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Copper sulfide nanoparticle-decorated graphene as a catalytic amplification platform for electrochemical detection of alkaline phosphatase activity

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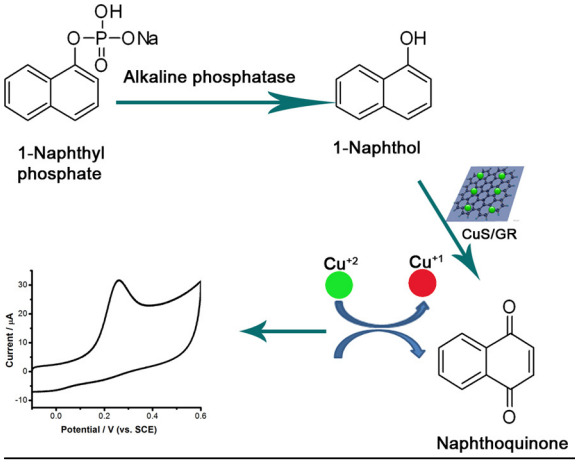
HIGHLIGHTS

- The copper sulfide nanoparticles–graphene nanocomposites were synthesized and used for electrochemical detection of alkaline phosphatase.
- The assay method showed a wide linear range and a lower detection limit of 0.02 U L^{-1}.
- This method was successfully applied to detection of alkaline phosphatase in clinical samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Copper sulfide nanoparticle-decorated graphene sheet (CuS/GR) was successfully synthesized and used as a signal amplification platform for electrochemical detection of alkaline phosphatase activity. First, CuS/GR was prepared through a microwave-assisted hydrothermal approach. The CuS/GR nanocomposites exhibited excellent electrocatalytic activity toward the oxidation of ALP hydrolyzed products such as 1-naphthol, which produced a current response. Thus, a catalytic amplification platform based on CuS/GR nanocomposite for electrochemical detection of ALP activity was designed using 1-naphthyl phosphate as a model substrate. The current response increased linearly with ALP concentration from 0.1 to 100 U L^{-1} with a detection limit of 0.02 U L^{-1}. The assay was applied to estimate ALP activity in human serum samples with satisfactory results. This strategy may find widespread and promising applications in other sensing systems that involves ALP.

1. Introduction

Alkaline phosphatase (ALP) is one of the most important enzymes that exists in different tissues. It can catalyze the dephosphorylation process of nucleic acids, proteins, and some

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small molecules [1]. As a widely distributed membrane-bound enzyme, ALP has an extensive substrate specificity, which can hydrolyze various phosphorylated compounds in vivo and in vitro [2]. ALP is produced differently on different tissues and with disease states such as hepatobiliary disease [3], adynamic bone disease [4], and prostate cancer [5]. Thus, sensitive ALP detection methods are urgently needed for many biomedical applications.

ALP assays generally utilize ALP substrates for detection of enzymatically-generated products via various monitoring techniques [6–9]. Current monitoring techniques include chemiluminescence [6], colorimetric [7], fluorometric [8], and electrochemical [9,10] approaches. Of these, electrochemical detection has drawn considerable interest due to its high sensitivity, low cost, and good portability [11,12]. In a recent effort to enhance detection sensitivity, a variety of strategies for signal amplification has been designed. These include the use of labels or polymerase chain reaction [13,14]. But these are expensive and complicated [15], which has limited their practical application. One alternative is nanoparticle-based signal amplification, which has attracted increasing attention for ultrasensitive, high selectivity, rapid, and miniaturized analysis platforms.

Inorganic nanomaterials, such as semiconductor nanocrystals, have captured tremendous attention for a wide range of biological applications including bioassays, drug delivery, and cell imaging because of their unique chemical and physical properties [16–17]. Monodisperse binary chalcogenides compounds such as ZnS, ZnSe, CdS, CdSe, PbS, and PbSe have drawn attention as “quantum dots”. However, these materials require toxic heavy metals, which have limited their application in biological systems. Copper sulfide (CuS) is a p type semiconductor [18] and has been extensively used as a promising material for lithium batteries, solar cells, light emitting diodes (LED), photocatalysis, and biological applications [19–22].

Recently, CuS nanoparticles have been integrated for the fabrication of sensors and biosensors due to their various morphologies, low cost, excellent catalytic properties, and the ability to enhance the electron transfer rate at the electrode surface [23]. However, these small nanoparticles easily aggregate and form large particles due to the strong attractive forces between the nanoparticles. This greatly weakens their catalytic activity [24]. In fact, CuS nanoparticles are not very good as electrode materials because of their semiconductor properties. Therefore, there is an urgent need to fabricate well-dispersed nanocatalysts with highly conductive supports to improve the conductivity and electron transfer rate for electrochemical applications.

Graphene has recently attracted enormous interest across many fields including nanoelectronic devices, sensors, biology, etc. [25–27]. Graphene offers superior electron conductivity, high heterogeneous electron-transfer rate at the edges and basal plane defect sites, and a large specific surface [28]. Graphene has been employed as an efficient atomic-scale scaffold for loading and stabilizing nanoparticles to prepare nanocomposites with better properties than the graphene or nanoparticles alone. Indeed, graphene nanosheets have been utilized as a robust support for immobilizing noble metal, metal oxide, or semiconductor nanoparticles [29–31] such as Au, Ag, Pt, Fe_3O_4, and ZnS, resulting in a variety of hybrid nanomaterials. Those nanocomposites exhibited improved catalytic, magnetic, or optical features [32]. To incorporate both the high conductivity and excellent catalytic activity into a single entity, the CuS nanoparticles anchored on a graphene nanosheets (CuS/GR) have been prepared [33]. They had promising applications in photocatalysis [34]. However, relatively little attention has been paid to the application of CuS/GR to measure ALP in biological samples.

Herein, CuS/GR nanocomposites were prepared by a facile one-pot method and used as a catalytic amplification platform for electrochemical detection of ALP. As shown in Scheme 1a, the CuS/GR nanocomposites were prepared by using graphene oxide (GO) sheets as the starting material via a microwave-assisted hydrothermal approach. In the microwave-assisted reaction process, the GO sheets were efficiently reduced to form graphene. The CuS nanoparticles were formed and uniformly distributed on the surface of the graphene nanosheets. The resulting CuS/GR nanocomposite had excellent electrocatalytic activity, high conductivity and a large surface area. These advantages made the nanocomposite an efficient catalytic amplification platform for electrochemical detection of ALP activity. The assay scheme is proposed in Scheme 1b. The CuS/GR nanocomposites greatly facilitated the oxidation of ALP-hydrolyzed products and resulted in a strong current response. Through this catalytic amplification strategy, the sensitivity of the ALP assay was dramatically improved. To the best of our knowledge, this is the first study to use CuS/GR nanocomposites as catalytic amplifiers for the high sensitive detection of ALP.

2. Experimental

2.1. Reagents

The graphite powder, $Cu(NO_3)_2$, $Na_2S{\cdot}9H_2O$, $NaH_2PO_4{\cdot}2H_2O$, $Na_2HPO_4{\cdot}7H_2O$, KH_2PO_4, NaCl, gelatin, 1-naphthol, and *p*-nitrophenol were purchased from Aladin Ltd. (Shanghai, China). The ALP and 1-naphthyl phosphate salt were from Sigma–Aldrich. All chemicals were used as received without further purification. The ALP ELISA kit was commercially from Beijing Biosynthesis Biotechnology Co., Ltd. Human serum samples were obtained from Ningxia Medical University, and used as received.

2.2. Apparatus

X-Ray powder diffraction (XRD) was obtained from an XRD-6000 (Shimadzu) using CuKα X-ray source. The TEM images were obtained on a JEOL JEM-200CX transmission electron microscope. The UV–vis spectra were collected on a UV-3600 (Shimadzu) UV–vis spectrophotometer. Electrochemical experiments were performed with a CHI660 workstation (Shanghai Chenhua, Shanghai, China) using a conventional three-electrode system with glassy carbon electrode (GCE) as the working electrode, a saturated calomel electrode (SCE) electrode as the reference, and a platinum wire as the counter electrode.

2.3. Synthesis of CuS/GR composites

The GO was prepared from graphite by a modified Hummers method [35]. The synthesis of CuS/GR nanocomposites were as follows: First, 0.025 mmol of $Cu(NO_3)_2$ was dissolved in 20 mL of an aqueous solution containing 2.0 mg GO and 8.0 mg gelatin. This was stirred for 10 min at room temperature. Then, 20 mL of the Na_2S aqueous solution (0.075 mmol) was added and stirred for 30 min. The resulting solution was sealed in an autoclave and heated to 180 °C for 1 h. The resulting suspension was centrifuged for 10 min at 12,000 rpm and washed with water three times. The CuS/GR composites were dried and dispersed in water at a concentration of 1.5 mg mL^{-1}.

2.4. ALP assay procedures

A 3 mm glass carbon electrode (GCE) was successively polished with 0.3 μm alumina slurry followed by rinsing thoroughly with water. Then, 5 μL of a 1.5 mg mL^{-1} CuS/GR solution was dropped on the pretreated GCE and dried. Electrochemical measurements of the ALP-catalyzed products (1-naphthol, *p*-nitrophenol) were

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