Research Progress on the Application of Nanozymes in Biosensors

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Abstract

Nanozymes refer to a class of nanomaterials endowed with enzyme-mimetic catalytic activity. Since the intrinsic peroxidase-like activity of Fe_3O_4 nanoparticles was discovered by the research group led by Academician Xiyun Yan in 2007, this research field has undergone rapid and vigorous development. This paper systematically reviews the developmental history, classification and catalytic mechanisms of nanozymes. It emphatically elaborates the latest research progress of nanozymes in bioanalytical detection, biomedical applications and environmental pollutant degradation, and prospects the future development directions of nanozymes in the field of bioanalysis. Endowed with the advantages of low cost, high stability, large-scale manufacturability and tunable structural functions, nanozymes demonstrate enormous application potential in biosensing, disease diagnosis and therapy, as well as environmental protection.

Keywords

Nanozyme, Biosensor, Catalytic mechanism, Disease diagnosis and therapy, Environmental monitoring

Introduction

As highly efficient and specific biological catalysts, enzymes play a pivotal role in various life activities. However, the industrial application of natural enzymes has long been limited by their inherent drawbacks, such as sophisticated preparation and purification processes, high production costs, and extreme sensitivity to environmental factors including temperature, potential of hydrogen (pH) values, organic solvents and protease digestion [1]. These deficiencies result in poor stability and easy inactivation of natural enzymes, thus restricting their extensive application in complex practical scenarios. A groundbreaking study conducted by the research group led by Academician Xiyun Yan in 2007 broke this bottleneck. They accidentally discovered that magnetite (Fe_3O_4) nanoparticles exhibit prominent peroxidase (POD)-like catalytic activity analogous to natural enzymes without any surface modification, and can efficiently catalyze the oxidation of various substrates by hydrogen peroxide (H_2O_2). This discovery not only revealed that inorganic nanomaterials themselves possess intrinsic "enzyme-mimetic" properties, but more importantly, it gave birth to a brand-new interdisciplinary research field - Nanozymology. Nanozymes are explicitly defined as artificially synthesized nanomaterials with catalytic activity mimicking that of natural enzymes. They ingeniously integrate the unique physicochemical properties of nanomaterials (e.g., high specific surface area, tunable energy band structure, magnetism, photothermal effect) with the catalytic functions of natural enzymes. They thereby exhibit remarkable advantages in production cost, large-scale manufacturing, stability and functional designability [2]. After more than a decade of rapid development, the research history of nanozymes can be clearly divided into four stages: The first stage (around 2007) was the period of accidental discovery and phenomenon confirmation. The second stage entered the era of rational design and performance optimization, where researchers realized the preliminary "programming" of catalytic activity and selectivity by regulating the size, morphology, composition and surface chemistry of nanozymes. The third stage was dedicated to the in-depth exploration of catalytic mechanisms, in which the electron transfer process and the intrinsic nature of active sites responsible for enzyme-mimetic activity were elucidated with the aid of advanced characterization techniques and theoretical calculations. At present, the research has stepped into the fourth stage, namely the intelligent design era. Artificial intelligence and machine learning methods are employed to mine the "structure-activity relationship" between material parameters and catalytic performance in a high-throughput manner, and to reversely guide the intelligent design and prediction of novel high-performance and multifunctional nanozymes. This has significantly accelerated the research and development process.

Today, the family of nanozymes has expanded tremendously, covering various systems such as single-atom nanozymes, metal oxide nanozymes and carbon-based nanozymes, and their catalytic mechanisms (e.g., oxidoreductase-like, hydrolase-like and lyase-like activities) have become increasingly clear. This has promoted their revolutionary applications in numerous fields. In the field of bioanalytical detection, nanozymes have evolved into the core catalytic component for constructing a new generation of highly sensitive and stable biosensors [3]. We are widely applied in disease marker detection, heavy metal ion monitoring, food safety analysis and other fields [4,5]. In addition, in the biomedical field, reactive oxygen species (ROS) regulation strategies based on nanozymes provide novel approaches for tumor catalytic therapy, anti-inflammation and antibiosis [6]. In the field of environmental governance, nanozymes are also utilized for the degradation of organic pollutants and the removal of toxic substances [9].

This paper aims to systematically sort out the developmental context, classification system and catalytic mechanisms of nanozymes, focus on reviewing the latest research progress and application examples of nanozymes in three major directions: bioanalytical detection, biomedical diagnosis and therapy, and environmental catalytic degradation, so as to provide a panoramic reference for researchers in related fields. Finally, this paper makes a prospective outlook on the future challenges and development trends of nanozymes, especially in the field of bioanalysis, such as intelligent design, multifunctional integration, in vivo real-time monitoring and clinical translation.

Classification of nanozymes

The core function of nanozymes is to mimic the catalytic behaviour of natural enzymes. According to the type of natural enzymes they mimic, nanozymes are mainly divided into oxidoreductase-like, hydrolase-like and other enzyme-like nanozymes [10]. This classification method is directly related to their biochemical functions and application scenarios.

Oxidoreductase-like nanozymes

This is the most abundant and widely studied category at present, which mainly catalyzes redox reactions involving electron transfer.

Peroxidase-like: Such nanozymes can efficiently catalyze the oxidation of various organic substrates such as 3,3',5,5'-Tetramethylbenzidine (TMB) and o-Phenylenediamine (OPD) in the presence of hydrogen peroxide, and their catalytic mechanism is often similar to the "Fenton" or "Fenton-like" reaction of natural peroxidases. The core lies in the metal active centers (e.g., Fe^{2+}/Fe^{3+}, Cu^{+}/Cu^{2+}) on the surface of nanozymes catalyzing the homolytic cleavage of H_2O_2 to generate highly reactive hydroxyl radicals (OH), or forming high-valent metal-oxygen intermediates (e.g., Fe(IV)=O), which in turn oxidize the substrates [11]. In addition to classic Fe_3O_4, nanomaterials such as FeS_2 and Cu single atom nanozymes are also recognized as highly efficient POD mimics. They have been widely used in the rational construction of colorimetric biosensors and promising tumor catalytic therapy. [12,13].

Oxidase-like: Different from POD, oxidase-like nanozymes can directly use dissolved oxygen in the environment as an electron acceptor without the participation of H_2O_2, catalyze the oxidation of substrates (e.g., TMB), and simultaneously reduce O_2 to H_2O or H_2O_2. The mechanism usually involves the adsorption and activation of O_2 on the surface of nanomaterials. For example, gold, platinum nanoparticles and some copper-based nanomaterials can promote the one-electron reduction of O_2 through their unique surface characteristics [14]. Such nanozymes have unique advantages in constructing sensing platforms in H_2O_2-free environments.

Catalase-like: Such nanozymes specifically catalyze the decomposition of H_2O_2 into water and oxygen ($2H_2O_2 \rightarrow O_2 + 2H_2O$). The catalytic pathway may include a heterolytic mechanism (producing water and hydrated high-valent metal species) or a redox cycle. Fe_3O_4, MnO_2, CeO_2 and other nanomaterials all exhibit obvious Catalase (CAT) activity [15]. This function is crucial in alleviating oxidative stress, providing oxygen to improve tumor hypoxia, or serving as a power source for H_2O_2-driven micro/nanomotors.

Superoxide dismutase-like: SOD mimics can catalyze the dismutation reaction of superoxide anion radicals: $2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$. Its activity is highly dependent on whether the redox potential of the material is between the standard potentials of the $O_2^{\cdot-}/H_2O_2$ and $O_2^{\cdot-}/O_2$ pairs. CeO_2 has become a classic SOD mimic due to its reversible Ce^{3+}/Ce^{4+} redox pair [16]. MnO_2 and some carbon-based materials also possess this function, which has broad application prospects in anti-inflammation, anti-aging and neuroprotection fields.

Hydrolase-like nanozymes

Such nanozymes mimic natural hydrolases and catalyze the hydrolysis of ester bonds, peptide bonds, phosphodiester bonds and other chemical bonds. The mechanism may involve surface atoms of nanomaterials acting as Lewis's acids to activate substrates, or nucleophilic attack through surface hydroxyl groups and other functional groups. For example, lanthanide-doped nanomaterials or MOFs with coordinatively unsaturated metal sites can mimic phosphodiesterase activity, hydrolyzing organophosphorus toxicants (e.g., sarin analogs) or cleaving DNA/RNA model substrates [17]. Functionalized gold nanoparticles have also been widely reported to possess excellent protease-like or esterase-like activity [18]. Such nanozymes have important application value in the degradation and protection of environmental toxicants and the development of novel biological catalysts.

Other enzyme-like nanozymes

With the in-depth research, the simulation range of nanozymes has exceeded the common oxidoreductases and hydrolases. For example, some zinc-containing nanomaterials have been found to mimic carbonic anhydrase activity, catalyzing the reversible conversion of CO_2 and water into bicarbonate and protons, which has great potential in carbon dioxide capture and conversion. In addition, nanomaterials with DNA photolyase-like activity can repair pyrimidine dimers under light irradiation, providing a new idea for ultraviolet damage protection [19]. There are also some nanomaterials that exhibit specific isomerase-like activity and can efficiently catalyze the isomerization reaction of various substrates [20]. These diverse enzyme-mimetic activities have greatly expanded the application boundary of nanozymes in interdisciplinary fields such as energy, environment and life sciences.

Applications of nanozymes in biosensor

The application of nanozymes in the field of biosensing is one of the most active research fields at present. As efficient signal amplification tags, nanozymes can significantly improve the sensitivity and selectivity of biosensors.

Colorimetric biosensors

Colorimetric detection based on nanozymes is the simplest and most intuitive detection method. Color changes are generated by catalyzing chromogenic substrates such as TMB, OPD and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), which can realize visual detection or spectral quantitative analysis of target substances.

Glucose detection: A glucose oxidase (GOx)-nanozyme cascade reaction system is utilized, in which GOx catalyzes the oxidation of glucose to generate H_2O_2, and the generated H_2O_2 is then catalyzed by nanozymes (e.g., Fe_3O_4, Au NPs) to oxidize chromogenic substrates, realizing the sensitive detection of glucose [21]. The latest research has developed a dual-mode sensor based on MOF nanozymes, such as NH_2-CuBDC, for the simultaneous detection of glucose and cysteine [22].

Heavy metal ion detection: Certain heavy metal ions can inhibit or enhance the catalytic activity of nanozymes, and specific detection methods are constructed based on this characteristic. For example, Hg^{2+} can combine with Au NPs to inhibit their enzyme-mimetic activity, which is applied for the sensitive detection of Hg^{2+} [23]. Cr(VI) can directly oxidize chromogenic substrates or be reduced by nanozymes by virtue of its strong oxidizing property, realizing the selective detection of Cr(VI) [24].

Biomarker detection: Immunoprobes labeled with nanozymes can be used to detect various protein biomarkers. For example, a novel sandwich-type electrochemical sensor based on $Cu@Cu_2O$ nanozymes is employed for the highly sensitive and selective detection of Mucin-1 [25]. $Fe_3O_4@Au$ core-shell nanoparticles labeled immunochromatographic test strips are applied for the rapid detection of cardiac troponin I (cTnI) [26].

Electrochemical biosensors

The high catalytic activity and excellent electrical conductivity of nanozymes make them ideal materials for constructing electrochemical biosensors.

Signal amplification strategy: Nanozymes can be used as electrochemical signal amplification tags to catalyze substrates to generate electrochemically active substances. For example, Fe_3O_4 nanozymes catalyze the reduction of H_2O_2 to generate stable current signals, which are used for the ultra-sensitive detection of biomolecules such as DNA and proteins [27].

Enzyme-free glucose sensors: The glucose oxidase-like or peroxidase-like activity of nanomaterials such as CuO and NiO is directly used to realize the electrochemical detection of glucose without the participation of natural enzymes. This fundamentally solves the problem of easy inactivation of natural enzymes in traditional glucose sensors [28,29].

Fluorescent and chemiluminescent sensors

Certain nanozymes possess the activity of catalyzing chemiluminescent reactions or regulating fluorescent signals, which provides a new strategy for the construction of fluorescent and chemiluminescent sensors.

Chemiluminescent detection: Nanozymes can catalyze classic chemiluminescent systems such as luminol-H_2O_2 to generate stable luminescent signals. For example, $CoFe_2O_4$ nanoparticles efficiently catalyze the luminol chemiluminescent reaction, which is applied for the sensitive detection of H_2O_2 and glucose [30].

Fluorescence regulation: Nanozymes regulate the fluorescence state (on or off) of fluorescent dyes by catalyzing specific redox reactions. For example, MnO_2 nanozymes can oxidize non-fluorescent dye precursors to generate fluorescent substances, or cause fluorescence quenching through electron transfer with fluorescent quantum dots, thus constructing sensitive “turn-on” or “turn-off” fluorescent sensors [31,32].

Nanozyme-CRISPR/Cas biosensors

The integration of nanozymes with the Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins (CRISPR/Cas) system is an important breakthrough in the field of biosensing in recent years. The CRISPR/Cas system has precise DNA recognition ability and strong trans-cleavage activity, while nanozymes provide efficient signal amplification functions.

Detection principle: When the CRISPR/Cas system recognizes the target nucleic acid, it activates its trans-cleavage activity and cleaves the reporter molecule labeled with nanozymes. The quantitative detection of the target is realized by detecting the signal change generated by nanozyme catalysis. This method can realize the ultra-sensitive detection of nucleic acids, proteins and small molecules, and even achieve single-molecule detection level without pre-amplification [33].

Typical application: The MnO_2 nanozyme-mediated CRISPR-Cas12a system is used for the sensitive detection of SARS-CoV-2 viral RNA. The MnO_2 nanosheets adsorb the Cas12a-crRNA complex and quench the fluorescent signal [34]. The presence of the target triggers the trans-cleavage of Cas12a to release MnO_2 and restore the fluorescent signal [35]. This method combines the efficient catalytic signal amplification of nanozymes with the precise specific recognition of CRISPR, greatly improving the detection sensitivity and specificity.

Conclusion

Nanozymes, as an interdisciplinary field of nanotechnology and enzyme engineering, have evolved from the initial Fe_3O_4 nanoparticles to a diverse family including single-atom and MOF-based nanozymes. Nanozymes have the advantages of high stability, low cost, tunable functions and multifunctional integration. They show important application value in biosensing, biomedical therapy and environmental remediation, especially in constructing high-sensitivity biosensors for the ultra-trace detection of disease markers and environmental pollutants. Although nanozymes face challenges such as low catalytic selectivity, unclear biological safety, and lack of standardization, the integration of AI, single-atom catalysis, and CRISPR technology will promote their development toward precise design, high selectivity, and green sustainability. In the future, nanozymes are expected to achieve a historic leap from basic research to large-scale clinical application and industrialization, providing important technical support for human health and environmental governance.

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Authors' contributions

Qijia Jiang and Jiaqi Xiao contribute equally to the article.

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Conflicts of Interest

The authors declare no conflict of interest.

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