Besides genetic functionality: DNAzymes, Aptamers, Aptazymes and their application as biological treatments

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Abstract: In the past several decades, DNA molecules have gained much attention beyond their genetic functionality to be utilized as powerful synthetic building blocks to create nanoscaled architectures and versatile programmable templates for nanomaterials assembly. Like the major types of functional DNA nano-structural basis, DNAzymes, aptamers, and aptazymes, the combination of DNAzymes and aptamers are thus reviewed in this paper. DNAzymes are catalytically active single-stranded DNA fragments produced in vitro via molecular evolution. Because of their superior programmability, stability, and activity, RNA-cleaving DNAzymes have gotten a lot of interest for applications in sensing, treatments, and nanodevices. Aptamers are a series of single-stranded nucleic acid molecules that bind to a specific target molecule. Aptamers are widely used in biosensors and some other nano-devices in therapeutic usage due to their high affinity, stability and safety. The aptazyme is an oligonucleotide composed of an aptamer and a ribozyme (or deoxyribose). Therefore, It has the properties of both, which are excellent selectivity and affinity for the binding to the target material, as well as catalytic activity. This review is dedicated to various bio-nano science applications of DNAzymes, aptamers and aptazymes, such as sensors and therapeutic devices. Moreover, it provides the foundation for future research.

1. Introduction

In 1982, T. cech discovered ribozyme in his research. The RNA that splice introns on itself have the catalytic function. This suggests that enzymes are not only proteins but also nucleic acids. Similarly, Gerald Joyce discovered in 1994 that deoxyribozyme (DNAzyme) catalyzes the Pb2+ dependent cleavage of single ribonucleotide phosphates. Dozens of Dnazyme species have been found so far. Due to their function, DNAzyme can be divided into seven categories: RNA cleavage activity, DNA ligase activity, porphyrin metalloproteinase and peroxidase activity, DNA hydrolysis activity, DNA kinase activity, N-glycosylase activity and DNA capping activity. On account of the different functions of DNAzyme, they can be used for several applications, such as biosensors and nano-devices. Aptamers are a class of functional oligonucleotides that have been engineered by artificial combinatorial methodologies of in vitro selection or systematic evolution of ligands by exponential enrichment that can bind a wide variety of specific targets with affinities and specificities that rival antibodies. With their high stability, low dimension and high affinity for small molecules, they have been quickly used in the sensor field as novel recognition elements. As the combination of aptamers and DNAzyme, aptazymes possess not only high specificity and affinity for the aptamer's binding to the target substance, but also the catalytic activity of deoxyribozyme. In this review, we discuss DNAzyme, aptamers, aptazymes and their applications.

2. DNAzymes

2.1. Brief introduction of DNAzym

Deoxyribozyme is a single-stranded DNA fragment with catalytic function synthesized by molecular evolution technology in vitro, which has high catalytic activity and structure recognition ability. Since 1994, Gerald. F. Joyce et al. First synthesized and discovered DNA. In 1995, cloud et al. Reported a DNA with ligase activity. Today, dozens of deoxyribozymes have been found. According to their functions, they can be divided into seven categories: RNA cleavage activity, DNA ligase activity, porphyrin metalloproteinase, and peroxidase activity, DNA hydrolysis activity, DNA kinase activity, N - and DNA glycosylase activity restrictions. Deoxyribozyme can catalyze specific ribonucleotides or deoxyribonucleotides to form phosphate diester bonds. It can be said that DNA with this catalytic activity is called deoxyribozyme or DNase. Although deoxyribozyme is not found in nature, it has been proved that DNA has enzyme activity. Because deoxyribozyme is more stable than ribozyme, and its production cost is relatively low, the research on deoxyribozyme has not only become a hot scientific research topic but also helps to understand the most basic problem about life, that is, how life evolved from RNA world to today's DNA and protein-based cell form. This discovery also reveals that the evolutionary pathway of RNA to DNA may also exist in other substances similar to nucleic acid, which is helpful to understand the basic structure of life and its evolutionary process.

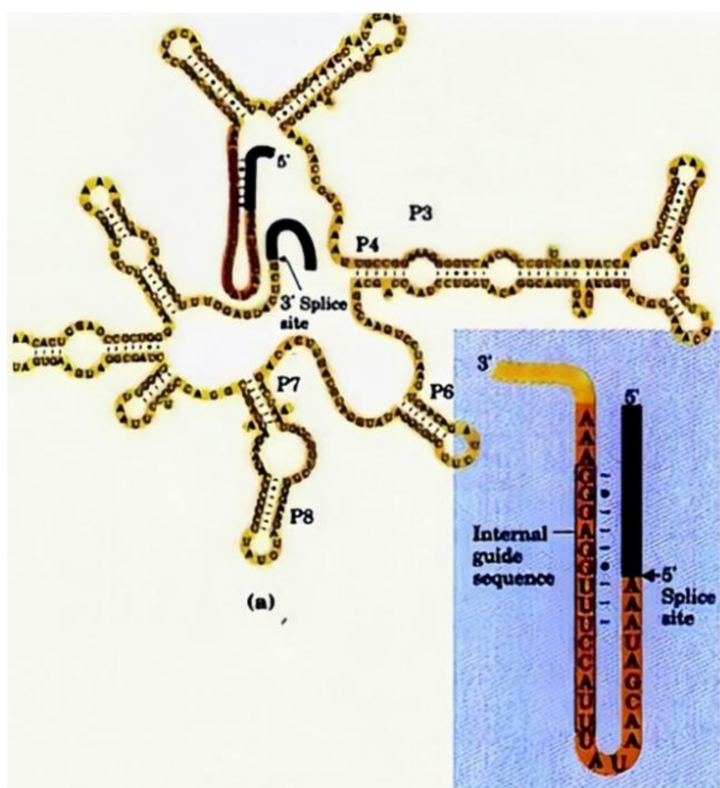

Figure 1. Common structural basis for DNAzymes [1]

2.2. Structure assembly for DNAzymes

Generally speaking, the structure of DNase is the same as that of DNA. It's a polymer of deoxynucleotides. Deoxynucleotides are made up of bases, deoxyribose, and phosphate.. There are four bases: adenine (A), guanine (G), thymine (T), and cytosine (C). Two polydeoxynucleotides are wound on a common central axis to form a double helix. The deoxyribose phosphate chain is located on the outside of the helix and the base is inward. The two polydeoxynucleotides are complementary to each other and are connected by base pairs formed by hydrogen bonds between bases to form a fairly stable combination. In addition to catalyzing the linear connection of RNA, Deoxyribonuclease can also activate 2'- hydroxyl in ribonucleotides and react with 5'- triphosphate to form 2', 5'- branched

ribonucleic acid. The design of the binding arm is to direct deoxyribozyme to the desired labeling site by direct hybridization between upstream and downstream of the target nucleoside. In a method called DNA catalytically labeled RNA, 17 NT long labeled RNA is attached to the RNA of interest. Recently described a general and general DNA catalytic labeling strategy that allows ribose-labeled mononucleotides to specifically attach to adenosine nucleotides needed for in vitro transcription of RNA. Fluorescent, spin biotinylated or cross. Because this labeling method can directly use long, natural or in vitro transcribed RNA, it can avoid the preparation and linking of RNA fragments. So far, Adenosine is the only internal nucleotide that this strategy can target, but in ongoing and future in vitro selection experiments, it is expected that deoxyribozymes can be obtained that respond similarly to other internal nucleotides (Guanosine, Cytidine, or Uridine). This will further expand the universal applicability of deoxyribonuclease as a post-transcriptional marker of RNA.[2]

2.3. Biomedical application of DNAzymes

2.3.1. DNAzymes as biosensors.

As a molecular tool, in vitro screening of deoxyribozymes has a broad application prospect in the design of biosensors and nano-devices. The catalytic activity of deoxyribozyme depends on cofactors and shows the conversion characteristics of many enzymes, which makes deoxyribozyme not only a multifunctional recognition element, but also an excellent signal amplifier.

2.3.2. DNAzymes as novel tools for catalytic detection.

DNA enzymes combined with nanomaterials have unique biological activities. Some DNA as functional units can also be used as a therapeutic means to supplement target genes or viral RNA, leading to down-regulation of protein expression. Deoxyribozymes have been widely used in sensing and biomedical fields due to their excellent catalytic activity. The application of these new discoveries provides hope for the design of novel catalytic DNA enzymes. Due to its good programmability, stability, and activity, RNA cleaved DNAzymes have attracted extensive attention in treatment and diagnosis. They can be designed to cleave a specific mRNA to down-regulate gene expression. At the same time, DNase can detect a wide range of analytes. Combining these two functions, we get the theoretical DNase. DNase has been used to cut RNA to treat various diseases, such as virus infection, cancer, inflammation and atherosclerosis. Some formulations have entered clinical trials. Next, the application of DNase in the detection of metal ions, small molecules, and nucleic acids related to disease diagnosis is also under discussion.

2.3.3. DNAzymes as functional nano-devices in diagnosis and therapeutic usage.

DNase are DNA molecules with catalytic activity. In order to find improved and better methods to treat cancer and other diseases, new molecules have been found and developed. Deoxyribozyme is such a molecule. 10-23 deoxyribozymes are special molecules, Characterized by its endonuclease activity, that is, the ability to cleave nucleic acid molecules after proper binding. Deoxyribozymes are used as drugs to treat cancer. It is speculated that with the development of a DNase intelligent drug delivery system, better pharmacodynamics and pharmacokinetics will become possible by accelerating the development of DNase technology in clinical research. Deoxyribozyme has the ability to produce photoinduced activation, Characterized by its endonuclease activity. Moreover, 10-23 deoxyribozyme has the ability to inhibit gene expression through sequence-specific gene cleavage. [3] There are several ways to control disease progression by inducing or inhibiting genes (including DNA based biological drugs):

1. Plasmid containing transgenes
2. Antisense oligonucleotides
3. Fitness
4. Ribozyme
5. Deoxyribozyme
6. Short interfering RNA

Wu and his colleagues first used DNase as a target cell f or cancer cells. The anticancer activity of DNase is not limited to the destruction of tumor blood vessels but cleavage of RNA substrate in a sequence-specific manner. Deoxyribozymes can produce better and safer drugs than existing drugs and can be used as potential therapies to target pathogenic genes.

3. Aptamers

3.1. Aptamers are single-stranded DNA or RNA sequencesfirst proposed in 1990.

Most of the aptamers are screened in vitro by systematic evolution of ligand by exponential enrichment. Firstly aptamers are a series of single-stranded nucleic acid molecules that bind to a specific target molecule. Its specificity is the same as antibodies, and it has a strict recognition ability and a high affinity for binding ligands. Compared to antibodies, aptamers show great stability under harsh conditions. And due to the simple chemical structures, aptamers are easily modified with functional groups by chemical coupling or physical adsorption. Finally, aptamers are safer in vivo than antibodies because of their non-immunogenic characteristics.[4] Therefore, nucleic acid aptamers are widely used in biosensors, new drug development, and nanotechnology.

3.1.1. Aptamers in biosensing.

Biosensors are integrated devices, which can convert a molecular recognition event into a detectible physiochemical signal. Generally, a biosensor contains a biological recognition element that interacts with a target species and a transducing element for signal detection. Aptamers have attracted the attention of scientists since their discovery. With their high stability, low dimension, and high affinity for small molecules, they have been quickly used in the sensor field as novel recognition elements. [5]. Hey-Mi So et al.[6] have established the first SWNT-FET-based biosensor includes DNA aptamers as the molecular reputation factors. The rapid reaction, high sensitivity, and relative simplicity of manufacture of these SWNT-FET sensors, coupled with the small length, economy, stability, and high selectivity of aptamers, can provide a low-cost, instant detection tool and a new approach to high-throughput screening.Iwona Grabowska et al.[7] shown an electrochemical aptamer-based sensor that is used to detect sensitive cardiovascular markers BNP and cTnI. The sensor is coated by EPD using PEI/rGO nanocomposite membrane. An NH2 group in PEI accelerated the binding of BNP-32 to cTnI ligands by grafting prophylactic acid ligands, followed by a complete one-click chemical linkage of Cu(I) -based azide terminal ligands. Using specific aptamers with dissociation constants ensures the ubiquity of targeted biomarkers that are quite sensitive in complicated substrates such as serum.

3.1.2. Aptamers as nano-devices in therapeutic usage.

There are many strategies for delivering drugs in cells, such as microspheres, liposomes, nanofibers, peptides, and antibodies. Nevertheless, these vectors have their limitations and are generally considered to be insufficiently specific or have low tissue penetration, especially in the case of antibodies and polypeptides. In this regard, aptamer has emerged as a new drug delivery tool with high specificity, lack of immunogenicity, and quite ideal tissue penetration, which are essential prerequisites for a good drug delivery vector.[8] The use of aptamers and nanoparticles has increased significantly over the past decade. During targeted delivery, specific cell surface proteins bind with aptamers, thereby increasing the specificity of the treatment. Therefore, drugs are only delivered to specific groups of cells carrying aptamer-specific targets, increasing the effectiveness of local delivery.[9]

4. Aptazymes

Aptazymes are made up of an aptamer (which contains an acceptor site) and a ribozyme or deoxyribozyme (containing a catalytically active site). The aptazyme is not like other enzymes. It is an oligonucleotide, not a protein. It possesses high specificity and affinity for the aptamer's binding

to the target substance and the catalytic activity of ribozyme or deoxyribozyme. The Breaker team created the first aptazymes by fusing an ATP-binding aptamer with the tiniest hammerhead ribozyme.[10] Their catalytic activity is regulated by allosteric effectors: when there are no allosteric effectors (some small molecules), they have no or only low activity, and when there are allosteric effectors, the target specificity binding would initiate, which can greatly change the activity of the enzyme. As a result, the aptamer enzyme performs the function of a complex chemical switch.[11]

4.1. Research status

An aptamer (DNA or RNA) and a nuclease (deoxyribozyme or ribozyme) are fused to produce a chemically regulated aptazyme. It has been used as a physical model for allosteric enzymes and microbial sensors and has long attracted the attention of chemists. Aptazymes based on RNA aptamers and self-cleaving ribozymes has been shown in synthetic biology to be effective for chemical regulation of gene expression in living cells and cell-free translation systems.[12] Although the aptamer enzyme performs its own cutting under the condition that its homologous aptamer coordination exists or does not exist, the integration with regulatory RNA elements such as the ribosome-binding site, 3'-untranslated region (UTR) in messenger RNA, tRNA, or pri-miRNA can chemically control of gene expression in various cellular and genetic contexts. Nevertheless, the types of aptamers and self-laser-cut ribozymes are very limited, and the countermeasures for connecting two RNA components to the engineer aptamers are also the same. Aptazymes are constructed using aptamers fused with theophylline, tetracycline, thiamine pyrophosphate and guanine to adjust the expression of genes in somatic and cell-free translation systems. Although aptamers based on the hammerhead ribozyme frame are more extensive, aptazymes based on other ribozyme types have been reported, including hepatitis delta virus (HDV) ribozymes and distortion ribozyme. The ease of use of ribozymes with a variety of secondary and tertiary structures, biochemical characteristics, and cutting site coding sequences has promoted the development and design of aptamers suitable for various applications. More importantly, basically, all of these aptazymes have a unified structure, in which the ligand aptamer is inserted into the stem-loop of the ribozyme frame. In the majority of cases, the allosteric adjustment of the activity of ribozyme-catalyzed reaction is due to the stability or instability of the partial stem structure in response to the aptamer-ligand fusion. The further expansion of the aptamer and ribozyme library and the development of new design solutions for aptazymes are expected to enhance the flexibility of engineering projects and the more general application in synthetic biology [13].

4.2. Applications

4.2.1. Aptazymes as regulators.

CRISPR-Cas9 and other sequence-specific genome editing agents have significantly improved our ability to manipulate the human genome. Although standard gene editing agents and programmable controller gene expression regulators are constitutively active, the precise time and space manipulation of gene editing and gene expression control theme activities will make this powerful technical application more selective and potentially safer. The Weixin Tang team has developed and designed a set of guide RNAs compatible with aptazyme-insertion. According to the fusion coordination response, the gene preparation of the small molecule manipulated nuclease receptor and the small-molecule manipulated. Molecule-dependent transcriptional activation in mammalian cells base preparation is completed by incorporating ligand-responsive self-cleaving catalytic RNAs (aptazymes) into guide RNAs.[14]

4.2.2. Aptazymes as biosensors.

The development and design of reliable sensors for testing adenosine triphosphate (ATP) has received more and more attention because of its leading role in various enzymatic activities and the process of biological. The Xia Li group has developed and designed a sensitive electrochemical sensor to test ATP using a cascaded, obvious data signal enlargement method, which involves aptazymes and catalytic hairpin assembly (CHA). The target ATP induces conformational changes in

the coding sequence of the aptazymes and fusion with the hairpin substrate to produce a viable aptamer enzyme, in which the hairpin substrate is cracked in the buffer solution and released by the circulatory system to release the enzyme coding sequence. The enzymatic sequences can also bind to the hairpin substrates to form active DNAzymes. As a result of the catalytic cleavage of the hairpin substrates by the aptazymes/DNAzymes, many intermediate coding sequences are generated. Following that, this intermediate coding sequence captures a lot of methylene blue-identified data signal coding sequences according to CHA in the electrical level surface catalytic reaction, resulting in a significantly larger current response, which is suitable for the sensitive detection of 0.6 nm ATP. Additionally, the sensor developed and designed can distinguish ATP from similar influencing molecular structures, and can be applied to human serum protein samples, making it a valuable addition to the arena of sensitive small molecule detection.[15] Cho et al. based on the hammerhead ribozymes stem I and stem III with the aptamer, a dual aptamer enzyme sensor was established for the hepatitis C virus replicate and helicase. It exhibits few blank signals (data signals caused by the subject activity of cutting in the absence of the target molecular structure), whereas the previously fused aptamer enzyme exhibits a significant blank signal.[16] The biosensor is a device that enlarges the data signal caused by the special interaction between the receptor and the target analyte. Aptamers, the RNA structural motifs, are found to be the receptor components of biosensors. Because they are easy to evolve outside the body, they fuse various Affinity with high specificity. According to the coupling reaction between the aptamer and the reactive catalytic RNA (such as the hammerhead ribozyme) as an allosteric control element, the coordination fusion is converted into a catalytic reaction. Rudel et al. Applicated Fluorescence Resonance Energy Transfer (FRET) to further enlarge the catalytic reaction induced by coordination into a fluorescent data signal that is convenient for inspection.[8] This sensor has a high signal-to-noise ratio, tens or even hundreds of times that of previous aptamer enzymes, which allows for an increase in the detection limit of target molecules and an increase in the sensor's sensitivity.

5. Conclusion

After continuous exploration and research by scientists, we found that DNA has another use as a multifunctional and programmable template for assembling nanomaterials besides genetic function. Enzymes are not only proteins, but also nucleic acids. Dozens of DNA enzymes have different functions and can be divided into seven categories. Therefore, DNase has been widely used in biosensors and nanodevices. Due to its excellent programmable ability, stability, and activity, deoxyribonuclease is widely used in sensing, therapy, and nanodevices. Aptamers, as a kind of functional oligonucleotides, have evolved into ligands through artificial selection. Because they can bind to a variety of specific targets, their affinity and specificity compete with antibodies. With the characteristics of high stability, low dimension and high affinity of small molecules, aptamers have been rapidly developed and applied in the field of new recognition sensor elements. Aptamer is a combination of aptamer and deoxyribozyme, which has high specificity and affinity for the binding of aptamer and target substance and has a deoxygenation effect. Through the exploration and research of DNase, aptamer, aptamer and other biological nano science applications, such as sensors and therapeutic devices, nano components, we have got more profound conclusions, which can lay a foundation for future research on DNA ribozyme, aptamer and aptamer application.

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