Biosensors and Bioelectronics 60 (2014) 112–117

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Enzymatic cascade based fluorescent DNAzyme machines for the ultrasensitive detection of Cu(II) ions

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ARTICLE INFO

Article history:
Received 1 January 2014
Received in revised form
20 March 2014
Accepted 31 March 2014
Available online 8 April 2014

Keywords:
DNAzyme machine
Polymerase
Endonuclease
Exponential amplification
Copper ions

ABSTRACT

A novel enzymatic cascade based fluorescent DNAzyme machine has been developed for the amplified detection of copper (Cu^{2+}) ions. This is the first attempt to carry out the combination of the self-cleaving DNAzyme and the polymerase/endonuclease reaction cycles involving cleaved substrate extension. In the presence of Cu^{2+} ions, the enzyme strand carries out catalytic reactions to hydrolytic cleavage of the substrate strand. The cleaved DNAzyme substrates act as primers and trigger the Klenow Fragment polymerization. Nb.BbvCI endonuclease cuts the double-stranded niking site and thus opens a new site for a new replication. The replication regenerates the complete dsDNA to initiate another cycle of nicking, polymerization and displacement. Finally the fluorescence dye, SG, inserts into the DNA double helix to generate a distinguishable fluorescence enhancement. The Cu^{2+} ions act as the activator for enzymatic cascade amplification generating multiple duplex structures in the nascent product. An increasing fluorescence is observed with increasing Cu^{2+} ions concentration. A good nonlinear correlation (R=0.9997) was obtained between fluorescence intensity and the cubic logarithm of the Cu^{2+} ions concentration over the range 0.50–200 nM. This nonlinear response phenomenon results in an efficient improvement of the sensitivity of our current proposed assay. The activation of such enzymatic cascades through analyte–DNAzyme interactions is not only valuable to activate the cooperation of enzyme networks, but also has a substantial impact on the development of amplified DNAzyme sensors.

1. Introduction

DNA machine are biomolecular assemblies that perform machine-like functions, where several consecutive functions at the molecular level are triggered by external chemical inputs, which lead to dynamic two-dimensional and three-dimensional structural changes, and to the formation of chemical by-products (Beissenhirtz and Willner, 2006; Xin et al., 2013). In Alberti and Mergny (2003), the G-quadruplex–duplex DNA transformations represent machine-like structural conversions driven by nucleic acid fuels. Shimron et al. (2013) introduced the Au nanoparticle-functionalized DNA tweezers as a functional machine for the switchable "mechanical" closure and opening with fuel and antifuel nucleic acid strands. Various enzymes that can catalyze serious reactions, such as cleavage (Yin et al., 2012; Bi et al., 2010), ligation (Tang et al., 2011; Lu et al., 2011), and polymerization (Cheglakov et al., 2008; Li et al., 2010), were applied as biocatalytic "nano-tools" to manipulate DNA machine (Beissenhirtz and Willner, 2006). DNA polymerase extended a primer on its completely hybridized template. Extension of these primers results in the generation of tandem repeats of a sequence that is complementary to the template (Litos et al., 2007). Ding et al. (2010) proposed an amplification strategy to convert the telomerase activity into fluorescence signals employing a hairpin probe and two primers based on primer elongation and polymerase-induced isothermal strand-displacement polymerization reaction. Sequence specific domains within double-stranded DNA provide instructive information for the selective binding of endonucleases or nicking enzymes that catalyze the cleavage of sequence-specific domains and facilitate the synthesis of new cDNA (Jung et al., 2010). Kong et al. (2011) reported a molecular beacon-based junction DNA machine using single-stranded DNA cleaved endonuclease with highly sensitive DNA detection and a strong capability to identify SNPs. Autonomous DNA-machine by multiple processes of replication, nicking, and strand displacement has attracted considerable attention for its excellent isothermal amplification capability (Connolly and Trau, 2010; Zhu et al., 2009). Li et al. (2008) utilized an aptamer-based DNA machine involving cooperation of polymerase and endonuclease to autonomously synthesize of the HRP mimicking DNAzyme units by repeated replication/nicking cycles.

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http://dx.doi.org/10.1016/j.bios.2014.03.065
0956-5663/

Liu et al. (2012) developed a similar DNA machine coupled with fluorescent DNA-scaffolded AgNCs to quantify miRNA expression levels. This approach was further extended to analyze heavy metal ions (e.g., Hg^{2+}) (Yin et al., 2013). A thymine-rich aptamer acts as a "track" for the recognition and operation of the machine leading to a two-step amplification of the sensing process. These biocatalytic transformations not only yield new DNA construction, but also generate new versatile components that can produce remarkable signals. Enzyme-mediate DNA machine hold great promise for the biosensing of low molecular-weight substrates or macromolecules due to their relative high sensitivity and specificity.

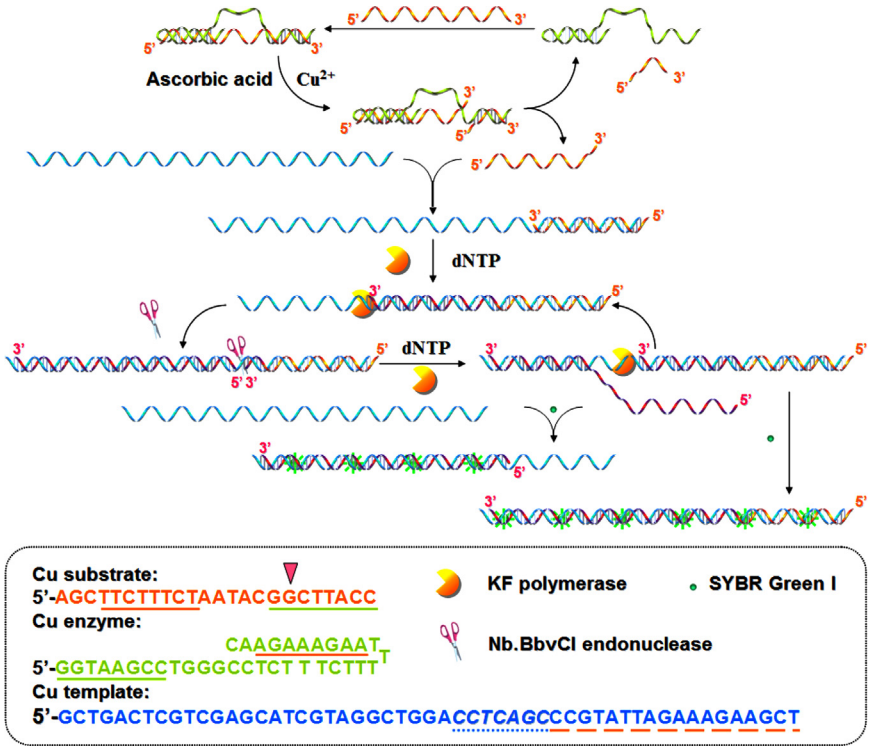
Heavy metal contaminations have posed significant environment and health concerns to the general public. Copper (Cu^{2+}) ion, as one of the heavy metal ions, is an essential element required as a cofactor and/or structural component of numerous enzymes (Tapiero et al., 2003). Normal-level copper is essential for biological activities such as enzyme regulation, metabolism and immune function (Murrell et al., 2000; Lutsenko et al., 2007). But excess of copper is related to neural disturbance (Zheng and Monnot, 2012) and liver or kidney damage (Arnal et al., 2012; Lavery et al., 2009). The U.S. Environmental Protection Agency (EPA) set 1.3 mg/L (20 μM) as the maximum contamination concentration for Cu^{2+} ions in drinking water (U.S. EPA, 2005). Developing sensitive biosensor is one way to minimize the chance of Cu^{2+} ions poisoning. The copper-dependent DNA-cleaving DNAzyme isolated by Breaker catalyzes oxidative cleavage with highly specificity for Cu^{2+} ions (Carmi et al., 1998). Some novel detection methods based on DNAzyme have been developed for the rapid detection of Cu^{2+} ions selectively (Liu and Lu, 2007; Li et al., 2013; Zhang et al., 2013). Fang et al. (2010) developed a lateral flow nucleic acid biosensor based on DNAzyme and gold nanoparticles for the visual detection of Cu^{2+} ions. Liu et al. (2011) designed a label-free fluorescent sensor based on self-assembled graphene/DNAzymes complex for real-time Cu^{2+} ions detection. In Yu's work (2013), graphene oxide introduced to an anisotropy DNAzyme-based strategy was identified to be effective for enhance the fluorescence anisotropy. In these Cu^{2+} ions sensing applications, DNAzymes exhibited the relatively high sensitivity and stability. Yin et al. (2009) described a dual DNAzyme allosteric unimolecule engineering design that utilizes the Cu^{2+}-dependent cleavage of substrate to initiate the formation of active HRP mimicking DNAzyme. This machine-like DNAzyme sensor opened up signal amplification approach for the detection of toxic metal ions in environmental samples.

Considering the powerful amplifying capability of DNA machine, we reasoned that the enzyme-mediate DNA machine should be used for DNAzyme based sensor as efficient nanoamplifier. To demonstrate the feasibility of this hypothesis, we report the first attempt to realize label-free fluorescence assay by employing conjugation of a DNA-cleaving DNAzyme and the polymerase/endonuclease reaction cycles. Cu^{2+} ions initiate catalytic reactions to hydrolytic cleavage of the substrate strand in DNAzyme. The cleaved substrate hybridizes with template strand as a primer, and thus triggers replication cycles of nicking, polymerization and displacement. The dsDNA-specific dyes insert into the replication products and stimulate the generation of fluorescence of the DNAzyme machine operation. Our current strategy opens up new possibilities of DNAzyme machines for amplified sensing of toxic metal ions.

2. Experiment section

2.1. Materials

All oligonucleotide samples used in this work were purified by Sangon Biotechnology Co. Ltd. (Shanghai, China), as listed in Scheme 1. The DNAzyme consists of the 24-mer Cu substrate (in orange) and the 35-mer Cu enzyme (in green). The 53-mer Cu template (in blue) contains the endonuclease recognizing site, which is expressed as an italicized region. Klenow fragment polymerase exo$^-$ (KF polymerase, 2–5 U/μL) and the deoxynucleotide



Scheme 1. Schematic illustration of procedures for enzymatic cascade based fluorescent DNAzyme machines.