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A New Voltammetric Enzyme Immunoassay System for the Detection of Alkaline Phosphatase

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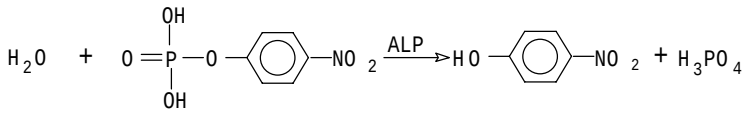
Abstract: A new voltammetric enzyme immunoassay system was investigated based on *p*-nitrophenyl phosphate (PNPP) as the substrate for alkaline phosphatase (ALP). PNPP is enzymatically hydrolyzed and the product *p*-nitrophenol (PNP) is detected by differential pulse voltammetry (DPV), which can be oxidized at +1.02 V (*vs.* Ag/AgCl) on bare glass carbon electrode (GCE). The conditions for enzymatic reaction and electrochemical detection were studied. According to this method, ALP can be detected with a detection limit of 2.8×10^2 mU/L and a linear range of $4.0\times10^2 \sim 1.0\times10^6$ mU/L.

Keywords: Alkaline phosphatase, *p*-nitrophenyl phosphate, *p*-nitrophenol, differential pulse voltammetry, voltammetric enzyme immunoassay.

Enzymes are widely used as labels in immunoassay and especially useful at low concentrations of the analyte in the sample. Most routine detection methods are based on the formation of color or fluorescent products, which are determined by spectrophotometry or fluorometry, respectively. Electrochemical detection has been applied to the enzyme immunoassay with the potentials of combining the low detection limits and good selectivity of electroanalytical chemistry with the specificity of immunoassay[1-2]. ALP is often used as an enzyme label in enzyme-linked immunosorbent assay (ELISA). It can catalyze the hydrolysis of orthophosphoric monoesters, delivering phosphate and corresponding alcohols[3]. If the phosphoric ester is electroinactive while the enzymatic product is electroactive in the working potential range, electrochemical methods can be used for detection[4-5].

In this paper a new system for the determination of ALP activity is induced into electrochemical immunoassay with PNPP as the substrate of ALP. The ALP-catalyzed reaction process is shown as follows:



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PNP, produced from ALP catalyzed-hydrolysis of PNPP, is electroactive and has an oxidizing peak at +1.02 V (*vs.* Ag/AgCl) in 0.2 mol/L Britton-Robinson (B-R) (pH 3.0) buffer solution on a bare GCE by DPV, while PNPP is electroinactive at this potential, so PNPP is inert in the potential region suitable for PNP detection. On the basis of this principle, this new system can be applied to the ALP-labeled immunoassay.

The electrochemical behavior of PNP on bare GCE was studied. The cyclic voltammetry of PNP shows an oxidation peak without corresponding reductive peak, so the process is chemically irreversible. The pH effect of supporting electrolyte on the electrochemical response of PNP was checked and in 0.2 mol/L B-R buffer (pH 3.0) is the best condition. The stability of PNP at pH 3.0 was measured and the oxidative peak remains stable within 24 hours. The relationship of pH with peak potential is a straight line with a slope of –0.052 in the pH range of 2.0 ~ 8.0, which implies that there is hydrogen ion participating in the oxidation reaction. The detection of PNP with DPV shows a high sensitivity, good reproducibility and wide measurable range. At scanning rate of 60 mV/s, a measurement time of 13 s was obtained when the potential range of the measurement was from 0.6 V to 1.40 V. The linear range of the calibration curve for the detection of PNP was from 4.0×10^{-6} to 6.0×10^{-4} mol/L. Comparing with often used FIA or HPLC, the DPV technique requires simple apparatus. It would be a choice for enzyme activity assessment.

The reaction conditions for ALP hydrolysis of PNPP were optimized. The pH 10.4 carbonate buffer was chosen as the enzymatic reaction medium, which is in accordance with the nature of ALP. Under the selected optimal conditions, as expected the peak current increased with the ALP concentration. After 30 min reaction, 0.2 mol/L B-R (pH 3.0) was added in order to stop the enzymatic reaction and to carry out the electrochemical detection. ALP can be determined in the concentration range from 4.0×10^{2} to 1.0×10^{6} mU/L. The relative standard deviation (RSD) for eleven determinations of 6.0×10^{2} mU/L ALP was 9.68%, with the detection limit of 2.8×10^{2} mU/L.

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