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Determination of Amino Acids in Single Human Lymphocytes after On-capillary Derivatization by Capillary Zone Electrophoresis with Electrochemical Detection

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Abstract: Amino acids in individual human lymphocytes were determined by capillary zone electrophoresis with electrochemical detection after on–capillary derivatization. In order to inject cells easily, a cell injector was designed. Four amino acids (serine, alanine, taurine, and glycine) in single human lymphocytes have been identified. Quantitation has been accomplished through the use of calibration curves.

Keywords: Capillary electrophoresis, electrochemical detection, amino acid, lymphocyte.

Sensitive and selective methods for the detection of amino acids (AAs) in single cells are of increasing importance. Capillary zone electrophoresis (CZE) with electrochemical detection (ED) is suitable for analysis of electroactive contents in single cells. However, most native AAs have no electroactivity. Derivatization with an electroactive tag is an attractive method. An on-capillary derivatization scheme was reported[1]. Naphthalene-2, 3-dicarboxaldehyde (NDA) can react with primary amines in the presence of cyanide to produce electroactive products. The electroactivity of the NDA-derivatives has been used to detect five AAs in a giant dopamine neuron of the snail (approximately 75 μm in size) by CZE with ED after on-capillary derivatization with NDA[2].

In this work, AAs in smaller single cells (human lymphocytes with *ca*. 7-18 μm in size) were analyzed by CZE-ED with on-capillary NDA-derivatization. Because the concentration of cell solution is more dilute, injecting single cells is difficult. The injection of a cell takes over 30-60 min or longer. For overcoming this difficulty, we designed a cell injector. Using it, a single cell was introduced easily. **Figure 1** shows the cell injector. A Plexiglas plate (1) (2 cm×1.5 cm×3 mm) with a round hole (2) of 2.5 mm in diameter was placed on a microscope slide (3). The plate had been machined to contain a small groove (around the hole) just the right size to accommodate a metal tubing (4) (400 μm ID, 680 μm OD). A hole was machined through the metal tubing with a diameter of 400 μm. Since both the hole of the metal tubing and the hole of the Plexiglas plate were concentric, the hole of the metal tubing can be observed under a microscope. The separation capillary (5) and the injection capillary (6) were inserted

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into the metal tubing from two ends. In order to see the opening of the injection end, a 5 mm section of the polyimide-coating at the injection end of the separation capillary was removed by burning. The distance between the two capillaries was about 5 μm. In order to insert the end of injection capillary outside the metal tubing into the small end of a trumpet-shaped glass tube for injecting cells (7) with a diameter of *ca*. 0.8 cm at the large end and a 375 μm ID, 800 μm OD at the small end, this end of injection capillary was etched to *ca*. 350 μm OD by 40% HF. The interface between the injection capillary (6) and the glass tube for injecting cells (7) was sealed by epoxy resin. There was a buffer reservoir (8) at the right corner of the microscope slide. In it an anode (9) and a Pt wire (10) were inserted, to inject the cells electrokineticly. The cell injector was placed on the inverted microscope. The separation capillary, the injection capillary and the buffer reservoir with an anode were filled with electrophoresis buffer. The other end of the separation capillary was inserted into a buffer reservoir (not shown in **Figure 1**) with a cathode. The lymphocyte suspension was transferred into the glass tube for injecting cells. When a cell appeared between the separation capillary and the injection capillary, an injection voltage of 2.0 kV was applied to transport the whole cell into the separation capillary tip. Once one cell was injected, the anode was manipulated up, out of the buffer reservoir. Then, the injection end of the separation capillary was taken out the cell injector. The cell injector on the inverted microscope was replaced by a microscope slide with a drop of lysing/derivatizing buffer (*ca*. 50 μL) used to lyse and derivatize the lymphocyte. The injection end of the separation capillary was gently immersed in the drop of the lysing/derivatizing buffer. The electromigration injection of this buffer at 2.0 kV for 10 s was carried out. In order to lyse and derivatize the lymphocyte, the lymphocyte was incubated for 20 min. Then the separation capillary was carefully immersed to a reservoir containing electrophoresis buffer with an anode for electrophoretic separation. The apparatus, the experimental procedure and the electrochemical detection are the same as in Ref. 3.

Figure 2 shows the electropherograms of the standard AAs and a lymphocyte. The relative standard deviations of the method for determining these standard AAs are between 0.80~2.3% for migration time and 1.4~6.4% for peak current, respectively. Four electrophoretic peaks in the single cell can be identified to be Ser, Ala, Tau and Gly on the basis of their migration times. In addition, three unknown peaks noted as U and a peak of the mixture of AAs noted as AA appear in its electropherogram. Based on their migration times, amino acids should not be responsible for these peaks. The external standardization can be used to quantification of AAs. AAs in 10 cells were analyzed. The electrophoretic peaks of Ser, Ala, Gly and Tau can be observed only in 4, 5, 8 and 10 cells, respectively. The amounts of Gly and Tau in the 10 cells are 0.43 and 2.5 fmol, respectively. The value of Gly is higher than 0.32 fmol and the value of Tau is lower than 4.1 fmol obtained from analyzing cell extract. If more cells are analyzed, the values from analyzing single cell and analyzing cell extract can be closer.

Figure 1 Schematic diagram of the cell injector

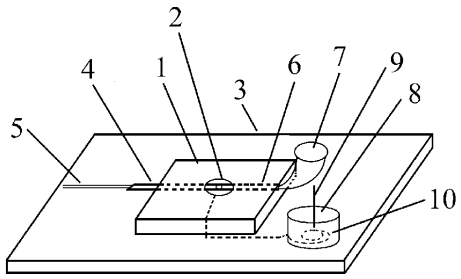
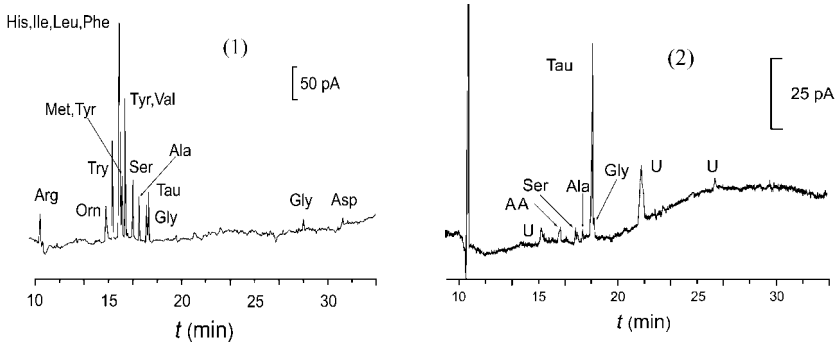


Figure 2 Electropherograms of (1) the standard AAs and (2) a lymphocyte



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