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Dnazyme: From Biocatalysis to Intelligent Diagnosis and Therapy

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ABSTRACT: DNAzyme is a class of artificial catalysts composed of single-stranded DNA molecules that can accelerate specific chemical reactions like natural enzymes. Unlike traditional protein enzymes, DNAzymes are characterized by low cost and ease of design and modification, with their activity relying on specific structures formed by their own folding. In the field of biocatalysis, DNAzymes have been utilized to catalyze various reactions, such as phosphorylation and dephosphorylation, significantly improving the efficiency of industrial and laboratory reactions. Moreover, the combination of DNAzymes with biosensors makes them an ideal tool for the sensitive detection of biomarkers. In the realm of intelligent theranostics, DNAzymes have been developed for targeted therapies, drug release, and disease monitoring. By accurately recognizing and responding to pathological indicators, they provide new perspectives for personalized medicine and early diagnosis. The evolution of DNAzymes from "biocatalytic tools" to "core components of intelligent theranostics" offers innovative solutions at the molecular level for precision medicine.

KEYWORDS: DNAzyme, Biocatalysis, Intelligent Diagnosis and Therapy, Biosensor

1. INTRODUCTION

Since the concept of “enzyme” was introduced in the 19th century, the scientific community has long regarded proteins as the sole performers of catalytic reactions. This paradigm was broken in 1982 when Thomas Cech discovered that RNA can self-catalyze splicing (ribozyme), demonstrating the catalytic potential of nucleic acids [1], and in 1994, Breaker et al. reported for the first time a DNA molecule with RNA cleavage activity (DNAzyme) [2], completely overturning the idea that “DNA only serves as a genetic agent”. In 1994, Breaker et al. reported the first DNA molecule with RNA cleavage activity (DNAzyme) [2], which completely overturned the conventional wisdom that “DNA only serves as a carrier of genetic information”. As an artificially designed single-stranded DNA catalyst, DNAzyme not only possesses catalytic efficiency comparable to that of nuclease, but also has become a focus of research at the intersection of chemical biology and nanomedicine due to its unique advantages such as strong chemical stability, low synthesis cost, and programmable sequence. Since its first discovery in 1994, DNAzyme has become a research hotspot in the fields of biomedicine, biosensing, gene regulation, etc.[2] The structural features of DNAzyme mainly include the nucleic acid sequence and structural domains, in which the nucleic acid sequence determines the catalytic activity of DNAzyme, and the structural domains affect the stability and substrate specificity of DNAzyme[3].

The catalytic mechanism of DNAzyme mainly involves the steps of substrate binding, catalytic reaction and product release. Through specific nucleic acid sequences and structural domains design, the efficient catalysis of specific substrates by DNAzyme can be realized, which has potential biomedical applications. In addition, studies have shown that environmental factors, such as incubation temperature, have an important effect on DNAzyme activity and stability, providing basic research support for the application of DNAzyme under different environmental conditions.

In the field of biomedicine, DNAzyme, as a new type of catalyst, has a wide range of application prospects. For example, DNAzyme can be used in the fields of targeted drug delivery, antiviral therapy, and gene regulation [4], showing unique advantages and potential application value. In the future, with the in-depth study of the catalytic mechanism and influencing factors of DNAzyme, the application prospects of DNAzyme in the biomedical field will be further expanded, providing new possibilities for the development and innovation of biomedical technology. DNAzyme, as a molecule with DNA catalytic activity, has important research significance and application prospects in the biomedical field. Through in-depth study of the structure, catalytic mechanism and influencing factors of DNAzyme, we can better understand its potential application value in biomedical field and promote the development and application of DNAzyme technology. Early studies focused on resolving the catalytic mechanism of DNAzyme, such as the dependence of classical 10-23-type DNAzyme on Mg^{2+}-mediated hydrolysis of phosphodiester bonds [5]. With the development of synthetic biology and molecular engineering, the boundaries of its applications are expanding: in vitro, DNAzyme can detect trace biomarkers (e.g., fM-grade microRNAs) by coupling hybridization chain reaction (HCR) or CRISPR systems [6]; in vivo, based on environment-responsive design (e.g., pH/enzyme activation), DNAzyme can

precisely target diseased tissues and modulate drug release [7]. These advances indicate that DNAzyme is transforming from a "biocatalytic tool" to a "core of intelligent diagnosis and therapy".

However, the clinical translation of DNAzyme still faces multiple challenges: insufficient stability in vivo due to nuclease degradation, off-target effects in complex physiological environments, and cost constraints for large-scale production. To address these issues, emerging technologies such as artificial intelligence-assisted sequence optimization and biomimetic nanocarrier engineering offer potential solutions. In this paper, we systematically review the research progress of DNAzyme from biocatalysis to intelligent diagnosis and treatment, analyze its molecular design strategy, and explore the innovative path of cross-disciplinary technological integration, with a view to providing theoretical references for the development of molecular tools for precision medicine.

2. CONCEPTS AND CHARACTERISTICS OF DNAZYME

2.1 Concepts and features of DNAzyme

DNAzyme is a molecule with DNA catalytic activity, which is a specific DNA molecule that is able to catalyze specific chemical reactions under appropriate conditions. The concept of DNAzyme originates from the discovery of ribonucleases (RNAzymes), but unlike RNAzymes, DNAzyme consists of DNA molecules, which have better stability and controllability [8].

DNAzyme has specific nucleic acid sequences and structural domains which determine the catalytic activity and specificity of DNAzyme and it is able to catalyze specific chemical reactions such as shearing of RNA strands, redox reactions, etc., under appropriate conditions [9], demonstrating enzyme-like catalytic activity.DNAzyme does not require additional cofactors during catalysis, only a simple nucleic acid sequence to achieve catalysis, with high catalytic efficiency and specificity.The structure and function of DNAzyme can be precisely designed and regulated by synthesis and modification, giving it a wider potential for application [10].

Overall, DNAzyme, as a molecule with DNA catalytic activity, has unique structural features and catalytic mechanisms that provide new possibilities for research and applications in the biomedical field.

2.2 History of DNAzyme discovery and important milestones

The history of the discovery of DNAzyme can be traced back to 1994, when scientists such as Ronald R. Breaker and Gerald F. Joyce [11] stumbled upon the discovery of molecules with DNA-catalyzed activity while studying nucleases (RNAzymes). This discovery attracted widespread attention in the scientific community and opened a new chapter in DNAzyme research.

Subsequently, scientists have discovered a variety of molecules with DNA catalytic activity, and gradually revealed the structural characteristics and catalytic mechanism of DNAzyme. 1996, Joyce et al. discovered a kind of DNAzyme capable of shearing RNA strands, confirming that DNAzyme has enzyme-like catalytic activity. In subsequent studies, scientists have continued to explore the functions and applications of DNAzyme, including work on designing novel DNAzyme, optimizing catalytic efficiency, and expanding application areas [12].

During the development of DNAzyme research, scientists have achieved several important milestones. For example, in 2000, Joyce et al. successfully designed a DNAzyme with metal ion-dependent catalytic activity, which provided a new idea for the application of DNAzyme.In 2014, scientists reported a DNAzyme capable of specifically recognizing and cleaving target RNAs, which provided a new tool for research in the fields of gene regulation and drug delivery.

Through these important milestones, DNAzyme research has gradually deepened and provided new possibilities for technological development in the biomedical field. The history of DNAzyme discovery and important milestones demonstrate scientists' relentless exploration and innovation in this field, laying a solid foundation for DNAzyme's application prospects.

3. STRUCTURAL FEATURES AND CATALYTIC MECHANISM OF DNAZYME

3.1 Nucleic acid sequence and structural domains of DNAzyme

DNAzyme is a molecule with DNA catalytic activity, and its nucleic acid sequence and structural domains play a key role in its catalytic activity and specificity. The nucleic acid sequence of DNAzyme usually consists of two parts: the catalytic nucleic acid sequence and the substrate recognition sequence. The catalytic nucleic acid sequence is the key part of DNAzyme that is responsible for catalyzing specific chemical reactions. This part of the sequence usually contains specific base sequences and structures that can bind specifically to the substrate and catalyze specific reactions, such as shearing the RNA strand, redox, and so on. The design and optimization of the catalytic nucleic acid sequence is critical to the catalytic activity of DNAzyme. The substrate recognition sequence is the part of DNAzyme used to recognize the substrate and is usually located around the catalytic nucleic acid sequence. This part of the sequence is able to bind to specific regions of the substrate, ensuring specific recognition and catalysis of the substrate by the DNAzyme. The design and optimization of the substrate recognition sequence can improve the specificity and selectivity of DNAzyme.

The structural domains of DNAzyme usually include the following features: single-stranded structure: DNAzyme is usually composed of single-stranded DNA molecules with a relatively simple structure. The single-stranded structure makes DNAzyme more controllable and tunable, which is easy to design and synthesize. Specific three-dimensional structure: The nucleic acid sequence and structural domains of DNAzyme determine its specific three-dimensional structure, which is

critical to its catalytic activity. The structure of DNAzyme can be precisely designed through synthesis and modification to achieve specific catalytic functions.

Adjustable active center: The active center of DNAzyme is the key site where its catalytic reaction occurs and usually consists of the catalytic nucleic acid sequence. This active center has high catalytic efficiency and specificity, and the catalytic activity of DNAzyme can be optimized by adjusting the nucleic acid sequence and structure [13].

The nucleic acid sequence and structural domains of DNAzyme are the key factors for its catalytic activity and specificity, and the regulation and enhancement of DNAzyme function can be achieved by precisely designing and optimizing these parts.

3.2 Catalytic mechanism and reaction steps of DNAzyme

DNAzyme is a DNA molecule capable of catalyzing specific chemical reactions in the absence of proteins.The catalytic mechanism of DNAzyme usually consists of the following key steps.Substrate binding is a process by which DNAzyme binds to a specific structural region of the substrate through its substrate recognition sequence to form a DNAzyme-substrate complex. This specific substrate binding ensures specific recognition of the substrate by the DNAzyme [14]. Active center formation: the catalytic nucleic acid sequence portion of the DNAzyme forms an active center, which usually undergoes a conformational change upon substrate binding, allowing the active center to form a specific structure that is catalytically active [15]. Chemical reactions: the active center catalyzes specific chemical reactions such as shearing of the RNA strand and redox. This reaction usually involves the breaking, joining or other chemical bond formation and breaking of nucleic acid strands [16]. Product release: the reaction product is released from the DNAzyme-substrate complex and the DNAzyme is restored to its initial state and can again participate in the catalyzed reaction [17].

The catalytic mechanism and reaction steps of DNAzyme are usually precisely designed and regulated by the nucleic acid sequence and structural domains of DNAzyme [18]. By rationally designing the nucleic acid sequence and structure of DNAzyme, the modulation of its catalytic activity and specificity can be achieved to catalyze specific chemical reactions [19]. This DNA-based catalytic mechanism offers new possibilities in biomedicine and biotechnology, such as gene regulation, drug delivery.

4. PROSPECTS FOR THE APPLICATION OF DNAZYME IN THE BIOMEDICAL FIELD

4.1 DNAzyme in drug delivery

DNAzyme is a catalytically active DNA molecule that specifically recognizes and cleaves specific RNA sequences [20]. Due to its high specificity and customizability, DNAzyme has a wide range of potential applications in drug delivery. In drug delivery, DNAzyme can mainly play the following roles [21]. In targeted therapy, DNAzyme can be designed to target specific RNA molecules, such as viral RNA or specific oncogene mRNA [22]. By designing a suitable DNAzyme, specific cleavage of these RNAs can be achieved, thereby inhibiting viral replication or oncogene expression and achieving targeted therapy [23].DNAzyme can be used as a drug carrier, which can be designed to bind to drugs to form complexes for drug delivery. Such complexes can deliver the drug precisely to the target cells by targeting the RNA, increasing the local concentration of the drug and reducing the toxicity of the drug to normal cells.DNAzyme can also be designed to control the release of the drug [24]. By designing DNAzyme with cleavage-specific sequences, it is possible to release drugs under specific conditions (e.g., in the presence of specific RNAs), thus realizing the function of smart release.DNAzyme can also be designed for gene regulation [25]. By designing specific DNAzyme, regulation of specific genes, such as inhibition or promotion of gene expression, can be realized, thus affecting cellular physiological processes.

In conclusion, the application of DNAzyme in drug delivery can improve the targeting and therapeutic effects of drugs, reduce the side effects of drugs, and provide new possibilities for personalized therapy and precision medicine. As the research on DNAzyme continues to deepen, it is believed that it will have a wider application and development in the field of drug delivery [26].

4.2 DNAzyme in gene regulation

The application of DNAzyme in gene regulation has wide potential. By designing specific DNAzyme, the regulation of gene expression can be realized, thus affecting the physiological process of cells [27].The applications of DNAzyme in gene regulation mainly include gene silencing, gene activation, and disease therapy, etc. DNAzyme can be designed to target specific mRNAs, and inhibit the expression of the target genes by cleavage of the targeted mRNAs. This technique is widely used in gene silencing research and therapy. By designing specific DNAzyme, target genes can be effectively silenced, thus realizing targeted regulation of genes. In addition to inhibiting gene expression, DNAzyme can also be designed to promote gene expression. By designing DNAzyme with activation function, the expression of target genes can be promoted under specific conditions, thus realizing the activation regulation of genes.The application of DNAzyme in gene regulation can also be used for disease treatment. By designing specific DNAzyme to target specific disease-related genes, the regulation of disease genes can be realized, thus achieving the purpose of disease treatment. For example, DNAzyme can be designed to target oncogenes for cancer therapy. DNAzyme can also be used as a research tool in gene regulation [28]. By designing specific DNAzyme, precise regulation of gene expression can be realized, thus helping researchers to study the function and regulatory mechanisms of genes.

In summary, the application of DNAzyme in gene regulation has a wide range of potentials and can be used in gene silencing, gene activation, disease therapy and research

tools. As the research on DNAzyme continues to deepen, it is believed that there will be more applications and developments in the field of gene regulation, providing new possibilities for gene therapy and gene research!

5. INNOVATION AND APPLICATION OF DNAZYME IN INTELLIGENT DIAGNOSTIC AND TREATMENT SYSTEMS

5.1 Construction of an integrated diagnosis and treatment platform

In recent years, the innovation of intelligent diagnosis and treatment systems has been mainly reflected in the construction of diagnosis and treatment integration platforms, among which the application of the Altered DNAzyme (ASLM)-coupled CRISPR-Cas system is particularly prominent. This integration not only improves the sensitivity and specificity of biomarker detection, but also realizes the seamless integration of early diagnosis and immediate treatment of diseases [29].

Variant DNAzyme, as a core component, has the ability to monitor biomarkers in vivo in real time. When ASLM binds to target molecules, its conformation changes, activating the release of downstream signaling molecules. This property enables ASLM to produce detectable signals even in the presence of low concentrations of biomarkers, greatly facilitating the early diagnosis of diseases such as hepatocellular carcinoma [30]. The detection capability of the platform is further enhanced through coupling with the CRISPR-Cas system. CRISPR technology is able to specifically cleave the target biomolecules, thereby lifting the inhibition of ASLM and enhancing the measurability of the signal. This strategy not only improves the detection sensitivity, but also provides strong support for personalized medicine [31].

5.2 Clinical validation of hepatocellular carcinoma marker testing and synchronized therapy

In terms of specific applications, an intelligent diagnostic platform based on variant DNAzyme and CRISPR-Cas system has demonstrated remarkable results in the detection of markers and treatment of hepatocellular carcinoma. The platform enables early diagnosis by detecting liver cancer-related biomarkers, such as alpha-fetoprotein (AFP), in serum samples in real time [32]. Studies have shown that the detection system incorporating ASLM has demonstrated high sensitivity in multicenter clinical trials, effectively helping physicians to make timely diagnoses in the early stages of liver cancer.

Once the biomarkers are confirmed, the platform will automatically activate the corresponding targeted therapeutic mechanisms. ASLM-based targeted DNAzyme can specifically target liver cancer cells, thereby reducing damage to normal cells during treatment. In addition, combined with CRISPR technology, this platform can also effectively inhibit the growth and spread of hepatocellular carcinoma cells. Clinically validated results have shown that patients using this integrated platform for early detection have significantly higher treatment response rates, demonstrating its potential in liver cancer management [33].

The smart diagnosis and treatment system not only enhances the ability of early diagnosis and precise treatment through the effective integration of variant DNAzyme and CRISPR-Cas system, but also provides new ideas and directions for the application of individualized medicine.

6. Conclusion and outlook

This thesis explores the unique properties of DNAzyme as a novel biocatalyst and its potential application in the field of smart diagnostics. Since its initial discovery, DNAzyme has rapidly become an important tool for molecular biology and biomedical research due to its high catalytic efficiency, chemical stability and programmability. Studies have shown that DNAzyme not only effectively catalyzes various chemical reactions, but also can be used for specific recognition and detection of biomarkers, providing new solutions for early disease diagnosis.

In recent years, the application of variant DNAzyme (ASLM) combined with the CRISPR-Cas system in smart diagnostic platforms has opened up new opportunities for precision medicine and personalized therapies.The introduction of ASLM has demonstrated significant clinical potential, both in the early diagnosis of malignant tumors such as hepatocellular carcinoma, as well as in the targeted therapies against diseased tissues. This combination not only enhances the therapeutic effect, but also improves the overall survival rate and quality of life of patients.

In the field of smart diagnosis and treatment, DNAzyme still has a promising future application. Through further research and technological advances, DNAzyme is expected to be combined with other biotechnologies (e.g., nanotechnology, biosensor technology, etc.) to drive continued innovation in smart diagnostic systems. In addition, further optimization for the stability, targeting and clinical application of DNAzyme will contribute to its wider utilization in disease management.

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