Journal of Chromatography B, 1053 (2017) 42–47

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Chemiluminescence noncompetitive immunoassay based on microchip electrophoresis for the determination of β-subunit of human chorionic gonadotropin

Shuting Li, Tingzheng Yang, Jingjin Zhao*, Yong Huang, Shulin Zhao*

State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection of Ministry Education, Guangxi Normal University, Guilin, 541004, China

ARTICLE INFO

Article history:
Received 28 September 2016
Received in revised form 23 March 2017
Accepted 26 March 2017
Available online 29 March 2017

Keywords:
Tumor marker
β-Subunit of human chorionic gonadotropin
Microchip electrophoresis
Chemiluminescence detection
Noncompetitive immunoassay

ABSTRACT

In this work, a microchip-electrophoresis chemiluminescence (MCE-CL)-based immunoassay method was established for the determination of β-subunit of human chorionic gonadotropin (β-HCG). This approach uses MCE-CL assay as a platform. First, the β-HCG antigen (Ag) binds to excess horseradish peroxidase (HRP)-labeled anti-β-HCG antibodies (Ab*) to form an immune complex (Ag-Ab*). Subsequently, the obtained Ag-Ab* complex and unreacted Ab* were separated by MCE, and detected by CL. The CL intensity (peak high) of Ag-Ab* was used to estimate β-HCG concentration. The calibration curve between the peak high and β-HCG concentration showed a good linearity in the range of 0.6–60 mIU/mL. Based on a signal/noise ratio (S/N) of 3, the detection limit for β-HCG was estimated to be 0.36 mIU/mL. The present method was successfully applied for the detection of β-HCG in human serum, and the serum content of β-HCG from three healthy subjects was found be in the range of 9.5–15.7 mIU/mL, while that from three ovarian cancer patients was found be in the range of 160.9–210.4 mIU/mL. These results suggest that cancer patients have higher contents of β-HCG than healthy individuals do, indicating that this method can be applied for assisting diagnosis of ovarian cancer in clinical application.

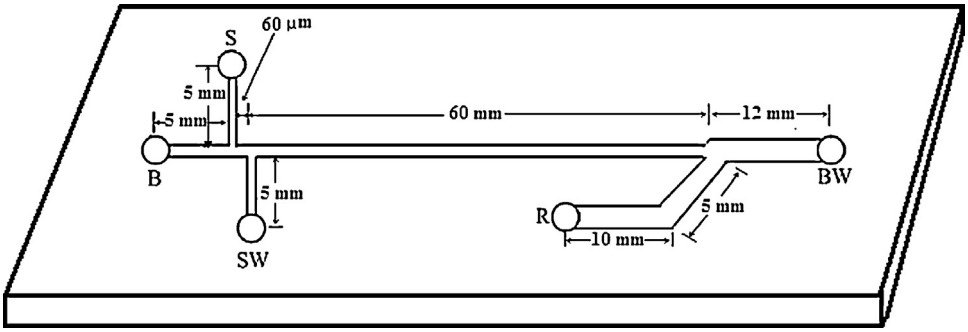
1. Introduction

The human chorionic gonadotropin (HCG) is a glycoprotein hormone, and is a dimer formed through a non-covalent bond between α and β subunit. The β-subunit of HCG (β-HCG) was often used as a biomarker for several disease-related conditions such as gestational trophoblastic diseases [1], nontrophoblastic neoplasms [2] and down's syndrome [3]. The β-HCG also has used as an important tumor marker to monitor choriocarcinoma therapy based on the β-HCG assay [4]. Also, American Society of Clinical Oncology Clinical Practice Guideline recommends measuring β-HCG before and after orchiectomy and before chemotherapy for those with extragonadal non-seminomas [5]. Thus, monitoring the levels of β-HCG, especially trace amounts of β-HCG in human serum, has become increasingly important.

Common methods for β-HCG detection include radioimmunoassay [6], enzyme-linked immunosorbent assay [7], and piezoelectric quartz micro-array immunoassay [8]. Recently, many new methods have been reported for β-HCG detection, such as immuno-MS spectrometry [9], fluorescent immunoassay [10] and visualized immunoassay [11]. Although these immunoassay methods are convenient and co-effective, the microchip-electrophoresis chemiluminescence (MCE-CL) method has rarely been reported for immunoassay [12–15]. In the past twenty years, the capillary electrophoresis (CE) technique has been applied in homogeneous immunoassays [16,17]. MCE is a further development of the CE technique, which allows integration of chemical and biological sample preparation, reaction, separation, and detection onto a chip with a size of a few square centimeters. A network is formed on the chip through microchannels that allow controllable fluids to run through the entire system and achieve conventional chemical or biological laboratory functions [18–21]. CL detection has many advantages such as no external light source required, low background noise, high sensitivity, wide linear range, rapid analysis, and simple equipment. Therefore, it has been very popular in MCE devices in recent years [22–25]. Furthermore, homogeneous immunoassays, which avoid complicated operation of solid-phase

* Corresponding authors at:. College of Chemistry and Pharmacy, Guangxi Normal University Guilin, 541004, China.
E-mail addresses: jzhao12@163.com (J. Zhao), zhaoshulin001@163.com (S. Zhao).

http://dx.doi.org/10.1016/j.jchromb.2017.03.031
1570-0232/



Scheme 1. Schematic diagram of the layout of the glass/PDMS microchip. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir; R: the oxidizer solution reservoir.

immunoassays, are easy to perform, can reduce errors in the washing process, and demonstrate improved accuracy.

In this work, the MCE-CL detection technique was utilized to establish a new homogeneous noncompetitive immunoassay for β-HCG detection. Briefly, the β-HCG antigen (Ag) binds to with excess horseradish peroxidase (HRP)-labeled anti-β-HCG antibodies (Ab*), followed by MCE-CL detection. The separation of free Ab* and immune complexes (Ag-Ab*) can be accomplished within 1 min. The proposed method has been successfully applied for the detection of β-HCG in human serum with satisfactory results.

2. Experimental section

2.1. Instruments

The MCE-CL detection system was designed by our laboratory as previously reported [26]. Double T-type microfluidic chips with a widened Y type CL detector were used in this study. The chip was constructed according to a previously published method [27], and the structure was illustrated in Scheme 1. The area of the chip was 9.5 cm × 2 cm, the width of the top of channel was 65 μm except the 250 μm-channel from the oxidizer reagent reservoir (R) to buffer waste reservoir (BW), the depth was 25 μm, the effective separation channel was 60 mm long, the distance from buffer reservoir (B) to the double-T cross point was 5 mm, the distance from sample reservoir (S) and sample waste reservoir (SW) to the separation channel was 5 mm, and the double-T cross point was 60 μm. The oxidizer reservoir (R) was 1.5 cm away from the Y-type cross point and the distance from the Y-type cross point to the BW was 1.2 cm.

2.2. Reagents and solutions

The β-HCG immunoassay kit and HRP-labeled anti-β-HCG antibodies (HRP-β-HCG) were purchased from Beijing Kemei Biological Technology Co. Ltd. (Beijing, China). Luminol was purchased from Fluka (Buchs, Switzerland). Tween 20, *p*-iodophenol (PIP) and hydrogen peroxide (H_2O_2, 30%) were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). All other chemicals used in this work were of analytical grade. β-HCG and HRP-β-HCG were both diluted to specified concentrations using 10 mM of phosphate solution (pH 7.4). The electrophoresis buffer solution used in this study was 25 mM borate buffer solution (pH 10.1) containing 0.07% (w/v) Tween 20 and 1.2 mM luminol. The post-separation oxidation reagent solution was 30 mM $NaHCO_3$ (pH 8.7) containing 0.1 M H_2O_2 and 1.1 mM PIP. Milli-Q water was used in all experiments requiring water.

2.3. Immunoreaction

The principle of noncompetitive immunoassay is as follows:

$$Ag + Ab*(excess) \rightarrow Ag\text{-}Ab* + Ab*$$

Different concentrations of 10 μLβ-HCG antigen solution or serum samples were mixed with 10 μL of 30 mIU/mL HRP-β-HCG antibody solution in a microcentrifuge tube. The mixture solution was then diluted to 50 μL with phosphate buffer solution (pH 7.4) and incubated at 37 °C for 35 min. The resulting solution was used for MCE-CL analysis.

2.4. MCE-CL assay procedure

Prior to and between two time electrophoresis, all channels of the chip were rinsed sequentially with 0.1 M NaOH, deionized water, and electrophoresis buffer solution for 10 min. Vacuum negative pressure was used to fill all the channels with electrophoresis buffer solution. After filling all the reservoirs with the appropriate solution (electrophoresis buffer solution for B, BW and SW; sample solution for S; oxidizer solution for R), the sample was introduced and separated. The pinched sampling method was adopted. A voltage of 550 V was applied to S, while SW was grounding. Meanwhile, B and BW were applied with 250 V and 400 V, respectively. The sample injection time was 25 s. The sampling volume was about 150 pL. During separation, 2500 V was applied to B, 550 V was applied to R, and BW was grounding. Meanwhile, 1550 V was applied to S and SW to avoid samples leaking into the separation channels during separation. The sample components were moved to the Y-type cross point in the separation channel and mixed with an oxidizer solution, and the CL then was detected through the eyepiece (placed directly at the cross point of Y-type channel), and transmitted to photomultiplier tube (PMT). Finally, the data was stored on a HW-2000 chromatography workstation.

3. Results and discussion

3.1. Optimization of electrophoresis separation conditions

To accurately quantify β-HCG, a variety of factors affecting electrophoretic separation were optimized in detail, including pH value of electrophoresis buffer solution, the concentration of electrophoresis buffer, Tween 20 concentration (w/v) and separation voltage.

The pH value of the electrophoresis buffer solution is an important factor affecting electrophoretic separation and protein adsorption. In this study, 25 mM borate solution was used as the electrophoresis buffer solution, the experiment examined the effects of pH values of borate solution on the separation of immune complexes (Ag-Ab*) and free labeled antibody (Ab*) in the pH range