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Journal of Chromatography A, 1156 (2007) 75–79
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Immunoassay by capillary electrophoresis with quantum dots

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Available online 23 December 2006

Abstract

The application of quantum dots in capillary electrophoresis immunoassay was studied for the first time. Quantum dots were conjugated with antibody and subsequently tested by electrophoretic separation of free antibody and antibody–antigen complex. Antibody was fluorescently labeled by quantum dots via conjugation procedures and its electrophoretic characteristics were effectively modified due to the attachment of quantum dots. The determination of human IgM by direct CE based immunoassay could be easily achieved by simply changing the pH value of separation buffer. Polymer additive influenced the separation too but the effect was not as significant as buffer pH adjustment. Satisfactory separation of complex from free antibody could be achieved with 20 mM sodium tetraborate as separation buffer, at pH 9.8. The immunoassay application of quantum dots in CE offers considerable advantages and can be readily applied to other large bio-molecules.

Keywords: Capillary electrophoresis; Immunoassay; Laser-induced fluorescence; Quantum dots; IgM; Nanoparticles

1. Introduction

Based on the specific reaction between antibodies (Ab) and antigens (Ag), immunoassays are developed to determine a wide variety of compounds [1,2]. Capillary electrophoresis immunoassay (CEIA), which is the combination of immunoassay with modern capillary electrophoresis, has become a new technique to carry out immunologic reactions in CE. With superior separation efficiency, CEIA can separate free Ab or Ag from Ab–Ag complex and enable the direct visualizations [3,4]. CEIA also requires relatively simpler procedures and less Ab samples, which are usually expensive. After the initial reports of CEIA [5,6], various applications have been conducted, such as specific recognition and determination of antigens [7–12], determination of binding constants of Ab and relevant antigens [13], and online affinity captures [14,15]. So far, most of the CEIA methods rely on laser-induced fluorescence (LIF) detection due to its high sensitivity and high selectivity for detection.

Though CE has high efficiency in separation, serious difficulties were sometimes encountered in the separation of Ab–Ag complex from free Ab or Ag. With regards to antigens with low molecular weights, the separation difficulty is expected since those antigens contribute very small amounts of mass or charge to the complex. In view of this problem, competitive CEIA can be employed for the determinations of small antigens. Unfortunately, the separation of Ab or Ag from Ag–Ab complex is also a challenge for some large antigens, such as proteins [3,4]. Proteins, like immunoglobulins, usually have relatively similar electrophoretic characteristics when compared to Ab (immunoglobulin G), and therefore the separation of complex peak from Ab peak was usually difficult.

Quantum dots (QD) are nanometer scale particles that consist of a semiconductor core, such as (CdSe) [16,17]. QD have narrow, symmetric, bright and photostable fluorescent emission. The emission spectrum of QD is tunable, from ultraviolet to infrared, by simply changing the size of CdSe cores. Unlike traditional fluorescent dyes, QD with different emission wavelengths can be excited simultaneously using a single excitation wavelength because of their broad absorbance bands. QD could provide a promising platform for the studies of immunofluorescence [18–23]. CE had been applied in the separation and characterization of QD in recent reports [24,25]. However, the application of QD in CEIA is still unexplored so far.

Nanoparticles had been applied successfully in the separation of long DNA molecules [26,27]. In this study, the effect of nanoparticle conjugation to Ab mobility in CEIA separation was investigated. Upon conjugation with QD, Ab is not only fluorescently labeled, but its electrophoretic mobility is also chemically modified due to the large mass of QD attached to the Ab.

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doi:10.1016/j.chroma.2006.12.077

2. Materials and methods

2.1. Reagents

Human IgM (purified immunoglobulin, 1 mg/mL), monoclonal anti-human IgM (IgG fraction of mouse ascites fluid, clone MB-11, 2 mg/mL), Tris(hydroxymethyl)aminomethane (TRIS), 2-amino-2-methyl-1,3-propanediol (AMPD) and *N*-[Tris(hydroxymethyl)-methyl]-3-aminopropanesulfonic acid (TAPs) were purchased from Sigma (St. Louis, MO, USA). Qdot®Antibody conjugation kit (525 nm) and Qdot@ 525 streptavidin conjugate solution were from Quantum Dot Co. (Hayward, CA, USA). Hydroxyethyl-cellulose (medium viscosity, WP-40) (HEC) was purchased from Fluka (Neu-Ulm, Switzerland). Human serum (909a-2) was from NIST (Gaithersburg, MD, USA). All reagents were of reagent grade and used without further purification. Water (≥18 MΩ) used throughout the experiments was generated by a NANOpure ultrapure water system (Barnstead, IA, USA).

2.2. Conjugation procedures

Conjugation was carried out according to the following protocol. There are briefly divided into four steps: (1) QD (125 μL) were activated using a hetero-bifunctional crosslinker, succinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate (SMCC). Excess SMCC was removed by size exclusion chromatography using a NAP-5 column. The QD with maleimide-nanocrystal surfaces were obtained. (2) Dithiothreitol (DTT) was added into Ab solution to expose free sulfhydryls. Excess DTT was removed by size exclusion chromatography. (3) Activated QD and reduced Ab solution were mixed and reacted for 1 h before quenching by β-mercaptoethanol. (4) The reaction mixture was centrifuged and purified further by size exclusion chromatography with Pierce column and Superdex 200 gel.

The final eluted solution, which contains approximately 1.5 μM QD and 60–70% of starting Ab, respectively, was stored at 4 °C.

2.3. Apparatus and CE experiments

A solution of 20 mM sodium tetraborate (pH 9.1) was used as CE separation buffer and adjusted by 1 M NaOH to desired pH. For the preparation of sample or standard, 5 μL solutions of QD conjugated with anti-human IgM were mixed with different amounts of IgM standard and further diluted by 1× PBS solution to 10 μL. The mixtures were kept at room temperature for 5 min before introducing into the capillary.

Except where otherwise noted, the total length of the 75 μm I.D. capillary used (Polymicro Technologies, AZ, USA) was 100 cm with effective length of 90 cm. When a capillary was firstly used, it was rinsed with 0.1 M NaOH, water and separation buffer, respectively, for 10 min. Hydrodynamic injection was performed by siphoning at 15 cm height differences for 10 s. The capillary was rinsed for 2.0 min with separation buffer between two runs. The separation buffer was replenished after each five runs. The separation was achieved at +22 kV and at room temperature of 25 ± 1 °C.

A LS55 luminescence spectrometer from Perkin Elmer (Boston, MA, USA) was used for fluorescence studies. The CE separations were performed with a CZE 1000R high voltage power supplier (Spellman, Plainview, NY, USA) and the detection systems employed were either a ZETALIF LIF detector (Picometrics, Ramonville, France) equipped with a 473 nm blue DPSS laser (Sintec Optronics, Singapore) or an SPD-10A UV–vis detector from Shimadzu (Kyoto, Japan). Data acquisition and recording of electropherograms were accomplished with a CSW Chromatography Station (Data Apex, Prague, Czech Republic).

3. Results and discussion

The wavelengths and intensities of QD fluorescent emission after conjugation were studied with a fluorescent spectrometer first. Fig. 1 indicated that conjugation has no effect on the fluorescent emission wavelengths. But the fluorescent intensity of QD was reduced around 10 times after conjugation. It might result from the dilution effect (from 125 to 500 μL) and loss or aggregation effects of QD during the conjugation procedures. Also, the fluorescent emission wavelength of QD after conjugation would not be influenced by the pH value change of solution. The emission wavelength was remained at 525 nm.

3.1. Ab–Ag complex

A slightly basic buffer was chosen for CE running to ensure strong Ab–Ag binding and minimize the protein adsorption on capillary inner wall. Usually, both proteins, Ab and Ag, were negatively charged and migrated out after EOF. UV detection was initially used to test 20 mg/L anti-IGM solution with 20 mM sodium tetraborate buffer (pH 9.1). Experimental results showed several peaks instead of a single peak. It indicated that there were multiple components in Ab standard. The complexity of the Ab standard makes the separation and determination of Ab

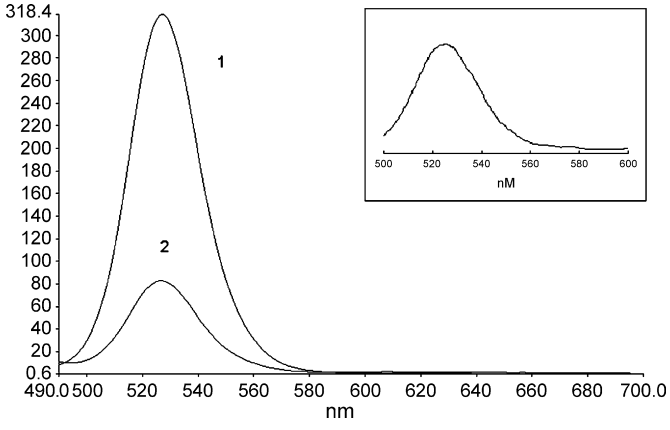

Fig. 1. Fluorescent intensities of original QD and QD after conjugation. Sample: (1) 200 times diluted original QD solution; (2) 100 times diluted QD solution conjugated with Ab. Excitation wavelength: 473 nm. The inset represents the emission wavelength of QD after conjugation in pH 9.8 buffer.