Study on the Characteristics and Application Value of Nanocatalysts Prepared by Enzymatic Cleavage

Qihariga[1], Tegxibaiyin Wang[2,*]
[1]*Inner Mongolia Medical University, Hohhot, China*
[2]*Affiliated Hospital of Inner Mongolia Minzu University, Tongliao, China*
[*]*Corresponding Author.*

Abstract: Nano-enzyme is a kind of functional nanomaterial with enzyme-like catalytic activity. Among them, Peroxidase-like nanozymes have attracted much attention due to their great potential applications in biosensing. This article provides an overview of the various types of nanozyme reactions, the factors influencing their activity, and their applications across different fields. Additionally, it briefly discusses the characteristics of the enzymatic cleavage method for preparing nanozymes, offering a theoretical foundation for the future clinical applications of nanozymes.

Keywords: Nanozyme; Enzymatic Cleavage; Nanozyme Applications

1. The History of Nanozyme Research

Nanozymes are organic or inorganic materials with enzyme-like catalytic activity at the nanoscale (1-100 nanometers). The ones that are typically formed of nanoparticles including metals, metal oxides, and carbon-based materials. In 2004, Manea et al. First proposed the concept of nanoenzyme.[1], who discovered gold nanoparticles exhibiting ribonuclease-like activity, thereby coining the term "nanozyme". This groundbreaking discovery sparked significant interest and increased research on nanozymes within the academic community. Nanozymes possess the unique properties of nanometer-scale dimensions while exhibiting catalytic activity akin to that of enzymes. In 2007, Academician Yan Xiyun's team further advanced the field by demonstrating that ferroferric oxide nanoparticles possess peroxidase-like activity [2]. This finding marked a pivotal moment in nanozyme research, drawing considerable attention from scientists worldwide. Over the years, research on nanozymes has rapidly progressed, with significant milestones achieved. Notably, in 2020, national standards for nanozyme terminology were established [3], providing critical guidance and standardization for future research in the field. As of now, more than 1,200 distinct types of nanozymes have been discovered. These include various metal oxide-based nanomaterials (including cerium oxide and iron oxide), metal-based nanoparticles (such as gold and platinum), bimetallic and other metal-based materials, as well as carbon-based nanomaterials. Nanoscale enzymes are broadly classified into different types based on their catalytic activity, including oxidoreductases, hydrolases, lyases, and isomerases. [4-6].

2. Classification of Nanozymes

2.1 Oxidoreductases

Oxidoreductases are enzymes that catalyze electron transfer or redox reactions. Four enzyme-like activities were included: catalase (CAT), oxidase (OXD), peroxidase (POD), and superoxide dismutase (SOD). Among these, CAT is one of the most important oxidoreductases in the body.It is an enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen, and is widely present in animals, plants, and microorganisms. Research has shown that gold nanoparticles (AuNPs)[7], silver nanoparticles (AgNPs) [8], platinum nanoparticles (PtNPs) [9], palladium nanoparticles (PdNPs) [10], cerium oxide (CeO_2) nanoparticles [11], and Fe_3O_4 nanoparticles [12] all exhibit CAT-like activity under different conditions. Substances exhibiting oxidase-like catalytic activity promote the oxidation of substrates under specific conditions, displaying catalytic characteristics similar to natural oxidases. The mechanism of oxidase activity typically involves the oxidation of the substrate and

electron transfer. During catalysis, the enzyme first binds to the substrate, forming an intermediate complex, then facilitates the oxidation of the substrate while transferring electrons to other molecules or acceptors. Studies have shown that molybdenum trioxide nanoparticles (MoO_3 NPs) [13] and cuprous oxide nanoparticles (Cu_2O NPs) [14] also display oxidase-like activity. POD, or peroxidase, plays a crucial role in biological systems. It not only participates in various physiological and biochemical processes but also maintains the redox balance within organisms. It catalyzes the decomposition of hydrogen peroxide (H_2O_2) or other peroxides, while oxidizing various substrates. Many metal nanomaterials, including gold, silver, palladium, platinum, and their multi-metallic nanoparticles, have been found to serve as peroxidase mimics [15]. SOD is a widely present metal enzyme in living organisms,SOD enzymes catalyze the disproportionation of superoxide anion radicals (O2--) to hydrogen peroxide (H2O2) and oxygen (O2). It plays a critical role as a free radical scavenger, efficiently eliminating superoxide anions and protecting cells from oxidative damage, thus maintaining redox balance in biological systems. Currently, cerium oxide (CeO_2) nanoparticles are considered potential candidates for SOD-like activity [16].

2.2 Hydrolases

Since the advent of nanozyme research, Various nanomaterials were developed to hydrolyze nanoenzymes. Six main types of hydrolytic nanoenzymes, including those based on AuNPs, Polymeric nanoenzymes, surfactant assemblies, peptide assemblies, metal and metal oxide nanoparticles, and MOFs have received extensive attention..Several types of metal oxide nanoparticles, such as cerium oxide (CeO_2), manganese oxide (MnOx), zirconium oxide (ZrO_2), also Cu_2O, have demonstrated highly efficient hydrolytic nanozyme-like activities [17].

2.3 Lyases

Lyases are enzymes that catalyze the dissociation of carbon atoms from other atoms, such as sulfur, oxygen, or other carbon atoms. They are involved in cellular processes such as the citric acid cycle and organic synthesis [18], where they break down complex biomolecules into smaller molecular fragments through specific catalytic mechanisms. In the field of nano-enzymes, modeling of lytic enzyme activity is equally important. In recent years, researchers have discovered that certain nanomaterials can mimic the activity of lyases, such as simulating protease activity by catalyzing the cleavage of peptide bonds. These nano-lyases hold potential application value in areas such as biomedicine, bioengineering, and drug development. For instance, nano-lyases can be used for the directed cleavage and modification of proteins, providing new tools for protein function research and drug design. Furthermore, nano-lyases can also be employed in disease diagnosis and treatment, intervening in diseases by catalyzing the cleavage of specific biomolecules.

2.4 Isomerases

Isomerases possess the unique ability to convert one molecule into another isomer, where the formation and breaking of bonds are catalyzed by the isomerase to facilitate an intramolecular rearrangement. The reaction products share the same molecular formula as the substrates but differ in their spatial arrangement of bonded atoms [18].

3. Factors Affecting the Activity of Nanoenzymes

3.1 Effect of Size on Nanoenzymes

The size of nanomaterials significantly influences the catalytic efficiency of nanozymes. As particle size decreases, the specific surface area increases, that is, the smaller the size of the nanoenzymes, The higher its specific surface area. Higher surface area exposes more active sites, promoting interactions with substrates, thereby enhancing catalytic activity [19-20]. Kalantari et al.[21] found that gold nanoparticles exhibit the highest catalytic activity when their size is approximately 1.9 nm. Valden et al.[22] Gold clusters prepared on the surface of single crystals of titanium dioxide exhibit maximum reactivity in the range of 3.5 nm, thereby demonstrating a strong correlation between nanozyme activity and particle size.

3.2 Effect of Morphology on Nanoenzymes

Catalytic properties of nanoenzymes are determined by their morphology, which is linked to the atomic coordination environment of the nanocrystals' surface. Different morphologies of nanozymes have different surface atomic coordination, leading to varied catalytic activities.

Singh et al. [23] comparison of nano-enzymatic activities of different morphologies of Mn3O4 by measuring the contents of SOD, CAT, and GPx. They found that cauliflower-like Mn_3O_4 exhibited the highest activity in all three enzyme mimic experiments, while other morphologies showed only SOD-like activity.

3.3 Effect of Surface Modifications on Nanoenzymes

Li et al. [24] synthesized semiconductor polymer nanozymes with photothermal activity. Upon near-infrared (NIR) irradiation, the modification of active sites on the nanozyme's surface increased its activity by 3.5 times. Fan et al. [25] also introduced histidine to the surface of Fe3O4 in order to improve the affinity of Fe_3O_4 for H_2O_2.

3.4 Effect of Temperature and pH on Nanoenzymes

Zhang Xin et al. [26] prepared molybdenum disulfide nanosheets (MoS_2 NSs) and found that the relative enzyme activity decreased when the temperature increased from 15°C-50°C, suggesting that low temperatures are more favorable for catalytic reactions. The enzyme exhibited higher activity at temperatures between 15°C and 30°C. The effect of pH on enzyme activity was also investigated, and it was found that the relative activity showed a tendency to increase and then decrease as pH was increased. The highest activity was observed at pH = 4.0. Wang Bo et al. [27] synthesized a manganese single-atom nanozyme (f-MnNC) with peroxidase-like activity, useful for alkaline phosphatase (ALP) activity. Their research revealed that, pH range between 3.0 and 6.0, the catalytic activity of f-MnNC increased and then decreased with increasing pH. High enzyme activity was observed between pH = 4.0–5.0, with the peak activity at pH = 4.4. Within a temperature range of 20–60°C, f-MnNC's catalytic activity initially increased and then decreased with rising temperature, maintaining high activity between 40°C and 60°C. These findings demonstrate that nanozyme activity varies under different pH and temperature conditions, exhibiting optimal activity within specific ranges.

4. Applications of Nanozymes in Various Fields

Nanozymes integrate multidisciplinary scientific research across materials science, chemistry, biology, and medicine.

Particularly, nanozymes have demonstrated immense potential and wide-ranging applications in tumor diagnosis and therapy. Their unique nanoscale size confers high surface area, excellent biocompatibility, and tunable catalytic activity, enabling precise targeting of tumor cells for efficient, low-toxicity therapeutic outcomes.

With the rapid advancement of nanotechnology, nanozymes, as a novel class of functional nanomaterials, have shown remarkable application potential due to their distinctive enzyme-like catalytic activity. In the biomedical field, nanozymes are not only utilized in biosensing and bioimaging but also play pivotal roles in disease diagnosis and treatment. Furthermore, nanozymes can serve as drug delivery carriers, precisely transporting medications to affected areas, thereby enhancing therapeutic efficacy while minimizing side effects.

In the realm of environmental protection, the application of nanozymes is equally promising. Due to their efficient catalytic properties, nanozymes can be employed to treat industrial wastewater, degrade organic pollutants, and help address environmental pollution challenges. Moreover, nanozymes can function as catalysts to accelerate chemical reaction rates, improving energy utilization efficiency.

Beyond biomedical and environmental applications, nanozymes also hold broad potential in industries such as food processing and agriculture. In the food industry, nanozymes can be used for food preservation and quality enhancement. In agriculture, they can aid in pesticide degradation and soil remediation, contributing to the sustainable development of agriculture.

In summary, nanozymes, as a novel class of functional nanomaterials with vast application prospects, are making significant contributions

across various fields. As research continues to deepen and technology advances, nanozymes are expected to further demonstrate their unique advantages and potential, contributing significantly to the development of human society.

5. Enzymatic Cleavage Method

5.1 Applications of the Enzymatic Cleavage Method

It has been observed that researchers have developed various methods for preparing carbon dots, such as synthesis methods, electrochemical approaches, laser ablation, strong acid dissolution, and hydrothermal one-step methods. During our team's comparative and optimization study of carbon dot preparation methods, we discovered an important technique—the enzymatic cleavage method. In this approach, traditional Mongolian pearl powder is first treated with gastrointestinal fluids, resulting in the formation of numerous oxygen-containing defects on its surface. Subsequently, a 37°C hydrothermal treatment follows, where pepsin selectively targets the defect regions on the surface of the carbon particles. This enzymatic process “cuts” or “clips” the carbon particles, producing smaller-sized quantum dots or even forming pore-like nanoparticles. Using the enzymatic cleavage method, we successfully synthesized fluorescent carbon dots from pearl powder and fluorescent nanomaterials from black ice flakes. The resulting particles, with diameters of approximately 5 nm, are enriched with functional organic groups such as hydroxyl and amino groups on their surfaces. It not only has good dispersibility and water solubility (in contrast to traditional carbon dots, which are poorly soluble in water) but also with ease of functional modification and biological conjugation. Moreover, they exhibit remarkable biocompatibility.

5.2 Advantages of the Enzymatic Cleavage Method

The small particle size and the abundance of functional groups such as hydroxyl and amino groups on the surface make carbon dots not only highly dispersible and water-soluble (unlike traditional carbon dots, which are poorly soluble in water) but also easily functionalized and biologically conjugated, all while exhibiting excellent biocompatibility.

Kalantari et al. [21] discovered that gold nanoparticles exhibit the highest catalytic activity at a size of 1.9 nm, while Valden et al. [22] found that gold clusters prepared on the single-crystal surface of titanium dioxide show maximum reactivity within the range of 3.5 nm. These findings suggest that particle size significantly influences enzymatic activity. This is consistent with the small particle size produced by the enzymatic cleavage method, specific surface area increases with decreasing particle size. That is, the smaller the size of the nano-enzymes, the higher their specific surface area, and a higher specific surface area exposes more active sites, facilitating enhanced interactions with substrates and thereby increasing catalytic activity[19-20].

Wang et al. [28] observed that nitrogen-doped graphene oxide (GO) nanoparticles incubated with Cu^{2+} ions form a Cu^{2+}-GO nanocomposite, where Cu^{2+} ions interact with the functional groups on the GO surface to form Cu^{2+}-carboxylate or mixed Cu^{2+}-carboxylate/amine complexes. This Cu^{2+}-GO nanocomposite demonstrates horseradish peroxidase (HRP)-like activity, capable of oxidizing dopamine to amine dyes in the presence of H_2O_2. In contrast, GO modified with other metal ions (Ni^{2+}, Co^{2+}, Pd^{2+}, Cd^{2+}) does not exhibit catalytic activity toward dopamine oxidation. This demonstrates that functionalization enhances or generates catalytic activity.

Further studies revealed that when comparing the typical UV-visible spectra of TMB(3,3',5,5'-tetramethylbenzidine) oxidation in the presence of H_2O_2, unmodified Fe_3O_4 nanoparticles show very low absorbance at 652 nm, while Fe_3O_4/Sm1 and Fe_3O_4/Sm2 nanoparticles modified with DNA show significant enzyme-like activity and higher the absorbency at a wavelength of 625 nanometers, suggesting enhanced resembling peroxidase activity compared to bare nanoparticles. Additionally, Fe_3O_4/Sm2 conjugates exhibit even greater absorbance than Fe_3O_4/Sm1 conjugates at 652 nm. This demonstrates that biological conjugation enhances enzyme activity.

In summary, the small particle size, ease of functional modification, and biological conjugation capabilities of nanoparticles prepared via the enzymatic cleavage method

significantly enhance enzymatic activity.

5.3 Limitations of the Enzymatic Cleavage Method

The enzymatic cleavage method is not effective in cutting metal nanoparticles, which are widely used in fields such as biomedicine and catalysis. However, due to their unique physicochemical properties—such as high surface energy and strong metal-metal bonding—enzymes are often unable to efficiently cleave and modify these particles. This limitation restricts the applicability of the enzymatic cleavage method in the preparation of metal nanoparticles.

6. Conclusion

Nanotechnology, as a cutting-edge scientific discipline, has become an essential tool for addressing a wide range of scientific challenges. Nanozymes are capable of imitating the functions of natural enzymes, also overcome many of the limitations associated with their natural counterparts. The enzymatic cleavage method, in particular, has proven effective in highlighting the unique characteristics of nanozymes, thereby facilitating a deeper understanding of their potential. Ongoing research into nanozymes continues to broaden their application areas. However, significant challenges remain in improving their catalytic activity, stability, and biocompatibility. Moreover, controlling the size, morphology, and surface modifications of nanozymes is crucial for optimizing their performance. The enzymatic cleavage technique plays a vital role in advancing these areas. Nevertheless, further in-depth research and optimization are necessary to ensure that nanozymes can be applied more stably and efficiently across various fields.

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