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Colorimetric detection of DNA hybridization based on a dual platform of gold nanoparticles and graphene oxide

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ABSTRACT

The unique property of gold nanoparticles (Au NP) to induce colour change and the versatility of graphene oxides (GO) in surface modification makes them ideal in the application of colorimetric biosensor. Thus we developed a label free optical method to detect DNA hybridization through a visually observed colour change. The Au NP is conjugated to a DNA probe and is allowed to hybridize with the DNA target to the GO thus causing a change in colour from pinkish-red to purplish blue. Spectrophometry analysis gave a wavelength shift of 22 nm with 1 μM of DNA target. Sensitivity testing using serially diluted DNA conjugated GO showed that the optimum detection was at 63 nM of DNA target with the limit at 8 nM. This proves the possibility for the detection of DNA hybridization through the use of dual nanoparticle system by visual observation.

1. Introduction

The recent interest in research and development of DNA biosensors was stimulated by innovations done in the utilization of nanoparticles as detection probes (Merkoçi, 2010). DNA biosensors are highly valued tools in several fields such as genetic analysis (Tothill, 2009), food safety (Sharma and Mutharasan, 2013), environmental protection (Farré and Barceló, 2003), forensic applications (Redshaw et al., 2007) and defence interest (Paddle, 1996). Hence numerous types and techniques of DNA biosensors based on colorimetry (Xiang et al., 2012b; Zheng et al., 2012), electrochemiluminescence (Gao et al., 2013; Lu et al., 2013), electrochemistry (Wang, 2002), fluorescence (Zhao et al., 2012), surface plasmon resonance spectroscopy (Eum et al., 2009; Tawa et al., 2005) and quartz crystal microbalance (Hao et al., 2011; Kleo et al., 2011) have been researched and developed for the detection of DNA. Colorimetric based DNA biosensor provides an accurate data in the detection of DNA hybridization in terms of high sensitivity in the range of nano molar concentration (Qi et al., 2009), low cost production (Xiang et al., 2012a), rapidity of result (Zhan et al., 2012), and finally its simplistic design in colour detection (Baptista et al., 2005). Colorimetric biosensor is a subset within the labelled optical biosensors in which the probes are mostly modified with either fluorescent tags (Tolley et al., 2003) or luminescence tags (Wood and Gruber, 1996) for the purpose of quantitative detection. The use of gold nanoparticles (Au NP) in optical biosensor (Zhan et al., 2012) gives a new detection angle approach in which results are visually observed without the use of any additional detection instruments, thus making such systems amenable for point of care testing through qualitative analysis.

Au NP has emerged as the choice in detection and identification of DNA in recent decade for colorimetric biosensor due to its chemical and physical properties (Zhou et al., 2009). The nano-optical properties of Au NP have been well illustrated by studies on surface plasmon band (SPB) (Lismont and Dreesen, 2012), surface-enhanced Raman scattering (SERS) (Yang et al., 2007), and Rayleigh resonance scattering (RRS) (He et al., 2005). The simple colour change from red to blue elicited by the aggregation of the Au NP due to the change in distance between particles provides a simple mechanism of manipulation in developing a biosensor. This phenomenon is explained through the change in its surface plasmon resonance (Huang and El-Sayed, 2010). It was also reported that the ability to immobilize single stranded DNA onto the surface of the Au NP provides a buffer against aggregation in high salt environment. Reversing the non-cross linking process through DNA hybridization forming double stranded DNA caused a change in colour of the Au NP solution (Sato et al., 2003). A second technique utilizing cross linking between two DNA probes immobilized onto Au NP through an unmodified complementary DNA target as a linker was reported

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(Baptista et al., 2005). With the introduction of the unmodified DNA target linker, an instantaneous colour change was observed within the Au NP conjugated DNA probe solution (Cao et al., 2005). This technique provided us a base reference in the development of an experimental system for the detection of DNA hybridization which would be discussed in this paper.

By utilizing the cross-linking method as a guideline, we designed a system consisting of two different nanoparticles immobilized with complementary DNA probe and target. The purpose was to measure its effectiveness in inducing a colour change through DNA hybridization. The primary nanoparticle chosen to be immobilized with the DNA probe was Au NP. Graphene oxide (GO) was chosen as the secondary nanoparticle to be immobilized with the DNA target. GO is used as the secondary nano-component because it is of a single layer atomic thickness sheet which provides a large surface area sufficient for the immobilization of higher concentration of DNA on a single plane, thus providing a larger hybridization area and higher sensitivity (Loh et al., 2010). The flat surface of the GO is littered with numerous functional groups such as carboxylic and hydroxyl groups which present an easy route for DNA immobilization (Wu et al., 2011). The appearance of GO sheets being transparent avoids interference in the colour change ability of the colorimetric system. It also uses 50% less Au NP when compared to conventional optical based Au NP biosensors. Thus this study was to design and produce a basic working model of a dual nanoparticle colorimetric system in the detection of DNA hybridization utilizing a pair of complementary modified DNA strands.

2. Materials and methods

2.1. Chemicals and instruments

Hydrogen tetrachloroaurate ($HAuCl_4$), trisodium citrate (Na_3-$C_6H_5O_7$), and graphite flakes were purchased from Acros Organics (USA) while 1-Ethyl-3-(3-dimethylaminopropyl) carobodiimide (EDC), N-hydroxy sulfosuccinimide (Sulfo-NHS), dithiothreitol (DTT), N,N-dimethylformamide (DMF), potassium permanganate ($KMnO_4$), sulphuric acid (H_2SO_4), and phosphoric acid (H_3PO_4) were bought from Sigma-Aldrich (USA). Conjugation of the modified DNA to the Au NP through purification and activation of the thiol linkages was done using the NAP-10 sephadex columns, (G-25, GE Healthcare). The preparation of Au NP conjugated DNA probe (Au NP-DNA) and GO conjugated DNA target (GO-DNA) was carried out with a high speed centrifuge (MIKRO 220R, HETTICH). Wavelength scans on the

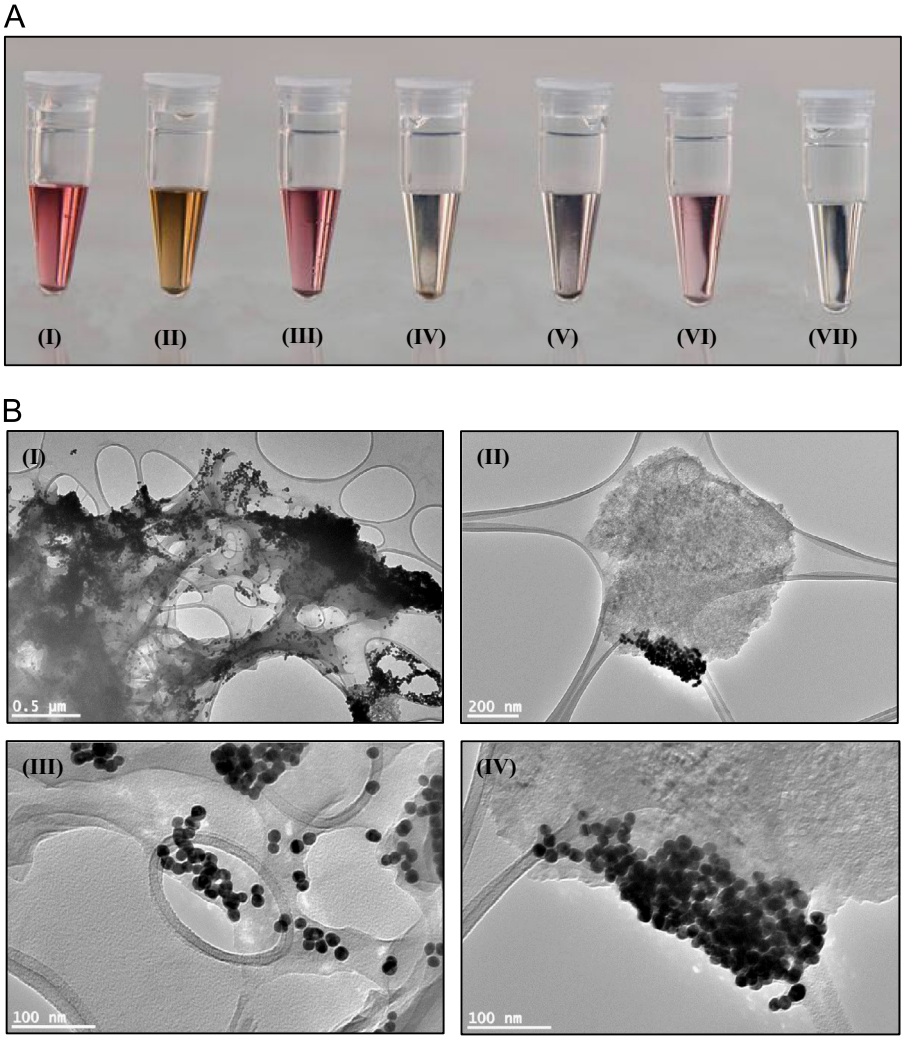

Fig. 1. (A) Visual observation of (I) ruby red Au NP solution, (II) golden-brown GO solution, (III) pinkish-red solution of Au NP-DNA, (IV) light brown solution of GO-DNA, (V) purplish-blue solution of the biosensor consisting hybridized Au NP-DNA and GO-DNA, (VI) pinkish-red solution of negative control consisting Au NP-DNA and Non-specific GO-DNA and (VII) transparent light brown solution of negative control with the mixture of GO-DNA and Non-specific probe DNA, (B) HRTEM images of (I) high conc. sample of the hybridized biosensor, (II) and (III) low conc. samples of the Au NP-DNA hybridizing on the edge of the GO-DNA sheets with no free floating Au NP-DNA observed. (IV) Higher magnification image of HRTEM image (II). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)