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Visual detection of cyanide ions by membrane-based nanozyme assay

Chia-Wen Lien[a], Binesh Unnikrishnan[b], Scott G. Harroun[c], Chih-Min Wang[b], Jia-Yaw Chang[d], Huan-Tsung Chang[a,e,*], Chih-Ching Huang[b,f,g,**]

[a] Department of Chemistry, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 10617, Taiwan
[b] Department of Bioscience and Biotechnology, National Taiwan Ocean University, 2, Beining Road, Keelung 20224, Taiwan
[c] Department of Chemistry, Université de Montréal, Montréal, Québec H3C 3J7, Canada
[d] Department of Chemical Engineering, National Taiwan University of Science and Technology, Taipei 10607, Taiwan
[e] Department of Chemistry, Chung Yuan Christian University, Taoyuan 32023, Taiwan
[f] Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung 20224, Taiwan
[g] School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 80708, Taiwan

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ABSTRACT

In this paper, we report a simple one-step synthesis of well-dispersed amorphous cobalt hydroxide/oxide-modified graphene oxide (CoO_xH-GO) possessing peroxidase-like catalytic activity, and its application for the detection of H_2O_2, glucose, and CN^- ions. CoO_xH is formed and deposited *in situ* on the GO surface through the reaction between GO (size ~ 240 nm) and Co^{2+} in basic solution at room temperature. We investigated the enzyme-mimicking activity of the CoO_xH-GO nanohybrid in detail *via* the H_2O_2-mediated oxidation of Amplex Red (AR) to form fluorescent resorufin. The peroxidase-like activity of CoO_xH-GO is utilized herein for the quantitation of H_2O_2 in a wide concentration range, from 100 nM to 100 μM. When coupled with glucose oxidase (GOD), the AR/CoO_xH-GO system can determine glucose level in blood samples. Interestingly, cyanide ions (CN^-) significantly inhibit the catalytic activity of the CoO_xH-GO nanohybrid, which allows for the construction of a probe for the detection of CN^- in water samples and laboratory wastes. We fabricated a membrane-based CoO_xH-GO probe for the visual detection of CN^- by preparing a thin film of CoO_xH-GO on a positively charged and porous nylon membrane (N^+M). The CoO_xH-GO/N^+M operates on the principle that CN^- inhibits the catalytic activity of CoO_xH-GO towards the H_2O_2-mediated oxidation of AR to form reddish resorufin on the membrane. The intensity of the red color of the membrane decreases with increasing CN^- concentration, which can be easily observed with the naked eye at the nanomolar level. This cost-effective sensing system allows for the rapid and simple determination of the concentrations of CN^- in complicated wastewater samples.

1. Introduction

Natural enzymes, with their substrate selectivity and immense catalytic efficiency, have been extensively investigated for their applications in the analytical, medical, biological, and food industry fields (Renata et al., 2015). Despite extensive developments, natural enzymes often suffer from inherent shortcomings such as high cost of preparation and purification, along with low storage and operational stability. Moreover, environmental conditions (pH, temperature, ionic strength, surfactants, and organic solvents) also affect the catalytic activity of enzymes. To address these problems, alternative candidates such as enzyme mimetic nanomaterials (nanozymes) have been explored, more specifically, bimetallic nanoparticles (NPs) and hybrid nanomaterials (Wei and Wang, 2013; Lin et al., 2014; Ragg et al., 2016). Enzyme-mimicking nanomaterials have generated much interest due to their intrinsic advantages, high stability under harsh conditions, low cost of preparation and purification, and easy modifications with diverse ligands for utilization in aptasensors, immunoassays, and for the detection of a wide range of molecules such as glucose, pesticides, proteins and DNA (Weerathunge et al., 2014; Gao et al., 2015; Wang et al., 2017a; Lin et al., 2017). Recently, graphene-based materials, such as graphene oxide (GO), reduced GO (rGO), and graphitic carbon nitride (g-C_3N_4), have been incorporated into nanocomposites with various enzyme-like activities, and have attracted much attention (Zhang et al., 2015a; Vázquez-González et al., 2017). These graphene-based materials can enhance catalytic activity by promoting electron transfer between the substrate and the catalytic NPs. In addition, GO or rGO can improve the dispersibility of catalytic nanocomposites, which have wide

* Corresponding author at: Department of Chemistry, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 10617, Taiwan.
** Correspondence to: Department of Bioscience and Biotechnology, National Taiwan Ocean University, 2, Beining Road, Keelung 20224, Taiwan.
E-mail addresses: changht@ntu.edu.tw (H.-T. Chang), huanging@ntou.edu.tw (C.-C. Huang).

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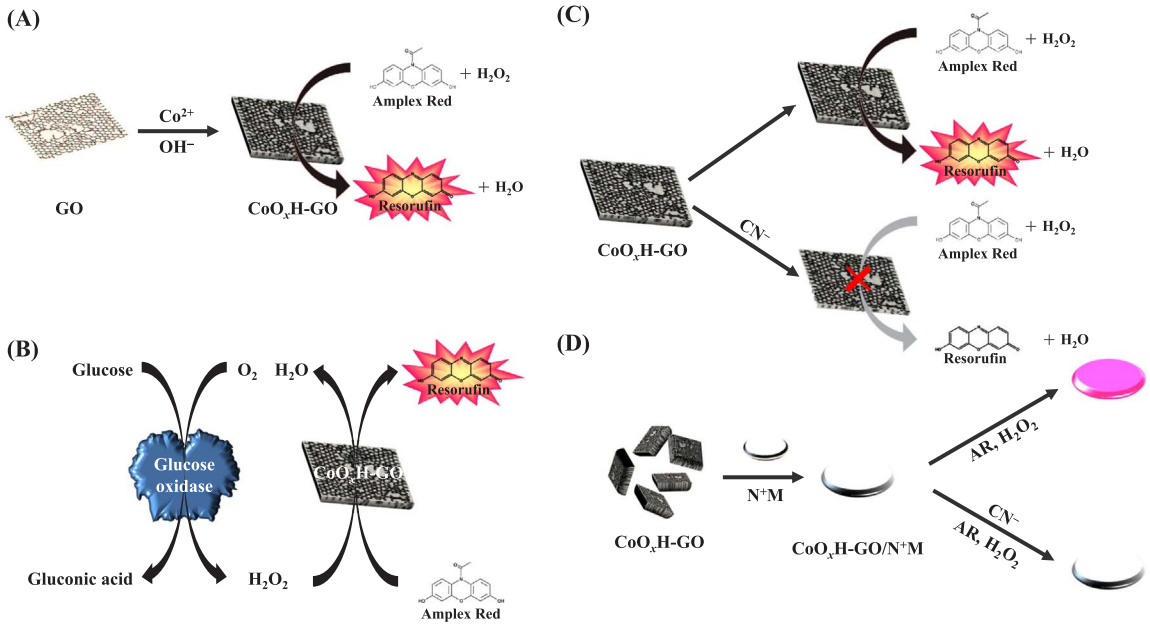

Scheme 1. Schematic representation of (A) the preparation of CoO_xH-GO nanohybrid for the catalysis of H_2O_2 mediated oxidation of AR to resorufin, (B) glucose oxidase coupled with peroxidase-like CoO_xH-GO nanohybrid for the detection of glucose, (C) detection of CN^- ions based on the inhibition of the enzymatic activity of CoO_xH-GO nanohybrid, and (D) the fabrication of CoO_xH-GO/N^+M for sensing of cyanide ions.

applications in aqueous phase reactions. Many graphene-supported and other forms of metal NPs or metal oxide NP hybrids, such as platinum NPs supported on rGO (Chau et al., 2016), GO-based Fe_2O_3 hybrid (Song et al., 2016), graphene–copper oxide nanocomposite (Ragavan and Rastogi, 2016), iron hydroxide/oxide immobilized on rGO (Hsu et al., 2014), and immobilized gold NPs on mesoporous silica-coated nanosized rGO (Maji et al., 2015), have been shown to act as artificial enzymes. These graphene-based hybrid nanomaterials have been employed for sensing applications, such as quantitation of DNA, bisphenol-A, and cancer cells, as well as for the catalytic degradation of dyes.

Cyanide ions (CN^-) are an extremely lethal poison to the human body. Strong binding of CN^- to one of the heme units of cytochrome c oxidase paralyzes cellular respiration, and causes serious damage to the central nervous system (Schubert and Brill, 1968). At excessive levels, intake of CN^- can lead to the death of human beings within a few minutes. The oral lethal dose for humans is 1.4 mg kg^{-1} body weight (calculated as hydrogen cyanide) (Simeonova and Fishbein, 2004). In industry, cyanides are widely employed for organic synthesis and metallurgy. Inevitably, accidental release of CN^- into the environment may occur. Studies reveal that under conditions of continuous exposure, adverse effects on the swimming and reproduction of fish usually occur between cyanide concentrations of 5.0 μg L^{-1} (190 nM) and 7.2 μg L^{-1} (280 nM) cyanide (Eisler et al., 1991). Concentrations of cyanide higher than 160 μg L^{-1} (6.1 μM) can cause acute health problems to most species of freshwater and marine fish as well as invertebrates (Eisler et al., 1991). Since CN^- is lethal to humans, the World Health Organization (WHO) regulates the permitted level of CN^- in drinking water at 2.7 μM (World Health Organization, 2008). Therefore, accurate quantification of CN^- in environmental samples is necessary. Many electrochemical and optical methods have been developed for CN^- detection (Ma and Dasgupta, 2010; Xu et al., 2010). Colorimetric and fluorometric approaches by using organic dyes and nanomaterials have become favorable techniques for the detection of CN^- due to their high selectivity, sensitivity, and simplicity (Promchat et al., 2017; Zhang et al., 2015b; Wang et al., 2017b). However, expensive reagents, complicated synthesis and purification procedures, use of organic solvents, and/or spectral interference are sometimes problematic. Therefore, there is a need to develop sensitive, selective, and simple optical approaches not only for qualitative analysis, but also for quantitation of CN^- in real samples at trace levels.

For many years, membrane-based tests have played a significant role in chemical analyses, including the well-known litmus paper test and paper chromatography, along with clinical tests at home for pregnancy, glucose, pH, and bilirubin (Doshi, 1986; Comer, 1956; Dobkin et al., 1990; Morgan and Wilson, 2004). Membrane-based sensing techniques have provided opportunities for the development of inexpensive, portable, and instrument-free analytical systems that are ease in manufacture and use. A membrane-based colorimetric assay is a simple sensor that can be used under field conditions, and at home without any special instruments. Color change assays have been employed for sensing applications in environmental samples, such as detection of toxins, heavy metal ions, and pesticides (Wang et al., 2011; Takahashi et al., 2006; Badawy and El-Aswad, 2014). However, only a few reports are available for membrane-based sensors in combination with nanozymes (Duan et al., 2015).

In this study, we prepared self-assembled cobalt hydroxide/oxide on GO sheets from GO (size ~ 240 nm) and cobalt ions (Co^{2+}) in 5.0 mM Tris-ammonium solution (pH 11.0). The Co^{2+} ions were mainly hydroxylated to cobalt(III) oxide-hydroxide [CoO(OH)] and cobalt(II) hydroxide [$Co(OH)_2$] to self-assemble on GO to form the CoO_xH-GO nanohybrid in a basic solution. The CoO_xH-GO nanohybrid exhibited high catalytic activity (peroxidase-like activity) for the H_2O_2-mediated oxidation of Amplex Red (AR; 10-acetyl-3,7-dihydroxyphenoxazine) to form fluorescent resorufin (7-hydroxy-3H-phenoxazin-3-one). We incorporated glucose oxidase (GOD) in to this system for the detection of glucose in two steps. We further applied the AR/H_2O_2 − CoO_xH-GO system for the sensing of CN^- based on the analyte-induced inhibition of the catalytic activity of the CoO_xH-GO nanohybrid. The practicality of this probe was validated separately through the detection of CN^- in tap water, river water, lake water, and sea water. Furthermore, we developed a simple membrane-based colorimetric sensor for detecting CN^- with a CoO_xH-GO nanohybrid-modified positively charged nylon membrane (CoO_xH-GO/N^+M). The CoO_xH-GO/N^+M was used for visual detection of CN^- down to nanomolar level in aqueous solutions in the presence of AR and H_2O_2. The CoO_xH-GO/N^+M was further applied to screen CN^- in wastewater samples. The reactions and mechanisms involved in the synthesis of CoO_xH-GO nanohybrid, detection of glucose, CN^-, and the application of CoO_xH-GO/N^+M are graphically