Analysis of Amino Acids in a Single Human Red Blood Cell by Capillary Zone Electrophoresis with Intracellular NDA-derivatization and Electrochemical Detection

Qian DONG, Xiao Lei WANG, Lan Lan ZHU, Wen Rui JIN*

School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100

Abstract: A novel method for determination of amino acids in individual human red blood cells has been developed. In this method, the derivatization reagents (NDA and CN^-) are introduced into living cells by electroporation. After completion of derivatization, the amino acids in a single cell is determined by capillary zone electrophoresis with end-column amperometric detection.

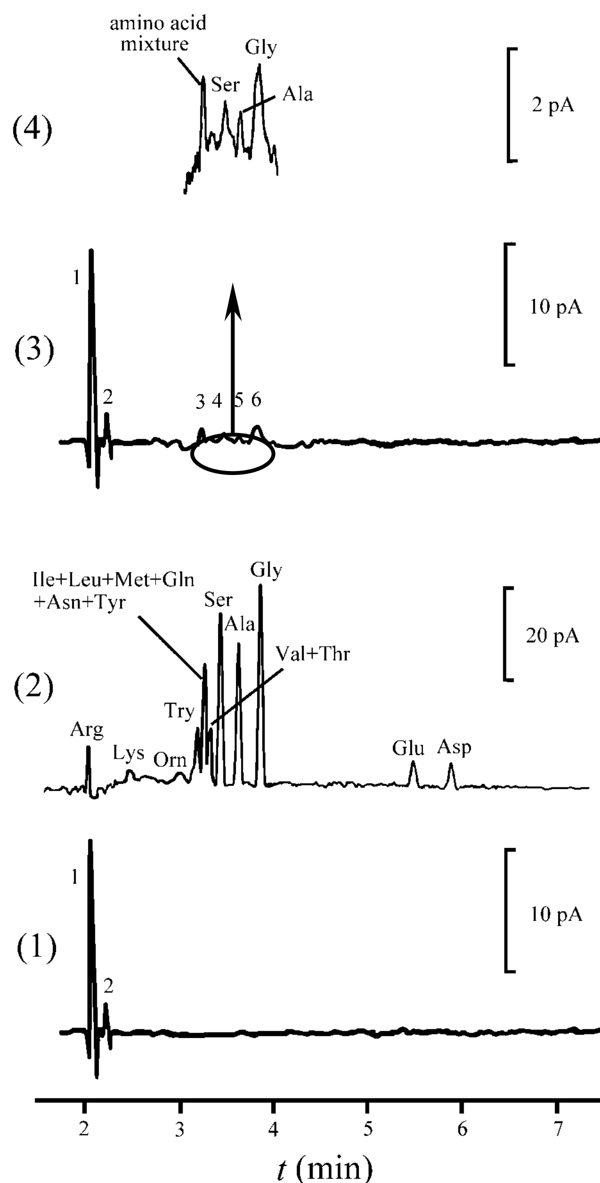
Keywords: Capillary electrophoresis, electrochemical detection, single cell analysis, amino acid.

Since most native amino acids (AAs) in single cells have no electroactivity, they are derivatized before determination. The critical problem is minimizing their dilution during derivatization. For this, an on-column derivatization scheme with a dilution factor of *ca.* 100 was described[1]. In this method, only five AAs in a giant dopamine neuron of the snail (*ca.* 75 μm in size) were quantitated by capillary zone electrophoresis (CZE) with electrochemical detection (ED).

In the present work, we developed a novel method of intracellular derivatization. Naphthalene-2,3-dicarboxaldehyde (NDA) and CN^- are introduced into human red blood cells with a diameter of *ca.* 8 μm by electroporation for derivatization. The preparation and electroporation of cells were described in Ref. 2. After completion of derivatization, a whole cell is drawn into the front of the separation capillary by electroosmotic flow. Then 0.1 mol/L NaOH as the lysing solution is introduced into the capillary by diffusion. Once the individual cell is lysed, the derivatized AAs from the cell are separated by CZE and detected by ED according to Ref. 3. Because the diameter of a cell changes to 10 μm after derivatization, the contents of a cell are diluted only by a factor of *ca.* 1.6. This method represents a significant reduction in dilution compared to on-column derivatization.

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

Figure 1 (1) Electropherogram of phosphate-buffered saline. (2) Electropherogram of standard AAs with NDA-derivatization. (3) Electropherogram of an cell with intracellular NDA-derivatization. (4) 5 times of a part of curve 3.



A typical electropherogram of the contents of an individual cell obtained using intracellular derivatization with NDA and CN^- is shown in **Figure 1**, curve 3. Four small peaks elute within 3 to 4 min following blank peaks 1 and 2. In order to distinguish the four electrophoretic peaks clearly, they are magnified 5 times and shown

in **Figure 1**, curve 4. Identification of the peaks is through comparison with the electropherogram shown in curve 2, indicating the peaks are Ser, Gly and Ala. Identification of peak 3 is difficult, because many AAs have the same migration time as that of peak 3. The external standardization can be used to quantifi- cation of AAs. Using this technology, six amino acids (Ala, Asp, Glu, Gly, Try, Ser) have been determined qualitatively and quantitatively.

Acknowledgment

This project was supported by the National Natural Science Foundation of China, the Natural Science Foundation of Shandong Province and the Key State Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences.

References

1. F. D. Swanek, B. B. Anderson, A. G. Ewing, *J. Microcolumn Sep.*, **1998**, *10*, 185.
2. Q. Dong, W. Jin, *Electrophoresis*, **2001**, *22*, 2786.
3. Q. Weng, W. Jin, *Electrophoresis*, **2001**, *22*, 2797.

Received 11 October, 2001