Chinese Chemical Letters Vol. 13, No. 9, pp 867 – 870, 2002 867
http://www.imm.ac.cn/journal/ccl/html

Electrochemical Determination of Cortisol by Capillary Electrophoretic Enzyme Immunoassay

Min JIA, Wen Rui JIN*

School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100

Abstract: An electrochemical method for detection of cortisol based on capillary electrophoretic enzyme immunoassay has been developed. A limit of detection of 1.7×10^{-9} mol/L was obtained.

Keywords: Capillary electrophoretic enzyme immunoassay, electrochemical detection, cortisol.

In recent years, capillary electrophoretic immunoassay (CEIA) has become a primary analytical tool in clinical diagnostics. The primary detection methods in CEIA are UV absorbance detection and laser induced fluorescence detection (LIF)[1]. The major disadvantages of the UV detector are the lack of sensitivity and not available for many antigens which are small molecules without strong UV absorbance. LIF is a more general approach to improve sensitivity. However, it is difficult to use most laser instruments because that their cost is high, complex maintenance is needed, and most biological fluids are strongly luminescent when excited by the laser in the blue or green region of the spectrum.

In this paper, we developed a novel capillary electrophoretic enzyme immunoassay with electrochemical detection (CE-EIA-ED) for determination of cortisol. In the assay, 3,3,5,5-tetramethyl-benzidine (TMB(Red)) and H_2O_2 are selected as the substrate of the labeled enzyme (horseradish peroxidase, HRP). The immunoassay is a competitive format in which the labeled cortisol competes with the cortisol in the sample for the limited number of anti-cortisol binding sites. After equilibrium is established, 6.00 nL of the incubate containing the free and bound HRP labeled cortisol is injected into the separation capillary. Then they are separated in the separation capillary and catalyze the substrate in the on-line catalytic reactor. The product (TMB(Ox)) of the reaction from the reactor is amperometrically detected on the carbon fiber microdisk bundle electrode.

Apparatus

A high-voltage power supply Model 9323-HVPS, Beijing New Technology Institute,

*E-mail: wenrujin@jn-public.sd.cninfo.net

Beijing, China) provided a variable voltage of 0-30 kV across the separation capillary with its outlet at ground potential. Poly(vinyl alcohol)-coated fused-silica capillaries (50 μm ID, 375 μm OD) were purchased from Hewlett-Packard Instruments. They were cut to a length of 20 cm as the separation capillaries and a length of 5 cm as the reaction capillaries. A high voltage of 10 kV was applied at the injection end. The products of the enzymatic catalysis reaction were introduced hydrodynamically into the reaction capillary. The electrochemical detection was performed at the outlet of the reaction capillary using the end-capillary amperometric approach with an electrochemical analyzer (Model CHI800, CH Instruments, Austin, TX, USA). The reaction capillary and the detection cell were housed in a Faradaic cage in order to minimize the interference from external sources of noise. The detection was carried out with a three-electrode system.

Procedure

For the immunoreaction, 25 μL of the cortisol standard or control, 2 μL of HRP-cortisol and 100 μL of rabbit anti-cortisol antiserum were added to a 0.6 mL microcentrifuge tube. The solution was incubated for 30 minutes at room temperature and was diluted at a ratio of 1 part solution into 1 part running buffer before injection. In the assay, samples were introduced hydrodynamically. After injection, the separation capillary was inserted into the running buffer reservoir. The enzyme substrate was introduced into the reaction capillary, which followed the separation capillary, at the same time. Then the separation high voltage was applied across the separation capillary and the detection potential was applied at the working electrode and the electropherogram was recorded. The procedure of electrochemical detection used here was similar to our previous description[2].

Results and Discussion

The optimum conditions of the method are 1.00×10^{-2} mol/L Na_2HPO_4 + 5.00×10^{-3} mol/L citrate phosphate + 2.50×10^{-3} mol/L H_2O_2 (pH 5.0) for the separation buffer, 2.00×10^{-2} mol/L Na_2HPO_4+ 1.00×10^{-2} mol/L citrate phosphate (pH 5.0) for the reaction buffer, 4.0×10^{-4} mol/L TMB(Red) for the substrate solution, 10 kV for the separation voltage, 0.00 mV (*vs*. SCE) for the detection potential. The electropherogram of HRP-cortisol is shown in **Figure. 1**. The typical electropherograms of the human serum without and with 60.0 μg/L standard cortisol are shown in **Figure 2**. Two peaks appear at 318 s and 350 s, respectively. Compared with **Figure 1**, peak 1 can be identified as peak of free HRP-cortisol because they had the same migration time. With the increase in the amount of cortisol, the area of peak 1 increases, while the area of the peak 2 decreases (see curve 2), which indicates the principle expected for a competitive immunoassay: an increase in the amount of cortisol standard leads to a decrease in signal for the bound HRP-cortisol and an increase in signal for the free HRP-cortisol. Thus,

peak 1 corresponds to the free HRP-cortisol and peak 2 corresponds to the bound HRP-cortisol. Since the peak 2 broadens, the peak 1 was used to establish the calibration curve in the range of 0.0 to 60.0 μg/L. The limit of detection for cortisol reaches 1.7×10^{-9} mol/L due to enzyme amplification, which is lower than that of CEIA with LIF detection method[3]. To verify the method, two cortisol controls (DSL-10-2000) from Diagnostic Systems Laboratories, Inc., Webster, TX, USA were determined. The results are listed in **Table 1**. The concentrations of cortisol in the two controls obtained by calibration curve method are 5.1 μg/L and 24 μg/L, respectively, which agree with the values (4.0±1.5 and 20±5 μg/L) given by the commercial company.

Figure 1 Electropherogram of 1.5% HRP-cortisol.

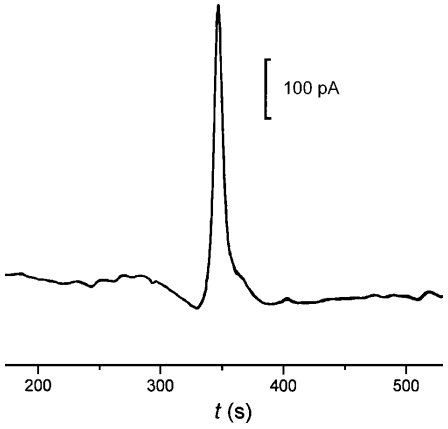

Figure 2 Electropherograms of the human serum (1) without and (2) with 60.0 μg/L cortisol.

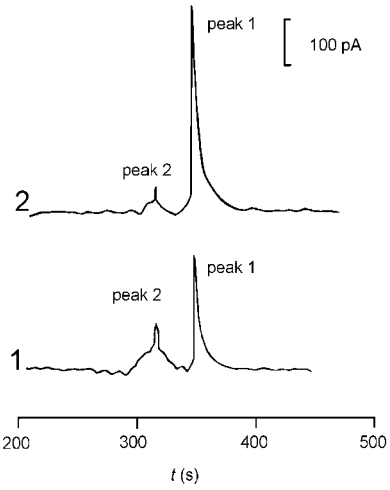

Table 1 The results detected and recovery of the cortisol controls.

Control	Determined value (μg/L)	Average value (μg/L)	Stated value (μg/L)	Added value (μg/L)	Observed value (μg/L)	Recovery (%)
I	5.4			1.5	6.8	93
	4.8	5.1	4.0±1.5	4.0	8.7	98
	5.1			10.0	15.5	104
II	23.5			10.0	35.1	116
	22.9	23.8	20±5.0	20.0	42.2	96
	25.0			40.0	62.9	95

Acknowledgment

This project was supported by the National Natural Science Foundation of China, the Natural Science Foundation of Shandong Province and the State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Science.

References

1. M. J. Gordon, X. Huang, S. L. Pentoney, R. N. Zare, *Science*, **1988**, *242*, 224.
2. W. Jin, D. Yu, Q. Dong, X. Ye, *Electrophoresis*, **2000**, *21*, 925.
3. J. J. Bao, *J. Chromagogr. B*, **1997**, *699*, 463.

Received 13 December, 2001