Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 792 (2003) 381–385
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of dopamine in single rat pheochromocytoma cell by capillary electrophoresis with amperometric detection

Liyao Zhang[a], Sanfu Qv[b], Zongli Wang[a], Jieke Cheng[a,*]

[a]*Department of Chemistry, Wuhan University, Wuhan 430072, China*
[b]*China Centre for Type Culture Collection, Wuhan 430072, China*

Received 20 December 2002; received in revised form 28 March 2003; accepted 2 April 2003

Abstract

A method is described for the direct identification of dopamine in single cultured rat pheochromocytoma cell by capillary electrophoresis (CE) with amperometric detection. The separation and detection conditions were optimized. The dopamine in single cell analysis was identified based on the migration time of standard dopamine and internal standard (epinephrine). The amount of dopamine in a single cell ranged from 0.29 to 1.28 fmol.

Keywords: Dopamine

1. Introduction

Capillary electrophoresis (CE) has emerged as a powerful separation tool for the analysis of chemical species. Owing to its ability to separate compounds based on their charge-to-size ratio, CE has been applied to species ranging in size from small metallic ions to biopolymers such as protein and DNA [1]. Especially, the high efficiency and resolving power of CE has led to the application of CE to the analysis of the chemical content of single cells [2–14].

Amperometric detection, as an inexpensive and sensitive detection method, has been successfully coupled to CE to detect electroactive compounds in single neuron [2–4], in cytoplasmic injection from snail neuron [5–8] and in single plant cell [10].

Rat pheochromocytoma (PC12) cells, as an immortal cell line isolated from a tumor on the adrenal gland of a rat by Greene and Tischler [15], has frequently been used as a model for the developing sympathetic nerve, since this clonal cell line exhibits many of the physiological properties of sympathetic ganglion neurons [16]. Swanek et al. [9] has used the CE with napthalene-2,3-dicarboxaldehyde (NDA) derivatization and electrochemical detection for the analysis of dopamine and amino acids from the single *Planorbis* neuron and PC12 cell. Gilman et al. [12] has detected dopamine and five amino acids of single PC12 cells by CE with on-column NDA derivatization and laser-induced fluorescence detection.

In this paper, a simple and rapid method for the analysis of dopamine in single PC12 cell without

*Corresponding author. Tel.: +86-27-8768-2291; fax: +86-27-8764-7617.
E-mail address: jkcheng@whu.edu.cn (J. Cheng).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00323-4

derivatization procedure using CE with amperometric detection is reported. The separation was performed in a 25 μm I.D. fused capillary and the amperometric detection was carried out at a carbon fiber electrode by means of a two-electrode system.

2. Materials and methods

2.1. CE Apparatus

The laboratory-built capillary electrophoresis and electrochemical detection system used for these experiments was similar to that described by our group previously [17]. The apparatus consisted of a 65–70 cm fused-silica capillary with dimension of 25-μm I.D., 375-μm O.D. (Yongnian Optical Conductive Fiber Plant, China) and a ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China). The high voltage was applied at the injection end, while the reservoir containing electrochemical detection system was held at ground potential. Injection was performed electrokinetically.

On the injection end of the capillary, a micro-injector was fashioned to facilitate the injection of whole cells. The end of capillary was etched in 40% aqueous hydrofluoric acid until its outside was about 100 μm and the inside about 70 μm. After etching, the tip was neutralized in NaOH and washed with water.

2.2. Amperometric detection apparatus

Amperometric detection was performed using a two-electrode configuration. A 8-μm O.D. carbon fiber with an exposed length of 150–300 μm was employed as the working electrode, which was constructed as we described previously [18]. The working electrode was inserted into the end of capillary about 20 μm deep with the aid of a micromanipulator. Amperometric detection was performed at a constant potential using by an HDV-7 potentiostat (Sanming Electric Factory, Fujian, China) and a picoammeter (Dept. of Chemistry, Wuhan University, Wuhan, China). The system was enclosed in a copper mesh Faraday cage to minimize the external noise.

2.3. Reagents and solutions

Dopamine (DA), epinephrine (E), norepinephrine (NE) were purchased from Sigma (St. Louis, MO) and used as received. 0.01 *M* DA, E and NE stock solution were prepared in 0.1 *M* perchloric acid and diluted to the desired concentration in running buffer, stored in a refrigerator. Cells were suspended in a balanced salt solution, which was composed of 150 m*M* NaCl, 4.2 m*M* KCl, 2.7 m*M* $MgCl_2$, 1.0 m*M* NaH_2PO_4, and 10 m*M* 4-(2-hydroxyethyl)-piperazineethanesulfonic acid (HEPES), pH 7.4. Other chemical reagents were of analytical grade and used without further purification. The electrophoresis buffer was 10 m*M* tris(hydroxymethyl)-aminomethane (Tris) solution, adjusted to pH 6.2 with H_3PO_4. Running buffer solutions were filtered through a polypropylene filter (0.22 μm) prior to use.

2.4. Cell preparation

The seed of PC12 cells were obtained from the American Type Culture Collection (ATCC) and cultured by the China Centre for Type Culture Collection (CCTCC). Growth medium was composed of 85% phenol red-free RPMI-1640 supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum. Before analysis, the cell was washed and suspended by the balanced salt solution.

2.5. Single cell injection and CE procedure

The injection end of the capillary was attached to a micromanulator and positioned at the bottom of the culture dish. A 5 kV injection voltage was applied for about 3–5 s to transport the whole cell into the etched capillary tip until the cell has been immobilized to the inner wall of capillary. Then, the injection end of the capillary was removed and immersed in 0.1% SDS solution. Then 5 kV injection voltage was applied for about 5 s to lyse the cell, with the visual confirmation of lysis through the microscope. After this, a potential of 5 kV for 10 s was applied to inject the internal standard (E), then a potential of 16 kV was applied to carry out an electrophoretic separation.

3. Results and discussion

3.1. Preparation of 0.1% SDS

The preparation of SDS is critical in the lysis of the cell. An interesting phenomenon was found in the experiment. When the SDS was prepared in water, the cell in the capillary would not be lysed by the electrokinetical injection SDS into capillary, but flowed backwards into the culture dish containing SDS, though the high voltage was applied at the injection end. The phenomenon may be due to negative charges on the cell surface, and the ionic strength of the water solution of 0.1% SDS lower than that of the running buffer. However, when SDS was prepared in running buffer, the above phenomenon would not be occurred. Thus, SDS must be prepared in running buffer for the lysis of cell.

3.2. Optimization of separation and detection conditions

The composition of the running buffer is an important factor influencing the peak current and the separation efficiency. Three common buffers, namely Tris–H_3PO_4 (Tris), Na_2HPO_4–NaH_2PO_4 (PBS), MES–NaOH (MES) were tested. It was found that the peak current and peak shape of the peak of dopamine are the same in the three different running buffers, when dopamine was prepared in running buffer. However, as the cell contains high ion strength buffer and is suspended in cell medium (high ion strength buffer) to keep the cell viability, the high ion strength buffer may influence the analysis of cellular dopamine. As shown in Fig. 1, it was found that the current and peak shape of the peak of dopamine in Tris buffer are the best. It might be due to the electrophoretic mobility of $(CH_2OH)_3CNH_3^+$ lower than that of sodium ion. Thus, Tris buffer is more suitable for the cell analysis.

The pH value of the running buffer is considered as one of the most important parameters in CE separation. As indicated in Ref. [14], pH 7.4 or beyond 7.4 is less suitable for the separation of catecholamines. Varying the pH value of the running buffer from 4.0 to 7.4, the separation of catecholamines was tested. When pH value is more than 7.0, dopamine is easy to be absorbed in the capillary inner surface and when pH value is less than 5.0, the migration time of dopamine is longer. After comparing the separation efficiency, pH 5.0–6.5 is more suitable for the separation of catecholamines, based on the higher peak current and separation efficiency.

In this work, the effect of potentials applied to the working electrode on peak current was investigated. It was found that the peak current detected increases with the applied potential until 0.7 V vs. SCE. However, applied the potential greater than 0.7 V vs. SCE would result in the higher background current, but not the higher peak current. Thus, 0.7 V is suitable for the detection of dopamine.

For a given capillary, the separation voltage would affect the migration time of the analytes. As ex-

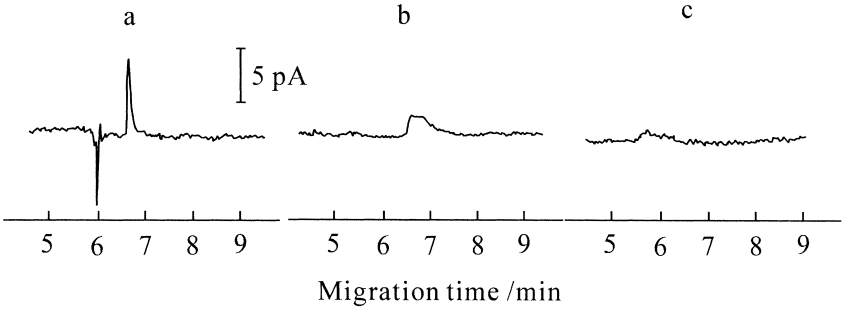

Fig. 1. Eletropherograms of 1.0×10^{-6} *M* dopamine prepared in PC12 cell balanced salt solution using three different running buffers: (a) 10 m*M* Tris–H_3PO_4 (pH 6.2); (b) 10 m*M* Na_2HPO_4–NaH_2PO_4 (pH 6.2); (c) 25 m*M* MES–NaOH (pH 5.7). Conditions: capillary, 65 cm×25 μm; injection, 15 kV×1 s; separation voltage, 15 kV; detection potential, 0.7 V vs. SCE.

pected, increasing the separation voltage makes the decrease of the retention time of dopamine. However, the higher electric field results in the larger background noise. Therefore, 15–16 kV is suitable for the separation with capillary length of 65–70 cm.

3.3. Importance of injecting less amount of cell medium

As shown in Fig. 1, the cell medium introduced would influence the separation. The importance of injecting less amount of cell medium was further demonstrated. Fig. 2 shows the effect of the different amount of the cell medium ahead of the standard dopamine sample in Tris buffer on the separation efficiency. It can be seen that the peak current and separation efficiency decrease with the increase of the amount of cell medium in the capillary. Fig. 3 shows the electropherogram of the standard mixture of DA, NE and E. Fig. 4 shows the electropherogram of a single-cell analysis using epinephrine as the internal standard and injecting larger amount of the cell medium. It was observed that the profiles of the peaks of dopamine and the internal standard were broadened. Thus, it is needed to reduce the amount of the cell medium in the capillary with short injection time and lower injection voltage during the injecting procedure of the whole cell.

3.4. Single cell analysis

Fig. 5 shows the electropherogram of a single-cell analysis using epinephrine as the internal standard.

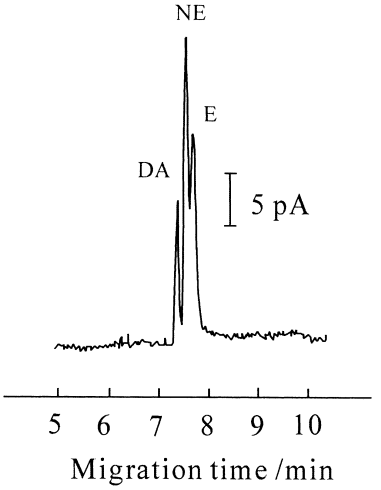

Fig. 3. Eletropherogram of the standard mixture of DA, NE and E prepared in CE buffer. Conditions: capillary, 70 cm×25 μm; CE buffer, 10 m*M* Tris–H_3PO_4 (pH 6.2); separation voltage, 16 kV; injection, 15 kV×1 s; detection potential, 0.7 V vs. SCE.

Based on the migration time of dopamine standard and internal standard, the dopamine from single cell was identified. After several runs, it was found that the retention times increased due to the adsorption of cell membranes and other species onto the capillary. However, this can be overcome by flushing the capillary with the running buffer, and the dopamine peak can be easily identified according to the migration time of internal standard. One of the important features of this method is that the lack of a chemical derivatization step could further facilitate the quantitative identification of dopamine in single cell.

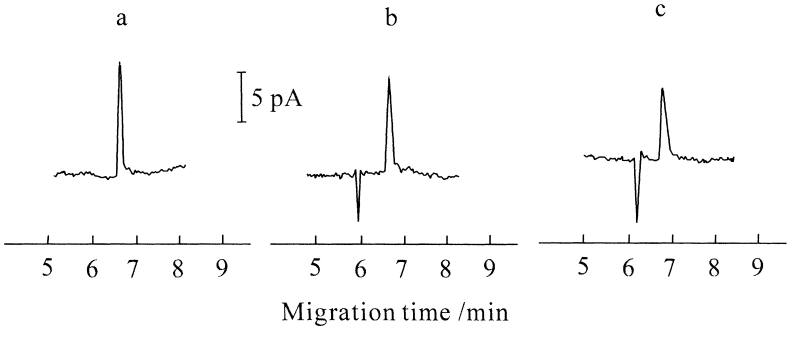

Fig. 2. Effect of cell medium plug on the peak current and the shape of the peak of dopamine prepared in running buffer by injection different length cell medium plug: (a) 3 s; (b) 6 s; (c) 12 s, respectively at 5 kV before sample introduction. Conditions: CE buffer, 10 m*M* Tris–H_3PO_4 (pH 6.2); other conditions as in Fig. 1.

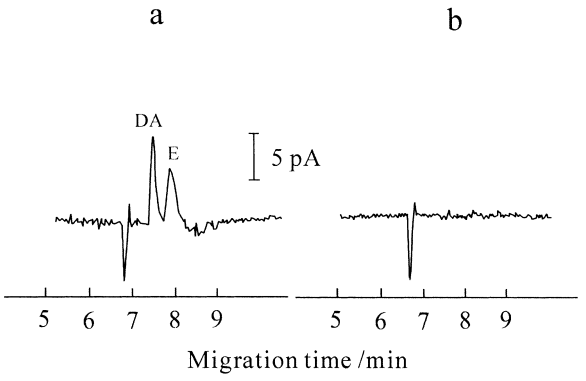

Fig. 4. (a) Typical electropherogram of a single cell analysis using epinephrine as the internal standard and injecting larger amount of the cell medium. Conditions: injection, about 12 s at 5 kV to pull cell into capillary; other conditions as in Fig. 3. (b) Electropherogram of a blank solution analysis with larger amount of cell medium injected. Other conditions as in Fig. 3.

Table 1 shows the amount of DA in ten individual PC12 cells. In contrast of the peak height of standard electropherogram, the average amount of DA in individual cell was estimated to be ~0.61 fmol, lower than that reported previously [12], and the reason might be due to the difference of cells. The blank test was also performed for the comparison (Figs. 4b and 5b), and dopamine is not detectable in the balanced salt solution.

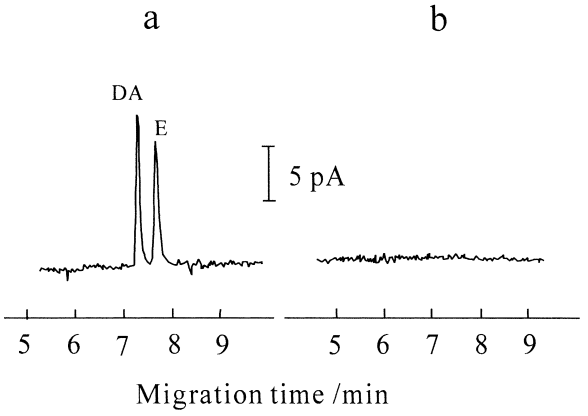

Fig. 5. (a) Electropherogram of a single cell analysis using epinephrine as the internal standard. Conditions: injection, about 3 s at 5 kV to pull cell into the capillary; 5 s at 5 kV to pull lysing reagent 0.1% SDS over cell; other conditions as in Fig. 3. (b) Electropherogram of a blank solution analysis, other conditions as in Fig. 3.

Table 1
Quantitation of dopamine content in individual PC 12 cells

Cell no.	Measured DA amount (fmol)	Mean±SD
1	1.28	
2	0.29	
3	0.96	
4	0.61	
5	0.38	0.61±0.30
6	0.64	
7	0.35	
8	0.58	
9	0.48	
10	0.48	

Acknowledgements

This project was supported by the National Natural Science Foundation of China.

References

[1] Y.Z. Deng, J.L. He, High Efficiency Capillary Electrophoresis, Science Press, China, 1996.
[2] T.M. Olefirowicz, A.G. Ewing, Chimia 4 (1991) 106.
[3] H.K. Kristensen, Y.Y. Lau, A.G. Ewing, J. Neurosci. Methods 51 (1994) 183.
[4] F.D. Swanek, G. Chen, A.G. Ewing, Anal. Chem. 68 (1996) 3912.
[5] R.A. Wallingford, A.G. Ewing, Anal. Chem. 60 (1988) 1972.
[6] T.M. Olefirowicz, A.G. Ewing, Anal. Chem. 62 (1990) 1872.
[7] T.M. Olefirowicz, A.G. Ewing, J. Neurosci. Methods 34 (1990) 11.
[8] J.B. Chien, R.A. Wallingford, A.G. Ewing, J. Nerurochem. 54 (1990) 633.
[9] F.D. Swanek, B.B. Anderson, A.G. Ewing, J. Microcol. Sep. 10 (1998) 185.
[10] J. Olsson, O. Nordstrom, A.C. Nordsreom, B. Karlberg, J. Chromatogr. A 826 (1998) 227.
[11] H.T. Chang, E.S. Yeung, Anal. Chem. 67 (1995) 1079.
[12] S.D. Gillman, A.G. Ewing, Anal. Chem. 67 (1995) 58.
[13] S.J. Lillard, E.S. Yeung, Anal. Chem. 68 (1996) 2897.
[14] W. Tong, E.S. Yeung, J. Neurosci. Methods 76 (1997) 193.
[15] L.A. Greene, A.S. Tischler, Proc. Natl. Acad. Sci. USA 73 (1976) 2424.
[16] K.D. Kozminski, D.A. Gutman, V. Davila, D. Sulzer, A.G. Ewing, Anal. Chem. 70 (1998) 3123.
[17] S. Hu, Z.L. Wang, P.B. Li, J.K. Cheng, Anal. Chem. 69 (1997) 264.
[18] W.H. Huang, D.W. Pang, H. Tong, Z.L. Wang, J.K. Cheng, Anal. Chem. 73 (2001) 1048.