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Encapsulated cholesterol oxidase in metal-organic framework and biomimetic Ag nanocluster decorated MoS_2 nanosheets for sensitive detection of cholesterol

Javad Hassanzadeh [a], Alireza Khataee [a,b,*], Habibeh Eskandari [c]

[a] Research Laboratory of Advanced Water and Wastewater Treatment Processes, Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, 51666-16471 Tabriz, Iran
[b] Department of Materials Science and Nanotechnology Engineering, Near East University, 99138 Nicosia, North Cyprus, Mersin 10, Turkey
[c] Department of Chemistry, Tabriz Branch, Islamic Azad University, Tabriz, Iran

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ABSTRACT

Herein, a nano-porous metal-organic-framework (MOF) was applied as a safe host for cholesterol oxidase (ChOx) enzyme to improve its stability and implement an efficient oxidation process for substrate molecules. Subsequently, a fluorometric method based on the conversion of non-fluorescent terephthalic acid (TA) to 2-Hydroxyterephthalate with a great fluorescence emission was used for fluorescence detection of the generated H_2O_2. On the other hand, Ag nanocluster (AgNC) decorated MoS_2 nanosheets (MoS_2-NS) nanocomposite as a new mimetic peroxidase catalyst was exploited in TA-H_2O_2 reaction. The synthesized enzyme encapsulated MOF and AgNC/MoS_2-NS nanocomposites were characterized using scanning electron microscopy, transmission electron microscopy, X-ray powder diffraction and some other analyzing techniques. The results showed a high porous MOF with a high capacity to uptake ChOx enzymes, improving their catalytic activity. The ChOx-MOF hybrid displayed good efficiency for oxidation of cholesterol. Also, an improved peroxidase-like activity was discovered for AgNC/MoS_2-NS composite, in comparison with each of the constituents or natural peroxidase enzyme (HRP). As a result, the performance of designed cholesterol probe based on ChOx-MOF and peroxidase-like AgNC/MoS_2-NS, was improved significantly, in which it presented a dynamic linear range of 0.06 μM–15 μM with a detection limit (3S) of 0.03 μM. The studied method showed a good selectivity and sensitivity for practical determination of cholesterol and its potential application for cholesterol detection in human blood samples.

1. Introduction

Cholesterol ((3β)-cholest-5-en-3-ol) is an essential constituent in the structure of cellular membranes which provide both the physical versatility and tissue indispensable integrity. Besides, cholesterol participates in the construction of steroid hormones and bile salts [1–4] which mainly act in fat transition process from small intestine into the blood or in discharge of waste products from liver. Furthermore, the main roles of cholesterol has been reported in the neural synapses and immune systems [3]. In contrast, high levels of cholesterol in blood and body tissues can cause perilous effects in human body such as, coronary heart and peripheral arterial diseases, diabetes, hypertension, cardiac arrest, and anemia [1,5–7]. Hypercholesterolemia has been recently known as one of the main reasons for human death. Accordingly, controlling the blood cholesterol content is a global challenge. The most of the cholesterol biosensors involve the enzymatic oxidation of cholesterol by cholesterol oxidase (ChOx) [1,5], resulting in the production of H_2O_2 which then is quantified by various procedures [2,4–6,8–13]. In this regard, fluorescence methods are more attractive owing to their excellent features. The great sensitivity and low detection limits with a simple and rapid practical procedure are precious traits of fluorometric techniques.

The general fluorometric cholesterol biosensors involve natural peroxidase enzyme (HRP) which can catalyze the oxidation of a specific substrate by H_2O_2 to produce a high fluorescent product. Recently, efficient alternative mimics have been developed to overcome the serious deficiencies of HRP, such as low

* Corresponding author at: Research Laboratory of Advanced Water and Wastewater Treatment Processes, Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, 51666-16471 Tabriz, Iran.
E-mail address: a_khataee@tabrizu.ac.ir (A. Khataee).

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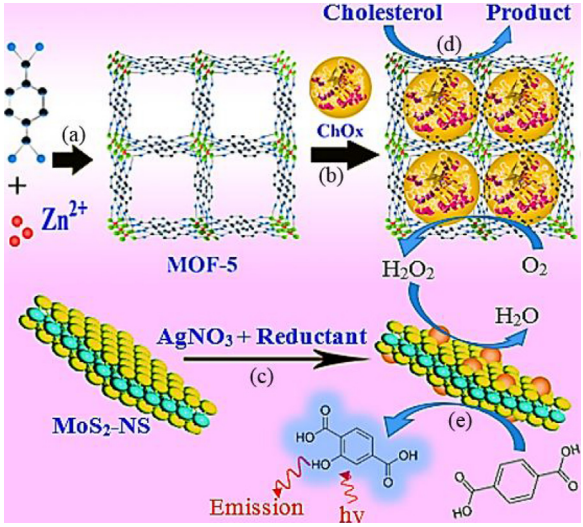
stability, degradable in robust conditions like strong acidic or basic environments and high temperatures, the laborious and high-cost preparation and purification process [14]. Nanomaterials are appealing artificial peroxidase mimetic which have been extensively used for catalysis of biologically important reactions [15]. Molybdenum disulfide nanosheets (MoS_2-NS) with a graphene-like 2D layered structure, has been reported as a novel artificial peroxidase-like nanomaterial. MoS_2-NS offers significant advantages over HRP, like great stability, high availability, simple preservation, low toxicity, simple and economical synthesis, and suitable optical features [16,17]. From the literature, Li et al. [18] have applied the hemin-capped MoS_2-NS for the spectrophotometric detection of H_2O_2. Likewise, Lin et al. [17] have reported the colorimetric detection of H_2O_2 and glucose using the peroxidase-like activity of MoS_2-NS. On the other hand, it was reported that the introduction of metal nanostructures in MoS_2-NS resulted in higher catalytic performances [19–21]. To the best of our knowledge, there is no report on the synthesis and peroxidase-mimic activity of Ag nanocluster/MoS_2-NS hybrid.

Meanwhile, some porous material-based enzymatic nanoreactors have been recently reported [22,23] as enzyme room which can preserve enzymatic reactivity and efficiency in harsh or poisonous media. In this regard, metal-organic frameworks (MOFs), a unique type of porous materials [23], have been widely used as helpful molecular traps which provide powerful interactions between the cage-MOF and the captured proteins [23–28]. Comparing to general porous species such as liposomes and mesoporous silica, the main advantages of MOFs for encapsulation of specific enzymes are the high enzyme encapsulation capacity, greater mechanical stability, higher permeability, and low enzyme leaching [29–31]. For example, Chen and coworkers have applied a water-stable metal-organic framework (MOF) to encapsulate horseradish peroxidases (HRP) for the fabrication of electrochemical H_2O_2 biosensor [32]. Feng research group have developed a series of stable metal-organic frameworks with rationally designed ultra-large mesoporous cages for the enzyme encapsulation [33].

In the present study, a unique bioassay was developed for cholesterol determination based on the encapsulation of ChOx into the MOF-5 and the enhanced peroxidase-like activity of Ag nanocluster/MoS_2 nanosheets nanocomposite (AgNC/MoS_2-NS). MOF-5 was synthesized by complexation reaction of Zn^{2+} and terephthalic acid, and ChOx enzyme was embedded into the so-prepared MOFs. The results showed great catalytic yield, suitable recycling performance and protecting role of MOF-5 nanoreactor. On the other hand, it was found that the modifying the MoS_2-NS by AgNC enhanced their mimetic activity for decomposition of H_2O_2. Based on these observations, a new biosensor was developed for the determination of cholesterol. After the enzymatic oxidation of cholesterol by using encapsulated ChOx in MOFs, the produced H_2O_2 was used for oxidation of terephthalic acid in the presence of AgNC/MoS_2-NS nanocomposite (Scheme 1). The developed system showed notable benefits in terms of sensitivity and selectivity for determination of cholesterol.



Scheme 1. Design for cholesterol sensing based on encapsulated ChOx in MOF-5 and AgNC/MoS_2-NS nanocomposite as peroxidase mimic: a) synthesis of MOF, b) enzyme immobilization in MOF, c) synthesis of AgNC/MoS_2-NS nanocomposite, d) enzymatic oxidation of cholesterol, and e) catalytic reaction of H_2O_2-TA in the surface of AgNC/MoS_2-NS nanocomposite.

2. Experimental

2.1. Apparatus and materials

Fluorescence intensities and spectra were obtained on a LS-45 spectrofluorometer (PerkinElmer, USA). UV–vis absorption spectra were documented by a Shimadzu spectrophotometer (UV-1800, Japan). Morphology studies for the produced nanomaterials were done by high resolution transmission electron spectroscope (HRTEM, JEOL, JEM-2100F, 200 KV) and scanning electron microscope (SEM, Mira3-FEG Czech Republic). X-ray diffraction (XRD) patterns were obtained by a Siemens-D5000 X-ray diffractometer (Germany) using Cu Kα exciting source (λ = 1.54056 Å). Fourier transform infrared (FTIR) spectra were achieved on a Bruker-Tensor 27 FTIR spectrometer (Germany). Investigations of Brunauer-Emmett-Teller (BET) surface area for prepared MOFs were carried out by N_2 adsorption/desorption isotherms measured at 77 K in a liquid nitrogen atmosphere using a Belsorp mini II analyzer (BEL, Japan).

All chemicals with analytical grade were used without additional purification. Deionized (DI) water (Kasra Co., Tabriz, Iran) was used for preparation of solutions. H_2O_2, terephthalic acid (TA), *N*,*N*-dimethylformamide (DMF), Zinc nitrate tetrahydrate, and Albumin were obtained from Merck Company (Darmstadt, Germany). Glutathione (GSH), α-lipoic acid (LA), silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), sodium borohydride ($NaBH_4$), MoS_2, Cholesterol, Cholesterol oxidase (ChOx, 100 UN), and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Sigma Company.

2.2. Preparation of ChOx encapsulated MOF-5

The synthesis of MOF-5 was carried out based on a simple method [34]. Briefly, 170 mg TA and 600 mg $Zn(NO_3)_2{\cdot}6H_2O$ were added in 20 mL DMF, continued by stirring to their dissolution. The mixture was heated in a sealed Teflon container (21 h at 120 °C). After separation of produced colorless crystal MOF-5, it was subsequently washed by DMF. Finally, the crystals were dried at room temperature. In order to immobilize the ChOx into the prepared MOF-5, 1 mL of ChOx solution (6 mg mL^{-1}) was added into 1 mL suspension of MOF-5 (5 mg) and, the mixture was incubated at 37 °C (32 h). The formed composites were separated by centrifugation and washed by phosphate buffer solution (0.05 M, pH 7.0). The mentioned ratio of ChOx:MOF was obtained after optimization experiments considering maximum enzymatic activity.

2.3. Synthesis of Ag nanocluster/MoS_2 nanosheet nanocomposites

Exfoliation of MoS_2 powder, to achieve its nanosheets, was performed using a recently reported method which was based on the sonication of bulk powder in 30% isopropanol/water solvent and in the presence of 4% N_2H_4 [35]. Subsequently, in order to hybrid