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Colorimetric detection of DNA damage by using hemin-graphene nanocomposites

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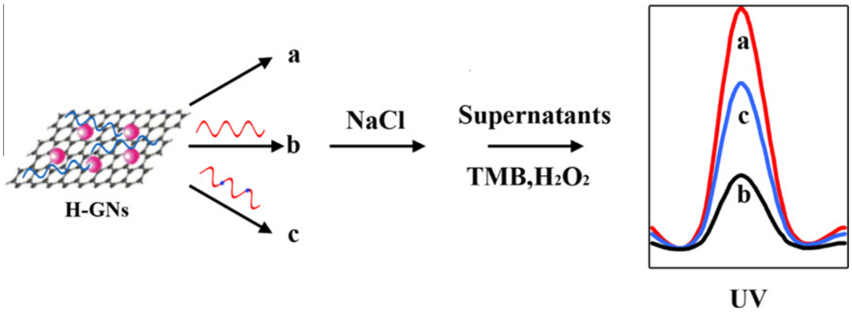
HIGHLIGHTS

- ▶ H-GNs can differentiate the damaged DNA from intact DNA.
- ▶ Damaged DNA could be detected by naked eyes or UV method.
- ▶ The method was simple and has good prospect in evaluation of genotoxicity of chemicals.

GRAPHICAL ABSTRACT



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ABSTRACT

A colorimetric method for detection of DNA damage was developed by using hemin-graphene nanosheets (H-GNs). H-GNs were skillfully synthesized by adsorping of hemin on graphene through π–π interactions. The as-prepared H-GNs possessed both the ability of graphene to differentiate the damage DNA from intact DNA and the catalytic action of hemin. The damaged DNA made H-GNs coagulated to different degrees from the intact DNA because there were different amount of negative charge exposed on their surface, which made a great impact on the solubility of H-GNs. As a result, the corresponding centrifugal supernatant of H-GNs solution showed different color in the presence of 3,3′,5,5′-tetramethylbenzidine (TMB) and H_2O_2, which could be discriminated by naked eyes or by ultraviolet (UV)–visible spectrometer. Based on this, the damaged effects of styrene oxide (SO), $NaAsO_2$ and UV radiation on DNA were studied. Results showed that SO exerted most serious damage effect on DNA although all of them damaged DNA seriously. The new method for detection of DNA damage showed good prospect in the evaluation of genotoxicity of new compounds, the maximum limit of pesticide residue, food additives, and so on, which is important in the fields of food science, pharmaceutical science and pesticide science.

Introduction

Vast amounts of new chemicals were produced in our world every year, playing an important role in our modern society. Unfortunately, some of them were proved to induce DNA damage and then cancer after being used for several years [1]. So, it is necessary to determine the genotoxicity of a new chemical before it is produced on a large scale.

Many analytic techniques have been employed to detect DNA damage, including biologic methods such as comet assay [2–4], unscheduled DNA synthesis test [5], sister chromatid exchange test [6], and SOS-chromotest [7]. However, these methods usually need to culture cells and fungus, which are complex, time consuming, low efficiency and low sensitivity. In recent years, many analytical methods such as high-performance liquid chromatography (HPLC) [8–10], electrochemical [11–16], electrogenerated

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chemiluminescence [17,18], photoelectrochemical [19,20], and fluorescence methods [21–24] have been developed to detect damaged DNA. However, these approaches have disadvantages either using complicated procedures or employing expensive instruments. Therefore, the method for detection DNA damage by the naked eyes is very promising.

Graphene oxide (GO) possess the ability to distinguish single-stranded DNA (ss-DNA) from double-stranded DNA (ds-DNA) because of its significant different adsorption affinities to ss- and ds-DNA [25,26]. ss-DNA can be stably adsorbed on GO due to the π–π stacking interactions between the ring construction in the nucleic acid bases and the hexagonal cells of the GO [27,28]. However, GO sheets tend to form irreversible agglomerates or even restack to form graphite through van der Waals interactions [29]. Thus, the prevention of aggregation is of particular importance to GO for its extensive application. Aggregation can be reduced by the attachment of other molecules or polymers onto the sheets through their covalent modification or nanocovalent functionalization. Among them the covalent bonding has attracted great interest because it cannot only improve its solubility but also can preserve the intrinsic properties of both GO and the molecules [30,31]. Organic soluble graphene was successfully prepared by hybriding with porphyry [32]. Geng et al. also reported a method to produce dispersible GO through π–π interaction between porphyrin and graphene [33]. Very recently, Dong et al. prepared hemin-graphene nanosheets (H-GNs) successfully through the π–π interactions [34]. H-GNs possess excellent properties of both hemin and graphene, that is to say, they possess not only the ability of graphene to distinguish ss- and ds-DNA but also the peroxidase-like activity of hemin that can catalyze the reaction of peroxidase substrate in the presence of H_2O_2. This novel material has been used for label-free colorimetric detection of single-nucleotide polymorphism.

In this paper, H-GNs were also synthesized by simple wet-chemical strategy through the π–π interactions according to Dong's method but with some revision. The well dispersed H-GNs coagulated completely in the presence of the optimum concentration of NaCl. As a result, the absorbance of the supernatant solution is very low. The addition of the ds-DNA increased its absorbance slightly, while the ss-DNA increased its absorbance largely. The reason is that ss-DNA can be stably adsorbed on H-GNs due to the π–π stacking interactions between the ring structure in the nucleobases and the hexagonal cells of the H-GNs, as a result, the exposed negatively charged DNA backbone enhances individual H-GNs electrostatic repulsion and resists salt-induced H-GNs coagulation. Based on this, a label-free colorimetric method was developed to detect ss-DNA. In addition, the method could be used to detect the damaged effects of chemicals such as SO and $NaAsO_2$ and physical radiation such as ultraviolet (UV) radiation on DNA. It was proved that their damage effects could be judged by naked eyes or by colorimetric method.

Experimental

Materials and reagents

Oligonucleotides of ss-DNA, complementary DNA (c-DNA) and single-base mismatched c-DNA were purchased from synthesized and purified using HPLC by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Their sequences were showed as follow:

ss-DNA: 5′-AGCGGATAACAATTTCACACAGGA-3′
c-DNA: 5′-TCCTGTGTGAAATTGTTATCCGCT-3′
Single-base mismatched c-DNA: 5′-TCCTGTGTGAATTTGTTATCCGCT-3′

The primitive samples were centrifuged at 5000 rpm for 5 min and then dispersed with 20 mM pH 7.4 Tris–HCl buffer solutions containing 100 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$ and 0.5 mM EDTA to a final concentration of 0.1 mM. This solution was protected form light and deposited at 0–4 °C and shaken gently prior to use.

Hemin and styrene oxide (SO, ⩾97%) were obtained from Sigma Aldrich (Shanghai, China). 3,3′,5,5′-tetramethylbenzidine (TMB) was received from Nanjing Sunshine Biotechnology Co. Ltd. (Jiangsu, China). Hydrazine hydrate (⩾85.0%) was obtained from Shanghai Lingfeng Chemical Reagent Co. Ltd. (Shanghai, China). Sodium arsenite was purchased from Aladdin reagent Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade. Double-distilled water was used throughout the study.

Safety Note: SO and $NaAsO_2$ were suspected human carcinogen and somewhat volatile. Gloves were worn, and all weightings and manipulations were done under a closed hood. All reactions were done in closed vessels.

Instruments

The morphology of H-GNs was analyzed with a transmission electron microscope (TEM, S-2400N, HITACHI, Japan). Fourier transform infrared spectroscopy (FTIR) was performed on a Bruker Tensor 27 instrument (Bruker, Germany) using dry KBr pellets. UV–visible spectra were recorded on a 2450 UV–visible spectrophotometer (Shimadzu, Japan). Raman spectra were measured with a Labram HR800 Laser Raman spectrophotometer (Jobin Yvon, France). The DNA damage with UV was performed with a ZF-7 UV analyzer (Yuhua, China) at 365 nm wavelength.

Synthesis of hemin-functionalized graphene nanosheets (H-GNs)

H-GNs were synthesized from graphene oxide nanosheets according to Dong's method but with some modification [34]. First, the grapheme oxide nanosheets were prepared by sonicated exfoliation of a 10 mg graphite oxide powder in 20 mL of H_2O for 120 min [35]. The graphene oxide was thus obtained by centrifugation of the resulting brown dispersion for 30 min at 3000 rmp to remove any unexfoliated graphite oxide followed by 1 week dialyzing with dialysis bag (8000–12,000) against double-distilled water to remove impurities of small molecules. Secondly, the as-prepared graphene oxide nanosheets were mixed with 20.0 mL of 0.5 mg/mL hemin solution followed by vigorously shaking for several minutes. Thirdly, 200.0 μL ammonia solution and 30.0 μL hydrazine were added to the solution. After being stirred for 1 h, the mixture was heated to 60 °C for 24 h. The dispersion was then centrifuged at 13,000 rpm for 30 min followed by rinsing with double-distilled water for several times to obtain H-GNs. The obtained H-GNs could be redispersed in water easily by ultrasonication to a final concentration of 1 μg/mL. As control, the graphene nanosheets were prepared by using the same method, just no hemin was added.

Preparation of graphene and H-GNs modified electrode

Prior to modification, GCE, diameter 3 mm, was polished with 1.0, 0.3, 0.05 μm alumina powder in sequence, and cleaned by sonicating in 1:1 nitric acid, acetone, 1:1 ethanol and double-distilled water successively. The graphene nanosheets modified GCE (GNs/GCE) and the H-GNs modified GCE (H-GNs/GCE) were obtained by casting 5 μL 0.25 mg/mL GNs or H-GNs dispersion onto the pretreated GCE, followed by drying in air.