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A sensitive sandwich-type electrochemical aptasensor for thrombin detection based on platinum nanoparticles decorated carbon nanocages as signal labels

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ABSTRACT

In this work, a novel and sensitive sandwich-type electrochemical aptasensor has been developed for thrombin detection based on platinum nanoparticles (Pt NPs) decorated carbon nanocages (CNCs) as signal tags. The morphological and compositional of the Pt NPs/CNCs were examined using transmission electron microscopy, X-ray diffraction, and Raman spectroscopy. The results showed that the Pt NPs with about 3–5 nm in diameter were well dispersed on the surface of CNCs. The thiolated aptamer was firstly immobilized on the gold electrode to capture the thrombin molecules, and then aptamer functionalized Pt NPs/CNCs nanocomposites were used to fabricate a sandwich sensing platform. Then, the high-content Pt NPs on carbon nanocages acting as hydrogen peroxide-mimicking enzyme catalyzed the reduction of H_2O_2, resulting in significant electrochemical signal amplification. Differential pulse voltammetry is employed to detect thrombin with different concentrations. Under optimized conditions, the approach provided a good linear response range from 0.05 pM to 20 nM with a low detection limit of 10 fM. This Pt NPs/CNCs-based aptasensor shows good precision, acceptable stability and reproducibility, which provided a promising strategy for electrochemical aptamer-based detection of other biomolecules.

1. Introduction

Protein detection has been the subject of increasing interest in both the scientific research and clinical diagnosis (Zhang and Cui, 2014a; Liu et al., 2014a; Song et al., 2014; Deng et al., 2009; Xu et al., 2015a; Xie et al., 2015). In many diagnostic methods, the molecular recognition of specific proteins is often accomplished through the use of antibodies (Shuman et al., 1976). Although these conventional strategies have been widely utilized, the developed methodologies still have some drawbacks, such as time-consumption use of radioactive substances in some cases, and enzyme labeling (Bichler et al., 1991; Zhu et al., 2000). To overcome these problems, aptamers based strategies have been developed as recognition elements in aptasensors fabrication. This technique has several advantages, including ease of preparation, excellent stability, outstanding reusability, and could be adapted for almost any given protein (Xue et al., 2012; Liu et al., 2014b; Zhang et al., 2014b, 2011).

In recently years, tremendous efforts have been devoted to the development of aptasensors based on fluorescence (Shrivastava et al., 2015; Zhu et al., 2011, 2010; Chu et al., 2016; Zhang et al., 2013a), colorimetric (Chen et al., 2016; Tian et al., 2012; Huang et al., 2013; Wang et al., 2015; Zheng et al., 2014; Zhang et al., 2012), electrochemiluminescence (Liu et al., 2015; Li et al., 2015; Wu et al., 2015), chemiluminescence (Bi et al., 2014; Zong et al., 2014), surface enhanced Raman spectroscopy (Wang et al., 2010a; Zengin et al., 2015), photoelectrochemical (Xin et al., 2015; Fan et al., 2015, 2014), and electrochemical (Zhao et al., 2014; Wu et al., 2013a; Chen et al., 2013) detection. In particular, the electrochemical methods have attracted substantial consideration due to their several benefits, such as fast response, high sensitivity, simpler operation, possibility of miniaturization, and relatively low cost (Ge et al., 2016; Yi et al., 2014). On the other hand, it is well known that, the sensitivity of the electrochemical aptasensors is closely related to the employed labels. Nanoparticles (NPs), such as metal NPs have been extensively used as signal amplifying molecules to enhance the sensitivities of sensors owing to their numerous advantages like elevated surface areas, unique electronic structures, enhanced catalytic properties, and excellent biocompatibility (Wu et al., 2013b; Han et al., 2013; Chen et al., 2015; Zhang et al., 2013b; Wang et al., 2016). However, the amplification efficiency with metal NPs depends on the amount of the immobilized metal NPs on the sensor's surface. Inspiringly, carbon

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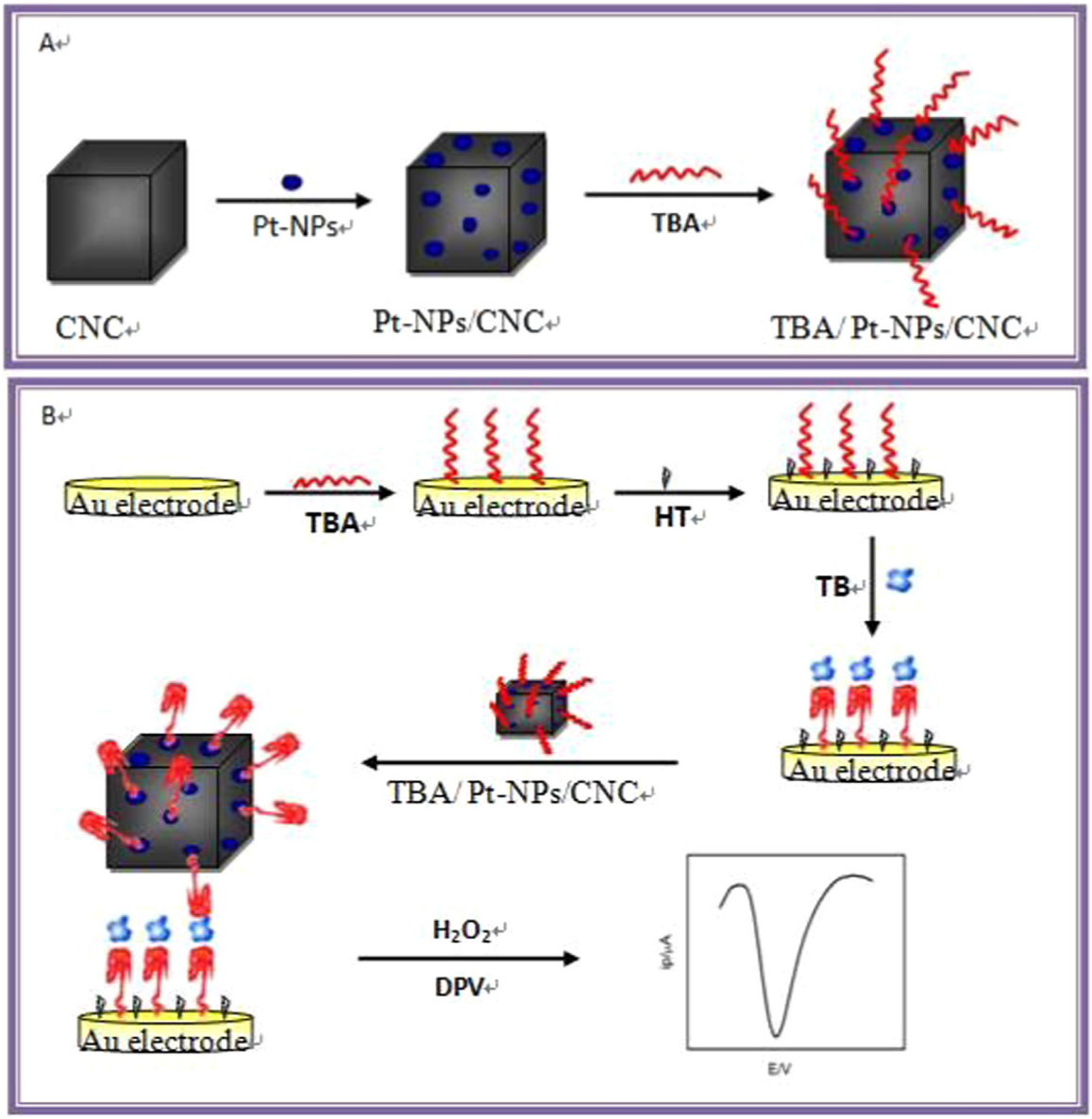
nanomaterials as metal nanoparticles nanocarriers, especially graphene (Li et al., 2016; Jing et al., 2015; Xie et al., 2012; Huang et al., 2015a) and carbon nanotubes (Xu et al., 2015b; Huang et al., 2014; Liu et al., 2013), have widely been applied in electrochemical-based biosensors, owing to theirs substantial electronic conductivities, large specific surface areas, and good biocompatibility. However, despite the extensive advances made in this field, obtaining novel aptasensors based a new type of carbon nanomaterials with increased sensitives and selectivities is still challenging.

Recently, carbon nanocages (CNCs), a category of three-dimensional carbon nanomaterial, are rapidly gaining interests in the materials science community due to their special well-defined structures, high degrees of graphitization and purity, superior electrical conductivities, elevated mechanical; and chemical properties, and suitable biocompatibility. Uniform CNCs were synthesized by direct-current arc-discharge for the first time in 1998 and until now these hollow nanomaterials are usually produced by template methods. CNCs are nanosized hollow particles with graphitic shells, which have extensively been investigated for various applications, including rechargeable batteries, hydrogen production and storage, catalysis, drug and gene delivery, and sensing (Li et al., 2011; Wang et al., 2010b; Tan et al., 2013). Importantly, except for the above advantages of carbon nanotubes and graphene, for example, the three-dimensional spherical structure with well-defined graphene, large surface area, is beneficial to interact with biomolecules by the homogeneous production of functional groups on their surfaces via oxidation (Chen et al., 2012; Huang et al., 2015b). To the best of our knowledge, CNCs nanocomposites were not yet utilized as both nanocarriers and amplifiers in the field of electrochemical aptasensors. In addition, Pt nanoparticles (Pt NPs), exhibiting a superior electroconductivity and significant electrocatalytic activity, have been extensively applied in the construction of electrochemical biosensors as catalyst of hydrogen peroxide (H_2O_2) for signal amplification (Wang et al., 2009; Bai et al., 2012).

This paper combined the advantages of CNCs with those of Pt NPs to design a novel sandwich-type electrochemical aptasensor based on Pt NPs/CNCs-catalyzed H_2O_2 process, where thrombin (TB) was employed as a model protein. As illustrated in Scheme 1,



Scheme 1. (A) Preparation procedure of TBA/Pt NPs/CNCs bioconjugate and (B) schematic illustration of the electrochemical aptasensor for detection of thrombin based on Pt NPs/CNCs as signal labels.