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Detection of C-reactive protein based on magnetic nanoparticles and capillary zone electrophoresis with laser-induced fluorescence detection

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ABSTRACT

A simple and fast method based on magnetic nanoparticles (MNPs) and capillary zone electrophoresis (CZE) with laser-induced fluorescence (LIF) detection was developed for the detection of C-reactive protein (CRP). To optimize the CZE conditions, several factors including buffer compositions, buffer ionic strength, buffer pH, applied voltage and capillary temperature have been examined. The optimal separation buffer selected was a 30 mM sodium phosphate (PB) buffer, pH 8.0. The optimal CE applied voltage and temperature selected were 20 kV and 35 °C, respectively. The CZE profile of the MNP-1°Ab-CRP-2°Ab/FITC bioconjugates showed good reproducibility. One major peak was observed for the MNP bioconjugates. The quantitative analysis also showed good results. The coefficient of variation (CV%) for the major peak area was 8.7%, and the CV% for the major peak migration time was 2.5%. The linear range for CRP analysis was 10–150 μg/mL, and the concentration limit of detection (LOD) was 9.2 μg/mL. Non-specific interactions between bovine serum albumin (BSA) and the system can be prevented by including 10% (v/v) of human plasma in the binding buffers. The CE/LIF method might be helpful for analyzing high concentrations of CRP in a patient's plasma after an acute-phase inflammation. This new method demonstrated the possibility of using MNPs and CE/LIF for the detection of proteins, and provided information for the establishment of appropriate CE conditions.

1. Introduction

CRP is a well known acute and chronic inflammatory biomarker. Many reports suggested that high levels of CRP strongly correlated with cardiovascular diseases [1–4]. Conventionally, CRP was measured by enzyme-linked immunosorbent assay (ELISA). In recent years, nanoparticles (NPs) have emerged as new and promising materials for analysis, diagnosis and imaging in the biomedical field. Among them, quantum dots (QDs), MNPs, and gold nanoparticles (Au NPs) have received lots of attention. Capillary electrophoresis (CE) is an analytical method which has the advantages of simplicity, high speed and high efficiency of separation. Meanwhile, it only needs minute volumes of sample and buffer for separation, and can be used to separate almost any kind of analytes. Recently, NPs have also been used as stationary phase, pseudostationary phase, or bioconjugates to characterize and separate biomolecules by CE. However, it is challenging to explore the applications of NPs in CE because many experimental conditions (*e.g.* material, structure and size of NPs, pH and salt of preincubation and separation buffers, electric field strength as well as the various biomolecules of interest) influence the aggregation of NPs and the reproducibility of CE electropherograms [5]. In this study, we have demonstrated the possibility of detecting CRP based on MNPs and CZE with LIF detection.

For employing bioconjugates of QDs in CE analysis, Feng et al. have used CE with LIF detection to analyze human IgM. QDs were bound to antibody and human IgM for CE analysis. It was found that pH value of CE buffer had significant influence on the separation of free antibody and antibody–antigen complex [6].

Huang et al. have used CE with LIF detection to separate free QDs from bioconjugates. It was found that the bioconjugates formed by QD and BSA can be purified by ultrafiltration [7]. Vicente et al. have used CE with LIF detection to separate bioconjugates formed by QD and streptavidin, biotin, as well as IgG. The electrophoretic mobilities of the bioconjugates differed with the bound biomolecule and the CE running buffer [8].

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For employing bioconjugates of Au NPs in CE analysis, Li et al. have used CE with LIF detection to study the distance dependence of Au-enhanced QDs fluorescence in solution. QD and Au NPs have been connected together by two complementary DNA strands which controlled the distance between the two NPs. The method was also shown to have high sensitivity for DNA analysis [9]. Zhang et al. have used CE with electrochemical detection to separate four shell fish toxins. The bioconjugates formed by Au NPs, horseradish peroxidase (HRP)-labeled antigen and antibody increased the resolution and sensitivity of CE analysis [10].

For employing bioconjugates of MNPs in CE analysis, Li et al. have used MNPs and Au NPs to study interactions between NPs and BSA by CE with UV–visible detection. The authors used CZE to separate stable NP–BSA complexes from free NPs and BSA. They used affinity capillary electrophoresis (ACE) to separate unstable transient complexes from free NPs. Dissociation constants (K_D) and cooperativity coefficients (n) were calculated to investigate the interactions of NPs and BSA. The CE separation buffer selected was a 10 mM sodium borate buffer (pH 8.3) [11]. Petr et al. has used CZE with UV–visible detection to characterize zwitterionic amino/polyethyleneoxide maghemite core/silica shell NPs which were functionalized with α-lactalbumin. The authors have found that the optimal CE conditions for separating functionalized from non-functionalized NPs were using a 25 mM of MES/NaOH buffer (pH 6.0), and a didodecyldimethylammonium bromide (DDAB)-coated capillary [12]. Wang et al. have used CE with LIF detection to investigate MNPs which were functionalized with BSA, streptavidin, and IgG. The NP-protein bioconjugates were derivatized with naphthalene-2, 3-dicarboxaldehyde (NDA) and then analyzed by CE. The selected CE separation buffer was a 100 mM sodium borate buffer (pH 9.2) [13].

The application of NPs in CE analysis is a relatively new field, and there is still a lack of knowledge of understanding the interactions of biomolecules on the NP surfaces, and the behaviors of bioconjugates as well as free NPs inside the open tubular capillary. Compared to other NPs, MNPs have lower toxicity and are more biocompatible since they are synthesized by Fe_3O_4 and Fe_2O_3. Meanwhile, superparamagnetic iron oxide NPs have been widely used as contrast agents for magnetic resonance imaging (MRI) to find diseased tissues. As a result, the study of bioconjugated MNPs by CE will help us understand the interactions of biomolecules with MNPs *in vivo*. It will also provide a new methodology for separating and characterizing biomolecules by CE. However, there were only a few studies of investigating the bioconjugated MNPs by CE so far. In this study, we demonstrated a new method using MNPs and CE/LIF analysis for the detection of CRP for the first time. This new method used protein G modified MNPs for non-covalent bindings with anti-CRP antibody 1, CRP and anti-CRP antibody 2/FITC. The interactions between protein G and magnetic particles were via the amide bonds. The MNP-1°Ab-CRP-2°Ab/FITC bioconjugates were directly analyzed by CE/LIF, without elution of CRP from the MNP surfaces.

2. Materials and methods

2.1. Chemicals

Protein G modified-MNP with inside diameter of 100 nm (Dynabeads Protein G; Invitrogen, Carlsbad, CA, USA), the first anti-CRP antibody (1°Ab; polyclonal Rabbit Anti-C-Reactive Protein; Merck KGaA, Darmstadt, Germany), CRP protein standard (1.0 mg/mL in Tris buffer, pH 7.40, CRP from human ascites; EMD Chemicals, San Diego, CA, USA), the second anti-CRP antibody labeled with FITC (2°Ab/FITC; polyclonal Goat Anti-C-Reactive Protein with FITC; Abcam, Cambridge, UK), phosphate buffered saline (PBS; Sigma Chemical, St. Louis, MO, USA), bovine serum albumin (BSA; Sigma Chemical), Tween-20 (Sigma Chemical), sodium phosphate dibasic (Na_2HPO_4; Sigma Chemical), sodium phosphate monobasic (NaH_2PO_4; Sigma Chemical), N-2-Hydroxy-ethylpiperazine-N′-2-ethanesulfonic Acid (HEPES; Merck KGaA), 2-(*n*-morpholino) ethanesulfonic acid monohydrate (MES; Avantor Performance Materials, Center Valley, PA, USA), Tris(hydroxymethyl) aminomethane (Tris; Usb, Cleveland, OH, USA), 3-(N-Morpholino) propanesulfonic acid (MOPS; Sigma Chemical) and deionized water (Millipore Simplicity; Millipore, Billerica, MA, USA) were used in this study.

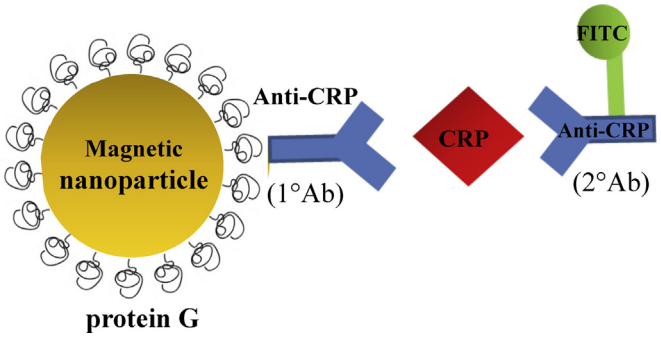

Fig. 1. Schematic of the modifications on the surface of MNP. Protein G modified MNP was bound to 1°Ab, CRP and 2°Ab/FITC non-covalently.

2.2. Preparation of the MNP-1°Ab-CRP-2°Ab/FITC bioconjugates

For the preparation of the bioconjugates, protein G modified-MNP with inside diameter of 100 nm was used. First, the protein G modified-MNPs were bound to the 1°Ab. The 1°Ab was diluted in PBS buffer (with 0.1% Tween-20, pH 7.40) to a final concentration of 0.035 mg/mL. The buffer of a 50 μL (0.05 mg) MNP solution for preserving the particles was eliminated before the binding reactions. The above MNPs and a 200 μL of the diluted 1°Ab solution were incubated at room temperature with shaking for 0.5 h. At the end of the reaction, the MNP-1°Ab bioconjugates and buffer were separated by magnetic separation. The bioconjugates were then washed three times with PBS buffer (with 0.1% Tween-20, pH 7.40). Second, the MNP-1°Ab bioconjugates were incubated with a 10 μL of CRP protein standard at various concentrations (in Tris buffer, pH 7.40) and a 90 μL of PBS buffer (with 0.1% Tween-20, pH 7.40) at room temperature with shaking for 2 h. At the end of the reaction, the bioconjugates and binding buffer were separated by magnetic separation. The MNP-1°Ab-CRP bioconjugates were then washed three times with PBS buffer (with 0.1% Tween-20, pH 7.40). Third, the 2°Ab/FITC was diluted in PBS buffer (with 0.1% Tween-20, pH 7.40) to a final concentration of 5.0 mg/mL. The above MNP-1°Ab-CRP bioconjugates were incubated with a 100 μL of the diluted 2°Ab/FITC solution at room temperature with shaking for 0.5 h. At the end of the reaction, the bioconjugates and binding buffer were separated by magnetic separation. The MNP-1°Ab-CRP-2°Ab/FITC bioconjugates were then washed five times with PBS buffer (with 0.1% Tween-20, pH 7.40). The bioconjugates were subsequently analyzed by CZE with fluorescence detection. Fig. 1 shows the MNP-1°Ab-CRP-2°Ab/FITC bioconjugate.

2.3. CZE analysis

The CZE analysis was carried out using a Beckman P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) which employed a LIF detector with a 488 nm argon ion laser. The acquired electropherograms were analyzed by 32 Karat software (version 8.0, Beckman). Uncoated fused-silica capillaries