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Highly sensitive immunoassay of carcinoembryonic antigen by capillary electrophoresis with gold nanoparticles amplified chemiluminescence detection

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ABSTRACT

A noncompetitive immunoassay based on gold nanoparticles (AuNPs) amplified capillary electrophoresis (CE) chemiluminescence (CL) detection was developed for the determination of carcinoembryonic antigen (CEA). In this method, citrate-modified AuNPs were conjugated with horseradish peroxidase (HRP) labeled CEA antibody (Ab*), and incubated with limited amount of CEA antigen. CEA-Ab*-AuNPs complex and excess of Ab*-AuNPs were then separated and quantified by CE with CL detection. Highly sensitive CL detection was achieved by means of p-iodophenol (PIP) enhanced luminol-H_2O_2-HPR CL reaction and AuNPs amplified. Under the optimal conditions, the CE assay was accomplished within 5 min. The linear range for CEA detection was 0.05–20 ng/mL with a detection limit of 0.034 ng/mL (signal/noise = 3), which is three orders magnitude lower than that of without AuNPs amplified. The current method was successfully applied for the quantification of CEA in human serum samples. It was demonstrated that the current CE-CL AuNPs amplified noncompetitive immunoassay was sensitive and highly selective. It may serve as a tool for clinical analysis of CEA to assist in the diagnosis of cancer.

1. Introduction

Immunoassay is known as one of the most important analytical methods and is widely used in clinical diagnoses and biochemical studies because of high selectivity and sensitivity for known determined analytes. Conventional immunoassays involve multiple steps including incubations, washing, and rinsing. This process is time and labor intensive [1]. Capillary electrophoresis-based immunoassay (CE-IA) was first demonstrated by Nielsen et al. in 1991 [2]. It combines the specificity of immune reactivity with the high separation efficiency and high-speed analysis that CE offers and has been widely used in the field of bioanalysis [3–9]. Compared to conventional immunoassays, CE-IA offer several possible advantages. Two important advantages of CE-IA are their ease of automation and their relatively fast separation of antibodies, analytes, and/or antibody/analyte complexes [10]. In addition, CE-IA tend to consume only small amounts of sample and reagents while still allowing the detection of trace amounts of analyte in a sample [11]. Unfortunately, CE-IA tend to give poorer concentration-based the limits of detection (LODs) than solid-phase immunoassay techniques such as an enzyme linked immunosorbent assay (ELISA) [12,13]. Therefore, the development of novel CE-IA methods for highly sensitive immunoassay is still an important task for medicinal, biological, and clinical requirements.

Gold nanoparticles (AuNPs) are one of the most extensively studied inorganic nanomaterials. Because of their unique optical, electrical, chemical and catalytic properties, AuNPs have attracted enormous interest for the possibility of versatile applications [14–16]. In particular, the high specific surface area and excellent biocompatibility of AuNPs can provide a suitable and promising platform for biological and biomedical applications [17–21]. All these made AuNPs become an excellent biological marker and have been used for the enrichment and extraction of analytes in complex matrices [22]. For example, Huo and co-workers [23] report a highly sensitive immunoassay for free prostate specific antigen detection using two different types of gold nanoparticle probes, which conjugated respectively with antibody and capture antibody, coupled with dynamic light scattering analysis. Recently, Liu et al. [24] developed a CE-based CL-IA method by using AuNPs conjugate with antibody (Ab) to form tagged antibody (Ab*), and then Ab* link to antigen (Ag) to produce an Ab*-Ag complex by a noncompetitive immunoreaction. AuNPs were used as a protein label reagent in the light of its excellent catalytic effect to the CL reaction of luminol and hydrogen peroxide (H_2O_2).

Carcinoembryonic antigen (CEA) is a glycoprotein often associated with certain cancers. It is hardly present in the serum of healthy adults; however, this protein may appear in the serum of patients who have certain kinds of cancers in a very high level. As the specificity tumor marker of the colorectal cancer, the

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positive rate of CEA usually reaches as high as 70–90%. Furthermore, the CEA level in human serum is also related to lung cancer [25], ovarian cancer [26] and breast cancer [27,28]. Therefore, the determination of CEA level in human serum plays an important role in giving an early diagnosis of disease progression and monitoring patient condition after therapy. Many methods have been investigated for the determination of CEA, including radioimmunoassay [29], enzyme immunoassay [30,31], electrochemical immunoassay [32], fluoroimmunoassay [33] and flow injection chemiluminescence immunoassay [34]. Although these methods are reliable and accurate, they suffer from the problems associated with the use of radioisotopes (for radioimmunoassay) and complex operation (for enzyme immunoassay). Therefore, developing highly specific and sensitive method for rapid determination of CEA in biofluids would be important and attractive.

In this study, a noncompetitive immunoassay based on AuNPs amplified CE-CL detection was developed for the determination of CEA. In this method, AuNPs was used as an amplified platform for immunoreaction, highly sensitive CL detection was achieved by means of p-iodophenol (PIP) enhanced luminol-H_2O_2-horseradish peroxidase (HPR) CL reaction and AuNPs amplified. The present method has been applied for determination of CEA in human serum. The results indicated that the method has a promise for simple and sensitive clinical assays of CEA.

2. Experimental

2.1. Apparatus

Experiments were carried out using a laboratory-built CE-CL system as described previously [35]. Briefly, a high-voltage supply (0–30 kV, Beijing Cailu Science Co., Ltd., Beijing) was used to drive the electrophoresis. A 50 cm × 75 μm i.d. uncoated fused-silica capillary (Hebei Optical Fiber) was used for the separation. The polyimide on 2.5 cm end section of the capillary was burned and removed. After etching with hydrofluoric acid for 1 h, the end of the capillary was inserted into a reaction capillary that was 320 μm i.d. (Hebei Optical Fiber). A four-way Plexiglas joint held them in place. The CL oxidizing solution was siphoned into a tee. The grounding electrode was placed in one joint of the tee. The CL solution flowed down to the detection window, which was made by burning 1 cm of the polyamide of the reaction capillary and was placed in front of a PMT (R374 equipped with a C1556-50 DA-type socket assembly, Hamamatsu, Shizuoka, Japan). The buffer reservoir at the high-voltage end was enclosed in a plexiglass box. CL emission collected by the PMT was recorded and processed with an IBM compatible computer using in-house written software.

Absorption spectra were recorded on a TU-1901 UV–vis spectrophotometer (Beijing Purkinje General Instrument Co., Ltd., Beijing) at room temperature. The scanning electron microscopy (SEM) image was obtained using FEI Quanta 200 FEG SEM (Philips Co., Ltd., Netherlands).

2.2. Reagents

CEA and HRP-labeled anti-CEA antibody (Ab*) were purchased from Beijing Yuande Biomedical Engineering Co., Ltd. (Beijing, China). Luminol was purchased from Fluka (Buchs, Switzerland). Bovine Serum Albumin (BSA) was purchased from Sigma Chemicals (St. Louis, MO). Tetrachloroaurate (III) tetrahydrate ($AuCl_3$·HCl·$4H_2O$, 48%, w/w), sodium citrate, PIP, Tween 20, hydrogen peroxide (H_2O_2, 30%), sodium dodecyl sulfate (SDS) and tris(hydroxymethyl) aminomethane (Tris) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used in this work were of analytical grade. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA), and used throughout the work.

The CEA and Ab* solutions were prepared by dissolving reagents in 0.01 M phosphate buffered saline (PBS; 0.1 M NaCl, 10 mM phosphate, pH 7.4). Blocking buffer solution consisted in a 0.01 M PBS solution (pH 7.4) with 1% (w/v) BSA (BSA in the PBS can block the active point of AuNPs and avoid unspecific adsorption of AuNPs). The electrophoresis buffer was 35 mM Tris–HCl solution at pH 9.5 containing 0.067% (v/v) Tween 20. CL reaction buffer was 50 mM sodium bicarbonate solution at pH 9.0 containing 0.1 mM Luminol, 30 mM H_2O_2 and 1 mM PIP.

2.3. Preparation and characterization of AuNPs

AuNPs were prepared according to the literature method [36] with slight modification. Briefly, 100 mL of 0.01% (m/v) $HAuCl_4$ solution was transferred to a flask and heated to boiling. With vigorous stirring, 3.5 mL of 1.0% (w/v) trisodium citrate solution was added quickly. The color of the solution changed from pale yellow to wine red in a few seconds. The solution was refluxed for 30 min. After cooling down, the solution was filtered through a 0.45 μm nylon membrane, and stored at the 4 °C before use. The size of AuNPs was verified by scanning electron microscope (SEM), and their concentration was estimated by UV–vis spectroscopy. The SEM images showed that the diameter of AuNPs was in the range of 10–15 nm, and with an average particle size of 13 nm.

2.4. Preparation of HRP-labeled antibody conjugated AuNPs

The HRP-labeled anti-CEA antibody conjugated AuNPs (Ab*-AuNPs) were prepared according to a modified procedure [37]. The Ab* (10% more than the minimum amount, which was determined using a gold aggregation test) was added dropwise to 1.0 mL of pH-adjusted to 9.0 AuNPs suspension followed by incubation at 4 °C for 3 h. The conjugate was centrifuged at 20,000 rpm for 20 min at 4 °C to remove the excess of Ab*. The precipitated conjugate was washed three times with 0.01 M PBS, with repetitive centrifugation and dispersion, and resuspended in blocking buffer solution to block any remaining active surface of AuNPs and stored at 4 °C until use.

2.5. Gold aggregation test

The gold aggregation test was preliminarily carried out according to the literature method [38]. AuNPs solution was adjusted to pH 9.0 with 0.01 M NaOH, then several solutions with different concentrations of Ab* were added into 100 μL of AuNPs solution, respectively. After placed 30 min, 10 μL of 10% NaCl solution was added, and the absorption spectrum of the solution and the absorbance at 519 nm was detected.

2.6. Preparation of human serum samples

Human blood samples from three healthy volunteers and three cancer patients volunteers were kindly provided by the No. 5 People's Hospital (Guilin, China). Human blood samples were centrifuged at 2000 rpm for 15 min to obtain serum. These samples were stored at −20 °C until analysis. According to the differences in CEA concentration between normal and cancer patients, the serum samples were diluted 1 fold for normal person and 150 folds for cancer patients with PBS before being injected into the CE-CL system.