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Highly selective and sensitive chemiluminescence biosensor for adenosine detection based on carbon quantum dots catalyzing luminescence released from aptamers functionalized graphene@magnetic β-cyclodextrin polymers

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ABSTRACT

In this work, a highly selective and sensitive chemiluminescence (CL) biosensor was prepared for adenosine (AD) detection based on carbon quantum dots (CQDs) catalyzing the CL system of luminol-H_2O_2 under alkaline environment and CQDs was released from the surface of AD aptamers functionalized graphene @ magnetic β-cyclodextrin polymers (GO@Fe_3O_4@β-CD@A-Apt). Firstly, GO@Fe_3O_4@β-CD and CQDs were prepared and characterized by transmission electron microscopy (TEM), scanning electron microscope (SEM), UV–Vis absorption spectra (UV), fluorescence spectra (FL), fourier transform infrared (FTIR) and X-ray powder diffraction (XRD). For GO@Fe_3O_4@β-CD, Fe_3O_4 was easy to separate, GO had good biocompatibility and large specific surface area, and β-CD further increased the specific surface area of the adenosine polymers (A-Apt) to provided larger binding sites to A-Apt. Then, A-Apt was modified on the surface of GO@Fe_3O_4@β-CD while CQDs was modified by ssDNA (a single stranded DNA partially complementary to A-Apt). The immobilization property (GO@Fe_3O_4@β-CD to A-Apt) and the adsorption property (GO@Fe_3O_4@β-CD@A-Apt to CQDs-ssDNA) were sequentially researched. The base-supported chain-like polymers – GO@Fe_3O_4@β-CD@A-Apt/CQDs-ssDNA was successfully obtained. When AD existed, CQDs-ssDNA was released from the surface of GO@Fe_3O_4@β-CD@A-Apt and catalyzed CL. After that, under optimized CL conditions, AD could be measured with the linear concentration range of 5.0×10^{-13}–5.0×10^{-9} mol/L and the detection limit of 2.1×10^{-13} mol/L (3δ) while the relative standard deviation (RSD) was 1.4%. Finally, the GO@Fe_3O_4@β-CD@A-Apt/CQDs-ssDNA-CL biosensor was used for the determination of AD in urine samples and recoveries ranged from 98.6% to 101.0%. Those satisfactory results illustrated the proposed CL biosensor could achieve highly selective, sensitive and reliable detection of AD and revealed potential application for AD detection in monitoring and diagnosis of human cancers.

1. Introduction

Adenosine (AD) is an endogenous nucleoside throughout of human cells and has aroused medical researchers' interest since it was discovered. AD is not only an important intermediate for the synthesis of adenosine triphosphate (ATP), adenine, adenylate and adenosine, but also can produce adenylate, participate in myocardial energy metabolism and expand the coronary blood vessels [1,2]. In addition, many studies have shown that AD can be used as a potential biomarker of cancers or tumors, which shows a good clinical practice value in broad-spectrum cancer diagnosis and surgical efficacy evaluation [3,4]. Therefore, the detection of AD is of great significance. At present, common methods for detection of AD have colorimetric [5], surface enhanced raman scattering (SERS) [6], electrochemical [7], fluorescence [8], fluorescence resonance energy transfer (FRET) [9], reversed-phase high-performance liquid chromatography (RP-HPLC) [10], resonance light scattering (RLS) [11], chemiluminescence (CL) [12,13]. But some of the above-mentioned methods also have some drawbacks, such as expensive instruments, complex operation, limited detection range and weak anti-interference ability. So, finding a more simple, cost-effective, sensitive and selective method to detect AD is significant important.

CL is defined as a kind of emissive light through chemical reactions [14]. Compared with other analytical methods, CL has many significant

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advantages, such as no need external light source, simple instrument, high sensitivity, wide detection range and easy to achieve automation [15]. Based on these advantages, CL has been used in biomedical [16], environmental monitoring [17] quality assessment [18], sensing analysis [19] and other fields. However, because the substances present in samples can easily affect the CL intensity, the application of CL technology is restricted by its low selectivity which could be improved by bringing some specific recognizable materials into the system, such as molecularly imprinted polymers [20], special frame materials [21], specific biomaterials [22].

The CL intensity (I) of the pure chemical reaction is quite weak, so catalysts are usually used in CL technology. Common catalysts used in CL are a variety of metal ions and enzymes (including some simulated enzymes) [23]. Among these catalysts, semiconductor nano-materials, also known as quantum dots (QDs), have attracted researchers' wide attention due to their unique optical properties [24]. Among these QDs, carbon quantum dots (CQDs) can be used as photoelectron transfer to the electron donor or receptor owing to its strong light energy conversion, so CQDs has been widely used in the field of photocatalysis [25]. CQDs with highly photoluminescent were successfully prepared and were applied to the construction of accurate ratiometric sensor for detection of Hg^{2+} and biological thiols by You et al. [26]. CQDs were used to the determination of cytochrome and displayed strong cathodic electrogenerated chemiluminescence (ECL) in neutral aqueous solution in the presence of potassium persulfate by Dong et al. [27].

Aptamers (Apt) are a kind of synthetic single-stranded oligonucleotides screened by SELEX technology [28]. Apt can efficiently and specifically bind to target molecules and its appearances provide an efficient and rapid identification of the research platform in the field of biology and biomedical sciences [29,30]. At present, biomolecule detection is usually based on the mode of antigen-specific antibody recognition, but the applications of antibodies are usually limited due to its easy inactivation and long preparation time. In contrast, Apt has its own advantages of good stability, easy to obtain, relatively simple and fast preparation synthesis, easy to functional modification and labeling. Therefore, Apt has been widely used in the construction of biosensors. Li et al. [31] proposed a novel ultrasensitive one-compartment enzyme biofuel cells (EBFCs)-based self-powered aptasensing platform for antibiotic residue detection. A reusable aptasensor of human thrombin was constructed based on an extension-contraction movement of DNA interconversion based on resonance light scattering technique using magnetic nanoparticles as the probe by Yue et al. [32].

Graphene (GR) is a two-dimensional carbon nanomaterial and its thickness is only 0.335 nm, known as the thinnest material in the world [33]. GR has a complete and stable six-membered ring structure to make it chemically inert and hydrophobic, so it difficult to disperse in a polar solvent or aqueous solution, which limits the application of GR. It is necessary to make modification to broaden its scope of application. Graphene oxide (GO) is an important derivative of GR, which has the basically similar structure to GR, and GO contains abundant carboxylic acid, epoxy group and hydroxyl group [34]. As an amphiphilic lamellar molecule, GO shows excellent biocompatibility that make it and its derivatives could be used in analytical detection [35], polymer materials synthesis [36], biomedicine [37], photoelectric applications [38] and other fields. Especially, GO and its composites have been widely used in the preparation of sensors [39]. For examples, Ling et al. [40] developed a simple, sensitive, selective turn-on fluorescent aptasensor for theophylline detection in serum by utilizing ligand-induced self-assembling RNA aptamers and two different interaction stages of the aptamers fragments with GO. GO was modified on the surface of glassy carbon electrode (GCE) and it was successfully used to construction of the new electrochemical sensor for acetaminophen detection by Song et al. [41].

Cyclodextrin (CD) is a cyclic oligosaccharide compound that formed by a number of D-glucopyranose units. The whole molecule of CD exhibits a narrow half-tapered cavity structure owing to the glucose units of CD with non-twisted chair conformations [42,43]. There are three kinds common CDs – α-CD, β-CD, γ-CD, of which β-CD is the most widely used because of its caliber moderate size, low production costs, raw material easy to get and product [44]. However, the unmodified β-CD still has some disadvantages, such as limited bonding ability, poor solubility in organic solvents and low catalytic activity, which limits its widely use. In order to change this situation, its derivatives or complex are composited [45] and has been widely used in environmental treatment [46], drug delivery [47], biosensing analysis [48] and other fields.

In this study, GO@Fe_3O_4@β-CD and CQDs were synthesized and characterized. A-Apt and CQDs-ssDNA were consecutively modified to the surface of GO@Fe_3O_4@β-CD to synthesize the final base-supported chain-like polymers – GO@Fe_3O_4@β-CD@A-Apt/CQDs-ssDNA and the polymers were applied to detect AD by CL method. When AD existed in solutions, CQDs-ssDNA was released from the surface of GO@Fe_3O_4@β-CD@A-Apt owing to the specific recognition ability between AD and A-Apt and the released CQDs-ssDNA catalyzed CL. Ultimately, a highly selective, sensitive and simple GO@Fe_3O_4@β-CD@A-Apt/CQDs-ssDNA - CL biosensor was successful constructed and realized the detection of AD.

2. Experimental

2.1. Materials

AD aptamers: 5′-GTCTCTGTGTGCGCCAGAGAACACTGGGGCAGATATG GGCCAGCACAGAATGAGGCCC-3′ (10 OD) and ssDNA : 5′-CTCC GGGCGGA GGAAGGTGTCACA-3′ (5 OD) were purchased from Sangon Biotech Company (Shanghai, China). Graphite powder was supplied by Hongyan Chemical Reagent Factory (Tianjin, China). β-CD was supplied by Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Ferrous chloride and ferric chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Epichlorohydrin (ECH), Aminopropyltriethoxysilane (APTS) and chitosan (CS) were provided by Shanghai Macklin Biochemicals Co., Ltd. (Shanghai, China). 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide Luminol (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sass chemical technology Co., Ltd (Shanghai, China). Adenosine was supplied by Mengyi beauty Biotechnology Co. Ltd. (Beijing, China). Bovine serum albumin (BSA), creatinine (Cr), glycine (Gly) and other biological interferences were purchased from Solebo Biological Technology Co. Ltd. (Beijing, China). Anhydrous toluene purchased from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Acetic acid, Sodium hydroxide, Potassium permanganate, ethanol and all other chemicals unless specified were analytical reagent grade and used without further purification. Redistilled water was used throughout the work. Phosphate buffer (PBS, pH = 7.4, 0.01 mol/L) solution was used to prepare all AD solutions in all the experiment.

2.2. Apparatus

The IFFM-E flow injection CL analyzer (Xi'an Remex Electronic instrument High-Tech Ltd, China) was equipped with an automatic injection system and a detection system. As shown in Fig. 1, in the flow injection system, luminol and NaOH flowed into the mixing reaction system through the two channels of main pump with the flow rate of 1.69 mL/min, respectively. Meanwhile, H_2O_2 and samples (or PBS) flowed through two channels of the vice pump with the flow rate of 1.45 mL/min, respectively. When AD samples were detected, a certain amount of GO@Fe_3O_4@β-CD@A-Apt/ CQDs-ssDNA dispersion was placed in AD sample and a magnet was placed in the bottom of sample tube to achieve magnetic separation. The supernatant after magnetic separation flowed into the IFFM-E flow injection CL analyzer system. It took only 311 s to complete a test and can yield five parallel data peaks simultaneously. All samples tests were carried out the optimized