Research on Activity Regulation of Nano-Enzymes and Biomedical Application

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Abstract. A particular class of simulated enzymes known as nanoenzymes performs biocatalytic activities in addition to having the distinctive characteristics of nanomaterials. Nanoenzymes offer distinct benefits over real enzymes and other artificial enzymes. On the one hand, nanoenzymes are endowed with several properties such as optical, electricity, and magnetism by the chemical makeup of nanomaterials. The building of precise biological analysis probes is made easier by the large specific surface area and rich surface chemical characteristics of nanomaterials, which enable nanoenzymes to chemically change and attach to biological recognition molecules. It's significant to note that the structure, content, morphology, surface modification layer, etc. of nanoenzymes are all directly connected to their catalytic activity. By regulating their activity, various aspects of the performance of nanoenzymes can be greatly improved, and it has become a research hotspot in multiple fields. Therefore, this article introduces the latest progress in the regulation of nanoenzyme activity from six aspects, with a focus on its applications in biomedical fields such as cancer treatment, antibacterial, and biosensing. In addition, this article also prospects the development prospects of nanoenzymes, aiming to provide more references for further research.

Keywords: Nanozymes, cancer therapy, biosensing, antibacterial.

1. Introduction

Enzymes are biomolecules with catalytic properties and complicated chemical structures that are used in a variety of processes, including industrial production, medicinal applications, biological research, and biosensing [1]. The majority of them are globular proteins, although some are also RNA ribozymes. Natural enzymes, however, have many disadvantages, including the fact that they are frequently expensive to produce and store, unstable to transfer or modify, and susceptible to challenging physicochemical conditions [2]. Modern chemistry's development of synthetic enzyme mimics with catalytic activity close to that of real enzymes has become crucial to address these issues. The advantages of nanozymes, a novel class of nanomaterials, include strong catalytic activity, gentle reaction conditions, good stability, low cost, and simplicity of mass manufacturing. Under moderate or harsh circumstances, they are capable of catalyzing the conversion of enzyme substrates into products that follow enzyme kinetics (such as the Michaelis-Menten equation).

The discovery of nanozymes breaks through the traditional concept that people used to regard nanomaterials as biologically inert substances, and it is the first time to discover another large class of substances with biocatalytic function besides proteins and nucleic acids, and it bridges the gap between nanomaterials, chemistry, enzymology, and medicine, according to which the nano-enzymes concept of catalytic medicine is being expanded from in vitro detection to the study of disease treatment, such as tumors, infectious diseases, degenerative lesions, and strokes, which proves the feasibility of the nano-enzymes to intervene in the pathological process, and it would lay a solid foundation for the nano-enzymes to serve in the health of human beings.

This article examines six techniques for controlling the activity of nanoenzymes, provides a current research update on the mechanism governing the catalytic activity of nanoenzymes, and discusses the uses of nanoenzymes in the treatment of cancer, the development of antibacterial agents, and biosensing.

2. Research on the mechanism of nano-enzymatic activity regulation

Numerous studies have demonstrated that new approaches, such as adjusting the size, morphology, crystallographic surface, valence, and coordination structure, can specifically regulate the catalytic performance of nanozymes. This opens the door to the search for more effective nanozymes and the investigation of their biocatalytic mechanism.

The size and morphology of nanozymes directly affect their specific surface area and surface energy, and nanozymes with more surface energy usually have higher activity. Changing the ratio of components in nanomaterials or compositing or hybridizing materials can specifically enhance some kind of activity of nanozymes or improve their catalytic performance. Additionally, surface modification of nanozymes and the state of the environmental medium in which they are located can affect their catalytic activity.

2.1. Size

The particle size of nanozymes has a substantial impact on their catalytic activity. Due to their increased specific surface area, smaller nanozymes have more exposed catalytically active sites, which increases their catalytic activity.

Yang et al. looked into the enzyme activity of Fe_3O_4 in 2007 using diameters of 30, 150, and 300nm. According to the findings, 30nm Fe_3O_4 has the maximum enzyme catalytic activity, whereas 300nm Fe_3O_4 has the lowest [3]. This is so that smaller Fe_3O_4 can bind more substrates since it will have more active binding sites.

2.2. Shape and morphology

Controlling the morphology and form of nanozymes can also affect their biocatalytic abilities. Pd octahedra and cubes were used as examples by Yin et al. to demonstrate this. The catalase and superoxide dismutase activity of Pd octahedra were reported to be greater than those of Pd nanocubes [4].

Qu et al. investigated the enzyme-like properties of CeO2 nanoparticles with different morphologies (particles, cubes, octahedrons, rods, mesopores) and found that mesoporous CeO2 had the highest enzyme activity due to its highest Ce3+ content and larger specific surface area [5].

2.3. Component

The catalytic activity of nanozymes may also be controlled by changing the ratio of the components in the nanomaterials. Doping certain components into the nanozymes has also proved successful in modifying the activity of the nanozymes.

Wei et al. found that the peroxidase-like activity of mesoporous carbon (MC) and reduced graphene oxide (rGO) was amplified by 100 and 60, respectively, when doped with heteroatomic nitrogen (N). N-doping only particularly increased the peroxidase activity while having no effect on the other catalytic activities of rGO and MC. By co-doping N and B, Lee et al. significantly improved the catalytic activity of graphene, which offered a fresh concept for the creation of highly active and particular carbon nanozymes [6].

2.4. Complex and hybridization

Material complexation/hybridization is also an approach to improve the catalytic properties of enzymes. For example, complexing heme/G-quadruplexes with Pt@Cu MOF significantly improved their peroxidase activity [7]. Also, some studies have used nano-enzymes such as CuO, Fe_3O_4, and Au in combination with graphene to form complexes whose catalytic activity is far superior to that of single-component catalysts due to their synergistic interaction, high conductivity, and good dispersibility.

Over a wide pH range, the graphene oxide-gold cluster complexes show strong peroxidase-like activity. The compounds somewhat lessen the impact of pH on activity while enhancing catalytic efficiency, which is significant for the investigation of the mechanism governing activity.

2.5. Surface modification

Most chemical reactions happen at the surface contact with nanozymes. The altered surface of nanozymes alters their attraction for substrates and catalytic activity by altering their surface charge and microenvironment, which in turn affects the exposure of their active sites.

The most extensive research on surface modification of nanozymes in recent years has focused on three types of nanomaterials: metal oxide nanomaterials, metal nanomaterials, and carbon nanomaterials. There are various ways of surface modification, which can combine nanomaterials with ions, molecules, and polymers, and the common types of modification are physical adsorption and covalent modification. Figure 1 shows some common ways of surface modification.

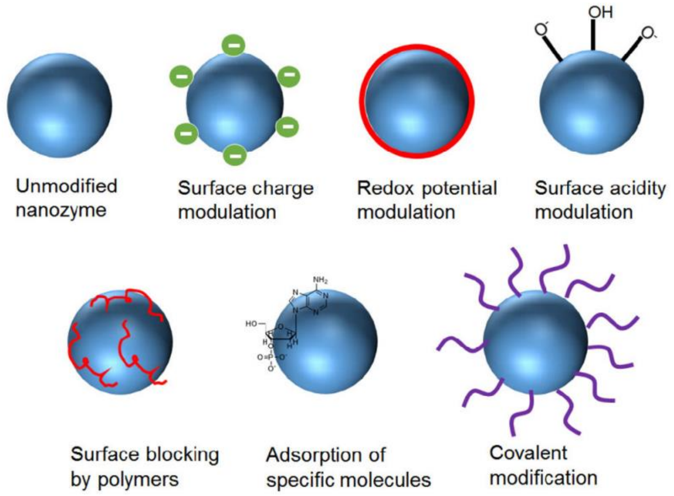

Figure 1. Strategies for surface modification to regulate nanoenzyme activity [7]

Important biomolecules called nucleoside triphosphates (NTPs) have been shown by researchers to work as coenzymes to boost CeO_2 nanoparticles' oxidase-like activity [8]. However, little research has been done on how NTPs affect the surface chemistry of CeO_2 nanoparticles.

Due to their multivalent binding action, polymers may adsorb relatively firmly compared to small molecules and offer higher colloidal stability. The biocompatible polysaccharide dextran is frequently used to modify nanoparticles, and when heated, nano CeO_2 modified with dextran demonstrated high colloidal stability. However, the results varied in different pH environments, which reflects the modulation of the nanoenzymatic activity by pH. In this case, where the nanoenzymatic activity is regulated by multiple factors simultaneously, the actual study is often more complicated, but at the same time, this complexity provides more possibilities for the design of the activity regulation mechanism.

Perez et al. also looked at how the polymer thickness affected the enzyme activity of CeO_2 surfaces. According to the findings, CeO_2 with a thin polymer covering exhibited more oxidase-like qualities. This could be a result of thin polymer coatings being more permeable, which makes substrate molecule transfer easier [8].

2.6. Environmental media

In addition to ambient temperature, pH, and environmental media including light, sound, electricity, and magnetism, nano-enzymes' catalytic activity is also influenced by these factors. Generally speaking, the activity of nano-enzymes will be the highest in the range of their optimal temperature and optimal pH value.

In 2022, He effectively regulated the redox capacity of the nanozymes by controlling the temperature to regulate the content of oxygen vacancies and improving the exposure ratio of active crystal planes to effectively regulate the nanoenzyme's redox capacity and prepared excellent multi-enzyme-like nanomaterials, which provides an idea for the regulation of the nanomaterial's redox capacity. In the same year, Hong et al. proposed that pH controls the ability of metal-free nanozymes to function as peroxidase or catalase [9]. They found that the selectivity of N- and B-doped fullerenes toward peroxidase- and catalase-like enzymatic activity, respectively, is modulated by pre-adsorbed H^* and OH^*.

In addition, some metal-based nanomaterials have unique physical properties, and magnetic field modulation of activity has become one of their current research hotspots. Magnetic field is an exogenous stimulus that can be adjusted easily and safely. It has unique advantages in the regulation of enzyme activity. Based on this, researchers created and analyzed a number of iron oxide nanozymes in 2021 and discovered that the rise in nanozyme catalytic activity with an applied magnetic field increased linearly with the magneto-thermal conversion efficiency of the particles. The development of magnetically sensitive nanozymes and tumor treatment based on magnetic nanozymes are anticipated to benefit theoretically from this study.

3. Nanozymes in biomedical applications

Nanozymes are currently being used in the fields of cancer therapy, antibacterial, and biosensing because they possess the characteristics of nanomaterials and enzyme-like catalytic activity, as well as high catalytic activity, stability, tunability, cheap and easy to obtain, and ease of storage for a long period.

3.1. Cancer therapy

The TME (tumor microenvironment) has an important impact on the functioning of nanozymes. It was found that during the rapid proliferation of tumor cells, the oxygen consumption increased, and at the same time, the oxygen supply of the abnormal tumor vascular system was insufficient. In the hypoxic environment, the glycometabolism of the tumor cells is altered, the pH becomes weakly acidic, and the H_2O_2 concentration increases.

Therefore, on the one hand, nanozymes can be used to improve anti-cancer properties by using oxygen supply and GSH depletion to change the conditions of the TEM and enhance other therapeutic effects. On the other hand, nanozymes can be used for catalytic therapies to directly kill tumor cells or inhibit tumor growth through their enzyme mimicry ability.

3.2. Antibacterial

2.2 Nanozymes combine their innate enzyme-mimicking activity with their physicochemical characteristics to effectively regulate reactive oxygen species. Reactive oxygen species overproduction can harm nucleic acids, proteins, and cell membranes, killing harmful microorganisms including bacteria, fungi, and viruses. It was discovered that the field of oral hygiene might greatly benefit from this novel class of nanozymes. Streptococcus mutans colonies may readily develop on the surface of teeth when sweets like sucrose and fructose are present in the mouth, which can harm the tooth's structural integrity.

Gao and his associates [10] discovered that Fe_3O_4NPs with POD-like activity could catalyze the in situ formation of free radicals from H2O2, which resulted in bacterial mortality in the periplasm and could also promote periplasmic matrix breakdown in Streptococcus mutans (Figure 2). Nanozymes are a novel class of antibacterial medications with significant potential since they are more stable, biocompatible, and less likely to develop drug resistance than conventional antibacterial treatments.

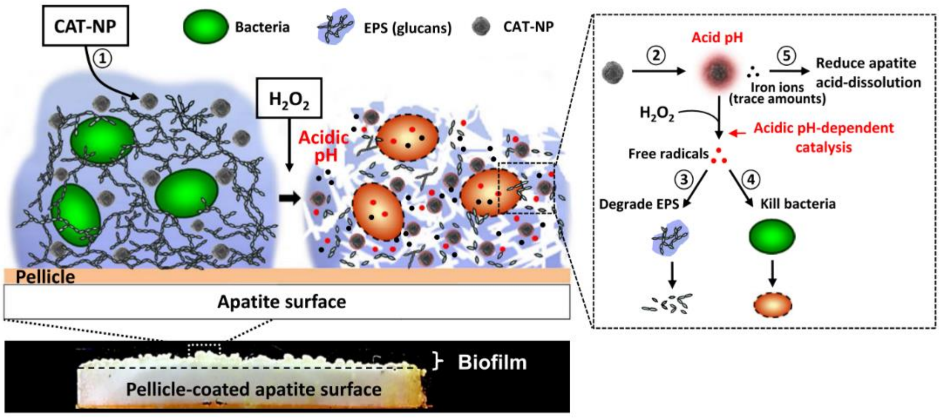

Figure 2. Schematics of biofilm disruption under acidic conditions by CAT-NP/H_2O_2 in situ [10].

3.3. Biosensing

Through biorecognition components and enzymic catalytic processes, nanozymes-based biosensors offer high-precision detection of a range of biomolecules (ions, small molecules, nucleic acids, proteins, cells, pathogens, etc.).

Shi et al. [11] created a multimodal nanozymes-linked immunosorbent test (named NLISA) for the in situ creation of Prussian Blue nanoparticles (PBNPs) to detect the flatoxin B1 (AFB1). Magnetic nanoparticles (MNPs), the loading carrier, were affixed to the 96-well plates. In the presence of HCl and $K_4Fe(CN)_6$, MNPs can be used as a precursor for the in situ synthesis of PBNPs. When exposed to near-infrared light, PBNPs may produce thermal energy that can be quantitatively measured by a thermometer and is easily portable. Additionally, PBNPs are good intrinsic peroxidase mimics that accelerate the oxidation of TMB to generate a colorimetric signal. This approach is more sensitive than other methods and aids in reducing false-positive/negative-positive findings brought on by random variables like modifications to the experimental setup and biochemical environment.

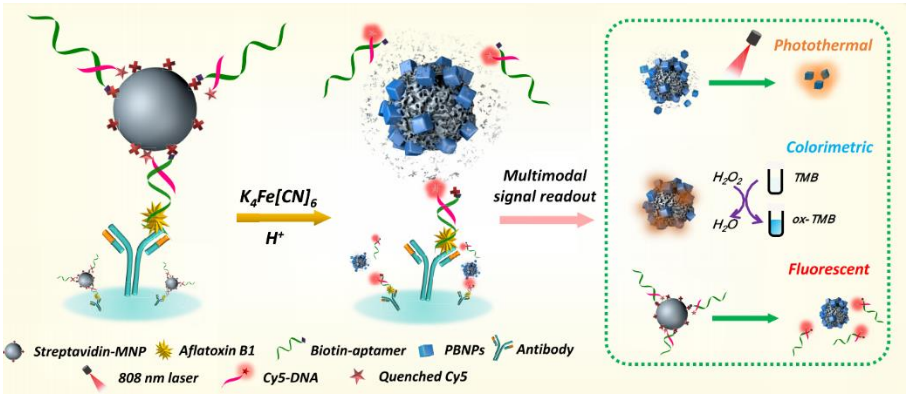

Figure 3. Novel Multimode NLISA Based on the In Situ Generation of PBNPs [11]

4. Conclusion

The development of nanozymes has made tremendous progress in recent years, but still faces great challenges, mainly in the following areas. (1) For the development of additional high-performance nanozymes, it is necessary to study specific structures related to the catalytic activity of nanozymes since, except for a few single-atom nanozymes, critical structural information on the catalytic sites of the majority of nanozymes is still poorly known. (2) The majority of current research on nanozymes concentrates more on improving catalytic activity than it does on identifying the catalytic mechanism. For the logical design and synthesis of high-performance nanozymes in the future, catalytic kinetics that apply to nanozymes must be established. (3) Currently, the application of nanozymes is concentrated in the field of medicine and health, but due to the short development time, the difficulty of clinical transformation of nanomedicine, low market recognition, and has not been put into actual production on a large scale. It is still necessary to explore how the existing research results can be transformed into practical productivity and its application can be extended to other fields. (4) The vast majority of nanozymes are synthesized in small laboratory systems, with less mass production of materials and a lack of quality control methods and standards for materials and enzymology. Therefore, a relevant large-scale production and quality control system needs to be established to lay the material foundation for translational applications.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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