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Application of chitosan/Fe_3O_4 microsphere–graphene composite modified carbon ionic liquid electrode for the electrochemical detection of the PCR product of soybean *Lectin* gene sequence

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ABSTRACT

In this paper a Fe_3O_4 microsphere, graphene (GR) and chitosan (CTS) nanocomposite material modified carbon ionic liquid electrode (CILE) was used as the platform for the construction of a new electrochemical DNA biosensor. The single-stranded DNA (ssDNA) probe was immobilized directly on the surface of the CTS/Fe_3O_4–GR/CILE, which could hybridize with the target ssDNA sequence at the selected conditions. By using methylene blue (MB) as the electrochemical indicator the hybridization reaction was investigated with the reduction peak current measured. By combining the specific properties such as the biocompatibility and big surface area of Fe_3O_4 microspheres, the excellent electron transfer ability of GR, the good film-forming ability of CTS and the high conductivity of CILE, the synergistic effects of nanocomposite increased the amounts of ssDNA adsorbed on the electrode surface and then resulted in the greatly increase of the electrochemical responses. Under the optimal conditions differential pulse voltammetric responses of MB were proportional to the specific ssDNA sequences concentration in the range from 1.0×10^{-12} to 1.0×10^{-6} mol/L with the detection limit as 3.59×10^{-13} mol/L (3σ). This DNA biosensor showed good stability and discrimination ability to one-base and three-base mismatched ssDNA sequences. The polymerase chain reaction (PCR) product of soybean *Lectin* gene sequence was detected by the proposed method with satisfactory result, suggesting that the CTS/Fe_3O_4–GR/CILE was a suitable sensing platform for the sensitive detection of specific gene sequence.

1. Introduction

Electrochemical DNA biosensor, which is based on the immobilization of ssDNA on the electrode surface, has been widely investigated and applied in different fields of the gene detection with the advantages such as high sensitivity, simplicity, low cost, small dimension and cheap instruments [1]. The performances of the electrochemical DNA sensor can be greatly influenced by the ssDNA immobilization methods such as covalent binding, adsorption and polymerization. In recent years, various kinds of nanoparticles have been utilized for the ssDNA probe immobilization due to their unique characteristics such as high surface area, excellent biocompatibility and strong adsorption ability [2]. For example Selvaraju et al. [3] fabricated a sandwich-type electrochemical DNA biosensor using streptavidin-coated magnetic beads and gold nanoparticles as the response magnifiers. The advances in the development of gold nanoparticles based electrochemical DNA biosensors were described in Pingarrón's review [4].

Graphene (GR) is a sheet of sp^2 bonded two-dimensional carbon atoms that are arranged into a honeycomb structure, which has attracted considerable attentions due to its unique and excellent properties, such as extremely high thermal conductivity, good mechanical strength, high mobility of charge carriers, high specific surface area, quantum hall effect and upstanding electric conductivity [5–7]. GR and its based composite materials had been widely used in the fields of electrochemistry and electroanalytical chemistry such as photovoltaic devices, lithium-ion batteries, electrochemical sensors and chemically modified electrodes. Due to the advantages such as wide potential windows, relatively inert electrochemistry and excellent electrocatalytic activities, GR based electrochemical sensors and nano-devices had been applied to the investigation on the DNA hybridization, protein electrochemistry and small electroactive molecules detection. GR also can be used efficiently as a conducting surface with a very high surface area for the deposition of different nanoparticles and consequent electrochemical sensing. For instance, Lim et al. [8] applied the epitaxial

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GR as an anode material for the simultaneous detection of all four DNA bases in double-stranded DNA (dsDNA) without a prehydrolysis step. Yang et al. [9] fabricated a GR-vaseline film modified glassy carbon electrode, which exhibited a good electrochemical activity and stability for fundamental studies on carbon-based electrochemistry. Li's group proved that GR possessed excellent electrocatalytic property towards the oxidation of dopamine and methanol [10,11]. In addition, GR can provide a favorable microenvironment for DNA and effectively accelerate the direct electron transfer rate of DNA at the electrode surface [12,13], which can be further applied to determine DNA with excellent sensitivity.

Magnetite (Fe_3O_4), which has a different valence state, has emerged as a promising supercapacitor material due to its low cost and environmentally benign nature [14]. Wu et al. [15] reported on the capacitive characteristics of nanostructured Fe_3O_4 as an electrode material with a pseudo-capacitance of 27 F/(g-Fe_3O_4). In addition, Fe_3O_4 magnetic nanoparticles (nano-Fe_3O_4) have also attracted an increasing interest in biotechnology and medicine [16]. Due to their properties such as good biocompatibility, strong superparamagnetic property, low toxicity, easy preparation and high adsorption ability, nano-Fe_3O_4 had been used as the modified material in biosensors [17]. Wei et al. [18] developed a dumbbell-like Au-Fe_3O_4 nanoparticles labeled electrochemical immunosensor for the detection of cancer biomarker prostate specific antigen. Tran et al. [19] detected the short HIV sequence with a chitosan/Fe_3O_4 modified screen printed electrode by using MB as redox indicator. Yang et al. [20] fabricated a glucose biosensor by using the intrinsic peroxidase-like activity of Fe_3O_4 nanoparticles in Nafion film with remarkably enhanced sensitivity and selectivity.

Ionic liquids (ILs), which are composed of organic cations and various anions, have been widely used in the fields of chemistry due to the unique advantages such as high chemical and thermal stability, negligible vapor pressure, high ionic conductivity, wide electrochemical windows, low toxicity and the ability to dissolve a wide range of organic and inorganic compounds [21]. Wei and Ivaska [22] reviewed the application of ILs in the electrochemical sensor. ILs can be used as not only the supporting electrolyte but also the modifier in chemically modified electrode. Safavi et al. [23] utilized octylpyridinium hexafluorophosphate as the binder to make a carbon ionic liquid electrode (CILE) for the electrochemical detection. CILE had showed the advantages including high electronic conductivity, remarkable electrocatalytic activity, inexpensive reagents and easily fabrication. Sun et al. applied the CILE as the basal electrode for the redox protein electrochemistry with different nanoparticles such as $CaCO_3$ nanoparticles [24] and CdS nanorods [25].

In this paper, a CILE was fabricated by the addition of 1-butylpyridinium hexafluorophosphate ($BPPF_6$) in carbon paste as binder and modifier, and further used as the basal electrode for the electrochemical DNA biosensor. Then Fe_3O_4 microspheres and GR were mixed together to form a novel nanocomposite material, which was casted on the surface of CILE. Chitosan (CTS) was further dropped on the electrode surface to form a stable film, which could fix the materials tightly on the electrode surface. To the best of our knowledge, the usage of CTS/Fe_3O_4–GR nanocomposite matrix as the DNA immobilization platform for the enlargement of the electrochemical signal of the DNA indicator has not been reported previously. The use of CTS in the composite material can increase the stability of the modifier on the electrode surface. At the same time the Fe_3O_4–GR nanocomposites in the CTS can form a porous structure to provide a specific loading interface for the ssDNA probe immobilization. By combining the advantages of the materials used and with the fabricated CTS/Fe_3O_4–GR/CILE as the basal electrode, ssDNA probe was successfully adsorbed on the modified electrode to get a new electrochemical DNA biosensor, which was applied to the detection of *Lectin* gene sequence fragment of soybean and further used to the polymerase chain reaction (PCR) product of soybean endogenous gene. The proposed DNA biosensor showed the advantages such as the simple preparation procedure, high selectivity and broad linear range.

2. Experimental

2.1. Apparatus and chemicals

All the voltammetric measurements were performed on a CHI 1210A electrochemical workstation (Shanghai CH Instrument, China). Electrochemical impedance spectroscopy (EIS) was performed on a CHI 750B electrochemical workstation (Shanghai CH Instrument, China). A three-electrode system was employed for the electrochemical detection, which was composed of a modified CILE as working electrode, a Pt wire as auxiliary electrode and a saturated calomel electrode (SCE) as reference electrode. Scanning electron microscopy (SEM) was obtained on a JSM-6700F scanning electron microscope (Japan Electron Company, Japan). Nitrogen sorption isotherms were measured with a BelSorp-Mini analyzer (BEL Japan, Inc.) at liquid nitrogen temperature by using a BJH (Barrett–Joyner–Halenda) models for porosity evaluation. The PCR amplification experiments were performed on an Eppendorf Mastercycler Gradient PCR system (Eppendorf, Germany).

1-Butylpyridinium hexafluorophosphate ($BPPF_6$, >99%, Lanzhou Greenchem ILS, LICP, CAS, China), chitosan (CTS, minimum 92% deacetylated, Dalian Xindie Co. Ltd., China), graphite powder (average particle size 30 μm, Shanghai Colloid Chemical Co. Ltd., China), methylene blue (MB, Shanghai Chemicals Plant, China) were used as received. Graphene (GR) was synthesized by the modified Hummer's method [26,27]. Fe_3O_4 microsphere was synthesized by the solvothermal reduction method [28]. Different kinds of buffers such as 10× reaction buffer B (Promega, Wisconsin, USA), Taq DNA polymerase (Promega, Wisconsin, USA), 1× TAE buffer (40.0 mmol/L Tris, 1.0 mmol/L EDTA, 40.0 mmol/L acetate, pH 8.0), 50.0 mmol/L Tris–HCl buffer solution (pH 7.4), 50.0 mmol/L phosphate buffer solution (PBS, pH 7.0) were used and all the solutions were prepared with doubly distilled water.

The 21-base oligonucleotides probe sequences (probe ssDNA), its target complementary sequence DNA (target ssDNA), one-base mismatched ssDNA, three-base mismatched ssDNA and noncomplementary sequence DNA (ncDNA) were synthesized by Shanghai Sangon Biological Engineering Technological Co. Ltd. (China), which were related to the *Lectin* gene sequence of soybean. Their base sequences were listed as below:

- probe ssDNA: 5′-GAA GCT GGC AAC GCT ACC GGT-3′;
- target ssDNA: 5′-ACC GGT AGC GTT GCC AGC TTC-3′;
- one-base mismatched ssDNA: 5′-ACA GGT AGC GTT GCC AGC TTC-3′;
- three-base mismatched ssDNA: 5′-ACA GGT AGC ATT GCC ATC TTC-3′;
- ncDNA: 5′-ACT ACA GCG TTA CGA CTT GTA C-3′.

The DNA samples for PCR amplification were extracted from soybean oil and arachis oil. The PCR reaction was performed on an Eppendorf Mastercycler Gradient PCR system using oligonucleotide primers for *Lectin* gene of soybean with the following sequences:

- Primer F: 5′-GCC CTC TAC TCC ACC CCC ATC C-3′;
- Primer R: 5′-GCC CAT CTG CAA GCC TTT TTG TG-3′.

Arabinose operon D gene of arachis was with the following sequences: