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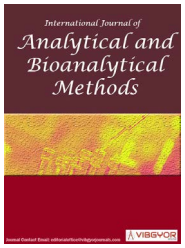
Biosensors and Nanodevices Based on DNAzymes



Lihao Wang[1], Jialin Wang[2] and Guifen Jie[2*]

[1]*College of Environmental Science and Engineering, Tongji University, China*

[2]*Qingdao University of Science and Technology, Qingdao, China*

Abstract

This review mainly report the principle and development of biosensors and nanodevices based on DNAzymes, including fluorescent DNAzyme biosensor, colorimetric DNAzyme biosensor, Surface Enhanced Raman Scattering (SERS) DNAzymes biosensor, electrochemical DNAzymes biosensor, and catalytic biosensor using DNAzymes as signal amplifiers.

Keywords

DNAzyme, Biosensors, Nanodevices, Surface enhanced raman scattering, Signal amplifier

Introduction

Biosensor is an analytical device that can quickly make a reversible response, it uses bioactive materials (such as enzymes, proteins, DNA, antibodies, antigens, biofilms, etc.) as detection substances, and combine various means such as chemistry [1], physics, medicine and electronic technology to achieve the purpose of detection. As an indispensable advanced means in the detection of biotechnology and monitoring, biosensor has important application value in the fields of biological analysis, medical detection and environmental monitoring.

As one of the most challenging fields at present, the design of nanodevice has attracted more and more researchers' attention [2]. DNA is often chosen as one of the most commonly used materials in the design of nanodevices for its unique physical properties. Good programmable sequences, rigid nanostructures, and memory devices with high fidelity have been constructed using DNA. DNA origami technology offers the possibility of instruments with resolution below 10 nm, which provide a new way to inspire the precise design of nanostructures. With the development of this technology, origami DNA nanostructures of different shapes, such as rectangles, smiley faces, maps, stars, honeycomb lattices, nanotubes, boxes and three-dimensional DNA structures, have been continuously created by people [3,4]. Inspired by the intermolecular driver proteins in biological systems, DNA nanodevices with dynamic functional structures have also been developed, such as tweezers and gears [5,6]. DNA walking device is one of the most complex in both design and manufacture [7]. Normal walking activities are initiated by chain hybridization [8], enzymatic reactions [9] and environmental stimuli [10]. The device has great application potential in sensors [11] and signal conversion media [12].

***Corresponding author:** *Guifen Jie, Qingdao University of Science and Technology, Zhengzhou Road 53, 266042, Qingdao, China*

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Biological technology has been well documented in the development of the past few decades. Nucleic acid is not only a carrier for storing genetic information, but also a versatile biomolecule as a proteinaceous substance, binding ligands, and biocatalysis. Such functionalized nucleic acid species, including DNA/RNA nucleic acid aptamers, ribozymes/deoxyribozymes have been widely used as molecular tools in various studies [13,14]. Due to the remarkable stability of DNA nucleic acid aptamers and deoxyribozymes, they are widely used in the identification and construction of catalytic units, and are applied to biological analysis and nanobiotechnology.

DNAzymes, as a class of catalytic nucleic acid materials, can achieve specific shearing of substrates in the presence of catalytic synergistic factors. Instead of requiring the evolution of the test tube for animals or cells, DNAzymes for identifying various analytes can be made by *in vitro* selection, which are inexpensive to assemble and easy to batch process. In addition, their modification and functionalization processes are easy to operate, which is suitable for signal amplification and output. Unlike ribozymes which are naturally available, DNAzymes are obtained by *in vitro* selection techniques, and show activity with the participation of metal ions [15]. In 1994, Break and Joyce discovered the first DNAzyme by *in vitro* selection process, the DNAzyme catalyzes the transesterification reaction in the presence of Pb^{2+}. This finding also suggests that single-stranded DNA can act as catalyst like nucleases and proteins [16]. Since then, a variety of DNAzymes have been isolated to catalyze various biological responses, such as RNA cleavage [17], DNA cleavage [18], ligation [19], or phosphorylation [20]. Collectively, this type of DNAzymes consists of a substrate chain and a single enzyme chain. The substrate chain contains a single-stranded RNA linkage (rA) as an enzyme cleavage site, and the enzyme usually consists of a catalytic core and two arms around it. The performance of DNAzymes requires different primary and secondary structures, different catalytic synergistic factors, dependence on the pH of the reaction environment, and the need for specific catalytic substrates. In the presence of a catalytic synergistic factor, the enzyme sequence can cleave the substrate sequence into two parts. Catalytic synergistic factors include different kinds of metal ions and amino acid species, which makes it possible to design biosensors that respond to different types of synergistic factors.

Moreover, DNAzymes show a high degree of specificity for their substrate chains, and even a mismatch in one base in the complementary strand of the arm will greatly reduce the shear activity. Therefore, the high flexibility of the binding arm design, the superiority of substrate recognition and multiple enzymatic conversion properties make DNAzymes not only have universal recognition and excellent signal amplification in biosensing, but also promise for mRNA targeting, DNAzymes therapy. Among all DNAzymes, 10-23 and 8-17 are two well-known DNAzymes, as long as their two binding arms form stable double-stranded structure with nucleotides, the whole RNA substrate can be sheared at appropriate conditions. Taking 10-23 DNAzyme as an example, the name "10-23" is the 23^{rd} clone of 10^{rd} round in the PCR selection process. It has been reported that the catalytic rate of 10-23 DNAzyme is 10^9 mol/min, which is 100 times higher than the most active nuclease cleavage activity and 10^5 times higher in physiological environment.

Another DNAzyme is called G-quadruplex-DNAzyme. In this DNAzyme, the guanine-rich (G-rich) sequence will fold into parallel or anti-parallel structure in the presence of K^+, Pb^{2+} or NH_4^+. The G4-DNAzyme structure can act as recognition devices in colorimetric biosensors for K^+ or Pb^{2+}, or as specific markers for signal labels.

Biosensors and Nanodevices Based on DNAzymes

All biosensors are formed from two partial groups. An identification device that selectively binds to the target molecule and a sensor device that converts the recognition activity into a detectable signal. As a functionalized DNA, DNAzymes possess two unique properties. Based on their recognition and shearing ability, they mainly construct recognition devices for synergistic factors, which are very suitable for multi-enzyme-catalyzed turnover performance of signal amplification.

DNAzymes with RNA cleavage capabilities typically require specific synergistic factors to catalyze the process of cleavage, including metal ions or amino acids. The presence of synergistic factors assists in folding the DNAzyme sequence into a specific structure to form a catalytic center for shearing. Using this property, many sensors based on

DNAzymes are used for highly specific detection of synergistic factors, including Pb^{2+} [21], Mg^{2+} [22], Zn^{2+} [23], Mn^{2+} [24], Cu^{2+} [25], and histidine [26].

In addition to being a versatile identification device for sensor design, DNAzymes are excellent signal amplifiers. By rational design, conformationally altered DNAzymes or simple aptamer enzymes can recognize a wide range of target substances other than synergistic factors. In these strategies, directed recognition binding activates the catalytic center or triggers the synthesis of DNAzymes, which in turn produces a cut that amplifies the signal. Therefore, DNAzymes are particularly well suited for the design of signal sensors and signal amplifiers for biosensors. The concept of conformational changes in aptamers and ribozymes has been used in the design of aptamer enzymes, in which DNA aptamers are integrated with DNAzymes so that DNAzymes can only be activated by binding to the target. Because the binding activities of aptamers and targets can activate DNAzymes, and the binding process can be translated into generating signals and amplifying signals, so the DNAzymes with conformation changes are well suited for the construction of biosensors.

Fluorescent DNAzyme biosensor

The most common method of designing RNA-cleaved DNAzymes for fluorescent detection of a target is to introduce a fluorophore-quenching group (F-Q). With this method, the fluorophore can be released after cleavage of the substrate sequence to detect the corresponding fluorescent signal. By clever design, the fluorophore and the quenching group are paired in pairs before shearing, and they are separated from each other after shearing. DNAzyme-based fluorescence sensors use organic molecules as quenchers and are often affected by incomplete fluorescence quenching. In addition, the substrate sequence that hybridizes with the DNAzymes is not easily removed. To overcome this problem, gold nanoparticles, gold nanorods, carbon nanotubes and graphene oxide are introduced into the sensor design as a fluorescent quencher. Fan and his collaborators have shown that graphene oxide (GO) and single-stranded DNA and double-stranded DNA will produce different fluorescence quenching efficiency, so the sensor with a dynamic range similar to GO-DNAzymes nanoprobes is developed for Pb^{2+} detection [27], Although DNAzyme-based sensors have been widely used, the intracellular application of DNAzymes sensors remains a major challenge. Lu and his collaborators demonstrated DNAzyme-gold nanoparticle probes for the detection of uranyl ions in living cells for the first time [28], They chose gold nanoparticles as a carrier for the transfer of DNAzymes between cells, because gold nanoparticles-DNAzymes have greater DNA loading efficiency and can increase their anti-enzymatic ability in cells or serum. First, the fluorophore and the corresponding quenching group are modified at both ends of the substrate chain respectively, forming a corresponding F-Q pair. A polyadenine spacer is designed between the two groups, thereby reducing the quenching effect of the nanogold. In the presence of the target, after shearing of the short chain, the fluorophore is released from the surface of the nanogold and leaved the quencher, resulting in an increase in the fluorescent signal. Moreover, Liu, et al. reported the first monovalent transition metal dependent RNA-cleaving DNAzyme for selectively sensing low concentrations of Ag^{+} ions, this study strengthens the idea of exploring beyond the traditional understanding of multivalent ion dependent DNAzyme catalysis [29].

Colorimetric DNAzyme biosensor

Metal nanoparticles, especially gold nanoparticles, have some special distance-determined surface plasmon properties. Moreover, they are biocompatible, easy to be functionalized with various ligands, and relatively stable under physiological conditions. Recent research interests in the field focus on RNA-based DNAzymes-gold nanoparticle sensing systems for sensitive colorimetric sensing studies. The surface plasmon resonance absorption of gold nanoparticles is affected by particle size and space. In general, gold nanoparticles appear red in a dispersed state. When aggregation occurs, the color of the nanogold solution changes to purple or even blue. The corresponding absorption wavelength will migrate from 520 nm to 650 nm, or even larger. According to this property, a series of colorimetric sensors for detecting metal ions based on RNA-cleavage-based DNAzyme-gold nanoparticles have also been proposed [30-33]. The unsheared substrate sequence leads to the aggregation of

gold nanoparticles. When the target metal ions are present, the substrate chain is sheared by the action of DNAzymes, so that the distance of the gold nanoparticles changes.

Surface Enhanced Raman Scattering (SERS) DNAzymes biosensor

The aggregation of gold nanoparticles will greatly increase the coupling of surface plasmon resonance between adjacent particles, called "hot spots". A hot spot can produce an enhanced electromagnetic field and thus cause a significant increase in the SRES signal. Using the unique distance-dependent surface plasmon properties of gold nanoparticles, highly sensitive SERS and metal ion-dependent RNA cleavage DNAzymes, Wang, et al. proposed a SERS biosensor based on DNAzymes for highly sensitive detection of Pb^{2+} [27].

Electrochemical DNAzymes biosensor

Electrochemical biosensors have drawn particular attention as a consequence of their high sensitivity, inherent simplicity, compatibility to miniaturization devices and without high cost. On the basis of DNAzyme and DNA molecules, a variety of electrochemical biosensors have been developed. Shao and his collaborators used DNA-Au conjugates as biocodes to amplify electrochemical signals [33]. In this study, $Ru(NH_3)_6^{3+}$ was used as a signal source to bind to the phosphate anion in the DNA backbone by electrostatic interaction. The detection limit of Pb^{2+} was 0.028 nM. Willner, et al. reported amplified biosensing for detection of DNA and AMP using the horseradish peroxidase-mimicking DNAzyme as an electrocatalyst [34]. Jie, et al. reported a novel silver nanocluster probe for versatile electrochemiluminescence and electrochemical detection of thrombin by multiple signal amplification strategy [18].

Conclusions

Different biosensors and nano-devices can be constructed by using DNA enzymes combined with various signal amplification methods to realize the detection of different kinds of targets, and improve the performance of biosensors. The optical sensor of DNA enzyme is more common than electrochemical sensor. Electrochemical sensor equipment is simple, small, and easy to operate. The development of DNA enzyme-based sensors will enrich the sensing strategies. DNAzymes are not only versatile identification devices for sensor design, but also excellent signal amplifiers. One of the challenges of DNAzymes-based amplification technology is the development of isothermal amplification methods triggered by new signals similar to polymerase chain reaction (PCR), such as rolling circle amplification (RCA), RNA autonomous transcription, etc.

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