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RESEARCH PAPER

Colorimetric Detection of Alkaline Phosphatase on Microfluidic Paper-based Analysis Devices

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Abstract: A low-cost, simple and sensitive colorimetric detection method for alkaline phosphatase (ALP) was developed on microfluidic paper-based analysis devices. In a typical colorimetric detection, 5-bromo-4-chloro-3-indolyl phosphate disodium salt (BCIP) and nitro blue tetrazolium (NBT) were firstly added onto the circle array zones of paper devices. Then ALP solutions were spotted to the array zones to perform the colorimetric reaction. The color reaction results were recorded by both Gel Documentation systems and a common digital camera, and analyzed with Quantity One software. All the reaction conditions were optimized. Under the optimal conditions, the colorimetric intensity showed a linear correlation to the concentration of ALP in the range of 1.5–20 U L^{-1} with a limit of detection (LOD) of 0.78 U L^{-1} (3σ), which was about two orders of magnitude lower than that of the reported method on μPADs. Besides, it was applied in the analysis of spiked real sample with a satisfactory result. In addition, the dual-color array-based paper strip was fabricated for the semiquantitative detection of ALP. The approximate activity of ALP could be simply distinguished by the color change with naked eyes.

Key Words: Microfluidic paper-based analysis devices; Colorimetric detection; Alkaline phosphatase

1 Introduction

Alkaline phosphatase (ALP) is a kind of enzyme catalyzing the hydrolysis of monophosphate esters. It exists in a wide variety of organisms, from bacteria to mammals[1]. Serum ALP activity is a common-used indicator of many diseases, such as hepatitis, liver dysfunction, cirrhosis, bone disease, endocrine disease, tumors and other disorders[2,3]. ALP is also a frequently-used labeling enzyme in enzyme immunoassay[4]. Significant research endeavor has been made for the detection of serum ALP. Traditional detection methods include colorimetry[5], electrochemistry[6], fluorescence[7], chemiluminescence[8] and surface enhanced Raman scattering (SERS) method[9]. However, these methods often require external instruments, electricity, and complicated manipulation.

Recently, as a new point-of-care testing system, microfluidic paper-based analytical device (μPADs) provides a promising platform for biochemical assays with the advantages of low cost, portablility, rapidness, simplicity, high throughput, and low consumption of samples[10–13]. Also, great efforts have been made on fabrication and functionality of μPADs[14–18]. As a suitable platform for bioassays, the μPADs combined well with colorimetric method as the direct outcome could be recorded by a simple camera. Colorimetric detection of ALP on μPADs offers a low-cost, rapid and simple way to help reduce the burden of health system and bring benefits in resource-limited areas.

In recent years, the color reaction of ALP has been introduced on μPADs because it produces an easy-observed

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color change from light yellow to dark purple. Li *et al*[19] utilized the 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium (BCIP/NBT) substrate system with ALP to evaluate the capability of performing analytical tests on plasma treated paper chips. Cheng *et al*[20] employed ALP-conjugated antibodies to demonstrate ELISA on paper-based devices for the detection of IgG and human HIV-1 antigen. Whitesides' group[21] fabricated a convenient micropatterned paper device containing a plasma separation membrane for the measurement of markers of liver disease, such as aspartate aminotransferase (AST), ALP, and total serum protein. Exponential calibration curves were obtained in artificial blood plasma solutions and ALP was detected with a color response in spiked whole blood. But the actual concentrations of the analyte were not measured. Besides, the detection limit of ALP was about 15 U L^{-1} only. So the improvement of sensitivity on μPADs is remarkably important and urgently needed.

In present work, a simple, rapid and high sensitive detection method for ALP was developed on μPADs and a dual-color paper test strip for semi-quantitative detection of ALP was fabricated. BCIP/NBT substrate system was used here because it produced a color change (from light yellow to dark purple) with strong contrast against the white background color of papers, showing an advantage over the conventional *p*-nitrophenyl phosphate substrate (with a color change from colorless to yellow). In an effort to improve the sensitivity, a drying method was used to reduce the background signal as described in our previous work[22]. Furthermore, human serum albumin (HSA) was used as a color enhancement reagent to further improve the sensitivity. It was found that the color signal was enhanced significantly when the ALP solutions were added with low concentration of HSA. Inspired by this, the inherently existing HSA in human serum was utilized in the detection of ALP, and real human samples were spiked and diluted for detection. In this way, the sensitivity for ALP detection was remarkably improved. On the other hand, a dual-color paper test strip was fabricated on μPADs which could be used for semi-quantitation of ALP by the naked eyes without any external equipment. In addition, real human serum samples were detected and the results were comparable with the clinical test results.

2 Experimental

2.1 Instruments and reagents

G-17 lithography machine (Chengdu Xin'nan Co. Ltd., China), PDC-M plasma cleaning machine (Chengdu Mingheng Scientific Instrument Co. Ltd., China), KW-4H-350 hot plate (Shanghai KMT Functional Ceramics Co. Ltd., China) were used in the fabrication of paper devices. HZQ-F160 vertical full-temperature incubator and DHG-9055 electric blast oven from Shanghai Yiheng Scientific Instrument Co. Ltd. were used to accelerate the solvent evaporation rate. Images were acquired by a ChemiDoc XRD system (Bio-Rad) or a digital camera (Nikon COOLPIX 5400). S25 Vortex (IKA) was used in the preparation of solutions. Ultra-pure water was prepared by a Milli-Q system (Millipore, Bedford, MA, USA).

Calf intestinal ALP was purchased from TaKaRa Biotechnology Co. Ltd. (Dalian, China). 5-Bromo-4-chloro-3-indolyl phosphate disodium salt (BCIP) was purchased from TCI Co. (Japan). Nitro blue tetrazolium (NBT) was purchased from JingKeHongDa Biotechnology Co. Ltd. (China). NaCl, $MgCl_2·6H_2O$ and tris(hydroxymethyl) aminomethane (Tris) were from Sigma Chemical Co. (USA). HCl was purchased from Xinyang Chemical Co. (China). Human serum albumin (HSA) was purchased from Biosharp Co. (Korea). Bovine serum albumin (BSA) was purchased from Wenhan Technology Co. (China). Glucose oxidase (GOD), trypsin and lysozyme were purchased from Amresco (USA). Horseradish peroxidase (HRP) was purchased from Boster (China). Papain was purchased from Jiehui Biotechnology Co. Ltd. (Kunming, China). SU-8 2010 photoresist was from MicroChem Corp. (USA). TX 609 Hitecloth was from Shenzhen Qing Gao Co. Ltd. (China). The fresh human serum samples were provided by Zhongnan Hospital of Wuhan University, and determined with a standard colorimetric test kit (4-nitrophenyl phosphate disodium salt, 2-amin-2-methyl-1-propanol method, Ningbo Rui Bio-technology Co. Ltd., China) as the reference values. All reagents were used as received.

2.2 Design strategy of microfluidic paper-based analytical devices

Photolithography was chosen to pattern the paper. The detailed procedures were as described in our previous work[22,23]. One-dimensional array paper devices were used here for the detection of ALP due to simplicity and exact control of volume. The diameter of circle detection zone was 2.5 mm, and 3 × 9 detection zones were on one piece of paper chip.

2.3 Preparation of detection reagents

BCIP/NBT substrate was used as the chromogenic indicator. The detection solution was composed of BCIP, NBT, $MgCl_2$, and NaCl which were dissolved in Tris-HCl buffer (0.1 M, pH 9.5). All the solutions should be kept away from light and stored at 4 °C. The detection reagents were freshly prepared every time and used immediately.

2.4 Detection procedure of ALP

2.4.1 Detection of ALP in buffer solutions

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