SAYAM Vol-II, Issue-I (June, 2024), Page No-08-12

Sayam

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SAYAM

Journal Homepage: https://sayamjournal.com/

Article

APPLICATION OF NANOZYMES IN THERAPEUTICS: PRESENT CHALLENGES

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ARTICLE INFO

Keywords:
Nanozymes, therapeutics, reversible regulation, target-selectivity, efficiency of catalysis.

Received : 06/12/2023
Accepted : 10/02/2024
Date of Publication: 02/06/2024

ABSTRACT

The nanozymes are the novel nanomaterials of size varying from 1 to 100 nm imbibed with the ability of mimicking the functions of natural enzymes. However, the activities of nanozymes can be modulated by the changes in size, morphology, composition, types and concentration of metal ions, pH, temperature, light and surrounding environment. Developing further types of nanozymes; elucidating their functional roles in the living systems and exploring their catalytic mechanisms are necessary in immediate future. Therefore, overcoming the shortcomings will assist in designing smart non-hazardous nanozymes with robust efficiency of catalysis. This short communication gives an insight into the enzyme-properties of the nanozymes, their mechanism of actions, application in biomedical fields and finally focuses on the challenges and limitations in use of nanozymes in biotechnology.

The nanozymes are the novel nanomaterials of size varying from 1 to 100 nm imbibed with the ability of mimicking the functions of natural enzymes (Zhu et al. 2018). A high firmness and resilience, huge surface-area and easy surface-modification with simple process of synthesis and profitable economic values of the nanozymes have overcome the intrinsic drawbacks faced during industrial applications of the natural enzymes such as their susceptibility to high pH, temperature, digestion by proteases, high cost and recycling difficulties. (Wang et al. 2018; Huang et al. 2019). This has resulted in the recent surge of use of nanozymes in the agricultural platforms, chemical industries, food-processing plants, environmental management and pharmaceutics as electrochemical/biological sensors for detecting numerous molecules including metal ions, glucose, proteins, nucleic acids, urea, vitamins (ascorbic acid), phosphates, peroxides, heparin, dopamine, carvedilol and cancer cells, for fluorescence detection, chemiluminescence analyses, imaging, phenol degradation, Hg removal, water purification, dilapidation of biological warfare agents, green synthesis and immunodetection and immunoassay studies and can act as antioxidants and antibacterial agents (Wang et al., 2023; Stasyuk et al., 2020; Wang et al., 2020; Tian et al., 2021; Fig. 1). In biomedical fields, the low-cost and stable nanozymes are being widely used (Zhu et al. 2018; Wang et al. 2018). Till date, the manufactured nanozymes belong to oxidoreductases and hydrolases such as catalase, oxidase and peroxidase and include metals and metallic oxides such as Fe_3O_4, Co_3O_4 CeO_2, CuO, V_2O_5, Pt, Pd, polypyrrole nanoparticles, MnO_2 and graphene oxide nanosheets, graphene quantum dots, metal clusters, metal sulfides (FeS, CuS), noble metals (Au, Pt) metal-organic frameworks (MOFs) and carbon-based nanomaterials (Zhu et al. 2018; Wang et al. 2018). This is possible due to the intrinsic oxidising properties of the metals like Ag, Au, Pd and Pt, and peroxidase, superoxide dismutase (SOD) and catalase (CAT)-mimic abilities of

CeO_2; glucose oxidase (GOx)-mimic abilities of Au nanoparticles to catalyse the formation of gluconic acid from glucose; oxidation of sulphites to sulphates by MoO_3 nanoparticles; peroxide-like activities of carbon-based nanoparticles; glutathione peroxidise (GPx)-like activity to detoxify harmful H_2O_2 with the assistance of glutathione (GSH) of V_2O_5 nanowires (Huang et al. 2019; Filippova et al., 2023). This latter is found to catalyze the production of hypohalous acids from halide ions (Cl- and Br- ions) resulting in oxidative damage on marine microorganisms; thereby protecting the ships from marine biofouling (Huang et al. 2019). Besides this, the nanozymes are also synthesized from natural enzymes or catalytic groups of the enzymes which are transformed on nanomaterials forming nanomaterial hybrid enzymes.

However, the activities of nanozymes can be modulated by the changes in size, morphology, composition, types and concentration of metal ions, pH, temperature, light and surrounding environment (Huang et al. 2019). In spite of wide range of applicability of nanoparticles; there are numerous stirring challenges and limitations that still remain to be overcome for complete utilization of the potential of the nanozymes for betterment of human living. The high durability of inexpensive nanozymes have made them a potential candidate for biomedicine. The modulation of pH, ionic state, and concentrations of hydrogen peroxide and glutathione result in changing the enzyme-mimetic properties of nanozymes which then can be used in biomedicine. This short communication gives an insight into the enzyme-properties of the nanozymes, their mechanism of actions, application in biomedical fields and finally focuses on the challenges and limitations in use of nanozymes in biotechnology.

The first ferromagnetic nanoparticles imbibed with horseradish peroxidase-like activity were discovered in 2007 (Fu et al., 2024). From then, the nanotechnology using biphasic approach of theoretical calculation and computer stimulation has contributed to the study of activities of novel nanozymes (Huang et al. 2019). In spite of the advancements, wide gaps still exist between the nanozymes and natural enzymes. Till date, two types of multifunctional nanozymes are developed based on iron and plasmonic metals and they target the functions of the oxidoreductases (Wu et al. 2019). However, the enzymes are classified into six groups based on the types of catalytic reactions and they take part in coordinated cascade of reactions in the living systems. Secondly, in comparison to the uniform natural enzymes, nanozymes are of various sizes and morphologies which affect their functions; make the synthesis of nanozymes and study their catalytic kinetics more complex (Huang et al. 2019). Therefore, developing further types of nanozymes; elucidating their functional roles in the living systems and exploring their catalytic mechanisms are necessary in immediate future. The specificity of the oxidase-mimics for selective catalysis of the oxidation of a given substrate is also very low compared to natural enzymes (Wang et al 2018). Not only this lack of specificity but the additional properties including imaging, multi-enzyme activity and photothermal therapy hinder the catalytic reactions of the nanozymes. Hence, both the improvement on the substrate-selectivity of the oxidase-mimics and the efficacy of catalyses of the nanozyme-based biosensors are important future challenges to be tackled by the nanotechnologists. Moreover, investigating these features of nanozymes will help to expand their applications in the arena of therapeutics, diagnostics, agriculture, food, industry, environment and other biotechnological fields.

The biocompatibility, bioavailability and biosafety with respect to toxicity of nanozymes are the vital factors to be considered while selectively targeting nanomaterial-based diagnosis/treatment of various diseases or detection of compounds in the body. The hyperaccumulation of metal nanozymes (eg. for gold) result in development of systemic toxicity, oxidative stress and damage of nucleic acids, resulting in normal tissue apoptosis.

In cancer therapy, nanozymes demonstrate great prospects in the form of nanocomposites by RNA-interference or by assisting in photodynamic therapy under hypoxic conditions (Huang et al 2019). However, their biological safety is still a concern and therefore is a great challenge to be solved in immediate future before their application in cancer therapeutics. Nanoparticles are extensively being used as antioxidant to quench the reactive oxygen species (ROS) in the living cells. Gpx, a glutathione-dependent antioxidant enzyme, distributed in the cytoplasm, mitochondria, nucleus, endoplasmic reticulum and extracellular fluid catalyses H_2O_2 to form nontoxic products (Satheesh and Pari, 2006; Daimond, 2015; Pei et al., 2023; Schamberger et al., 2016). Till date, GPx mimicking is performed by selenium-based organic molecules for ultrasensitive detection of H_2O_2, glucose and other compounds and in immunoassay kits (Wang et al., 2018; Satheesh and Pari 2006). However, this suffers from shortcomings related to complicated preparation process, high toxicity and low cycling efficiency indicating a necessity of developing more biocompatible GPx mimics.

The bioorthogonal reactions with transition metal catalysts (TMCs) that aid to the bio-transformations for generation of imaging and therapeutic agents *in situ* becomes a challenging effort due to restricted biocompatibility, poor water solubility/stability and prompt cellular efflux (Wei and Wang 2013). Therefore, with respect to bio-orthogonal reactions future studies are necessary to elucidate the pharmacokinetic properties of the nanozymes including their distribution, degradation, clearance (particularly the heavy metal conjugates) and the accumulation in the biological systems (Wei and Wang 2013). This information will be beneficial for drug designing and its subsequent administration in the living organisms. The new drug development strategies will provide mechanisms (1) for spatio-temporal activation of drugs in the target cells and (2) to produce programmable nanozymes with the allosteric mechanisms to self-modulate the catalytic reactions including switching mechanisms on the basis of the environmental stimuli (Zhang et al. 2020). The latter is important because in spite of ability of catalysis of various biochemical reactions by various types of the same nanozyme, they lack "regulatory switches" to regulate reversible catalysis (Huang 2019). Oxygenated-group-enriched carbon nanotubes (o-CNTs) displaying high peroxidase-like activities for biocatalysis over a wide range of pH make it suitable for antimicrobial application (Wang 2018). However, the lack of targeting to bacteria cells and the difficulty to directly deliver it to the infected sites limit its further application.

The low dispersive capability with high rate of precipitation is a great challenge for the nanozymes. Additionally, the nanozymes have limited catalytic powers and low selectivity towards substrates adding difficulty towards their traditional methods of preparation. Also, the reactions mediated by the nanozymes mostly are redox reactions and hence are less dynamic than natural enzymes (Zhang et al., 2019). Therefore, the alleviation and/or reduction of adverse effects of nanozymes and thereby enhancing the diagnostic efficiency and precision in nano therapeutics is a crucial hitch for the sustainable development of nanozymes (Luo 2023). Another challenge is the limitation in the post-administration monitoring of the nanozymes in the living systems. The effective conversion of the nanozymes clinically is hindered due to the factors including the uptake of the nanozymes as drugs, systemic distribution, cellular toxicity and immune-histocompatibility (Luo 2023).

Recent developments in nano/micro motor fabrication and their association with targeted drug delivery is of immense importance. However, the main challenge lies in the designing of the motors with respect to the size, shape, propulsion forces, portability, toxicity, biological safety, biological barriers (cell walls, membranes, biomolecules), propellant fuels and developing complexities in adjacent tissues. The in-vivo imaging and/or video simulation of the used nanoparticles for targeted drug delivery is the greatest challenge at present (Falahati 2022).

The nanozymes are also applied as inhibitors of enzymes [eg. β-galactosidase (β-Gal), β-lactamase, mitochondrial F0F1-ATPase and R-chymotrypsin (ChT)] in biomedicine. However, the inability of recognition of specific enzymes by the small enzyme-inhibitors fail to develop any chemical interactions. Even, the blocking of nanozymes that act as inhibitors with distinct proteins diminish the activity of the nanozymes. The difficulty of the nanozymes in crossing the membrane-barriers to act upon the intracellular target enzymes is another challenge to use them as enzyme-inhibitors in biomedical applications (Huang 2021). Therefore, enhancement in the structure of the nanozymes and their optimization in application as enzyme-inhibitors are the recent foci of research in nanomedicine.

Therefore, the nanozymes, novel nanomaterials of nanoscale sizes imbibed with the ability of mimicking the functions of natural enzymes are crucial for their stability, durability, greater area and easy modifiability. These features along with profitable economic values have overcome the intrinsic drawbacks faced during industrial applications of the natural enzymes such as their susceptibility to high pH, temperature, digestion by proteases, low operational stability, high cost and recycling difficulties. This has resulted in the recent surge of applications of nanozymes in the targeting therapeutics. However, challenges regarding their low activity and target-selectivity as compared to natural enzymes, reversible regulation and development of human and environmental toxicity are huge. Therefore, overcoming these shortcomings will assist in designing smart non-hazardous nanozymes with robust efficiency of catalysis.

ACKNOWLEDGEMENT

The author thanks to Department of Biochemistry, Asutosh College for providing the infrastructure during preparation of the manuscript.

CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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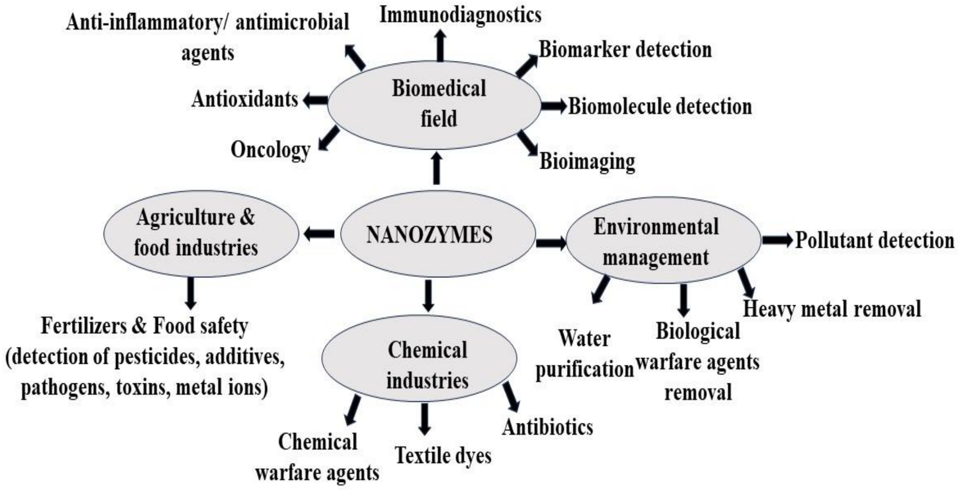

Figure 1 Schematic presentation of application of nanozymes in science