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Analysis of Ascorbic Acid in Single Human Neutrophils by Electrochemical Detection

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Abstract: Ascorbic acid in individual human neutrophils was determined by capillary zone electrophoresis with electrochemical detection. In order to overcome the influence of the adsorption of the substances in cells on the inner surface wall of the capillary on the migration time and the number of theoretical plates, a procedure for treating capillaries has been described.

Keywords: Electrochemical detection, ascorbic acid, capillary electrophoresis, neutrophil.

Ascorbic acid (AA) is an important component of many biological systems. Analysis of AA in a single pea plant protoplast (a plant cell without cell wall) with *ca*. 5 pL by capillary electrophoresis (CE) and electrochemical detection (ED)[1] has been reported.

In this work, we developed a technology for determination of AA in single smaller cells with cell wall (human neutrophils with *ca*. 0.3 pL) by CE-ED. The preparation of cells was done according to **Ref. 2**. The experimental procedure was described in Ref. 3. The optimum conditions of detecting AA were 1.88×10^{-2} mol/L Na_2HPO_4-1.20×10^{-3} mol/L NaH_2PO_4-1.00×10^{-3} mol/L Na_2H_2EDTA (pH 8.0) for buffer, 25 kV for separation voltage, 0.90 V for detection potential. The mass LOD of 1.7×10^{-16} amol was obtained for the injection voltage of 1 kV and the injection time of 10 s. In order to observe if AA was oxidized under the experimental conditions, the electrophoretic peak currents of AA were measured at different times for the same solution. It was found that the peak currents were constant within 40 min. Therefore, it can be concluded that the oxidation of AA can be neglected during determination of AA by CE-ED for the running time of 10 min. Since neutrophils were suspended in phosphate-buffered saline (PBS), PBS was injected into the capillary with a neutrophil. The electropherograms of PBS, an individual neutrophil and standard AA are shown in **Figure 1**. There are two peaks on the electropherogram of the single neutrophil (curve 2). By comparison to the electropherograms of PBS (curve 1) and the standard AA (curve 3), it can be concluded that the first peak is the peak of PBS and the second peak is the peak of AA based on their migration times (t_m). Under our experimental conditions, no peak of glutathione for a concentration of 1.00×10^{-4} mol/L was detected. The peak of AA was well separated from the peaks of those easily oxidized compounds such as glutathione, dopa, dopamine, serotonin and epinephrine. In almost all experiments of single cell

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analysis for whole cell injection reported in literatures, t_m was prolonged and the number of theoretical plates (N) decreased with increasing run number. Adsorption of the substances in the cells on the surface of the capillary wall is responsible for this. In order to solve this problem, after analyzing a cell the injection end of the capillary was treated as follows: The injection end was cleaned in water by a supersonic wave cleaner. Then the capillary was flushed with 1 mol/L NaOH, doubly distilled water, and the corresponding separation electrolyte for *ca*. 3 min, successively. The results of analysis for 7 single neutrophils are listed in **Table 1**. It can be found that the migration time and the number of theoretical plates do not change with increasing run number. External standardization was used for quantitation of AA in a neutrophil. Quantitation of AA present in the neutrophils is also shown in **Table 1**, which shows that the amounts of AA in single neutrophil differ from cell to cell. The amounts of AA determined in seven cells are 0.557 ± 0.236 fmol (mean ± SD), which is in the range of 0.42-0.77 fmol reported in literatures[4,5].

Figure 1 Electropherograms of (1) PBS; (2) a single neutrophil and (3) 1.00×10^{-4} mol/L AA.

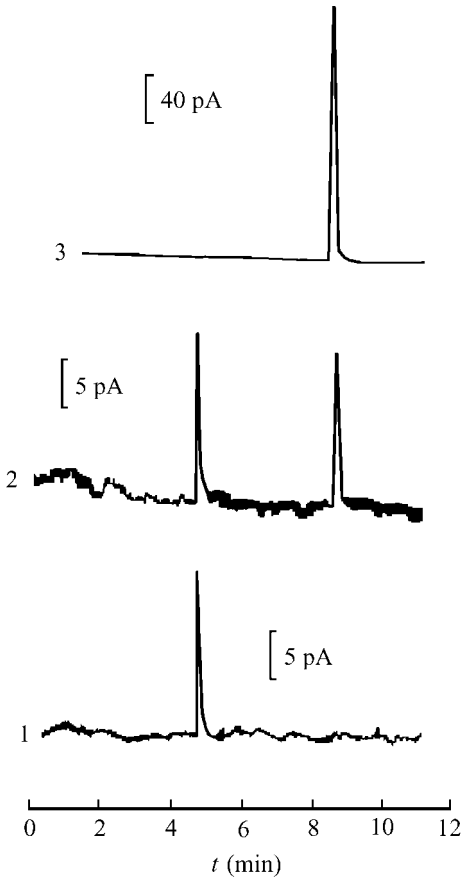

Table 1 Migration time, t_m, number of theoretical plates, N, peak current, i_p, and amount of AA, q, in single neutrophils.

Run number	t_m (s)	$10^{-4}N$	i_p (pA)	q (fmol)
1	527	4.0	4.51	0.413
2	526	4.0	3.54	0.338
3	520	3.9	7.83	0.672
4	525	4.0	7.82	0.671
5	523	3.9	11.7	0.973
6	526	4.0	6.02	0.531
7	526	4.0	3.10	0.303

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References

1. J. Olsson, O. Nordström, A-C. Nordström, B. Karlberg, *J. Chromatogr. A*, **1998**, *826*, 227.
2. L. Bei, T. Hu, X. Shen, *Prog. Biochem. Biophys.*, **1998**, *25*, 359.
3. W. Jin, Q. Dong, X. Ye, D. Yu, *Anal. Biochem.*, **2000**, *285*, 255.
4. W. W. Philip, O. H. William, L. Mark, *Anal. Biochem.*, **1989**, *181*, 270.
5. M. Wang, S. Ma, S. Xu, *et al*., *Translation of "Review of Physiological Chemistry" by Harper, H. A., Rodwell, V. W., Mayes, P. A.*, p 257, Science Press, Beijing, **1985**.

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