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As a new peroxidase mimetics: The synthesis of selenium doped graphitic carbon nitride nanosheets and applications on colorimetric detection of H_2O_2 and xanthine

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ABSTRACT

Selenium has been successfully doped into graphite phase carbon nitride (g-C_3N_4) by a simple thermal condensation method through calcinating the mixture of dicyandiamide, cyanuric acid and selenium dioxide. The morphology and composition of thus-prepared selenium doped graphite phase carbon nitride (Se-g-C_3N_4) nanosheets were characterized by transmission electron microscopy (TEM), high resolution TEM, X-ray diffraction (XRD), fourier transform infrared (FT-IR) spectra and X-ray photoelectron spectroscopy (XPS). Characterization results revealed that the resultant products have typical ultrathin lamellar structure with the uniform lateral diameter of 20 nm. The amount of Se dopant is *ca.* 2.1 at% based on the XPS result. For the first time, ultrathin Se-g-C_3N_4 nanosheets have been demonstrated to possess an intrinsic peroxidase activity by following the Michaelis–Menten kinetics and even have higher affinity to peroxidase substrates TMB and H_2O_2 in comparison to that of HRP. Se-g-C_3N_4 nanosheets is conducive to mediating electron transfer and thus to enhancing the catalytic oxidation of the substrate TMB in the presence of H_2O_2 to produce a blue color change in aqueous solution. More importantly, a sensitive and selective method for xanthine detection was developed using xanthine oxidase and the as-prepared Se-g-C_3N_4 nanosheets. On the basis of the high catalytic activity of Se-g-C_3N_4 nanosheets, a rapid, sensitive, and convenient approach was developed for colorimetric detection of xanthine with detection limit 1.6×10^{-8} mol L^{-1}. Taking the advantages of this, the novel Se-g-C_3N_4 nanosheets represent a promising candidate as an enzyme mimic and may find a wide range of new applications in diagnostics and biotechnology fields.

1. Introduction

Graphitic carbon nitride (g-C_3N_4) as an organic semiconductor consists of carbon and nitrogen, which are among the most abundant elements in our planet, and is thus environmentally friendly, sustainable and can be produced on a large scale with low cost [1,2]. It was described that g-C_3N_4 has attracted increasing attention because of its high thermal and chemical stability, semiconductivity, intrinsic fluorescence property and special optical features [3,4]. Nevertheless, the optical band gap of pure C_3N_4 is ~2.7 eV, absorbing only blue light up to 450 nm, and has high recombination rate of photogenerated electron-hole pairs [5], which lead to the purest g-C_3N_4 being not always the suitable candidates for certain applications. Therefore, to enhance the performance, the development of innovative strategies to modify of the pure g-C_3N_4 is playing an indispensible role in developing technologies. Indeed, many strategies have been devoted into g-C_3N_4 modification, *e.g.* doping with metal/nonmetal elements [6,7], protonation with HCl [8], dye sensitizing [9], hybridization [10], copolymerization [11] and nanostructuring [12]. Among various strategies, there is little doubt that doping, especially anion doping pioneered by Asahi et al., is most widely investigated for its effectiveness in broadening the light responsive range of wide-bandgap semiconductors [13]. This concept of thermolysis of heteroatom rich precursors for the synthesis of doped C_3N_4 has been applied frequently. Boron doped carbon nitride materials with graphite like structure have been reported by Bartlett et al. in 1987, who used a chemical vapor deposition method with boron trichloride, ammonia and acetylene as the precursor mixture [14]. Le et al. reported successful attempt to introduce oxygen atoms into g-C_3N_4 by a simple H_2O_2 hydrothermal procedure [15]. Zhang et al. reported a "structural doping" strategy, in which the precursors of g-C_3N_4 and a heteroatom source (specifically for phosphorus) were mixed first and then polycondensed together into the final products [16].

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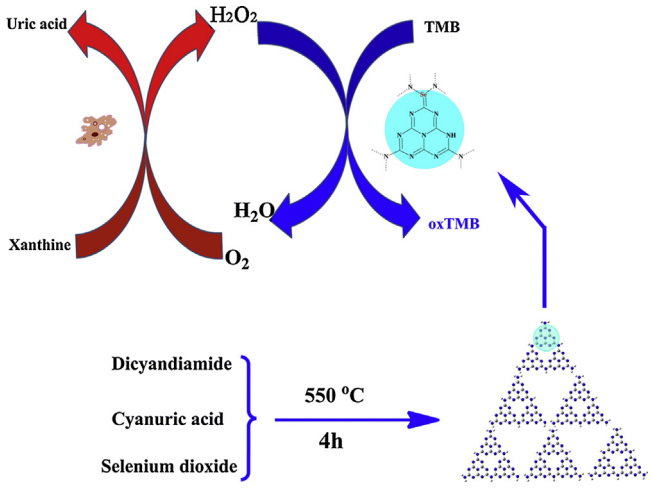
Through doping, the activity of the resulting catalysts has been improved to some extent. Undeniably, doping g-C_3N_4 through nonmetal elements is becoming a hot research topic, and it builts up a new method to design and fabricate more practicable and efficient catalysts. Thus, they promise access to the widespread application in energy related fields, such as fuel cells, batteries, hydrogen storage or supercapacitors, most of which mainly focused on photocatalysis in recent years. Nevertheless, to the best of our knowledge, there have been few attempts to investigate the enzyme mimetic activity of doped g-C_3N_4 through nonmetal elements.

Natural enzymes, due to high substrate specificities and high efficiency under mild conditions, have significant practical applications in medicine, chemical industry, food processing and agriculture [17]. They, however, also suffer from some intrinsic drawbacks such as sensitivity of catalytic activity to environmental conditions and relatively low stability (denaturation and digestion). In addition, high costs in preparation and purification also limit their large scale applications [18]. Thus, the creation of efficient, simple, and sustainable materials as peroxidase mimetics represents a central challenge of this field [19]. Recently, the increasing availability of nanomaterials has created widespread interest in their use as high-efficiency catalysts due to their large surface-to-volume ratio. Indeed, a series of nanostructured materials, such as Fe_3O_4 nanoparticles [20,21], carbon nanotubes [22], carbon dots [23], Au NPs [24], BSA-stabilized Au clusters [25], exfoliated layered double hydroxides nanosheets [26,27], graphene oxide [28,29], graphitic carbon nitride [30,31] and other nanomaterials [32–36] have been demonstrated to possess peroxidase-like catalytic activities and be used in biomedical and environmental detection.

Selenium is an element with unique and basically incomparable properties within the periodic table. [37] It is thus a highly interesting candidate worhy exploring for the doping of carbon nitride materials. In this work, to demonstrate the above considerations, selenium, which has a smaller electronegativity than nitrogen (2.55 *vs* 3.04), was chosen as dopant into C_3N_4. We demonstrated that using selenium-containing inorganic compounds SeO_2 together with triazine compounds as the precursors, can produce Se doped g-C_3N_4 (Se-g-C_3N_4) nanosheets through a simple calcination treatment. It reveals that ultrathin Se-g-C_3N_4 nanosheets possess superior peroxidase mimetics activity than undoped counterparts, which can catalyze the reaction of peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H_2O_2 to produce a blue color reaction. Moreover, many oxidases can react with substrates to generate H_2O_2 as a product. Therefore, measuring the color variation of TMB catalyzed by peroxidase may provide an indirect method to develop optical sensors for substrates, *i.e.*, glucose oxidase and glucose [20].



Scheme 1. Schematic illustration of the colorimetric detection of xanthine using XOD and Se-g-C_3N_4 nanosheets.

Xanthine is a major metabolite intermediate of the purine nucleotide and deoxynucleotide metabolism in animals, and is produced after adenosine triphosphate (ATP) decomposition [38]. Extreme abnormal levels of xanthine will lead to gout [39]. Therefore, it is of medical and biological importance to fabricate a simple and cheap sensor for xanthine. Based on the peroxidase mimetics of Se-g-C_3N_4 nanosheets, and the selective catalytic oxidation of xanthine by xanthine oxidase (XOD), a colorimetric method for H_2O_2 and subsequent xanthine detection has been developed (Scheme 1). The results indicate that this assay is rapid, simple, cheap and highly sensitive with a pretty low detection limit and high selectivity. This assay has also been used successfully in diluted serum samples.

2. Experimental

2.1. Reagents and apparatus

3,3′,5,5′-tetramethylbenzidine (TMB), cyanuric acid ($C_3N_3H_3O$), xanthine ($C_5H_4N_4O_2$), uric acid ($C_5H_4N_4O_3$), ascorbic acid ($C_6H_8O_6$) were purchased from Aladdin (Shanghai, China). Selenium dioxide (SeO_2), hydrogen peroxide (30 wt%,H_2O_2), dicyandiamide ($C_2H_4N_4$), acetic acid (HAc), sodium acetate (NaAc), acetic acid and other chemical reagents were purchased from Kay Tong Chemical Reagents Co. Ltd (Tianjin, China). Xanthine oxidase (XOD, 10 U/mL) were purchased from Sigma–Aldrich (Shanghai, China). Acetate buffer solution (100 mM, pH from 2.0 to 11.0) were used in this work and double distilled deionized water was applied throughout the experiment. All chemicals used in this work were of analytical grade and used as received without further purification. Drinking coffee was purchased from marketplace. Serum samples and urine samples were obtained from Hospital of Shandong Agricultural University.

The morphology and size distribution of the nanoparticles were studied by using a JEM-2010 transmission electron microscope (TEM, Japan). The crystal phase was investigated by a Rigaku DLMAX-2550 V diffractometer (40 kV, Cu Kα (λ = 1.54056 Å), 2θ range 5–80°; scan speed of 6°/min). The chemical state and percentage of selenium were measured by X-ray photoelectron spectroscopy (XPS) analysis in a Thermo ESCALAB 250XI. Fourier transform infrared (FT-IR) spectra were obtained on a Thermo Nicolet-380 IR spectrophotometer (USA). Kinetic measurements and UV–vis absorption spectra were carried out on a UV-2450 Shimadzu Vis-spectrometer (Japan).

2.2. Preparation of selenium-doped-g-C_3N_4 nanosheets

The selenium-doped g-C_3N_4 (denoted as Se-g-C_3N_4) was prepared by heating the mixture of dicyandiamide, cyanuric acid and selenium dioxide in the semiclosed system for preventing sublimation of dicyandiamide. In detail, 0.8587 g of dicyandiamide, 0.6499 g of cyanuric acid and 1.0 g selenium dioxide was mixed in a agate mortar, after grinded for 10 min, the mixture was transferred into the alumina crucible with a cover, and then heated at 550 °C in the flow of N_2 gas for 4 h with a ramping rate of 2.3 °C/min, leading to brown powder Se-g-C_3N_4. For comparison, native graphitic carbon nitride (g-C_3N_4) was prepared by pyrolysis of the mixture of cyanuric acid and dicyandiamide at 550 °C for 4 h through the same method.