Chinese Chemical Letters Vol. 14, No. 9, pp 952 – 954, 2003
http://www.imm.ac.cn/journal/ccl.html

Determination of Amino Acids in an Individual Erythrocyteby Capillary Electrophoresis with Intracellular FITC-derivatization and Laser-induced Fluorescence Detection

Hua ZHANG [1], Wen Rui JIN [2]*

[1] State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100
[2] School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100

Abstract: A novel approach for analysis of amino acids in individual erythrocytes was established. In this method, the derivatization reagent was introduced into the living cells by electroporation. After derivatization, the amino acids in a single cell were determined by capillary electrophoresis with laser-induced fluorescence detection.

Keywords: Capillary electrophoresis, laser-induced fluorescence detection, single cell analysis, amino acid.

The ability to accurately determine the amino acids (AAs) in individual cells is important in the field of biochemistry. AAs have to be derivatized before determination because of lacking native fluorescent properties. The obstacle is minimizing their dilution during derivatizaion of amino acids in a single cell. On–column derivatizaion reported is effective for reducing the dilution[1]. However, the dilution factor is still high (*ca.* 100). Using this method, AAs in a PC12 cell with *ca.* 20 μm in size were quantitated. In our previous work[2], we developed a method of intracellular derivatization, in which a dilution factor of *ca.* 1.6 was achieved. However, AAs in a single erythrocyte with a diameter of *ca.* 8 μm could not be detected because an electrochemical detection with low sensitivity was used.

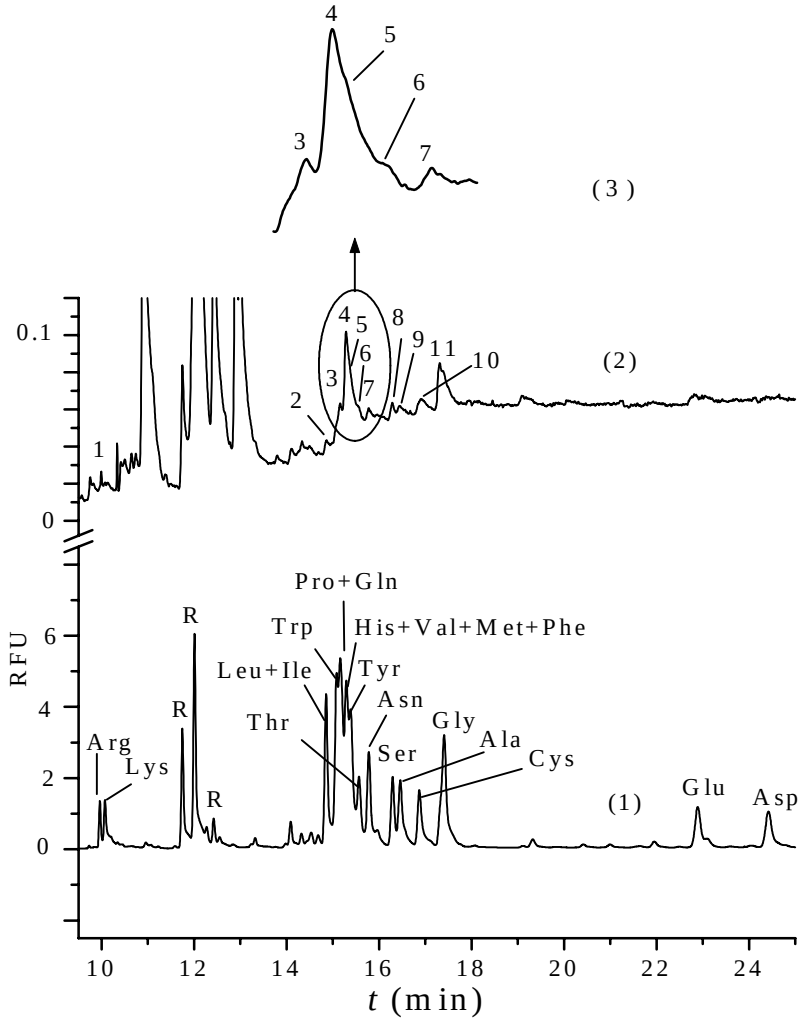
In the present work, we used a sensitive laser-induced fluorescence (LIF) detection for determination of AAs in single erythrocytes with capillary electrophoresis (CE) and the intracellular derivatization. In this method, a derivatizaion reagent, fluorescein isothiocyanate (FITC), was introduced into cells by electroporation described in Ref. 3. After derivatization for 42 hour, a cell was transferred into the capillary. Then 0.1 mol/L NaOH as the lysing solution was dynamically introduced into the capillary.

Once the cell was lysed, the derivatized AAs released from the cell were separated by CE and detected by LIF. CE-LIF was performed using a CE system (P/ACE MDQ, Beckman Coulter, Fullerton CA, USA).

* E-mail: wenrujin@jn-public.sd.cninfo.net

A typical electropherogram of AAs in an erythrocyte is shown in **Figure 1** (curve 2), and peaks 3-7 in the electropherogram are magnified and shown in **Figure 1** (curve 3). Peak identification was carried out through comparison with the standard AAs electropherogram (curve 1). It was found that Lys (peak 1), Trp (peak 3), Tyr (peak 5),

Figure 1 (1) Electropherogram of 20 standard AAs, (2) Electropherogram of one erythrocyte with intracellular derivatization, (3) Amplification of a part of curve 2.



R is a derivatization reagent. 25×10^{-2} mol/L borax-1.25×10^{-2} mol/L NaOH-1.20×10^{-4} mol/L spermine; Capillary, 60.2 cm×25 μm I.D., Effective length, 50 cm; Coolant tubing, 42.8 cm; Separation voltage, 25 kV; Coolant, 25 ℃.

Thr (peak 6), Asn (peak 7), Ser (peak 8), Ala (peak 9), Cys (peak 10) and Gly (peak 11)

in an erythrocyte could be detected. However, identification of peak 2 and peak 4 was difficult because more than one AA had the same migration time as that of peak 2 or peak 4. Although peak 5 and 6 could be identified, quantification of peak 5 and peak 6 was difficult because they were not well separated. The external standardization could be used for quantification of AAs. Using this technology, seven amino acids (Lys, Trp, Asn, Ser, Ala, Cys, Gly) in an individual erythrocyte have been determined qualitatively and quantitatively.

Acknowledgment

This project was supported by the National Natural Science Foundation of China, the State Key Laboratory of Microbial Technology, Shandong University.

References

1. S. D. Gilman, A. G. Ewing, *Anal. Chem.*, **1995**, *67*, 58.
2. Q. Dong, X. Wang, L. Zhu, W. Jin, *J. Chromatogr. A*, **2002**, *959*, 269.
3. Q. Dong, W. Jin, *Electrophoresis*, **2001**, *22*, 2786.

Received 11 September, 2002