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ssDNA-tailorable oxidase-mimicking activity of spinel $MnCo_2O_4$ for sensitive biomolecular detection in food sample

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ABSTRACT

Nanozyme technique with efficient modulating strategies is a promising tool for biocatalytic and biosensing applications, yet insights into target-responsive controllability of oxidase nanozymes remain largely unknown. In this contribution, we report a state-of-art oxidase-like and ssDNA-tunable nanozyme for biomolecular recognition. In detail, the spinel-type manganese cation substituted cobalt oxide ($MnCo_2O_4$) submicrospheres can imitate oxidase for efficiently catalytic oxidation of colorimetric substrate, superior to current metal oxide-based oxidase nanozymes. More importantly, the nanozyme activity of spinel $MnCo_2O_4$ can be reversibly inhibited by the attachment/detachment of aptamer strands on $MnCo_2O_4$ surface via the aptamer-target binding event, enabling a new colorimetric access for detecting biomolecules. Taking highly toxic ochratoxin A (OTA) as model molecule, we can selectively determine OTA in maize samples with a limit of detection ($3\delta/S$) of 0.08 ng/mL. The unique nanozyme property of spinel $MnCo_2O_4$, together with the principle of our approach, reveals the potential of ssDNA-tailorable oxidase nanozymes in biocatalytic and biosensing fields.

1. Introduction

Horseradish peroxidase (HRP), alkaline phosphatase, and their analogues play central roles in various biosensing systems based on biocatalysis, while they are vulnerable to inactivation under severe conditions. To overcome the limitations, tremendous research interests have been devoted to explore the imitation of such natural enzymes. Lately, functional nanomaterials with enzymatic activities, called as nanozymes, have been emerging alternative to natural enzyme due to their unique nanoscale properties, such as high specific surface area, high stability, comparable activity, biocompatibility, and low cost [1,2]. Many metal oxide nanoparticles have been found to exert peroxidase, oxidase and other enzyme-like activities and used in versatile enzyme-catalytic systems for medical, environmental, and bio-analytical applications [3]. Despite continuous progresses in nanozyme technology, the insufficient catalytic activity is still one of the significant drawbacks of nanozymes yet to be solved in analytical applications [4].

Of particular interest, spinel-type metal oxides with the formula of AB_2O_4 (where A^{2+} and B^{3+} are metal ions), possess controllable composition, structure, valence, and morphology [5–7], making them superior in promoting nanozyme performance over other metal oxides. Generally, the incorporation of foreign-metal cations can enrich the catalytic sites and endow the catalyst with a better or even new catalytic activity [2,8]. Following this strategy, several mixed-metal spinels such as MFe_2O_4 (M = Co, Mg, Mn, Ni, Cu, Zn) [8–10] and $NiCo_2O_4$ [11] can exert superior peroxidase or oxidase activities over their monometallic oxide components. Thus, the evolution of mixed-metal spinels towards highly efficient oxidase nanozymes facilitates enhancing nanozyme performance for analytical applications. As commonly used nanozymes, Mn_3O_4 and Co_3O_4 have many in common that they both possess spinel structures, own redox cycles of metal ions, and exhibit oxidase-like activity [12,13], implying the potential of materials that combine redox active Co and Mn species in a spinel structure. Besides, spinel $MnCo_2O_4$ material has exhibited excellent catalytic activities for oxygen reduction/evolution and CO_2 reduction [14,15], while the potential of $MnCo_2O_4$ for enzyme-mimicking and biosensing applications remains to be thoroughly explored.

Beyond the rational design of nanomaterial for enhanced activity, equal attention has been focused on the target-responsive

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modulation of nanozyme in pursuing exquisite nanozyme biosensing techniques [2]. Particularly, target-responsive nanozymes exhibit tunable activity varying with the concentration of target molecule, which endows new avenue to selective recognition of analyte, such as biomolecules. Up to now, bioactive molecules such as enzymes [16], small biomolecules [17], DNA strands [18–21], and even inorganic ions [22,23] have been found to regulate catalytic activities of nanozymes. Among them, ssDNA-regulated nanozymes are especially promising and attractive because it enables nanozyme into the biomolecular detection, by bridging the nanozyme activity and the target-specific aptamers (specific ssDNA strands) [24]. To our knowledge, most nanozyme-based methods for biomolecule detection are established on ssDNA-regulated peroxidase mimics (noble metal nanoparticles [25,26], graphene oxide [27], carbon nanotubes [28], and g-C_3N_4 nanosheets [29], etc.). However, compared with peroxidase species, oxidase that can oxidize the substrates by simply employing molecular oxygen rather than unstable hydrogen peroxide as the electron acceptor are more practical, economic and eco-friendly in biosensing applications [30]. Except for the aptaswitch approach using nanoceria [31], the promising applications of oxidase nanozymes for biomolecular detection are scarcely reported due to the rarity of ssDNA-tailorable oxidase mimics.

In this contribution, we report that manganese cobalt oxide ($MnCo_2O_4$) submicrospheres exert oxidase-like activity that can be reversibly regulated by ssDNA strands, which happens to spinel metal oxides for the first time. The truth that $MnCo_2O_4$ spheres display high activity and controllability makes them superior to most reported metal oxide-based oxidase mimics. It is demonstrated that the inhibition of oxidase-like activity of $MnCo_2O_4$ nanozymes depends on the DNA sequence, length and concentration of ssDNA strands. The reversible inhibition of $MnCo_2O_4$ nanozyme can be realized by the aptamer-analyte recognition, due to the disassembly of aptamer strands from $MnCo_2O_4$ surface. Moreover, the key factors that determine reversible assembly of ssDNA on $MnCo_2O_4$ surface have also been deeply studied. As a proof of concept, we designed a simple "turn-on" colorimetric method for biomolecular determination based on the aptamer-regulated oxidase activity, taking ochratoxin A (OTA), one of the most prevalent mycotoxins in foods, as a model biomolecule. Our assay exhibits good sensitivity and selectivity to OTA, which can be well applied in real samples.

2. Experiment section

2.1. Reagents and apparatus

Manganese sulfate hydrate ($MnSO_4{\cdot}H_2O$), cobalt sulfate hydrate ($CoSO_4{\cdot}7H_2O$), and NH_4HCO_3 were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, http://www.sinoreagent.com/). All DNA oligonucleotides (Table S1) were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China, http://www.sangon.com/). The 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 3,3′,5,5′-tetramethylbenzidine (TMB), and o-phenylenediamine (OPD) were purchased from Sigma Aldrich (Shanghai, China, http://www.sigmaaldrich.com). Ochratoxin A (OTA), ochratoxin B (OTB), aflatoxin B1 (AFB1), and zearalenone (ZEN) were obtained from Qingdao pribolab biotech co., Ltd (Shandong, China, http://www.pribolab.cn/). Ochratoxin C (OTC) was purchased from Shandong Lvdu Bio-science & Technology Co., Ltd (Shandong, China, http://www.lvdu.net/). All of these reagents were analytical grade and used as received. Ultrapure water (18.2 MΩ cm) produced by a Milli-Q system was used throughout this work.

The UV–vis absorption data was recorded with a UV-2550 spectrophotometer (Shimadzu, Japan) and a microplate reader (Thermo Scientific Multiskan MK3, USA) at room temperature. Field emission scanning electron microscope (SEM) image was taken by an S-4800 (Hitachi, Japan). The fluorescence data was measured with a F-7000 fluorescence spectrophotometer (Hitachi, Japan) in time-scan mode. Powder X-ray diffraction (XRD) patterns were obtained using a powder diffractometer (Bruker D8 Advanced Diffractometer System, Germany) with a Cu Kα (1.5418 Å) source. X-ray photoelectron spectroscopy (XPS) data was collected using an Axis Ultra DLD X-ray photoelectron spectrometer equipped with an Al Kα X-ray source (1486.6 eV). All pH measurements were performed with a PB-10 digital pH-meter (Sartorius, Germany) with a combined glass-calomel electrode.

2.2. Synthesis of $MnCo_2O_4$ submicrospheres

$MnCo_2O_4$ submicrospheres were prepared via a hydrothermal reaction followed by an annealing process [14,15]. Briefly, 1 mmol of $MnSO_4{\cdot}H_2O$ and 2 mmol of $CoSO_4{\cdot}7H_2O$ were dissolved into 65 mL of ethylene glycol (EG) and 10 mL of distilled water. After magnetically stirring the mixture for an hour to obtain a pink solution, 30 mmol NH_4HCO_3 powder was added slowly. After stirring for another 30 min, the resulting homogeneous solution was transferred into a 100 mL Teflon lined stainless-steel autoclave and heated at 200 °C for 20 h. The precipitates were collected by centrifugation and washed with distilled water and ethanol three times, followed by vacuum drying at 40 °C overnight. Finally, the precursor was calcined at 600 °C in air for 10 h with a ramping rate of 2 °C min^{-1} to harvest $MnCo_2O_4$ products.

2.3. Oxidase-mimicking activity of spinel $MnCo_2O_4$

The time-dependent kinetics of spinel $MnCo_2O_4$ was studied as follows: A reaction system with different concentration of $MnCo_2O_4$ (from 0 to 50 μg/mL), 0.5 mM TMB was monitored at 652 nm right after the reagents were mixed. The operation of the leaching experiment is the same as the above, except that the $MnCo_2O_4$ solution is replaced with leaching solution.

The reaction kinetic assays were performed under standard reaction conditions (10 μg/mL $MnCo_2O_4$ at room temperature in pH 4.0 acetate buffer) by varying concentrations of TMB. All the reactions were monitored in kinetic mode at 652 nm using a microplate reader. Catalytic parameters were determined by fitting the absorbance data to the Michaelis-Menten equation (Eq. (1)).

$$V = \frac{\nu_{max}[S]}{K_m + [S]} \quad (1)$$

The Michaelis-Menten equation describes the relationship between the rates of substrate conversion by an enzyme and the concentration of the substrate. In this equation, ν is the rate of conversion, ν_{max} is the maximum rate of conversion, [S] is the substrate concentration, and K_m is the Michaelis constant.

2.4. SsDNA-regulated activity of $MnCo_2O_4$ oxidase mimics

All oligonucleotides were heated at 92 °C for 10 min and subsequently placed at room temperature for 3 min before use. To explore the effect of DNA base on the oxidase properties of $MnCo_2O_4$, $MnCo_2O_4$ (10 μg/mL) were firstly incubated with 500 nM 15-mer homo DNAs (A15, T15, C15, G15) at pH 4 (acetate buffer, 100 mM) for 10 min, followed by adding the substrate TMB. The influence of DNA length on the oxidase activity of $MnCo_2O_4$ was examined by 500 nM poly-A DNAs with different lengths (15, 25, 35, and 45 bases). The regulation of DNA on $MnCo_2O_4$ oxidase mimics and the optimization of aptamer concentration were conducted by monitoring the TMB oxidation catalyzed by $MnCo_2O_4$