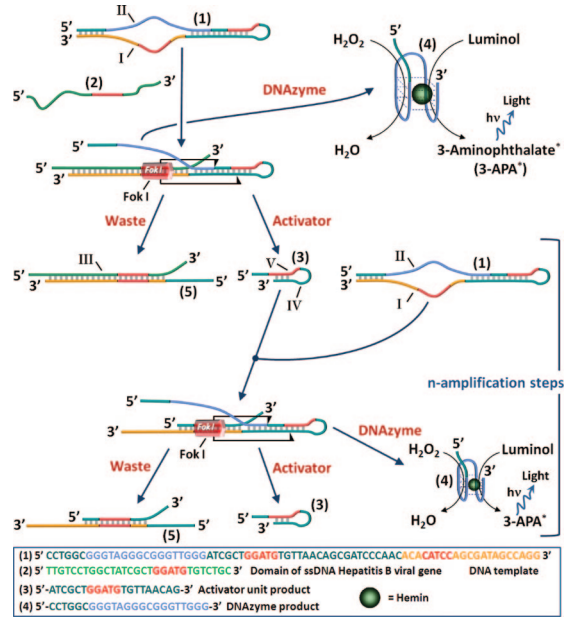
Received August 7, 2008; E-mail: willnea@vms.huji.ac.il

The structure and composition of nucleic acids dictate their recognition properties,[1] their catalytic functions,[2] and their sensitivity toward biocatalytic transformations. Recent research efforts use the structural information encoded in DNA to develop DNA-based machines,[3] amplified sensors,[4] and biomolecule-based logic gates.[5] Different sensor systems employed nucleic acids as an active structure for the detection of DNA,[6] low-molecular-weight substrates,[7] proteins,[8] or ions.[9] For example, the recognition of cocaine by its aptamer, or the association of Hg^{2+} to an oligothymine-functionalized nucleic acid, led to the activation of replication/scission reactions that enabled the optical detection of cocaine or Hg^{2+} ions, respectively.[7,9] Similarly, catalytic nucleic acids were extensively used for the amplified detection of DNA or metal ions. For example, the hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme[10] was used for the colorimetric or chemiluminescent detection of DNA,[11] and the Pb^{2+}-dependent DNAzyme was used for the amplified optical detection of Pb^{2+} ions.[12] Also, "DNA machines" that amplify the detection of DNA were developed. These included the use of a DNA track that, upon binding the target DNA, activated the autonomous replication/scission reactions on the template and the synthesis of the HRP-mimicking DNAzyme as a reporter.[13] Similarly, the recognition of a target DNA by a structurally designed DNA activated the biocatalytic cleavage of the resulting duplex and the initiation of the autonomous scission of a "fuel" substrate, a process that enabled the optical reporting of the primary recognition event.[14]

In the present study, we describe the use of a DNA template that recognizes a target DNA, which represents a sequence nucleic acid being a part of the Hepatitis B viral gene, that leads to the amplified analysis of the target gene. Amplification is accomplished by the activation of an autonomous biocatalytic process that degrades the template and yields a nucleic acid that includes the gene-fragment sequence acting as an activator for the cleavage of the fuel (**1**) and a HRP-mimicking DNAzyme. The latter product acts as a reporter for the gene sequence by the generation of chemiluminescence.

The method for the amplified detection of the DNA is schematically depicted in Scheme 1. A hairpin nucleic acid (**1**) was designed as a capture unit for the target DNA (the fragment of the hepatitis B gene) that activates, as a result of hybridization with the target DNA, the autonomous synthesis of the HRP-mimicking DNAzyme. The region I in **1** includes a complementary sequence to the target DNA (**2**), while region II includes the HRP-mimicking DNAzyme sequence. The formation of the active DNAzyme unit is, however, prevented because of partial hybridization between sequences I and II. The hybridization of **2** with the region I of **1** yields the duplex structure that includes the sequence-specific binding domain for Fok I (red). The association of the biocatalyst to this region results in the remote nonsymmetrical scission of the partially opened structure of **1** and to the release of the HRP-mimicking DNAzyme that yields, in the presence of hemin, the biocatalytic G-quadruplex/hemin structure, which catalyzes the oxidation of luminol by H_2O_2,

Scheme 1. Amplified Analysis of a Gene Domain by a Tailored Nucleic Acid Structure Acting As a "Fuel" for the Autonomous Activation of a Fok I/DNA Machine That Yields Reporter DNAzyme Units



and the generation of chemiluminescence. The scission process yields also the duplex structure III and the hairpin (**3**). The latter product, **3**, acts as the "key" for the activation of the autonomous cleavage of **1** and the generation of the DNAzyme units. The hairpin, **3**, includes a domain IV (blue) that is complementary to domain I (orange) of **1**. In fact, the loop domain and the duplex stem IV in **3** consist of the base sequence of the analyte, the hepatitis B gene. That is, the recognition of the analyte duplicates the gene domain embedded in the structure of **1** that acts as fuel. The interaction (hybridization) of the activator (**3**) with the fuel (**1**) gives then, rise to the Fok I-stimulated autonomous regeneration of the "gene fragment" and the release of the HRP-mimicking DNAzyme that acts as a reporter. It also includes a sequence V (red) that, upon the formation of the duplex with **1**, yields the Fok I binding site. Accordingly, the formation of **3** activates the autonomous cleavage of **1**, accompanied by the synthesis of the HRP-mimicking DNAzyme and the reformation of **3**. One may consider **3** as a "key" that cleaves **1** while yielding the reporting DNAzyme and a new "key" for the cleaving element. Several points should be clarified: (i) The catalytic activity of the DNAzyme sequence is blocked by the hairpin structure (**1**). (ii) Following our design, the hairpin domain (IV) in structure **3**, and also structure (**1**), includes the sequence of the hepatitis B gene. This region could, in principle, hybridize with domain I of the template (**1**), a process that would open the hairpin structure of **1** and release the DNAzyme, even in the absence of the analyte (**2**). This redundant path is, however,

10.1021/ja806222e CCC: $40.75

prevented by the tight and stable duplex structure of **1** that cannot be opened by hybridization with the recognition sequence. (iii) The design of the cleavable substrate **1** seems to be complex, and eventually, special for a specific gene. The method is, however, very general and could be applied for almost any gene. One has to identify the Fok I specific sequence GGATG in the target gene and to tailor subsequently the template and the fuel substrate.

Figure 1A shows the time-dependent increase in the chemiluminescence intensities, using a constant concentration of **2**, by operating the Fok I-activated system for different time intervals. As the autonomous generation of the HRP-mimicking DNAzyme is prolonged, the resulting chemiluminescence signals are intensified. The depletion of the DNA template upon its time-dependent scission by **3** was further confirmed by gel electrophoresis experiments (Supporting Information (SI), Figure 1S).

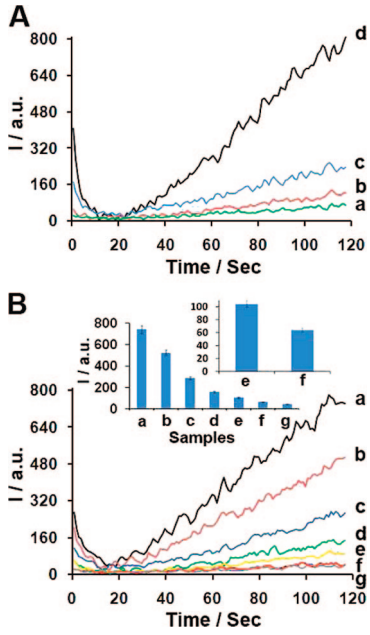


Figure 1. (A) Chemiluminescence intensities observed upon the oxidation of luminol by H_2O_2 by the DNA-based machine as depicted in Scheme 1, at different time intervals and at the fixed concentrations of (**1**) 1×10^{-6} M and (**2**) 1×10^{-6} M: (a) 0 min, (b) 30 min, (c) 90 min, (d) 120 min. (B) Chemiluminescence intensities observed upon oxidation of luminol by H_2O_2 and the DNAzyme synthesized by the DNA-based machine as depicted in Scheme 1. Different concentrations of **2** were used: (a) 1×10^{-6}, (b) 1×10^{-8}, (c) 1×10^{-9}, (d) 1×10^{-12}, (e) 1×10^{-14} M; (f) analysis of the single-stranded M13 phage DNA, 1×10^{-9} M and (g) absence of the target DNA **2**. Inset: chemiluminescence intensities upon analyzing different concentrations of **2** in a fixed time interval of 120 min. In all systems, fixed concentrations of **1** (1×10^{-6} M) were employed.

Accordingly, the quantitative analysis of DNA was performed by interacting template (**1**) with variable concentrations of **2** and allowing the system to yield the transducing HRP-DNAzyme units (by the autonomous scission) for a fixed time interval of 120 min. The chemiluminescence generated by the different systems was then recorded (for experimental details, see SI). Figure 1B depicts the time-dependent chemiluminescence intensities upon analyzing different concentrations of **2**. As the concentration of **2** increases, the resulting chemiluminescence signals are intensified (see also Figure 1B, inset). Detailed analysis of the chemiluminescence intensities confirmed the cyclic formation and release of the reporting DNAzyme units (see SI, Figure 2SA and B). Control experiments revealed that the system yields a minute chemiluminescence signal in the absence of the analyte (**2**), Figure 1B curve (g), implying that **1** is opened only in the presence of the analyte and that the DNAzyme sequence is tightly blocked in the hairpin structure. Similarly, challenging of the template (**1**) with the single-stranded M13 phage DNA does not lead to any significant chemiluminescence, Figure 1B curve (f), indicating that appropriate design of the hairpin structure leads to specific analysis of the target (**2**). The system enabled the analysis of **2** with a sensitivity that corresponded to 1×10^{-14} M, a value that is comparable to the sensitivities of recently reported "DNA machines".

In conclusion, the present study has introduced a new approach to amplify the detection of DNA. The concept is based on the design of a template DNA that, upon the recognition of a DNA, activates the biocatalytic generation of a "key" nucleic acid that autonomously leads to the cleavage of the template and to the generation of HRP-mimicking DNAzyme units as reporter units.

Acknowledgment. This study is supported by the Israel Science Foundation and by the Canadian Institute for the Advancement of Research (CIFAR).

Supporting Information Available: Experimental protocols for the enzymatic processes, chemiluminescence measurements, and gel electrophoresis experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Breaker, R. R. *Curr. Opin. Chem. Biol.* **1997**, *1*, 26–31. (b) Luzi, E.; Minunni, M.; Tombelli, S.; Mascini, M. *Trends Anal. Chem.* **2003**, *22*, 810–818. (c) Ellington, A. D.; Szostak, J. W. *Nature* **1990**, *346*, 818–822. (d) Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505–510. (e) Famulok, M.; Mayer, G.; Blind, M. *Acc. Chem. Res.* **2000**, *33*, 591–599.
(2) (a) Lu, Y. *Acc. Chem. Res.* **2007**, *40*, 315–323. (b) Lu, Y.; Liu, J. *Curr. Opin. Biotechnol.* **2006**, *17*, 580–588. (c) Mao, C. D.; Sun, W. Q.; Shen, Z. Y.; Seeman, N. C. *Nature* **1999**, *397*, 144–146. (d) Yurke, B.; Turberfield, A. J.; Mills, A. P.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605–608.
(3) (a) Bath, J.; Turberfield, A. J. *Nat. Nanotechnol.* **2007**, *2*, 275–284. (b) Beissenhirts, M. K.; Willner, I. *Org. Biomol. Chem.* **2006**, *4*, 3392–3401.
(4) Willner, I.; Shlyahovsky, B.; Zayats, M.; Willner, B. *Chem. Soc. Rev.* **2008**, *37*, 1157–1165.
(5) (a) Stojanovic, M. N.; Mitchell, T. E.; Stefanovic, D. *J. Am. Chem. Soc.* **2002**, *124*, 3555–3561. (b) Frezza, B. M.; Cockroft, S. L.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2007**, *129*, 14875–14879. (c) Benenson, Y. *Nature* **2001**, *414*, 430–434. (d) Seelig, G.; Soloveichic, D.; Zhang, D. Y.; Winfree, E. *Science* **2006**, *314*, 1585–1588.
(6) Xiao, Y.; Pavlov, V.; Niazov, T.; Dishon, A.; Kotler, M.; Willner, I. *J. Am. Chem. Soc.* **2004**, *126*, 7430–7431.
(7) Shlyahovsky, B.; Li, D.; Weizmann, Y.; Nowarski, R.; Kotler, M.; Willner, I. *J. Am. Chem. Soc.* **2007**, *129*, 3814–3815.
(8) Li, D.; Shlyahovsky, B.; Elbaz, J.; Willner, I. *J. Am. Chem. Soc.* **2007**, *129*, 3814–3815.
(9) Li, D.; Wieckowska, A.; Willner, I. *Angew. Chem., Int Ed.* **2008**, *47*, 5804–5805.
(10) (a) Travascio, P.; Witting, P. K.; Mauk, A. G.; Sen, D. *J. Am. Chem. Soc.* **2001**, *123*, 1337–1348.
(11) (a) Tian, Y.; He, Y.; Mao, C. D. *Chem BioChem* **2006**, *7*, 1862–1864. (b) Weizmann, Y.; Beissenhirtz, M. K.; Cheglakov, Z.; Nowarski, R.; Kotler, M.; Willner, I. *Angew. Chem., Int Ed.* **2006**, *45*, 7384–7388. (c) Cheglakov, Z.; Weizmann, Y.; Basnar, B.; Willner, I. *Org. Biomol. Chem.* **2007**, *5*, 223–225.
(12) (a) Liu, J.; Lu, Y. *J. Am. Chem. Soc.* **2003**, *125*, 6642–6643. (b) Elbaz, J.; Shlyahovsky, B.; Willner, I. *Chem. Commun.* **2008**, 1569–1571.
(13) Cheglakov, Z.; Weizmann, Y.; Beissenhirtz, M. K.; Willner, I. *Chem. Commun.* **2006**, 3205–3207.
(14) Weizmann, Y.; Cheglakov, Z.; Pavlov, V.; Willner, I. *Angew. Chem., Int Ed.* **2006**, *45*, 2238–2242.

JA806222E