Nanobio Bionano 2014 (2) 30-34 Correspondence

Catalytically Active Nanomaterials:

Promising Candidates for Artificial Enzymes

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Keywords:
Catalyst
Nanomaterials
Enzymes
Enzyme mimickers
Nanozyme

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received: 2014.6.26
revised: 2014.12.27
accepted: 2014.12.29

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Enzymes are highly efficient biomolecules that speed up biochemical reactions. They usually exhibit high specificity to their distinctive substrates with enzymatic activities normally taking place in mild conditions [1]. Enzymes provide significant number of assistance from mediating cellular metabolisms to the utilization in industrial processes such as pharmaceutical, food and agrochemical industries.

However, 'natural limitations' hinder the usage of enzymes to its maximum trajectory e.g. harsh environmental condition that affects catalytic capability (i.e. by protein denaturation or enzyme being digested). In addition, there are many difficulties that affect enzyme recovery and industrial operation often requires exorbitant cost of preparation for purification [1].

While the advancement in biotechnology leads to the exploration of biomolecules with enzymatic properties including cyclodextrins, metal complexes, porphyrins, polymers, and dendrimers, nanobiotechnology explores nanomaterials. There are varieties of nanomaterials that have been extensively explored to mimic the structures and functions of naturally occurring enzymes, namely; gold nanoparticles, platinum nanomaterials, nanoceria, iron and copper oxides, manganese dioxide and others. These nanomaterials (with artificial enzyme mimicking activities) were successfully utilized in immunoassays, cancer diagnostics and therapies, neuroprotection, stem cell growth, pollutant removal and biosensing applications [1].

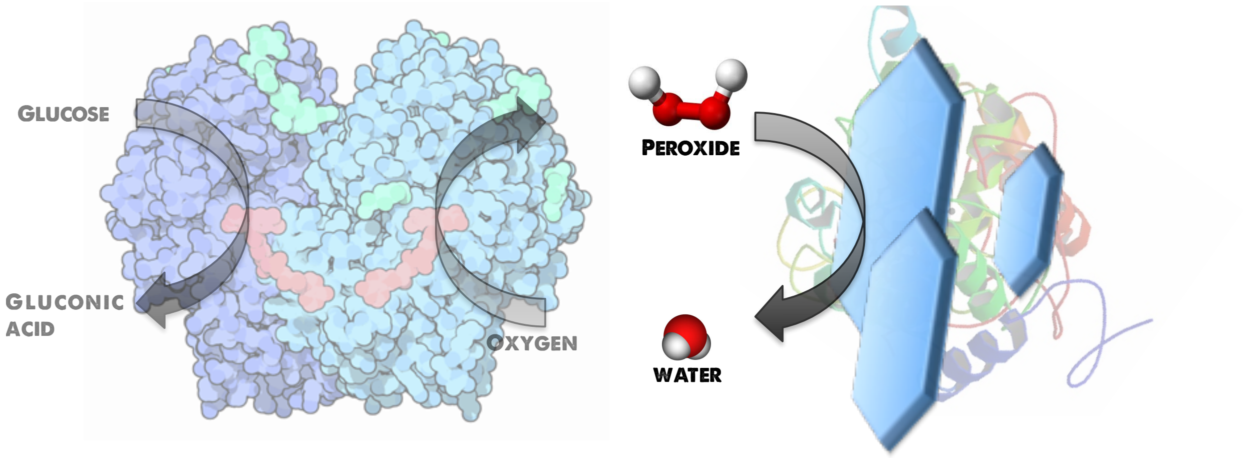

Figure 1: Nanozyme as peroxidase mimic for colorimetric sensing of H_2O_2 and glucose when combined with glucose oxidase

The term "nanozymes" is used for the nanomaterials with enzyme-like activities [1,2]. From the name, it can be unserstood that the word "nano" stands for nanomaterials and "zymes" stands for enzymes and together gives the meaning "nanomaterials with enzyme-like activities".

Size

There are several factors that affect catalytic activities of nanomaterials. Wei & Wang reported that catalytic activities of the nanomaterials depend on their sizes with smaller particles exhibiting higher activities than the relatively larger ones. This is most likely due to a higher surface area to volume ratio. It was reported that Fe_3O_4 magnetic nanoparticles (MNPs) with the sizes of 30, 50 and 300 nm all oxidized TMB but also show variation in the catalysis according to their sizes [1]. Lin and coworkers also showed that the catalytic activities of AuNPs depend strongly on their sizes and also reported that small and stable AuNPs may exhibit much higher activity [2].

Shape

Shape is another factor on which the activities of the nanozymes depend on. Wei and Wang reported that when the oxidase-like activities of manganese oxide nanomaterials with different shapes (sheet, sphere, wire, complex, stick) were compared, nanospheres and nanowires showed highest activities. It was also reported that when the effect of shape of Fe_3O_4 MNPs on their peroxidase activities studied, the sphere shaped nanomaterials showed highest activities as compared to the triangular plates and the octahedrons and the increased catalysis was attributed to their higher surface area [1].

Surface Coating

Size, thickness and packing density of the of the coating layer can all affect the activities of the nanozymes. This is because surface coating minimizes the contact between the core of the nanozyme

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and the substrate which lead to a decreased activity. Thickness and molecular weight of the coating material are reported to be inversely proportional to the activity of the nanozyme [1]. Gao et al (2007) reported that the peroxidase activity of Fe_3O_4 MNPs decreased when coated with SiO_2, 3-aminopropyl-triethoxysilane, polyethylene glycol and dextran, however, the dextran-modified Fe_3O_4 MNPs exhibited highest performance [3].

Mode of Catalysis

The mode of catalysis of nanozymes follow the ping-pong mechanism where one substrate is converted to the product and dissociates before the other one binds without the formation of an intermediate. Yan and coworkers suggested that the Fe_3O_4 MNP-based peroxidase exhibits this mechanism which is also similar to that of the native horse radish peroxidase reaction [1].

Classification of Nanozymes

Lin et al. (2014) classified the nanozyme (nanomaterials with enzymatic properties) into three classes according to their structural characteristics; (i) carbon-based, (ii) metal-based and (iii) metal-oxide-based nanomaterials [2]. It was further shown that different materials from each of the classes exhibit different unique enzymatic activities and this knowledge was used to develop different kinds of assay methods.

Carbon-based nanomaterials.

Carbon-based nanomaterials have been reported to mimic peroxidase and superoxide dismutase activities [2]. One of the examples of carbon-based nanomaterials used as enzymes is carboxyl-modified graphene oxide (GO−COOH) which was reported to have peroxidase activity oxidizing 3, 3, 5, 5-tetramethylbenzidine (TMB) to glow blue in the presence of H_2O_2. This is similar to horseradish peroxidase reaction [2]. The idea was used to design a simple and cheap, yet highly sensitive and selective colorimetric technique that can detect glucose concentration as low as 1μM in blood samples and commercial fruit juices [2].

Xue et al. used these materials to develop a colorimetric method for the detection of cancer biomarker for prostate specific antigen. In addition to GO-COOH, graphene-hemin (GH) nanocomposite also exhibits high peroxidase activity [4, 5] and was harnessed in the development of colorimetric assay for rapid quantitative cancer cells detection [6]. GH was used to develop biosensors for the detection of different targets like DNA, metal ions e.g. copper.

A label-free colorimetric system for DNA analysis was also developed. The difference on affinities of ssDNA and dsDNA toward nanozymes (such as CNTs and graphene) is usually taken advantage of. This is because ssDNA with higher affinity towards (GH) than dsDNA can stabilize salt-induced aggregation therefore leaving a majority of the GH, which exhibits high nanozyme activity in the supernatant after centrifugation. This principle was combined with DNA hybridization to differentiate a target and a single base mismatch DNA [1]. The method could be used in the detection of single nucleotide polymorphism (SNPs) in human DNA

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through the diagnosis of SNPs-associated diseases like sickle cell anaemia [7].

Metal-based nanomaterials

Gold, which was known to be relatively inert, was found to possess enzymatic property when incorporated or modified with something else. Modified gold nanomaterial like mesoporous silica-encapsulated gold nanoparticles and gold nanoclusters were reported to exhibit glucose oxidase (GOx) and peroxidase catalytic activities [8, 9].

Lin et al. (2014) also reported that the GOx-mimicking potential of gold-nanoparticles (Au-NP) was used by Fan and co-workers in constructing a sensor for DNA and microRNA detection. Another interesting breakthrough is the construction of a nanozyme cascade system (Figure 2) with the ability to mimic dual enzyme catalysis (GOx- and peroxidase-like catalytic activities) [2]. Based on the peroxidase activity of Au-NP, Lin et al. also developed easy and simple fluorometric and colorimetric dual analysis for dopamine using BSA-AuNP.

Metal oxide-based nanomaterials

One of the most commonly used metal oxide nanoparticles as enzyme mimic is cerium oxide [10,11]. CeO_2 exhibits superoxide dismutase (SOD) [11], catalase [9], oxidase and phosphatase activities [2]. Due to their different oxidation states of Ce (III) and Ce (IV), cerium oxide with oxidase-like activity has been effectively utilized to oxidize intracellular and extracellular components to induce cancer cell's apoptosis [1, 2]. Meanwhile, Wei and Wang (2013) reported that iron oxide could mimic peroxidase [1]. This plays a critical role in biological systems especially detoxification of reactive oxygen species and defence against pathogens like myeloperoxidase [3]. Similar activity was also reported for Fe_3O_4 magnetic nanoparticles (MNPs) [1].

Enhancing the catalytic activities of nanozymes.

Modulators that can enhance catalytic activities of these nanomaterials are explored in order to have the ideal nanozymes. Graphene oxide (GO), ionic liquid and nucleoside triphosphates (NTPs), are three modulators that are being used to enhance the performance of nanozymes [2]. Gold nano-clusters (AuNcs) which reacts in highly acidic pH shows a high activity at neutral pH only with the presence of GO. This condition makes it feasible for its utilization in biological systems. Ionic liquid, which is known as a liquid salt of charged molecules like Na+ and Cl- are used to provide thermal stability to the nanozymes. This was proven in the peroxidase activity of AuNPs. NTPs act as coenzymes and have been found to regulate the catalysis of nanozymes. It is used as a modulator of nanoceria oxidase-like activity. However, the increase in nanozymes performance by NTPs is highly dependent on the type of NTPs used. E.g., in nanoceria with oxidase and phosphatase-like activities, it was shown that different NTPs used reflected in different improvement of the catalysis of nanoceria signifying the influence of different types of NTPs on the nanozymes.

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Challenges

The artificial enzymes have low cost of preparation compared to the natural enzymes, as they exhibit high operational stability, withstand more stringent conditions, and easy to recycle and modify. However, low in efficiency, specificity and selectivity are challenges that need to be addressed in order to construct ideal enzymes. Another issue that also needs to be looked into is the the stduy of the exact mechanism of action of the nanozymes because it is likely that not all the artificial enzymes exhibit the ping pong mechanism. Biosafety of the nano materials is to be considered as well, especially when the nanomaterials are to be used in biological systems. It is hoped that in the near future, nanozymes can efficiently mimic natural enzymes and thus can be used as alternatives.

Conclusion

Engineered nano-materials are shown to be able to mimic the activities of natural enzymes. It holds huge potential for future breakthroughs in bio-catalysis as nanozymes can be utilized as highly stable, efficient and low-cost alternatives in a wide range of industrial, biomedical, genetic applications and assay methods. This can be fully harnessed especially after overcoming the above-mentioned challenges associated with nanozymes utilization.