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Quantum dot-modified aptamer probe for chemiluminescence detection of carcino-embryonic antigen using capillary electrophoresis

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ABSTRACT

A new method is proposed for determination of carcino-embryonic antigen (CEA) based on quantum dot (QD)-modified aptamer probe by capillary electrophoresis-chemiluminescence (CE-CL) detection system. An oligonucleotide sequence A contains two parts, one is CEA aptamer and the other is bridging segment. Horseradish peroxidase (HRP) conjugates with sequence A (HRP-DNA_A) and then mixes with QD labeling complementary bridging segment (QD-DNA_B), forming HRP-DNA_{A-B}-QD probe. When CEA exists, the specific combination of the aptamer in sequence A and CEA can form CEA/HRP-DNA_{A-B}-QD complex. After separation of CEA/HRP-DNA_{A-B}-QD complex and HRP-DNA_{A-B}-QD probe by capillary electrophoresis (CE), the chemiluminescence (CL) catalyzed by CEA/HRP-DNA_{A-B}-QD complex can be detected without any interference, and the content of CEA can be estimated by the CL intensity. It has been proved that the separation efficiency of HRP-DNA_{A-B}-QD and CEA/HRP-DNA_{A-B}-QD complex is improved greatly after QD modification, then the interference issue resulted from free HRP-DNA_{A-B}-QD probe is solved well; and the specific combination of HRP-DNA_{A-B}-QD and CEA leads to a closer distance of HRP and QD, then chemiluminescent resonance energy transfer (CRET) occurs, which confirms the validity of the strategy. Results show that the CL intensity has a linear relationship with the concentration of CEA in the range from 0.0654 to 6.54 ng/mL, and the limit of detection is approximately 8 pg/mL ($S/N = 3$). This proposed method with high specificity has been applied for the accurate analysis of content of CEA in patient's serum.

1. Introduction

Because of its simplicity, cheapness, high sensitivity and no external source needed, chemiluminescence (CL) method has been widely applied in clinical examination, biomedical detection and other fields [1–5]. Capillary electrophoresis (CE) is a powerful liquid phase separation technique, which has been widely applied in separation of biomolecule owing to its advantage in efficiency and sample consumption [6–8]. It can simultaneously achieve efficient separation and sensitive detection of trace components in complex sample by combination of sensitive CL detection with effective CE separation, and the interference issue of coexisting component in CL detection can be solved, which can realized a good combination of high efficient separation in microscale and high sensitive detection in a simple way [9,10].

Unfortunately, many studies have found that the separation efficiency of different types of protein or different length of DNA is unsatisfying by CE, even serious overlap peaks can be observed and can't realize separation [11–13]. In generally, in the separation of protein or DNA by CE, the separation efficiency can be improved by optimizing the experiment conditions, like the pH of running buffer and separation voltage or adding additives [13–15].

Recently, the discovery of new nanomaterials has a great influence in many fields. In CE, some kinds of nanoparticle have been added in running buffer as pseudostationary phase to improve the separation efficiency of protein or DNA [13–17]. For example, gold

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nanoparticles have been used to provide pseudostationary phase in the separation of protein and DNA [14,15]. SiO_2 nanoparticles are one of the most popular auxiliary materials for separation of biomolecules because of their great biocompatibility and chemical stability [13]. Inorganic quantum dot (QD) is a fluorescent nanomaterial, which has good chemical stability, broad absorption and narrow emission wavelengths, and has been applied in CE separation. On one hand, in separation of biomolecules by CE, QD conjugates with proteins or DNA and forms QD-protein or QD-DNA, which can be separated well by CE after combination with target molecules, indicating the separation efficiency of protein or DNA is improved after conjugation with QD [18]. On the other hand, QD can be also used as fluorescent acceptor in fluorescence resonance energy transfer (FRET). According to the fluorescent intensity of QD, molecular binding events can be detected sensitively. Our group had studied immunological recognition reaction using antibody and antigen labeled with different kinds of QD to form immunocomplex according to the fluorescent electrophoretic peak of acceptor [19].

Carcino-embryonic antigen (CEA), as a tumor marker, has an important role in the diagnosis and screening for colon cancer and other malignancies, and its level indicates the curative effect, progression of disease and the prognosis estimate for colorectal cancer, breast cancer and lung cancer [20–22]. Therefore, accurate determination of CEA has a very important significance in clinical medicine. However, some of current detection methods of CEA are less sensitive [23–25]; some need stable excitation source [26]; some are easy interfered by external environment [27–30]; some are complex testing process [31,32]. In a word, it is particularly important to develop new way for determination of CEA.

Aptamers are the artificial single-stranded DNA or RNA sequences. Owing to their high affinity and thermostability, convenient synthesis and modification, and extremely high specificity with certain targets, aptamers have sparked tremendous interest in biomedical analysis. At present, aptamer that can specially recognize CEA has been screened out, and applied to analyze the content of CEA [33,34]. In this paper, a new method was proposed for determination of CEA based on QD-modified aptamer probe by combining CE and CL detection system. An oligonucleotide sequence A contains two parts, one is CEA aptamer and the other is bridging segment. Horseradish peroxidase (HRP) conjugates with sequence A (HRP-DNA_A) and then mixes with QD labeling the complementary bridging segment (QD-DNA_B), forming HRP-$DNA_{A\text{-}B}$-QD probe. When CEA exists, the specific combination of the aptamer in sequence A and CEA can form CEA/HRP-$DNA_{A\text{-}B}$-QD complex. After separation of CEA/HRP-$DNA_{A\text{-}B}$-QD complex and HRP-$DNA_{A\text{-}B}$-QD probe by capillary electrophoresis (CE), the chemiluminescence (CL) catalyzed by CEA/HRP-$DNA_{A\text{-}B}$-QD complex can be detected without any interference, and the content of CEA can be estimated by the CL intensity. It has been proved that the separation efficiency of HRP-$DNA_{A\text{-}B}$-QD and CEA/HRP-$DNA_{A\text{-}B}$-QD complex is improved greatly after labeling QD, then the interference issue resulted from free HRP-$DNA_{A\text{-}B}$-QD probe is solved well; and the specific combination of HRP-$DNA_{A\text{-}B}$-QD and CEA leads to a closer distance of HRP and QD, then chemiluminescent resonance energy transfer (CRET) occurs, which confirms the validity of the strategy. This proposed method with high specificity offers a new way for separation and determination of biomolecule, and has good potential in application of biochemistry and bioanalysis.

2. Materials and methods

2.1. Chemicals and materials

Selenium powder (99.99%), zinc acetate, chromium acetic, sodium tetraborate (99%), boric acid, hydrogen peroxide (30%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tri-n-octylphosphine (TOP, 90%), tri-n-octylphosphine oxide (TOPO, 90%), glutathione (GSH), thrombin were supplied by Sigma–Aldrich Fine Chemicals (St. Louis, MO, America). 1-ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl, 98.5%), N-hydroxy succinimide sulfur generation (Sulfo-NHS), luminol, *para*-iodophenol (*p*-Ip), and horseradish peroxidase were purchased from Aladdin Chemistry (Shanghai) Co., Ltd. (Shanghai, China). Ultrafilter was supplied by Millipore (Bedford, MA, America). CEA was purchased from Meridian Life Science, Inc. (Memphis, America). Human immune globulin (IgG, 99%), human immunoglobulin (HSA, 99%) were obtained from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). All high performance liquid chromatography (HPLC)-purified oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai. China) and their sequences were shown as follows. Sequence A: 5′-NH_2-$(CH_2)_6$-ATACCAGCTTATTCAATTAAAAAAAA-3′; sequence B: 5′-TTTTTTTT-$(CH_2)_6$-NH_2-3′. All oligonucleotides were diluted to 100 μM by PBS (0.01 M, pH 7.4) and stored in the dark at −20 °C. Fused-silica capillary (75 μm of inner diameter) was supplied by Yongnian Optical Fiber Factory (Hebei, China). All other materials and reagents were of analytical grade, and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water (≥18.2 MΩ) from a Milli-Q system (Millipore, Bedford, MA, America) was used for all solutions.

2.2. Apparatus

The absorption spectra were measured by UV–vis spectrophotometer (UV-2550, Shimadzu, Japan). A chemiluminescent immunoassay system (LIASON, DiaSorin S.p.A, Italy) with standard CEA kit (DiaSorin S.p.A, Italy) was used to standardize the concentrations of CEA in patient's sample. CE analyses with chemiluminescent detection were carried out on a home-built system, consisting of a high-voltage supply (0–30 kV) (Shanghai Nuclear Research Institute, China), DHL-B computer constant flow pump (Shanghai Huxi Analysis Instrument Factory Co., Ltd., China), a QE65000 fiber-optic spectrometer (Hamamatsu S7031-1006 back-thinned CCD) (Ocean Optics, U.S.A.) with a SI 600/720 fiber (diameter of 80 mm, length of 2 m, Yangtze Optical Fiber and Cable Co., Ltd., China), a four-port plastic tube with inner diameter of 1.6 mm, and also a personal computer. All other instrumentations included Eppendorf 5415 D rotary concentration meter (Gene Co., Ltd., Germany), KQ5200 ultrasonic cleaner (Kunshan ultrasonic instrument Co., Ltd., China), and a Milli-Q system (Millipore, Bedford, MA, America).

2.3. Preparation of HRP-DNA_A and QD-DNA_B probe

Water-soluble CdSe/ZnS core–shell quantum dot were synthesized according to previous reports [35]. Water-soluble CdSe/ZnS QDs (30 μL) was put into EDC solution (30 μL, 5 mg/mL) followed by addition of Sulfo-NHS (20 μL, 1.5 mg/mL), and the solution was kept stirring for 0.5 h. After that, sequence B (50 μL, 100 μM) solution was added and the mixture was whirled for 2 h. Similarly, HRP (20 μL, 2 mg/mL) was put into EDC·HCl solution (30 μL, 5 mg/mL) followed by addition of Sulfo-NHS (20 μL, 1.5 mg/mL), and the solution was kept stirring for 0.5 h. After that, sequence A (50 μL, 100 μM) solution was added and the mixture was whirled for 2 h. Then, both of the mixture solution was centrifuged respectively by ultrafilter to obtained HRP-DNA_A and QD-DNA_B for future experiments.