

Current Review of Nanozymes

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Abstract—Nanomaterials along with enzyme-like activities are referred as nanozymes. Since Fe₃O₄ magnetic nanoparticles were described to employ peroxidase like activity in the year 2007, widespread research development in this area with deep investigation of various nanozymes has been made. As promising substitutes for native enzymes, nanozymes have established outstanding work in biopharmaceuticals, medicine, environmental observation and numerous other fields. The past few decades have supported the quick expansion of metal, metal oxide-based nanozymes due to their high stability, low cost, and easy storage. The investigation of the reaction mechanism is the basic need for understanding the catalytic response of nanozymes. In this his review, we elaborated the recent advances in expanding the types of nanozymes, activity and catalytic reaction mechanism of the metal, metal oxide-based nanozymes, and conclude the current challenges in future research in front of researchers.

Index Terms— Nanozymes, Metal and metal oxide nanoparticles, catalytic reaction mechanism.

I. INTRODUCTION

Most of the natural enzymes are mainly composed of proteins and are environmentally friendly bio-catalyst that catalyze the biochemical reactions[1, 2] with great substrate selectivity with remarkable catalytic efficiency and yields under mild conditions, and these reactions are the basis of the metabolism of all living organisms, and offer great opportunities for industry to carry out well-designed, well-organized biocatalytic adaptations. Natural enzymes are used since ancient times in the production of food products, such as cheese, wine, beer, vinegar and in the manufacture of leather, indigo and linen. In the widest sense enzyme technology can be understood as the applications of enzymes in the manufacture of goods and services. The previous four decades have observed the widespread progress of biological enzymes in drug, chemistry, biological and industrial fields. However, enzymes undergo denaturation and degradation under harsh conditions (low operational stability) such as high temperature, high pressure, organic solvents, extreme pH, difficulty in storage, complications in recovery and recycling, and high costs in extraction and purification of natural enzymes greatly constrained their practical applications. These limitations of natural enzymes stimulated the researchers to explore artificial enzyme mimics. These are also called as artificial enzymes. Among them, Nanozymes have emerged as the next age group of enzyme mimics then the unexpected detection of ferromagnetic particles (Fe₃O₄) with intrinsic peroxidase like activity was first reported in 2007 by Gao et al. After that a lot of nanozymes have been illustrated as enzyme mimics. Ex, Au@Co-Fe hybrid NPs[3], CdS NPs, CeO₂ NPs[4], Co₃O₄ NPs[5], MnO₂ nanowires[6], Au NPs[7], Pd NPs[8], VO₂ NPs[9], CuCo₂S₄ NPs[10], Carbon based NPs like carboxyl-modified graphene oxide (GO-COOH), CNTs[11] have been reported as peroxidase mimics.

Nanozymes are inspired by natural enzymes but are advantageous than natural enzymes, generally they are low cost, commonly retained better stability and robustness over long range of reaction conditions like temperature, pressure, pH, than natural enzymes[12]. Furthermore, physicochemical properties such as high surface energy and area, superior optical, and photothermal conversion properties, in addition easiness in preparation and storage also extend their applications[13]. Nanomaterials with enzyme mimicking ability are recognized as a class of functional nanomaterials, they have unique properties of both nanomaterials and enzymes. From the past five years, rapid development in the field of nanotechnology, biotechnology and catalysis science, substantial developments have been achieved in synthesizing the nanozymes with different enzymatic activities with high performance of nanomaterials. Interestingly, many metal and metal oxide nanoparticles are showing different enzymes activity[14]. Surface modification of nanomaterials exposed as promising approach to enhance the biocompatibility of these nanozymes. The structure and catalytic efficiency are flexible with suitable control of synthesis conditions. Metal and metal oxide based nanozymes progressively extended from environment to chemical, agriculture, biomedicine industries and so on. further elevation of nanozymes is still facing difficulties even though numerous efforts were dedicated. Hence, a complete review is needed to summarize and investigate the progress. Such reviews are helpful for researchers to understand the nanozyme well and elevation of the field. In this review, we proposed to cover the past five years reported nanozymes and their activities.

II. TYPES OF NANOZYMES

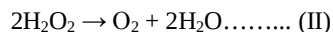
In order to get a clearer understanding, Nanozymes can be classified as oxidoreductase family and hydrolase family nanozymes. oxidoreductase nanozymes includes peroxidase, catalase, oxidase and superoxide dismutase and reductase nanozymes includes bilirubin reductase, nitrate reductase, 5 α -reductase, 5 β -reductase, ribonucleotide reductase etc.

A. peroxidase Like Activity:

peroxidases are generally found in plants, animals, fungi and microorganisms that act as oxidoreductase[15]. Most of the peroxidase enzymes are heme based (Fe⁺)[16, 17] with ferric protoporphyrin as prosthetic group e.g., horseradish peroxidase, myeloperoxidase, lignin peroxidases. Peroxidase with selenium (glutathione peroxidase), vanadium (Bromo peroxidase), and manganese (manganese peroxidase)[18-21], as active centers are also reported. With the expense of H₂O₂, it oxidizes the wide range of substrate. In 2007, Gao et al accidentally discovered ferromagnetic particles (Fe₃O₄) had a similar properties of horseradish peroxidase which have an ability to oxidize three substrates such as TMB (3,3',5,5'-tetramethylbenzidine), o-phenylenediamine, and diazoaminobenzene to their respective colored products using H₂O₂[22]. The kinetics studies recommended ping-pong mechanism of Fe₃O₄ nanozymes, and Michaelis–Menten constants determined specifies higher affinities of the Fe₃O₄ nanozymes for TMB, lower affinity for H₂O₂ than that of the HRP. Later Wei and Wang used the peroxidase property of Fe₃O₄ NPs for the detection of both H₂O₂ and glucose[23]. Inspired by this work iron oxide based nanozymes were extensively discovered and studied, such as Fe₃O₄ (magnetite)[24, 25], Fe₂O₃ (hematite)[26-28] and [29, 30]. Nanomaterials including metal organic frame works[31], conducting polymers[32] carbon-based nanomaterials[11] are also unraveled to serve as peroxidase mimics. Search for the catalytic mechanism has become one of the key tasks in this evolving research area. Particularly, of Fe₃O₄ nanozymes is one of the wildly studied. Peroxide and catalase activity of Fe₃O₄ nanozymes normally determined by environmental pH[33]. They exhibit peroxidase like activity under acidic pH (3-6.5)[34], in which H₂O₂ is disintegrated to produce free radicals (hydroxyl radicles) and then hydrogen donor is oxidized to form the oxidized donor and water as shown below. (HA refers to hydrogen donor and A refers to oxidized donor).

1. H₂O₂ → free radicals (mainly ·OH)
2. OH + HA → A + H₂O.....(I)

They also exhibit catalase activity under neutral and basic pH (7-10) and this activity leading to decomposition of H₂O₂ to oxygen and water[35].



Usually, equation (I) was to be catalyzed only by the release of iron ions in solution by Fenton reaction (Haber-Weiss reaction). However, Xiyun Yan et al. first reported that this process could also catalyzed by Fe₃O₄ itself[35]. This stimulating discovery promoted the researchers to further discovery of the difference between Fe₃O₄ and the Fenton reaction. This distinguishes from Fenton mechanism mainly because the Fe₃O₄ nanozyme reaction occurs on the surface of crystallized nanozymes, while Fenton mechanism happens in homogeneous system[25]. Overall, the mechanism of Fe₃O₄ nanozymes can be recognized as heterogeneous Fenton reaction. Therefore, it is easy to understand the dependency of catalytic activity of Fe₃O₄ nanozymes on multiple factors

like morphology, size, valence, composition, and surface modification, because this factors mainly affects the surface properties to large extent, which closely relay to active site[36]. It is also interesting that Fe_3O_4 nanozymes are have ability to imitate some properties of usual enzymes' like Michaelis–Menten kinetics and ping-pong mechanism and also showing pH and temperature-dependent natures[36].

Previous studies have confirmed that the shape and morphology of nanomaterials play an important role in the enzyme activity of nanozymes to a large extent. Tian et al. prepared three different morphological VO_2 NPs such as nanofibers, nanosheets and nanorods with peroxidase like activity in which VO_2 nanofibers are best with colorimetric assay of H_2O_2 and glucose due to their large surface area[9].

Since nanomaterials with smaller size, due to their high surface to volume ratio shows more active sites, size of the nanoparticles has significant effect on catalytic property of nanozymes. Generally, nanoparticles with smaller size tend to have more nanozyme characters in catalytic reactions attributed to the larger specific surface area. Xiaohu Xia et al. studied size dependence of peroxidase like activity of Pd-Ir Core-Shell Nanoparticles. They synthesized and studied the catalytic activity of different size Pd-Ir nanozyme (3.3, 5.9, 9.8 and 13.0 nm) but with identical shape and morphology. They found the catalytic activity increases with particle size. Using enzyme-linked immunosorbent assay (ELISA), biosensing application was studied, where smaller sized Pd-Ir nanoparticles were found to have lower detection limit. Higher catalytic property of smaller size Pd-Ir nanozymes is attributed to their diffusivities and reduced steric effect[37]. Table I shows some surface modified nanozymes with peroxides activity and their catalytic parameters.

B. Catalase like Activity

Catalase is a type of enzyme that consists of iron porphyrin ring as a prosthetic group[38]. Catalase in living tissue could efficiently catalyze the decomposition of hydrogen peroxide into oxygen and water and hence protect the tissues from hydrogen peroxide. Up to now, many metals and metal-oxide nanomaterials have been demonstrated as catalase activity like platinum (Pt)[39], gold (Au)[40], Co_3O_4 [5], [41], Mn_3O_4 [42]. Generally, these nanozymes processes other enzyme mimicking activities along with catalase like activity, and pH and temperature can affect the dominance of the enzyme mimicking activity. As we discussed above, Fe_3O_4 nanozymes under neutral or basic medium catalyze H_2O_2 on the surface of the metal to produce H_2O^* and O_2^* . Furthermore, Pd and Pt were verified to possess better catalase activity than Au and Ag. Using benefit of such extremely efficient oxygen generation by Pt, biological sensing and photodynamic therapy were established. Li et al. confirmed that the adsorbed OH group on the outer surface of nanomaterials served as the active sites for catalase like catalytic reaction[43]. Most of the reported catalase like nanozymes are favored under neutral and alkaline environment, Liu et al. first reported that AuNCs-NH₂ nanoclusters shows catalase mimicking property over physiological pH range (i.e., pH 4.8–7.4)[44]. Wang and co-workers, when studying the peroxidase activity of Co_3O_4 NPs, observed that Co_3O_4 NPs are also showing weak catalase activity[45]. They demonstrated that catalase activity would be increased by increasing the pH from acidic to neutral and even basic medium, and deeply suggested that hole mechanism process as one, Co (II) can activate the H_2O_2 on the surface to decompose into $\text{HO}^\cdot$, second, formation of $\text{HOO}^\cdot$ via reaction of H_2O_2 with $\text{OH}^\cdot$, and then it reacts with Co (III) to generate. $\text{O}_2\text{H}^\cdot$. The reaction between the two radicals formed produces H_2O and O_2 as the final products[46]. Stimulated by these samples stated above, other nanozymes with peroxidase activity could also be examined to their catalytic activity. And the elaborate mechanism should be used to further widen their applications. G.mugesh and coworkers prepared different shaped Mn_3O_4 NPs such as nanoflowers, flakes, cubes, polyhedral, and hexagonal plates and studied their catalase, Glutathione peroxidase and superoxide dismutase like activity. They found that Mn_3O_4 nanoflowers shows better catalytic activity for all the three enzymes namely SOD, CAT and GPx and is used for the neuroprotection application[42]. Table II shows some nanozymes with catalase activity and their catalytic parameters.

C. Oxidase-Like Activity

Natural oxidases have much attention because of their vital roles in the development of aerobic organisms on earth by effective and careful consumption of O_2 [56, 57], and have an ability to catalytically oxidize the electron donor substrate to produce H_2O or H_2O_2 in presence of oxygen or other oxidizing agents, which generally acts as the electron acceptor. This enzyme has extensive application in food, pharmaceuticals, biotechnology and other industries. But the application of natural enzyme is limited because of their low yield and high purification cost and less stability at environmental conditions. Till date, number of engineered metal and metal oxide-based nanomaterials are reported to act as oxidases, like CuO [58], Au NPs[59], $\text{Au/Al}_2\text{O}_3$ [60], MnFe_2O_4 [61], Au/TiO_2 [62, 63], and Pt@MnO_2 [64]. This oxidase nanozyme family can be classified according to the donor

TABLE I. PEROXIDASE ACTIVITY OF METAL, METAL OXIDE BASED NANOZYMES AND THEIR CATALYTIC EFFICIENCY

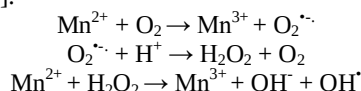
Nanozyme	Surface modification	K _m [mM] {TMB}x	K _m [mM] {H ₂ O ₂ }	V _m [10 ⁻⁸ Ms ⁻¹] {TMB}	V _m [10 ⁻⁸ Ms ⁻¹] {H ₂ O ₂ }	Ref.
Fe ₃ O ₄		0.098	154	3.44	9.78	[22]
GO-COOH	COOH	0.43	0.71	13.08	13.08	[47]
Ir NPs	Citrate	0.0906	0.27	1.7	1.5	[48]
Rh NPs	Citrate	0.198	0.38	0.0678	0.241	[49]
NiPd NPs		0.11	0.66	0.0152	0.2618	[50]
Pt NPs	Bovine serum albumin	0.119	41.8	0.21	0.167	[51]
Co ₃ O ₄ nanoflowers		0.283	5.9322	0.1052	0.0985	[52]
Co@Fe ₃ O ₄		1.17	0.19	0.379	0.715	[53]

TABLE II. CATALASE ACTIVITY OF METAL, METAL OXIDE BASED NANOZYMES AND THEIR CATALYTIC EFFICIENCY

Nanomaterial	k _{cat} (s ⁻¹)	substrate	K _m (mM)	K _m (mM),	Ref
Co ₃ O ₄ NPs	1.63 × 104	H ₂ O ₂	34.3	11.2	[54]
Au ₂ Pt		H ₂ O ₂	7.7066	0.9018	[21]
AuNCs-NH ₂		H ₂ O ₂	16.0	0.452	[44]
Ir NPs		H ₂ O ₂	21.09		[55]

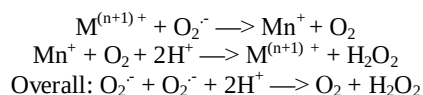
group. Including amino groups, Ph-OH group, CH-OH group, ferrous ions and sulfur group[65]. Among them, nanozymes that acting on amino groups with oxidase like activity were largely studied.

Mn based NPs (MnO₂ and Mn₃O₄) are one of the extensively studied nanomaterials as oxidase mimics. For instance, MnO₂ NPs with different nanostructures like nanorods, nanospheres, nanowires and nanosheets, can catalyze the oxidation of TMB and OPD substrates in presence of O₂ to produce colored products[66-69]. Along with these nanostructures, Mn₃O₄ NPs also exhibit outstanding oxidase like activity due to their mixed valence state [Mn (II), Mn (III)] and high affinity towards TMB substrate. Conceivable mechanism was proposed in below equation. First electron transfer takes place from Mn (II) to O₂ resulting in the formation of O₂^{•-}, then which is responsible for the formation of H₂O₂, and O₂ via non enzymatic dismutation., produced H₂O₂ would reacts with Mn²⁺ ions and disintegrated into OH[•]. Finally, Mn³⁺ and intermediate OH[•]/ O₂^{•-} would oxidize the TMB substrate to colored products[70].



D. Superoxide Dismutase-Like Activity

Superoxide dismutase, it is a type of metalloenzyme that mainly found in microorganisms, animals and plants[71]. Reactive oxygen species means reduced products of oxygen in the body produced by incomplete reduction of oxygen molecules, that includes oxygen radicals (H₂O[•], HO[•] and O₂^{•-}) and some oxidizing agents (ozone, H₂O₂)[72]. Among them O₂^{•-} is the prime one. Increase in production of reactive oxygen species leads to oxidative stress can cause oxidative cellular structural damage exclusively membrane damage, and protein and DNA oxidative damage and is the significant factor in aging and disease[73]. On one side it destructs the normal tissues, on other side, oxidative damage property of superoxide dismutase is able to induce cell senescence and apoptosis. This property can be used as anti-tumor and antibacterial applications. Since superoxide dismutase could convert superoxide anions (O₂^{•-}) one of the reactive oxygen species into H₂O₂ and O₂ through the dismutation reaction as shown in the below equation. Hence it is selected as a promising means to anti-aging and anti-oxidation.



In the above reactions, M⁽ⁿ⁺¹⁾⁺ denotes the oxidized metal and Mn⁺ denotes the reduced metal[74]. As this mechanism involve the consecutive reduction and oxidation of metal center, this mechanism is also known as the ping-pong mechanism[74]. Hence it is selected as a promising means to anti-aging and anti-oxidation. Several nanomaterials have been reported as superoxide dismutase mimics, like fullerene, CeO₂[75], Mn₃O₄[76], Au[77], etc. Most of them consists of transition elements and elements like carbon, nitrogen, oxygen, sulfur, etc. Due to low toxicity and high biocompatibility, cerium oxide nanoparticles (CeO₂) are most studied nanozyme currently

with superoxide dismutase like activity and this was first reported by Korsvik et al. in 2007[75]. The coupled electron-transfers model was first used to explain the mechanism of Superoxide dismutase like activity of CeO₂ nanoparticles[78]. But this model was questioned since Cafun et al. confirmed the nonappearance of spin-unpaired Ce³⁺ sites in colloidal solution by high-energy resolution X-ray spectroscopy[79]. Wang et al. proposed a refined catalytic cycle mechanism for nanoceria as Superoxide dismutase mimics[80]. It is primarily associated with the conversion between Ce³⁺ and Ce⁴⁺. This change in valence of cerium results in the formation of defects or vacancies in the lattice structure of the CeO₂ by losing oxygen and electrons. These defects or vacancies in the lattice structure were critical to the superoxide dismutase like activity of CeO₂ nanoparticles in this model. The quick formation and vanishing of oxygen vacancies in CeO₂ lattice structure deliver the capability to store and release of oxygen. Due to these defects CeO₂ release oxygen on the surface to stabilize the linear oxygen vacancy clusters, the main defects on reduced surfaces. First HO₂[·] is adsorbed onto the surface valences of CeO₂ lattice structure to form an intermediate. Again, this intermediate reacts with another HO₂[·] radical to release H₂O₂ and O₂, and nanoceria returned to the initial state[81]. The decrease in size of CeO₂ nanoparticles increases the surface area and discloses large oxygen vacancies on CeO₂ nanoparticle. Hence size of nanoparticles has great effect on Superoxide dismutase like activity of CeO₂. Hence, small sized, usually less than 5nm cerium oxides, were widely explored as Superoxide dismutase mimics[75]. Mn₃O₄ Flake like morphology nanoparticles shows comparable SOD like activity with Mn₃O₄ nanoflowers. Ramming nanoparticles exhibits only SOD like activity almost 50-60% of nanoflowers. In recent trends Surface side effect becomes the main focus in morphology control because it determines its surface reactivity[42]. As we discussed above, the size of the nanozymes affects the catalytic activity of nanozymes. Ivana Celardo et al. prepared different size cerium oxide nanoparticles and studied their superoxide dismutase activity. This study shows that superoxide dismutase activity of cerium oxide nanoparticles is almost inversely proportional to its size[82]. Most of the catalytic reactions takes place on the surface of the nanozymes. Surface coating or modification of nanozymes can affect the catalytic activity of nanozymes through the change of surface charge and exposure of active sites. Yang et al. studied the SOD-like activity of cerium oxide nanozymes with different shape and morphology. They found that, the SOD-like activity of ceria nanochains (CNHs) is strongest than ceria nanoclusters (CNLs), last ceria nanoparticles (CNPs)[83].

III. CONCLUSION

The wealth of nanotechnology and biology constructed sequence of new artificial enzymes. nanozymes have established outstanding performance in biopharmaceuticals, clinical medicine, environmental observation and many other fields. Herein, we accurately elaborated the recent advances in expanding the types of nanozymes, activity and catalytic reaction mechanism of the metal- and metal oxide-based nanozymes. Even though nanozymes have been exposed to conquer several drawbacks of natural enzymes such as denaturation and degradation under harsh conditions such as high temperature, high pressure, complications in recovery and recycling, and high costs in extraction and purification there are still many challenges for upcoming researches. Metal- and metal oxide-based nanozymes appear to lack the substrate specificity when compared with most of the natural enzymes. Researchers have discovered numerous numbers of factors that affect the enzymatic properties, even though the exact control of catalytic performance, particularly for the multi-enzyme activity nanozymes, there still remains plenty of room for advancing. The investigation of the catalytic mechanism is the basic need for understanding the catalytic reaction of nanozymes. Peroxidase mimics are the most studied with enzyme activity related applications, particularly in analysis and detection field. While other oxidoreductase enzymes such as catalase, oxidase, superoxide dismutase mimics are yet to be developed. Looking for substitutes for noble metal nanozymes has slowly received increasing attention, bearing in mind the cost control in large-scale preparation. For clinical employment, the long-lasting in vivo toxicity of nanozymes is still left over a task. Precise mechanisms of toxicity and analogous solutions are in urgent need, even though huge number of studies have involved the argument about the biocompatibility.

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