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A New Immunoassay Method by Capillary Electrophoresis with Enhanced Chemiluminescence Detection

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Abstract: This paper described a new immunoassay method by capillary electrophoresis with enhanced chemiluminescence (CL) detection system based on luminol-hydrogen peroxide reaction catalyzed by horseradish peroxides (HRP). Using *para*-iodophenol as a CL enhancer, the detection limit of about 1×10^{-12} mol/L for HRP was achieved, which corresponded to 1.32×10^{-5} U/mL. In optimal conditions, the free HRP-labeled CA125 antibody (Ab*) and the bound enzyme-labeled complex (Ab*-Ag) were well separated by capillary electrophoresis within 4 min. The assay was successfully used to determine the contents of CA125 in human sera, which were associated with ovarian cancer, and the recoveries of the standard addition experiments were 96 to 109 %.

Keywords: Capillary electrophoresis, chemiluminescence detection, CA125, immunoassay.

Immunoassay based on capillary electrophoresis has recently been paid a great attention due to its excellent separation power, high analysis speed, small sample volume requirements, and automation [1,2]. Since the injection volume is too small, immunoassay by CE needs an extremely sensitive detector. Laser-induced fluorescence[3] and electro-chemical detections [4] have been used for this purpose. In fact chemiluminescence (CL) is also a highly sensitive method for detection, and is widely used for detection in conventional immunoassay and bioassay. And recently, CL detection has been used for CE separation due to its simple optical system and low background nature. CE combined with CL (CE-CL) has been successfully used for analysis of amino acids, proteins, catecholamines, ATP, catalytic and non-catalytic metals ions, and polyamines [5,6]. These results also demonstrated that CL was a very sensitive detector for CE separation. Just now, CEIA based on chemiluminescence detection was successfully applied for determination of bone morphogenic protein-2 in rat vascular smooth muscle cells [7]. To our knowledge, there has been no report on the use of CE-CL in immunoassay of real sera samples in clinical diagnosis. In this paper we explored the possibility to develop a highly sensitive immunoassay for quantification of CA125 by capillary electrophoresis with chemiluminescence detection.

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Experimental

Reagents

Luminol was purchased from Fluka. HRP was purchased from Dongfeng biochemical Ltd. (Shanghai, 300 U/mg). Hydrogen peroxide was from Taopu Chemical Factory (Shanghai). *Para*-iodophenol was product of Sigma Co., and the CA125 kit (no. 400-10) was from CanAg Diagnostics AB (Gothenburg, Sweden). All chemicals were of analytical grade, ultra-pure water (18.2 MΩ), double-distilled and purified on Millipore Simplicity, was used for preparation of all aqueous solutions. All solutions were filtered through 0.22 μm membrane filters *prior to* use.

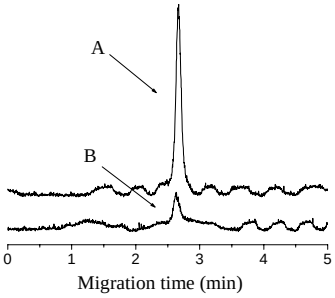
Capillary Electrophoresis Procedure

The electrophoresis was performed on a commercial capillary electrophoresis instrument (Prince Technologies, Emmn, The Netherlands) equipped with an in house-built CL detector [6]. Chemiluminescence buffer was delivered by a syringe pump (MD-1001, BioAnalytical System Inc., W. Lafayette, IN). The new capillary (44 cm effective length, i.d.=50 μm) was rinsed successively with 0.1 mol/L NaOH for 15 min, water for 3 min, 0.1 mol/L HCl for 15 min, then with water for 3 min, and running buffer for 5 min. Between consecutive analysis, the capillary was flushed with 0.1 mol/L NaOH for 2 min, running buffer for 3 min. The sample was introduced by electrokinetic injection with 10 kV for 6 s, and electrophoresis was run at positive polarity under 25 KV.

Results and Discussion

Detection of HRP

Figure 1 Electropherograms of HRP by CE-CL detection



Running buffer: 3.75 mmol/L borate (pH=10.2.); chemiluminescence buffer: 50 mmol/L $NaHCO_3$ (pH=9.0), 7.5×10^{-3} mol/L H_2O_2, 7.5×10^{-4} mol/L Luminol, 1.25×10^{-4} mol/L EDTA ; sample: 5×10^{-11} mol/L HRP; **(A)** in the presence of 6.0×10^{-4} mol/L *para*-iodophenol and **(B)** in the absence of *para*-iodophenol.

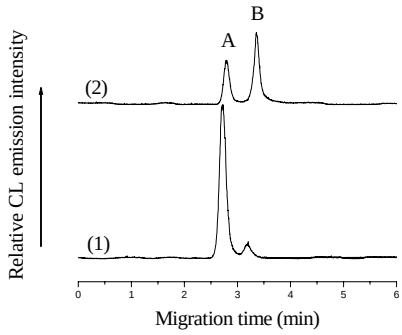
Since HRP was generally used to label antibody or antigen in the immunoassay, firstly, we systematically optimized the chemiluminescence detection system using HRP as a model sample. We found that chemiluminescence signals were associated with the concentrations of reagents (luminol, H_2O_2, *para*-iodophenol), and pHs of reaction and separation buffers. **Figure 1** shows that typical electropherograms of HRP (5×10^{-11} mol/L) in the presence of *para*-iodophenol and in the absence of *para*-iodophenol. We can see *para*-iodophenol markedly enhanced CL intensity in CE-CL system. The detection limit of about 1×10^{-12} mol/L for HRP was achieved, which corresponded to 1.32×10^{-5} U/mL.

Immunoassay of CA125 in sera

CA-125 is an antigen associated with ovarian cancer. The immunoassay protocol was a noncompetitive format. The different concentrations of CA125 standards (Ag) and the HRP-labeled anti-CA125 antibody (Ab*) were added to a microcentrifuge tube (200 μL) and then diluted with water (18.2 MΩ), the solution was incubated at 37 °C for 2 hours. The free antibody and antibody-antigen complex were separated by capillary electrophoresis, some typical electropherograms were shown in **Figure 2**.

The peak A and peak B in **Figure 2** were derived from the free Ab* and Ab*-Ag complex. The area of peak B was used for quantification of CA125, and it was directly proportional to the concentrations of CA125. The regression equation was y=0.0619 +0.00999 C (y: the area of peak B; C: CA125 concentration (U/mL)). The linearity ranged from 0.2 to 50 U/mL (R=0.998). In capillary electrophoresis of proteins, covalently coated or dynamically modified capillaries were normally used in order to eliminate adsorption of protein, however, we found that these modified capillaries were not suitable for our analysis system. Very low or even no signal of the free antibody and antibody-antigen complex were observed using covalent-coated or dynamically modified capillaries. We investigated the electrophoretic migration of HRP and Ab* and Ab*-Ag complexes in alkaline buffer ranging from pH 9.0 to 11, and found that the

Figure 2 Electropherograms of CA125 by CE-CL detection



Concentrations of CA125 (U/mL): (1) 0; (2) 50. Other conditions are the same as **Figure 1 (A)**.

HRP, Ab* and Ab*-Ag complexes were efficiently separated within 4 min and sensitively detected using the borate solution pH 10.2 as separation buffer, as shown in **Figure 2**. In our system, high pH buffer (pH 10.2) was used as running buffer to eliminate adsorption of proteins, and good resolution and reproducibility were achieved.

In order to evaluate the feasibility of the immunoassay method, three female serum samples were analyzed using capillary electrophoresis with enhanced CL detection, and the contents of CA125 were shown in **Table 1**. The recoveries of standard addition experiments were between 96 and 109 %.

Table1 Results detected and recovery of CA125 in serum samples

Sample	Determined value (U/mL)	Average value (U/mL)	Concentration in serum (U/mL)	Added value (U/mL)	Observed value (U/mL)	Recovery (%)
1	1.20	1.225	24.5	2.0	3.47	108
	1.25			2.0	3.52	109
2	1.11	1.065	21.3	2.5	3.51	98
	1.02			2.5	3.41	96
3	0.887	0.899	18.0	2.0	2.88	99
	0.911			2.0	2.94	101

Acknowledgments

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