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Short communication

A high-performance DNA biosensor using polyhydroxylated fullerenol as 3D matrix for probe immobilization

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ABSTRACT

A novel electrochemical DNA biosensor has been developed using water-soluble polyhydroxylated fullerenols as 3D matrix platform for probe DNA immobilization. Owing to the multiple merits including the unique spherical 3D nanostructure, rich −OH on the outside surface, and good water-solubility of fullerenol platform, the developed biosensor revealed high probe loading density (2.24×10^{13} strands cm^{-2}) and fast hybridization kinetics. Also, a wide linear range from 1.0 fM to 1.0 nM with a detection limit down to 0.17 fM in target DNA detection was obtained.

1. Introduction

In order to meet the need of the fast development of bioscience and bioengineering, many novel materials and strategies have been utilized in electrochemical biosensor constructions [1,2]. For example, many semiconductive nanoparticles [3], three-dimensional (3D) functional matrix-based organic polymer [4], and highly conductive carbon materials [5] have been used as probe DNA immobilization matrix, through which the DNA loading amount, electrochemical sensing signal as well as the analytical sensitivity were greatly improved. Besides, in order to prevent the non-specific adsorption of DNA sequences or signal labels, many efficient blockers such as casein [6] and bovine serum albumin [7] had been modified on the electrode surfaces. Also, it was found that the immobilization method has significant effect on the performance of the biosensors. Usually, the covalent attachment or self-assembly of probe DNA via one terminal of probe DNA is favorable to improve the hybridization efficiency of the biosensors because the loading density, structural flexibility and orientation control of the probe chains were well assured by this way [8,9].

Fullerenol (FLN) is a water-soluble fullerene derivative with polyhydroxy groups on its 3D spherical surface. In recent years, with the better acquaintance to the property of the material, the research and application of FLN in electrochemical field received more and more concerns. For example, Zhuo et al. [10] recently fabricated a FLN salt modified glassy carbon electrode (GCE) through simple electrodeposition, and they found that the FLN on the electrode surface presented surprising electrocatalytic performance for hydrogen evolution reaction. Sun et al. [11] constructed a FLN-based hemoglobin (Hb) biosensor. The analytical assay showed that the presence of FLN in the sensing film not only successfully realized the direct electron transfer of Hb, but also reduced the oxidation damage of Hb by H_2O_2. However, the application of FLN as a functional platform for the grafting of the biomolecules as well as the electrochemical sensing analysis has not been reported yet.

In this communication, a novel electrochemical DNA biosensor has been developed through covalent grafting of DNA probe on a FLN modified electrode (Fig. 1). The hybridization experiments showed that the developed biosensor holds high performance in hybridization sensitivity and hybridization kinetics due to the multiple merits including the unique spherical 3D nanostructure, rich active sites, and good hydrophilic of the FLN platform.

2. Experimental

2.1. Reagents and apparatus

The polyhydroxylated FLN was synthesized according to the literature [12]. The 18-base oligonucleotides were purchased from Shanghai Sangon Biotech Co., Ltd. (China). Their sequences were: probe sequence (S1): 5′-PO_4^{3-}-TCT TTG GGA CCA CTG TCG-3′; complementary sequence (S2): 5′-CGA CAG TGG TCC CAA AGA-3′; one-base mismatched sequence (S3): 5′-CGA CAG TGG ACC CAA AGA-3′; three-base mismatched sequence (S4): 5′-CGA CAA TGG CCC CAA CGA-3′; non-complementary sequence (S5): 5′-GCA TCG AGC GAG CAC GTA-3′. Stock solutions of all the oligonucleotides were prepared with TE buffer solution (10 μM Tris–HCl, 1.0 mM EDTA, pH 8.0) and kept frozen. All the other reagents were all of analytical reagent grade and were purchased commercially.

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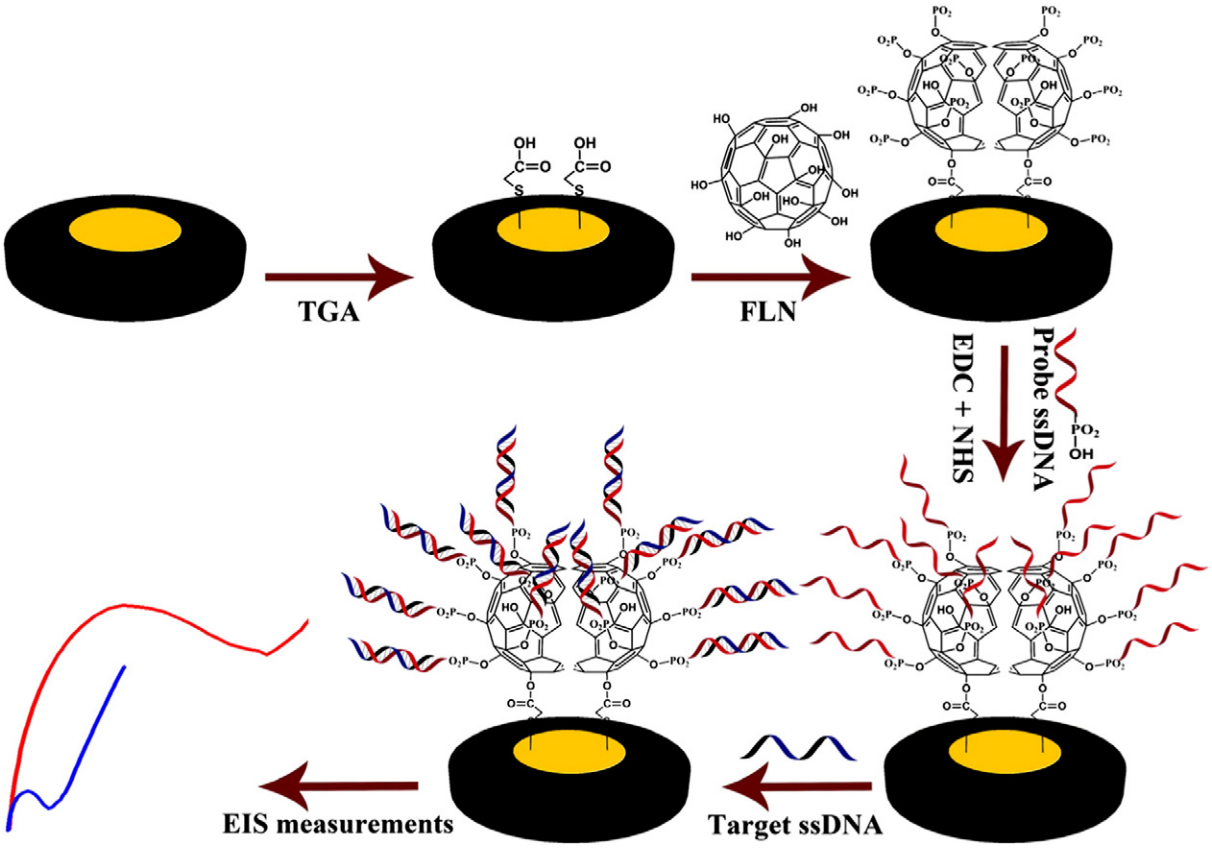

Fig. 1. Illustration for the construction and detection strategy of the FLN-based DNA biosensor.

Fourier translation infrared spectroscopy (FT-IR) was recorded on a Nicolet 360 spectrometer (USA) using pressed KBr plates. Electrochemical experiments were measured on a CHI 650C electrochemical workstation (China). A three electrode system was employed with a platinum wire as the counter electrode, Ag/AgCl/(3 M KCl) as the reference electrode and a bare or modified gold electrode (AuE) as the working electrode.

2.2. Fabrication of the biosensor

The assembly of thioglycolic acid (TGA) was carried out by immersing a freshly cleaned AuE in an ethanol solution containing 10 mM TGA for 24 h in the dark, and then rinsed with double distilled water (DDW) to remove the physically absorbed TGA. The obtained electrode was denoted as TGA/AuE. Then, 10 μL phosphate buffer saline (PBS) containing N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC, 5 mM)/N-Hydroxysuccinimide (NHS, 8 mM) and 10 μL 1 g L^{-1} FLN water solution were successively dropped on TGA/AuE. After dryness, the electrode was rinsed with PBS and water, respectively. Followed by, 10 μL 1.0 μM probe DNA (S1) that has been pre-mixed with EDC (5 mM)/NHS (8 mM) for 2 h was cast on the FLN/TGA/AuE for dryness at room temperature. After further washing with TE buffer and DDW to remove the non-specifically adsorbed DNA, a DNA biosensor termed as S1/FLN/TGA/AuE was fabricated. The DNA surface density was determined according to classical chronocoulometry (CC) using $[Ru(NH_3)_6]^{3+}$ as the probe [13].

3. Results and discussion

Fig. 2A shows the FT-IR spectrum of synthesized FLN, and the electronic photo (inset) of aqueous dispersion of C_{60} and FLN. For the dispersion of C_{60}, the black precipitate could be observed less than 1 h after the dispersion was prepared (left vial), indicating very poor solubility of original C_{60}. However, the aqueous solution of the synthesized FLN showed a clear pale yellow in color (right vial), and not any precipitate appeared after the solution was placed for 1 month, suggesting that the synthesized FLN had excellent water-solubility. In the FT-IR of FLN, the broad absorption peak centered at 3441 cm^{-1} could be assigned to the stretching vibration of –OH, and the peak at 1085 cm^{-1} was the characteristic absorption of C–O bond. These two peaks suggested that –OH had been formed on fullerene cage. In addition, the peaks at 1634 cm^{-1} and 537 cm^{-1} corresponding to the characteristic absorption of C═C and fullerene cage indicated that the synthesized FLN remained the basic backbone of C_{60}.

The fabrication process of the biosensor was monitored by electrochemical impedance spectra (EIS, Fig. 2B) and cyclic voltammetry (CV, Fig. 2C) exploiting the solution-based redox probe of $[Fe(CN)_6]^{3-/4-}$. As seen, when TGA was self-assembled on AuE via Au–S bond, the electron transfer resistance (R_{et}) value that reflected by the semicircle diameter in the high frequency region was dramatically increased from 200 Ω to 2700 Ω. However, when the TGA layer was further grafted with the FLN molecules, the R_{et} decreased significantly to about 825 Ω, indicating that the electron transfer kinetic of redox-active species on the electrode surface was greatly improved by the FLN molecules. Furthermore, when the DNA probe (S1) was immobilized to FLN/TGA/AuE through reaction of the 5′-PO_4^- with the rest –OH groups on FLN, the R_{et} value was increased again, because the negatively charged phosphate backbone on the immobilized DNA prevented the approaching of $[Fe(CN)_6]^{3-/4-}$ to electrode surface [14]. The CV results were in good agreement with those from above EIS studies. All these results demonstrated that the FLN-based DNA sensing interface had been fabricated successfully.

Fig. 2D shows the typical CCs of S1/FLN/TGA/AuE in the absence (a) and presence (b) of 50 μM $[Ru(NH_3)_6]^{3+}$ in 0.01 M Tris–HCl buffer. According to the method proposed by Steel et al. [13], the DNA surface density (Γ) of S1/FLN/TGA/AuE was calculated to be 2.24×10^{13} strands cm^{-2}. For comparison, a control electrode was