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Graphite-like carbon nitrides as peroxidase mimetics and their applications to glucose detection

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ABSTRACT

g-C_3N_4 was found to possess intrinsic peroxidase-like activity, and could catalytically oxidize 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to produce a color reaction. Using g-C_3N_4 peroxidase-like catalytic activity and glucose oxidase (GOx), a colorimetric method for glucose detection in serum samples has been developed. The linear range for glucose was from 5 to 100 μM (R^2=0.9987) and the limit of detection was as low as 1.0 μM. The proposed method was applied to detect glucose in serum samples by the naked eyes.

1. Introduction

Enzyme mimetics have attracted great attention because they possess many advantages over nature enzymes such as low cost, more stable against denature or protease digestion. Nanoparticles (NPs), due to their large surface areas and controlled catalytic potentials, have been exploited as peroxidase mimics in catalyzing H_2O_2-mediated reaction. In 2007, Fe_3O_4 magnetic nanoparticles (MNPs) were firstly discovered to exhibit an intrinsic peroxidase-like enzyme mimetic activity (Gao et al., 2007; Wei and Wang, 2008). Thereafter, several nanomaterials such as cerium oxide (Asati et al., 2009), FeS nanosheets (Dai et al., 2009), V_2O_5 nanowires (André et al., 2011), Co_3O_4 NPs (Mu et al., 2012), TiO_2 nanotubes (Zhang et al., 2013), Au NPs (Long et al., 2011), Pt nanotubes (Cai et al., 2013), MIL-53(Fe) (Ai et al., 2013), and bimetallic or hybrid nanomaterials such as Au@Pt nanostructures (He et al., 2011), CuPt nanorods (Kwon et al., 2011), Au@PdPt nanorods (Zhang et al., 2011), Bi–Au NPs (Lien et al., 2012), Fe–Co alloy NPs (Chen et al., 2013), $ZnFe_2O_4$ MNPs (Su et al., 2012; Zhao et al., 2013), Au@Pd NPs (Chen et al., 2011), PtPd–Fe_3O_4 (Sun et al., 2013), and Fe_3O_4 NPs-carbon nitride nanotube hybrids (Lee et al., 2012) have demonstrated the peroxidase-like activity in the presence of H_2O_2. Other than metal nanomaterials, carbon nanomaterials such as single-walled carbon nanotubes (Song et al., 2010b), graphene oxide (Song et al., 2010a), carbon nanodots (Shi et al., 2011), and carbon nitride dots (Liu et al., 2012) have also reported to possess the intrinsic peroxidase-like activity, which was not related to the trace amount of metal catalyst in the sample but instead was caused by their own intrinsic property (Shi et al., 2011; Song et al., 2010a, 2010b). With advantages of low cost, high stability and tunability in catalytic activities, these nanomaterials based peroxidase mimetics have been applied in bioassays and medical diagnostics.

Graphite-like carbon nitride (g-C_3N_4), an analog of graphite, is a fascinating semiconductor material with a band gap of ca. 2.7 eV (Wang et al., 2008) and has been explored for various applications, especially in the field of photocatalysis (Chen et al., 2009; Cui et al., 2012; Maeda et al., 2009; Niu et al., 2012; Wang et al., 2012, 2009, 2008; Yan et al., 2009, 2010; Yang et al., 2013). For instance, taking advantage of the large band gap to overcome the endothermic character of the water-splitting reaction g-C_3N_4 was used as efficient catalysts for hydrogen evolution under visible light (Maeda et al., 2009; Niu et al., 2012; Wang et al., 2008; Yang et al., 2013). g-C_3N_4 has been used as photocatalysts to decompose organic pollutant rhodamine B, methyl orange, etc. (Cui et al., 2012; Wang et al., 2009; Yan et al., 2009, 2010). Compared with traditional heterogeneous catalysts or catalyst supports, g-C_3N_4 has demonstrated many advantages such as the graphite-like structure, metal-free, high thermal and chemical stability, tunable electronic structure, and abundant and inexpensive (Wang et al., 2012). Recently, g-C_3N_4 due to its unique structure and excellent properties such as electrocatalytic activity (Tian et al., 2013c),

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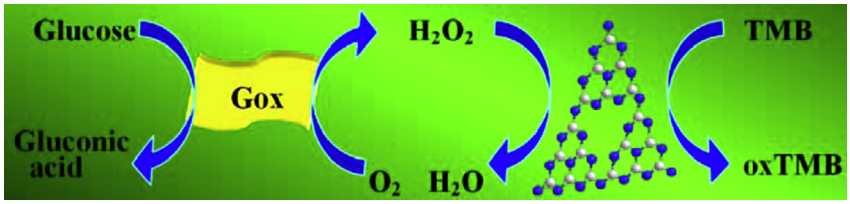

Scheme 1. Schematic illustration of colorimetric detection of glucose by using glucose oxidase (GOx) and g-C_3N_4 peroxidase-like catalytic reactions.

luminescence performance (Cheng et al., 2012; Lee et al., 2010; Tang et al., 2013; Tian et al., 2013a; Zhang et al., 2012), photo-induced electron transfer (Wang et al., 2013) has been applied for sensing and imaging.

Herein, we demonstrated the intrinsic catalytic activity of g-C_3N_4 in the catalysis of oxidation of a peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 to produce a blue color product. Using g-C_3N_4 peroxidase-like catalytic activity and glucose oxidase (GOx), a colorimetric method for glucose detection was developed (Scheme 1). This method was simple, cheap, and highly sensitive and selective, and was successfully applied for glucose detection in serum samples.

2. Experimental section

2.1. Chemicals and materials

Melamine, H_2O_2, ferrous sulfate ($FeSO_4$), sodium nitrite ($NaNO_2$), fructose and maltose were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Glucose oxidase (GOx), glucose and lactose were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). 3,3′,5,5′-Tetramethylbenzidine (TMB) was bought from Bio Basic Inc. (Ontario, Canada). Sodium hypochlorite (NaClO) was bought from Guangdong Guanghua Sci-Tech Co., Ltd. (Guangdong, China). Potassium dioxide (KO_2, 96.5%) was obtained from Aladdin. All chemicals are used as received and without further purification. The solutions were prepared using ultrapure water which was purified by Millipore Milli-Q (18 MΩ cm).

2.2. Synthesis and characterization of g-C_3N_4

g-C_3N_4 was prepared as described previously with a slight modification (Cheng et al., 2012; Tian et al., 2013a; Yan et al., 2009). In brief, 10.0 g of white melamine powder was placed into a tube furnace and heated at 600 °C for 4 h under N_2 with a heating rate of 3 °C/min, leading to pale yellow products. Then, the products were ground to powder and dispersed into 2.5 mg/mL water. The g-C_3N_4 suspension was treated ultrasonically for 24 h before further use.

g-C_3N_4 samples were characterized by X-ray diffraction (XRD) for phase identification on a Panalytical X'Pert PRO MPD X-ray diffractometer (Philips, Netherlands) equipped with Cu Kα radiation (40 kV, 40 mA). Fourier transform infrared (FTIR) spectra were recorded on a Spectrum 2000 spectrometer (Perkin-Elmer, USA) using the KBr method in the range between 4000 and 400 cm^{-1}.

2.3. Detection of H_2O_2 and glucose using g-C_3N_4 as peroxidase mimetics

Peroxidase catalytic activity of g-C_3N_4 was performed by recording the UV–vis absorption spectra of oxidation product of TMB on a Lamda 750 UV–vis–NIR spectrometer (PE, USA). H_2O_2 detection was carried out as follows: 300 μL g-C_3N_4 (2.5 mg/mL), 50 μL TMB (12 mM) and 200 μL phosphate buffer (25 mM, pH 3.0) were mixed and incubated at 60 °C for 15 min in dark with slight shaking (400 rpm). Then 200 μL H_2O_2 with different concentrations was added and the mixture was further incubated at 60 °C in dark with slight shaking (400 rpm) for 60 min. The mixture was centrifugated at 13,000 rpm for 10 min; the absorbance of supernatant was recorded.

Glucose detection was realized as follows: 20 μL GOx (10 mg/mL) was added into 180 μL phosphate buffer (5 mM, pH 5.72) containing different concentrations of glucose, and the mixture was incubated at 37 °C for 40 min to produce H_2O_2. The other detection procedure was the same as that of H_2O_2.

Before the detection of glucose in serum, the proteins in serum were removed by precipitation. 30 μL of serum sample was diluted with 20 μL water, and then 500 μL $Ba(OH)_2$ (0.11 M) and 500 μL $ZnSO_4$ (0.0765 M) were added and blended; the mixture was centrifugated at 3880 rpm for 10 min. 200 μL of supernatant solution was taken and diluted with phosphate buffer (5 mM, pH 5.72) to 1000 μL. 180 μL of above solution was mixed with 20 μL GOx (10 mg/mL); the mixture was incubated at 37 °C for 40 min to produce H_2O_2. The other detection procedure was the same as that of H_2O_2.

3. Results and discussion

3.1. Characterization of g-C_3N_4

The bulk g-C_3N_4 was prepared by direct pyrolysis of melamine under N_2 atmosphere; the solid sample and suspension of g-C_3N_4 are shown in Fig. S1 (in Supporting information). The phase structure of the as-synthesized g-C_3N_4 sample was identified by XRD measurement. The XRD pattern (Fig. S2 in Supporting information) presented a strong peak at 27.4°, characteristic of the stacking peak of π-conjugated layers and indexed for graphitic materials as the (002) peak (Cheng et al., 2012; Cui et al., 2012; Tian et al., 2013c; Yan et al., 2009). FTIR spectroscopy (see Fig. S3 in Supporting information) supported the existence of a graphite-like structure of carbon nitride (Cui et al., 2012; Yan et al., 2009; Zhang et al., 2012). The band at 810 cm^{-1} belonged to the characteristic breathing mode of triazine ring and bands at 1240–1630 cm^{-1} were characteristic of aromatic carbon nitride heterocycles. The broad absorption band around 3250 cm^{-1} could be assigned to the stretching modes of secondary and primary amines.

3.2. Peroxidase-like activity of g-C_3N_4

Very recently, Sun et al. reported that ultrathin graphitic carbon nitride nanosheets possessed peroxidase activity and Fe doping of the nanosheets led to great enhancement of the catalytic performance (Tian et al., 2013b). However, visible-light photocatalysis was involved in the catalytic reaction. Metal-free g-C_3N_4 has been found to activate H_2O_2 to generate reactive $OH^{\bullet}$ under visible light irradiation (Cui et al., 2012). To exclude the photocatalytic activity and avoid the influence of the visible light on the peroxidase-like catalytic activity of g-C_3N_4, all experimentals of catalytic reaction were performed in dark. The catalysis of peroxidase substrate TMB