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A sensitive immunoassay for determination of hepatitis B surface antigen and antibody in human serum using capillary electrophoresis with chemiluminescence detection

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Abstract

A sensitive and homogeneous immunoassay (IA) based on capillary electrophoresis (CE) with enhanced chemiluminescence (CL) detection has been developed for the determination of hepatitis B surface antigen (HBsAg) and antibody (HBsAb) in human serum. The conditions for the CL reaction and electrophoresis were investigated in detail using horseradish peroxidase (HRP) labeled HBsAg (HBsAg*) as a marker because of its catalytic effects on the luminol–hydrogen peroxide reaction. The CL reaction was enhanced by para-iodophenol and the CL detector was designed uniquely without any dead volume or diluents effect. The present method has been used for assaying HBsAg and HBsAb in human serum using a competitive format and a non-competitive format, respectively. Under the optimal conditions, the linear ranges were from 1 to 400 pmol/L ($R = 0.9988$) for HBsAg and 2 to 200 mIU/mL ($R = 0.9981$) for HBsAb. The detection limits were 0.4 pmol/L and 1 mIU/mL for HBsAg and HBsAb, respectively. The relative standard deviations of peak area were 4.2% and the errors of it were from −0.03% to +0.05% for 80 pmol/L HBsAg* ($n = 7$). In this study, the free HBsAg* and the bound HBsAg* (HBsAg*–HBsAb) were separated in the separation capillary within 6 min using a borate run buffer. To verify the experimental reliability, the result was comparable with that of enzyme linked immunosorbent assay (ELISA) and demonstrated the feasibility of the CE–CL immunoassay method for clinical diagnosis.

Keywords: Immunoassay; HBsAg; HBsAb; Capillary electrophoresis; Chemiluminescence

1. Introduction

The human B hepatitisis is one of the most widespread infectious diseases and it can induce chronic or acute hepatitis. There are approximately 300 million people that are still suffering from this detrimental or fatal disease around the world. In China alone, about 60% of above-mentioned people have been infected with hepatitis B or about 10% of Chinese people have been infected with it [1]. It is generally accepted that the diagnosis of infection by hepatitis B virus (HBV) is based on the presence of the HBsAg in the blood, since that it can generally be detected while still in the incubation period [2]. To patients, HBsAb is the symbol for restoring to health after infecting with HBV and to healthy people, injecting the hepatitis B bacteria, as successful immunoreactions should generate HBsAb. On the fast development of the clinical diagnostic technologies, there were some case reports on detection of HBV DNA by polymerase chain reaction (PCR) technology of molecular biological methods [3,4] as well as the chip-based detection method [5]. Currently, surface plasmon resonance biosensor [6] and immunovoltammetry with nano-magnetic microsphere [7] technologies were reported, while enzyme-linked immunosorbent assay (ELISA) was still the main means to clinically diagnose HBV [8,9]. However, the shortcomings of ELISA method are indirect, long analytical time and low sensitivity. Therefore, it is necessary to develop a simple, rapid, and effective method to detect HBV in real samples.

Compared to conventional ELISA and radioimmunoassay (RIA) [10], CE combined with IA is characterized by high resolution and less sample, and will become a powerful tool in clinical diagnosis, environmental and food analyses. There were detailed reviews of CEIA in 1997, 2000 and 2003 [11–13].

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High-performance liquid chromatography (HPLC) is the regular means in comparison with ELISA due to its broad application in pharmaceutical analysis and clinical diagnosis [14–18], while its applicability in IA including sensitivity isn't comparable to that of CEIA. Therefore, no reports were found using HPLC methods for determination of HBV. Laser induced fluorescence (LIF) detection is still the usual choice in CEIA because of its high sensitivity, commercially available apparatus and the ease in the preparation of fluorescent-labeled tracer [19–27]. Apart from LIF detection, CL detection has received much attention due to its high sensitivity, low cost, wide linear range and having no use for excitation sources, while few reports have been found using CEIA with CL detection [28,29] including the successful application of it to quantification of CA125 in human sera and determination of bone morphogenic protein-2 in rat vascular smooth muscle cells.

In the present paper, a sensitive and homogeneous IA based on CE–CL detection has been developed for the determination of HBsAg and HBsAb in human serum. The conditions for the CL reaction and electrophoresis were investigated in detail using HBsAg* as a marker because of its catalytic effects on the luminol–hydrogen peroxide reaction with the para-iodophenol as a CL enhancer. Since this CL reaction is a fast and weakly luminescence reaction, it is necessary that the location of this CL reaction should be controlled accurately at the detection windows. The zone of enzymatic marker which was discharged from the end of the separation capillary and at the center of the detection window could be reacted immediately with the CL reagents, which were transported continuously in the reaction capillary. Thus a unique CL detector was designed so extraordinary that it can obtain the most luminescent intensity. The HBsAg* reacted with a limited amount of HBsAb, and then formed a non-competitive CEIA. The determined HBsAg was added to above fixed mixture and thus unlabeled HBsAg competed with HBsAg* for binding to a limiting amount of HBsAb, and this was a competitive CEIA. Separation of the mixture by CE with CL detection produced two distinct peaks corresponding to HBsAg* and HBsAg*–HBsAb complex, the intensities of which can be related to the original concentration of HBsAg or HBsAb. In CE separations performed in fused-silica capillaries coated with polyimide, the adsorption of the capillary significantly affected the separation efficiencies of proteins. Therefore, the use of high-pH buffer was adopted for suppression protein adsorption [30], in which the proteins were changed into negatively charged species.

The present method has been employed to determination of the HBsAg and HBsAb in human serum. The result was comparable with that of enzyme linked immunosorbent assay (ELISA) and in CE–CL, the sensitivity was higher than that of ELISA method [31]. Its detection limit is 0.4 pmol/L for HBsAg and 1 mIU/mL for HBsAb, and this value is also lower than that of time resolved fluoroimmunoassay with 0.2 ng/mL for HBsAg and 5 mIU/mL for HBsAb [32]. The relative standard deviations of peak area were 4.2% and the errors of it were from −0.03% to +0.05% for 80 pmol/L HBsAg* ($n = 7$). Thereby, this method demonstrated the feasibility of the CE–CL immunoassay method for clinical diagnosis. In the future, the analytical system of CE–IA based on CL detection can be integrated into an array capillary electrophoresis chip and thus, a large number of samples could be synchronously determined along with overall improved analytical efficiency.

2. Materials and methods

2.1. Apparatus

A MPI-A CE–CL detection system including a 0–20 kV high voltage (HV) power supply and analytical system of multimember data collection (Ruimai electronic technological corporation, Xi'an, China) was used throughout the experiment (Fig. 1A). A fused-silica capillary (50 cm × 75 μm I.D.) coated with polyimide (Polymicro Technologies, Phoenix, AZ, USA) was used for separation, and then inserted into a reaction capillary (18 cm × 620 μm I.D.; Chongqing Optical Fiber, Chongqing, China). The detection window was formed by burning 8 mm of the polyimide of the reaction capillary and setting it in front of the photomultiplier tube (PMT; Hamamatsu, Japan), and synchronously putting a reflector on the other side. A section of the end of the separation capillary was inserted in the middle of the detection window (Fig. 1B). The CL reaction proceeds immediately in the detection window and there is no dead volume or diluents effect in the reaction capillary in such elaborate device. The distance between the reaction capillary detection window and the PMT was 3 mm. The PMT of the detector was operated at 700–800 V. CL reagents were delivered by a double microsyringe pump (Shanghai Instrument Plant, Shanghai, China) and flowed through a reagent capillary (22 cm × 250 μm I.D.; Lanzhou Institute of Chemical Physics, China) to the reaction capillary. CL reagent solutions were fed at a rate of 15–20 μL/min. The end section of the reaction capil-

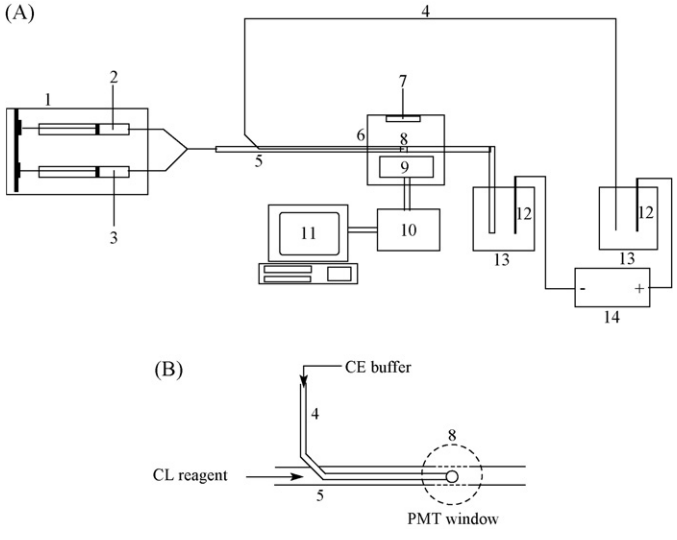

Fig. 1. Schematic diagram of the capillary electrophoresis instrument with chemiluminescence detection. (A) (1) double syringe pumps; (2) luminol solution; (3) H_2O_2 solution; (4) electrophoresis separation capillary; (5) reaction capillary; (6) black box; (7) reflector; (8) PMT window; (9) PMT; (10) signal amplifier; (11) personal computer; (12) Pt electrodes; (13) electrolyte reservoirs; (14) high-voltage power. (B) Schematic of CL detection interface.