Chinese Chemical Letters Vol. 15, No. 2, pp 212 – 213, 2004
http://www.imm.ac.cn/journal/ccl.html

Electrochemical Detection of Alkaline Phosphatase in BALB/c Mouse Fetal Liver Stromal Cells with Capillary Electrophoresis

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Abstract: A method for determination of alkaline phosphatase (ALP) in BALB/c mouse fetal liver stromal cells has been described based on the catalytic reaction. After the cell extract is incubated with the substrate disodium phenyl phosphate, the reaction product phenol generated by ALP is determined by capillary electrophoresis with electrochemical detection.

Keywords: Alkaline phosphatase, capillary electrophoresis, electrochemical detection.

Alkaline phosphatase (ALP) is an important enzyme for clinical chemistry and an indicator of liver function[1]. Besides colorimetry, fluorimetry and chemiluminescence, electrochemical detection (ED) has been used to determine ALP[2-4]. In order to decrease the mass limit of detection (LOD), capillary electrophoresis (CE) has been used for determination of ALP. Wu and Regnier[5] used CE with UV detection to measure ALP with a mass LOD of 5.2×10^{-20} mol. In the present work, we developed a more sensitive method for determination of ALP. In this method, ALP catalyzed the conversion of an electroinactive substrate disodium phenyl phosphate (DPP) to an electroactive product phenol. ALP could be determined through measuring the generated phenol by CE with electrochemical detection (ED). CE-ED system used in this work was similar to that described previously[6]. The linear range of activity was 3.0×10^{-4}-3.0×10^{-1} U/mL with a correlation coefficient of 0.995. The activity LOD and concentration LOD (*S/N*=3) were 1.0×10^{-4} U/mL and 2.4×10^{-13} mol/L, respectively, which corresponded to the mass LOD of 3.6×10^{-22} mol according to its injected volume of 1.5 nL. The relative standard deviation of the method is 5.2% for a series of six injections of 3.0×10^{-3} U/mL ALP.

The cell extract was prepared by lysing the BALB/c mouse fetal liver stromal (BMFLS) cells using an ultrasonicator. After the cell extract was reacted with DPP for 30 min, the solution was injected into the capillary and the product phenol was detected.

The elctropherograms of phenol and the standard ALP are shown in **Figure 1.** It could be found that phenol produced a peak at 479 s (curve 1). When the standard ALP was incubated for 30 min with DPP, the peak of phenol also appeared (curve 2). Other chemical species, which can be oxidized at the working electrode, can be separated well

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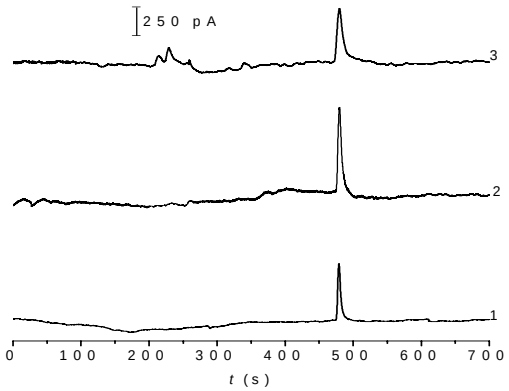
from phenol. Therefore, they did not interfere with the determination of ALP. The electropherogram of the BMFLS cell extract is shown in **Figure 1**. Phenol generated by ALP can be detected (curve 3). ALP in the cell extract can be quantified by the calibration curve. Based on the cell amount counted before analysis, the activity of one BMFLS cell could be calculated to be 4.4×10^{-9} U. The recovery is between 95% and 108%. This is the first report about the ALP activity in BMFLS cells.

Figure 1 The electropherograms of (1) phenol, (2) standard ALP and (3) BMFLS cell extract.



5.0×10^{-2} mol/L $Na_2B_4O_7$-3.0×10^{-2} mol/L NaCl, (pH=9.8), incubation time: 30 min.

Acknowledgment

This project was supported by the National Natural Science Foundation of China (No. 20235010).

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Received 20 January, 2003