Talanta 131 (2015) 379–385

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Electrochemiluminescence immunosensor for highly sensitive detection of 8-hydroxy-2′-deoxyguanosine based on carbon quantum dot coated Au/SiO_2 core–shell nanoparticles

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ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form
1 August 2014
Accepted 6 August 2014
Available online 19 August 2014

Keywords:
Core–shell nanoparticle
8-hydroxy-2′-deoxyguanosine
Electrochemiluminescence immnuosensor
Carbon quantum dot

ABSTRACT

An electrochemiluminescence (ECL) immunosensor using Pt electrode modified with carbon quantum dot (CQDs) coated Au/SiO_2 core–shell nanoparticles was proposed for sensitive detection of 8-hydroxy-2′-deoxyguanosine (8-OHdG) in this work. Rabbit anti-8-OHdG antibody was covalently bound to CQDs on the surface of Au/SiO_2 core–shell nanoparticles. Through signal amplification of Au/SiO_2 core–shell nanoparticles, 8-fold enhancement of the ECL signals was achieved. Under optimal conditions, a good linear range from 0.2 to 200 ng mL^{-1} with a low detection limit of 0.085 ng mL^{-1} (S/N=3) for 8-OHdG detection was obtained. Interfering substances tests showed that the corresponding ECL intensity (ΔECL) of 8-OHdG is 8–18 times higher than that of guanine, uric acid (UA) and ascorbic acid, demonstrating its good selectivity for 8-OHdG detection. The ECL immnuosensor exhibits long-term stability with a relative standard deviation (RSD) of 8.5% even after 16 cycles of continuous potential scans. The result of analytical detection of 8-OHdG in real samples was satisfactory. The proposed ECL immnuosensor shows good performance with high sensitivity, specificity, repeatability, stability and provided a powerful tool for 8-OHdG monitoring in clinical samples.

1. Introduction

Reactive oxygen species, such as superoxide radical, singlet oxygen, hydroxyl radicals, superoxide anion and peroxyl, are considered as harmful species due to their effect on oxidative DNA damage [1,2]. 8-hydroxy-2′-deoxyguanosine (8-OHdG, also known as 8-oxo-7, 8-dihydroguanine and 8-oxo-dGuo) (Scheme 1), one of the main products of oxidative DNA damage, can mispair with adenine, leading to G:C→T:A transverse mutation [3]. Many researches show that 8-OHdG has a much closer relationship with some disease, such as cancer, diabetes and neurological disorders [4–6]. Therefore, 8-OHdG is concerned as the most investigated biomarker of oxidative DNA damage [7].

Traditional methods for 8-OHdG determination, such as high performance liquid chromatography with electrochemical detection [8], liquid chromatography combined with electrospray ionization tandem mass spectrometry multiple reaction monitoring [9], high performance liquid chromatography/positive electrospray ionization tandem mass spectrometry [10], enzyme-linked immunosorbent assay [11] and ^{32}P-labeling [12], are pretreatment complex, specialized skills required and unavailable for in-situ detection. Electrochemistry sensors are widely applied in detecting various pollutants due to its rapid and high sensitivity [13,14]. It also been developed for 8-OHdG detection. For example, electrochemical method based on poly(3-methylthiophene) (P3MT) and polyethylenimine dispersed carbon nanotubes modified glassy carbon electrode (GCE) used as working electrode allowed the sensitive determination of 8-OHdG. But the results of electrochemistry methods above were likely to be affected by other biomolecules or matrix effects [15,16]. Fluorescent sensor is the another method for 8-OHdG detection which developed in recent years. Zhang et al. [2] reported a DNA aptamer fluorescent sensor for 8-OHdG determination using two triple-stranded DNAs as the scaffolds, which shows inherently insufficient binding constants between aptamers and 8-OHdG due to the small molecule of 8-OHdG.

Electrochemiluminescence (ECL) is a special kind of chemiluminescence (CL) with its light emission preceded by electrochemical reactions [17]. The ECL method presents widespread prospect due to its high sensitivity and good controllability compared to the conventional electrochemical and CL technique [18]. Its performance mainly depends on the favorable luminescent characterization of ECL materials. Recently, as a new group of ECL luminophores, quantum dots (QDs) attracted more and more

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http://dx.doi.org/10.1016/j.talanta.2014.08.024
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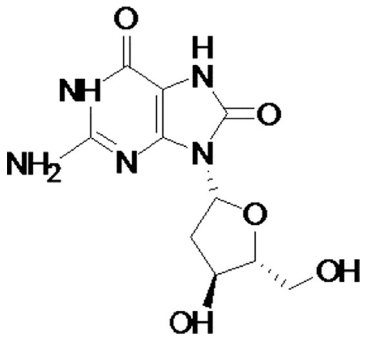

Scheme 1. Chemical structure of 8-OHdG.

attention [19–21], since they not only exhibit excellent ECL activities but also have the merit of easy labeling. However, reports on QD-based ECL assay for 8-OHdG detection are relatively scarce since the inherently toxicity and low ECL intensity due to the poor stability of conventional QDs used in ECL at high electrochemical potentials [22]. Therefore, nontoxicity and reproducibility are urgently needed for the development of QD-based ECL sensors in 8-OHdG monitoring.

Carbon quantum dots (CQDs), a newly emerging ECL luminophores, have several significant advantages over the previously reported QDs, such as high stability, low toxicity, easy labeling and good biocompatibility [23–25]. To the best of our knowledge, the research report of CQD-based ECL 8-OHdG is hampered by the low ECL intensity of CQDs and the difficulty in reuse of CQDs in an aqueous system [26,27]. Thus, how to effectively amplify ECL signal and solve the reusability simultaneously is the key point in designing a CQDs-based ECL for 8-OHdG detection.

A structure of "core–shell" based on concentric multilayer semiconductor nanoparticles has recently attracted great attention in sensor field due to its large surface-to-volume ratio for modifying QDs. Liu et al. [28] reported a well-defined hybrid structures that comprised a gold core coated with a silica shell (Au/SiO_2), followed by the modification of CdSe-QDs (Au/SiO_2/CdSe-QD). The research on the photoluminescence intensities of CdSe-QD and the Au/SiO_2/CdSe-QD nanoparticles prove that the Au/SiO_2 core–shell nanoparticles exhibit excellent ability of signal amplification on photoluminescence. Moreover, due to its robust chemical stability, uniformity and biocompatibility of the SiO_2 shell coating, and ease of surface functionalization, we consider Au/SiO_2 core–shell can be used as suitable platform for CQDs immobilization to improve its ECL signal and reusability.

Here in, an advanced ECL immunosensor based on the hybrid of CQDs and Au/SiO_2 was fabricated for the supersensitive, rapid, and selective detection of 8-OHdG upon the amplification of CQD ECL signal by Au/SiO_2 core–shell. In the sensor Au/SiO_2 core–shell nanoparticles serve as the platform for CQDs immobilization, which can not only enhance the detection sensitivity but also achieve good recycle of the sensor through avoiding the loss of CQDs. Meanwhile the rabbit anti-8-OHdG antibody serves as the immunosorbent of antigen 8-OHdG. Compared to the previously reported methods, this approach provides a promising tool for the routine detection of 8-OHdG.

2. Experimental

2.1. Reagents and chemicals

8-OHdG (CAS: 88847-89-6), ethyl-3-(dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma (America). Rabbit anti-8-OHdG (Ab) was obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). $HAuCl_4 \cdot 3H_2O$ (99%), tetraethoxysilane (TEOS), poly(vinylpyrrolidone) (PVP) and trisodium citrate (99%) were bought from Xilong Chemical Co., Ltd. (Guangdong, China). NH_4OH was purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Uric acid (UA) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Guanine and (3-Aminopropyl) trimethoxysilane (APS) were supplied by J&K Scientific Ltd. (Beijing, China). Bovine serum albumin (BSA) was purchased from Beijing Aoboxing Bio-tech Co., Ltd. (Beijing, China). Ascorbic acid, $K_2S_2O_8$ (potassium persulfate) and KCl were bought from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). Phosphate buffer solution (PBS, 0.1 mol L^{-1}, pH = 7.4) was prepared by mixing the stock solutions of Na_2HPO_4 (0.1 mol L^{-1}) and NaH_2PO_4 (0.1 mol L^{-1}). Tris–EDTA (TE) buffer was used as dilution solvent of 8-OHdG. Ultrapure water purified by a Millipore water system (resistivity > 18.0 MΩ cm^{-1}, Laikie Instrument Co., Ltd., Shanghai, China) was used throughout the experiments.

2.2. Apparatus

The ECL emission was detected by a MPI-B multi-parameter chemiluminescence analysis test system (Xi'an Remax Analysis Instrument Co., Ltd., Xi'an, China) with a three electrode system consisting of a bare or modified Pt electrode (1 mm-diameter) as the working electrode, the standard calomel electrode (SCE, saturated KCl) and a Pt wire were used as the reference electrode and the auxiliary electrode, respectively. Electrochemical impedance spectroscopy (EIS) was performed on a CHI 660 electrochemical analyzer (Shanghai Chenhua Instrument, Shanghai, China). Unless noted, the photomultiplier tube (PMT) was 600 V. The morphology of the samples was observed by transmission electron microscopy (TEM) (FEI Tecnai G2 20, America) and scanning electron microscopy (SEM) (Hitachi S-4800) equipped with an X-ray energy dispersive spectroscopy (EDS) (Japan). Photoluminescence (PL) spectra were obtained on a Hitachi F-4500 fluorescence spectrometer (Japan). UV–visible spectra were carried out on a Jasco V-550 spectrometer (Japan). Sonications were carried out on a KQ5200B-type ultrasonic cleaner with the cleaning slot size 300*240*150 mm^3 (Kun Shan Ultrasonic Instruments Co., Ltd., Jiangsu, China).

2.3. Synthesis of Au/SiO_2/CQDs core–shell nanoparticles

Preparation of CQDs: ascorbic acid was used as carbon source of CQDs. The mixed liquor of ascorbic acid and ethanol was heated at 180 °C for 4 h in a Teflon®-lined stainless steel autoclave and then cooled to room temperature. The dark brown solution was obtained, followed by extracting with dichloromethane. Then a yellow aqueous solution containing CQDs was obtained. The specific processes refer to previous literature [29].

Preparation of Au nanoparticles (AuNPs): The AuNPs core was synthesized through the seeded growth synthesis process according to literature [30]. Briefly, 2.2 mmol L^{-1} sodium citrate solution (150 mL) was added into a 250 mL round-bottomed flask. The solution was heated reflux under vigorous stirring. When the solution start boiling, 1 mL of $HAuCl_4$ (25 mmol L^{-1}) was injected and kept boiling for 10 min. Then cooled the mixtures to 90 °C. 1 mL of sodium citrate (60 mmol L^{-1}) and 1 mL of $HAuCl_4$ solution (25 mmol L^{-1}) were sequentially injected, then kept reacting for 30 min at 90 °C. After repeating this process steps (sequential addition of 25 mmol L^{-1}) $HAuCl_4$ (1 mL) and 60 mmol L^{-1} sodium citrate (1 mL) 5 times, a vinicolor aqueous solution containing AuNPs was finally obtained.

Preparation of Au/SiO_2 core–shell nanoparticles: The Au/SiO_2 core–shell nanoparticles were synthesized by Stöber processin accordance to literature [31]. An aqueous solution of poly