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Short communication

Indirect determination of pyruvic acid by capillary electrophoresis with amperometric detection

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Abstract

A method of indirectly measuring pyruvic acid (PA) by capillary electrophoresis with amperometric detection is proposed for the first time. It is based on the oximation reaction between PA and hydroxylamine (NH_2OH), and the quantification of PA was performed by direct and sensitive amperometric detection of excessive NH_2OH after the oximation reaction. This method displayed a good sensitivity, and the detection limits of NH_2OH and PA are 1.76×10^{-7} and 3.88×10^{-7} mol/L, respectively at S/N = 3. The linear relationship between the peak current and PA concentration is exhibited over the range from 4×10^{-6} to 1×10^{-4} mol/L. This method has been applied to determine PA in rat plasma with satisfactory results.

Keywords: Capillary electrophoresis; Amperometric detection; Carbon fiber microelectrode; Hydroxylamine; Pyruvic acid

1. Introduction

Pyruvate is an important chemical compound in biochemistry. It is the output of the metabolism of glucose known as glycolysis, and an intermediate compound in the metabolism of carbohydrates, proteins, and fats [1–3]. Exceptional change of pyruvic acid (PA) concentration in the body could influence health. The determination of PA can give valuable information as to the process of metabolism and reactions in clinic analysis and bioanalysis [4–6].

Capillary electrophoresis (CE) has become a powerful tool for rapid and automated analysis of complex mixtures in biological samples [7–10], but there were only a few reports on CE for PA detection. Simonet et al. [11] has measured pyruvate according to the absorptivity decrease based on the pyruvate/lactate dehydrogenase/β-NADH reaction systems. Yeung's group has indirectly determined lactate and pyruvate in single erythrocytes by capillary electrophoresis with fluorescence detection [12]. Compared with the optical detection, CE-amperometric detection (CE-AD) was proved to be a highly sensitive and simple method for most of electrochemical compounds [13–16], but pyruvate itself has no absolute redox peak appearing in its voltammograms at −0.2–1.0 V. An approach for electrochemical detection of PA was performed by using enzyme biosensors [17–19], but the instability and relative low anti-disturb performance of the biosensors confine their applications in real sample detections. Another approach for direct detection of PA has been achieved by oxidizing PA at a high potential, and the detection limit is 8.0×10^{-6} mol/L at 1.6 V [20]. But high current noise and unstable current resulted from high working potential would also restrict the detection sensitivity and selectivity.

The indirect electrochemical determination methods of PA can be developed based on detecting the electroactive reactants or products associated with the PA chemical reactions. By oximation reaction that has been widely used to measure the content of carboxyl [21], PA can react with NH_2OH and forms oxime derivative, PA could be measured by detection of the electroactive compounds, hydroxylamine (NH_2OH) or oxime derivative. Similar principle has been applied by Yu et al. to measuring ρ-tyrosine aminotransferase with HPLC-AD by detecting the oxime derivative [22]. However the approach of PA measurement based on NH_2OH detection has not been reported.

In this study, we have developed, for the first time, a method of indirect measuring PA based on the concentration change of NH_2OH which was sensitively and accurately detected by

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CE-AD. There exists a perfectly linear relationship between the peak current and NH_2OH concentration ranged from 4×10^{-6} to 1×10^{-4} mol/L, and the detection limit is 1.76×10^{-7} mol/L. PA concentration has been investigated by monitoring the excessive NH_2OH concentration, this method exhibits a higher sensitivity and the detection limit of PA is 3.88×10^{-7} mol/L. The PA in rat plasma was measured by this method and the results showed good agreements with the value previously reported.

2. Materials and methods

2.1. Reagents and solutions

All chemicals were of analytical reagent grade and used as received without further purification. PA was prepared by dilution of 100 mM stock solution obtained by dissolving sodium pyruvate (biochemical reagent, Sinopharm group chemical reagent Co., Ltd., Shanghai, China) in 0.1 mol/L HCl. Hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, analytical pure, Shanghai No. 4 reagent & H.V. chemical Co., Ltd. Shanghai, China) was prepared by dilution of 100 mM stock solution with the buffer solution. The standard solution of both PA and NH_2OH were daily prepared before using. All electrochemical and CE experiments were conducted in the pH 8.0 phosphate buffer saline (PBS) solution. All solutions were prepared with ultrapure water and sterilized by filtering through 0.2 μm membrane filter units.

2.2. Apparatus

The laboratory-built capillary electrophoresis-electrochemical detection (CE-ED) system used for the experiments was similar to our group's previously described [23]. High voltage for the CE experiments was supplied by a +30 kV high voltage power source (Shanghai Institute of Nuclear Research, Shanghai, China), a fused-silica capillary (25 μm i.d., 360 μm o.d., length 65–70 cm, Yongnian Optical Fiber Factory, Hebei, China) was used throughout the CE experiments. The high voltage was applied in the injection end, while the reservoir containing electrochemical detection system was held at ground potential.

The end-column amperometric detection was performed by a two-electrode configuration. A carbon fiber microelelctrode (CFME) with diameter of 8 μm and exposed length of 100–200 μm, which was prepared as described previously [24], was employed as the working electrode, a SCE and Ag/AgCl electrode worked as reference electrode in end-column amperometric detection and cyclic voltammetry, respectively. The working electrode was inserted into the end capillary about 5 μm deep with the aid of a micromanipulator. All electrochemical detections were performed with a CHI660A elelctrochemical workstation (CH Instruments, Shanghai, China) in conjunction with a computer. The system was enclosed in a copper mesh Faraday cage to minimize the external noise.

2.3. Sample preparation

1 mL blood sampled from a rat that had not been fed for 8 h was then put into a 1 mL centrifugal tube before adding 15 μL 4% EDTA anticoagulate. Shake it to well distribution, quickly cool it in ice water (0 °C) for 15 min, and spin in a centrifuge at 2000 rpm for 15 min, supernatant liquid (the plasma) was collected into another centrifugal tube and stored in the refrigerator at −18 °C. The plasma was put into ice water prior to being added in the NH_2OH solution for oximation reaction.

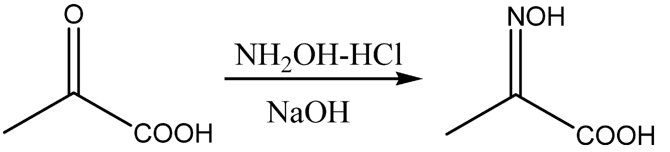
2.4. Electrophoresis method

New separation capillary was treated with 0.1 mol/L NaOH, 0.1 mol/L HCl and doubly distilled water in sequence prior to use and balanced with electrophoresis buffer overnight, the capillary was washed with buffer during every run. Injection was performed electrokinetically at 15 kV for 8 s; separations were carried out at an applied voltage of 15 kV.

3. Results and discussions

3.1. Reaction mechanism

PA (CH_3COCO_2H) is an alpha-keto acid and its carboxylate anion is known as pyruvate. PA can form the oxime derivative as the reaction scheme below:



From the chemical equation above, PA can react with NH_2OH, a compound that can be directly electrochemically detected, to form oxime as the product. The PA could be quantified by CE separation followed by amperometric detection of the excessive NH_2OH, and the indirect methods of measuring PA could therefore be developed.

3.2. Detection of NH_2OH

3.2.1. Detection potential of NH_2OH

Detection of NH_2OH is very important, since NH_2OH is not only the key reactant but also the final detected reagent in the whole process of this method. No obvious oxidation peak at carbon fiber microelectrode (CFME) was observed from the cyclic voltammetry of NH_2OH in the potential range from −0.20 to +1.60 V (Fig. 1). The current shows an increase caused by the NH_2OH oxidation on the CFME when the applied potential above 0.30 V, and the oxidation current increases rapidly with the positive shift of applied potential from 0.60 V, until the sharp rising of the current from the oxygen evolution when the potential is over 1.40 V. The electrophoresis peak current of NH_2OH at different working potential ranging from 0.60 to 1.40 V were investigated and the optimum detection performance was obtained at the voltage of 1.2 V with a compromise between high sensitivity and low background current.