ELSEVIER

Journal of Chromatography B, 779 (2002) 347–352

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Short communication

Measurement of histamine in individual rat peritoneal mast cells by capillary zone electrophoresis with electrochemical detection

Qianfeng Weng, Fangquan Xia, Wenrui Jin*

School of Chemistry and Chemical Engineering and State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, China

Received 12 March 2002; received in revised form 23 May 2002; accepted 23 May 2002

Abstract

Capillary zone electrophoresis was employed for the analysis of histamine in single rat peritoneal mast cells using an amperometric detector with a carbon fiber microdisk bundle electrode. In this method, individual mast cells and then 0.02 mol/l NaOH as a lysing solution are injected into the front end of the separation capillary by electromigration with an aid of a inverted microscope. A cell injector was constructed. Using it, the cell suspension was static, when a voltage for injecting single cells was applied. Histamine in single rat peritoneal mast cells have been identified. Quantitation has been accomplished through the use of calibration curves. The mean amount of histamine for nine cells is 95.8 fmol, which is consistent with the literature value.

Keywords: Histamine

1. Introduction

Histamine is one of the chemical mediators in connection with allergies. Mast cells can synthesize, store, and release histamine. HPLC [1] and capillary electrophoresis (CE) [2] have been used for determination of histamine in mast cells. In these assays, histamine in supernatants and cellular extracts of mast cells were derivatized before injection or histamine in single peritoneal mast cells lysed was derivatized in-capillary. Only Pihel et al. [3] reported a method for detection of histamine at isolated mast cells by HPLC with electrochemical detection. In this method, single cells were removed and transferred to 300-nl microvials. After an internal standard solution was added into the microvials, the microvials were centrifuged. A part of the supernatant from individual vials was injected into the chromatography column. Single cell analysis is one of the more challenging areas for analysts. Four features at least are noted for single cell analysis. They are ultra-small size or extremely small volume, ultra-trace of species, a large number of species with similar structure and instability of the structure with time for some species. Capillary zone electrophoresis (CZE) has emerged as a powerful new method for rapid separation and detection of biological substances [4,5]. It is a method well suited to the analysis of single cells [6], because it can satisfy the requirements mentioned above. CZE can be used to sample volumes as low as nl to fl. It can also detect

*Corresponding author. Fax: +86-531-856-5167.
E-mail address: wenrujin@jn-public.sd.cninfo.net (W. Jin).

1570-0232/02/$ – see front matter
PII: S1570-0232(02)00374-4

the analytes with high sensitivity. When a electrochemical detector or laser-induced fluorescence detector are used, fmol to zmol components can be determined. The extremely high separation efficiency of CZE (exceeding 300 000 theoretical plates) allows for the resolution of many constituents present in a single cell. Finally, species separations can be achieved on a relatively short time scale. Ewing, Yeung, Jorgenson and Sweedler with their co-workers have used the method for single cell analysis, respectively [7,8].

Laser-induced fluorescence (LIF) and amperometry are the most popular detection method for analysis of single cells by CZE because of their sensitivity. However, LIF often requires pre- or postcolumn derivatization of the sample of interest. Amperometric detection with microelectrodes has been shown to be well suited for the determination of electroactive neurotransmitters, such as dopamine, serotonin, epinephrine, and norepinephrine in cytoplasmic injections from snail neurons [9–12], single whole snail neurons [13–15] and single sympathetic nerve cells [16]. Glutathione in single human erythrocytes [17] and in individual mouse peritoneal lymphocytes [18] has also been detected in our laboratory. However, the analysis of histamine in individual rat peritoneal mast cells using CZE with amperometric detection has not been reported to date. In this paper, we have developed a method for the determination of histamine in single rat peritoneal mast cells by using CZE with amperometric detection at a carbon fiber microdisk bundle electrode. In this scheme, a whole cell was injected into and lysed in the front end of the separation capillary. CZE was performed in neutral NaH_2PO_4–Na_2HPO_4 solution (pH 7.0). No derivatization is needed. The method is simple, sensitive and relatively easy.

2. Experimental

2.1. Apparatus

Details of the CZE separation system used in this work were similar to our description previously [19]. Briefly, a reversible high-voltage power supply (Model NT-9123, Beijing Institute of New Technology, Beijing, China) provided a variable voltage of 0–30 kV across the capillary, with the outlet of the capillary at ground potential. Fused-silica capillaries (25 μm I.D., 360 μm O.D.), from Yongnian Optical Conductive Fiber Plant (Yongnian, China), were cut a length of 46 cm and placed between two buffer reservoirs. A high voltage was applied at the injection end, while the reservoir containing the electrochemical detection cell was held at ground potential. Separations were carried out at an applied voltage of 12.5 kV. The amperometric detection at a constant potential was performed using the end-capillary approach with an electrochemical analyzer (Model CHI800, CH Instruments Austin, TX, USA). The detection cell and detector were housed in a Faraday cage in order to minimize the interference from external sources of noises. Electrochemical detection was carried out with a three-electrode system. It consisted of a carbon fiber microdisk bundle electrode (about 30 carbon fibers with 6 μm diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a coiled Pt wire (0.5 mm diameter, 4 cm in length) placed at the bottom of the cell as the auxiliary electrode. The arrangement of the electrochemical detection cell was illustrated in [19] in detail. The carbon fiber microdisk bundle electrodes used here were described previously [20].

2.2. Preparation of rat peritoneal mast cells

Rat peritoneal mast cells (RPMCs) were isolated from a female rat by the method described in Ref. [21]. The rat was anesthetized with ether and then decapitated. Approximately 30 ml phosphate-buffered saline (PBS) buffer was injected into the peritoneal cavity. After the abdomen was massaged for 2 min, the peritoneal cavity was cut open, ca. 25 ml of the buffer containing cells was withdrawn and collected in three 10-ml centrifuge tubes. The buffer was centrifuged for 10 min (1000 rpm, 4 °C) and the supernatant discarded. Cell mixtures at the bottom of the three centrifuge tubes were transferred into one centrifuge tube. Then the cell mixture was suspended in ca. 2 ml PBS buffer. The PBS buffer was added into another centrifuge tube containing ca. 3 ml mast cells separation solution. Then it was centrifuged for 15 min at 2500 rpm and 4 °C. The supernatant was

removed and the cells were washed twice with PBS buffer. After the supernatant was discarded, the cells were resuspended in ca. 2 ml PBS buffer. This was the suspension of mast cell. The suspension was stored at 4 °C for use.

2.3. Injection and lysis of whole cell

Fig. 1 shows the cell injector. A transparency (1) with two small holes, one (2) for the suspension of mast cell and another (3) for the lysis solution, was glued on a microscope slide (4). There was a buffer reservoir (5) at the right of the transparent slide. In it an anode for injection cell (6) and two Ni wires (7) were inserted. The Ni wires were connected to the two holes, respectively. The cell injector was placed on the inverted microscope (Chongqing Optical Instrument Factory, Chongqing, China) with a magnification of ×640. The separation capillary and the buffer reservoir were filled with electrophoresis buffer. The suspension of mast cell was put in the hole (2). The injection end of the separation capillary was gently inserted in the suspension. The other end of the separation capillary was inserted into a buffer reservoir (not shown in Fig. 1) with a cathode. In order to see the opening of the injection end, a ca. 5-mm section of the polyimide coating at the injection end of the capillary was removed by burning before use. As soon as the mast cell was drifting towards the injection end, an injection voltage of 2.0 kV was applied to the anode to transport the whole cell into the capillary tip. Once one cell was injected into the separation capillary tip, the anode was manipulated up, out of the buffer reservoir. The entire process of cell injection typically took 30–120 s. Since the anode for injecting single cells was not directly inserted in the cell suspension, the cell suspension was static, when a voltage for injecting single cells was applied. Then, the injection end of the separation capillary was taken out the suspension of mast cell and was gently immersed in the lysis solution (0.02 mol/l NaOH) in the hole (3). The electromigration injection of this solution at 2.0 kV for 5 s was carried out. After the cell was lysed, which could be observed under the microscope, the separation capillary was carefully immersed to a reservoir containing electrophoresis buffer with an anode for electrophoretic separation.

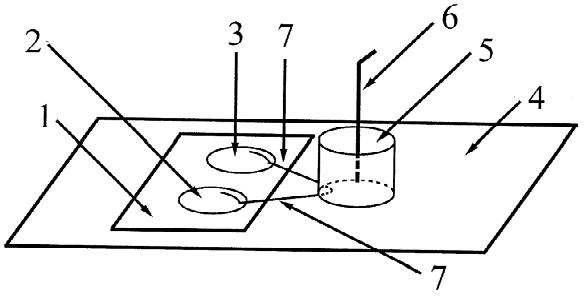

Fig. 1. Schematic diagram of the cell injector. (1) Transparency; (2) hole for the suspension of mast cell; (3) hole for the lysis solution; (4) microscope slide; (5) buffer reservoir; (6) anode for injection cell; (7) Ni wires.

2.4. Single cell analysis

Single cell analysis was similar to our previous work [22]. To prepare a CZE-electrochemical detection system, the carbon fiber microdisk bundle electrode was placed over a home-made XYZ micromanipulator and glued in place. The position of carbon fiber microdisk bundle electrode was adjusted (under a microscope) against the end of the capillary so that the electrode and the capillary were in contact. This arrangement allowed one to easily remove and realign both the capillary and the electrode. The other end of the capillary was inserted into a plastic syringe tip (the metal needle was previously removed) and glued in place with a small amount of epoxy glue. Before each run, the capillaries were flushed with 0.1 mol/l NaOH and double-distilled water, and the corresponding separation electrolyte for ca. 1 min, respectively, by means of a syringe. During the experiments the separation voltage was applied across the capillary and the detection potential was applied at the working electrode. After the electroosmotic flow reached a constant value, the electromigration injection of the whole cell mentioned above was carried out. Then a separation voltage of 12.5 kV was applied across the capillary and the detection potential of 1.30 V was applied at the working electrode, and the electropherogram was recorded.

2.5. Reagents and solutions

Ficoll-400 was obtained from Pharmacia, Sino-

American Biotec. (Beijing, China). Lymphocytes separation medium (density 1.077±0.002 g/ml) was purchased from Shanghai Henxing Chemical Reagent (Shanghai, China). Histamine dihydrochloride (content>99%) was purchased from Sigma (St. Louis, MO, USA). The physiological buffered saline (PBS) was consisted of 0.135 mol/l NaCl and 0.02 mol/l NaH_2PO_4–NaOH (pH 7.4). A 1.00×10^{-2} mol/l stock solution of histamine was prepared by dissolving an appropriate amount of histamine dihydrochloride in water and was stored at 4 °C in a refrigerator. Dilute solutions were obtained by serial dilution of the stock solution with 1.56×10^{-2} mol/l NaH_2PO_4–2.44×10^{-2} mol/l Na_2HPO_4 (pH 7.0). 40% Ficoll-400 solution (density 1.120 g/ml) was prepared by dissolving an appropriate amount of Ficoll-400 in water and was stored at 4 °C in a refrigerator. The mast cell separation solution (density 1.085 g/ml) was prepared by mixing 1.86 ml of 40% Ficoll-400 and 8.14 ml of lymphocytes separation medium. Other reagents were of analytical grade. All solutions were prepared with double-distilled water.

3. Results and discussion

3.1. Lysis of the mast cells

Usually, cell lysis is accomplished by injecting a plug of a lysis solution around the cell in the injection end of the separation capillary. Lysis of human erythrocytes and macrophages has been investigated in our laboratory. Erythrocytes can be lysed in CZE running buffer solution (pH 7.0) easily, but macrophages cannot. Macrophages can be lysed in NaOH. It was noted that mast cells could not be lysed in CZE running buffer solution (pH 7.0) and could be lysed in 0.1% SDS between 5 and 9 min. However, they could be lysed in NaOH easily. The higher the concentration of NaOH is, the faster the cells are lysed. In 0.02 mol/l NaOH, the mast cells are lysed quickly (less than 60 s). This solution is selected to lyse mast cells.

3.2. Identification of quantification histamine in individual mast cells

The conditions of separating and detecting histamine are: 1.56×10^{-2} mol/l NaH_2PO_4–2.44×10^{-2} mol/l Na_2HPO_4 (pH 7.0) for the buffer solution, 12.5 kV for the separation voltage, 5 kV and 10 s for the injection voltage and the injection time, and 1.30 V versus SCE for the detection potential. Fig. 2 shows the electropherograms of the lysis solution, a mast cell and the standard solution of histamine, serotonin and histidine. Three peaks with the migration times of 240, 270 and 400 s, respectively, appear in the electropherogram of the single cell (curve 2). By comparison with the electropherogram of the lysis solution shown in curve 1, the lysis

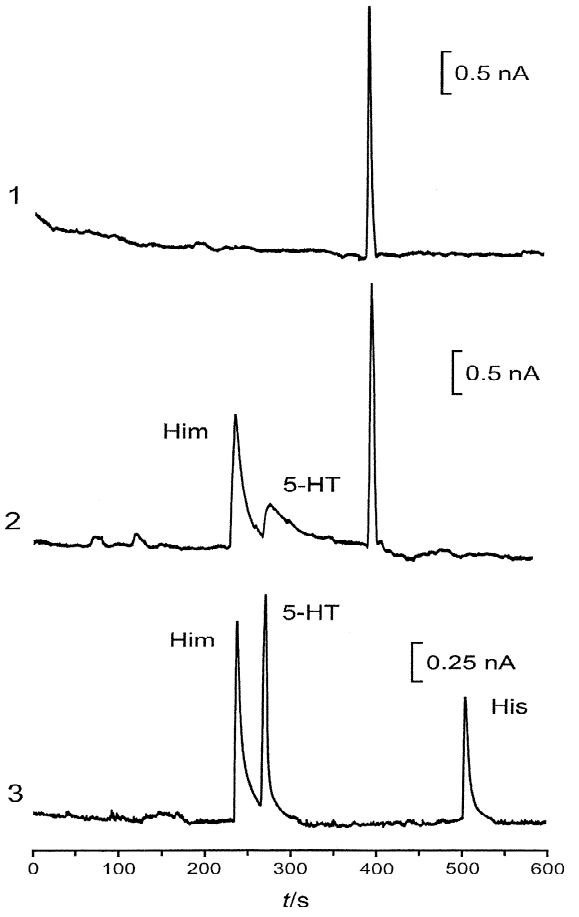

Fig. 2. Electropherograms of the lysis solution (curve 1), the contents in a single mast cell (curve 2) and the standard solution containing histamine (Him), serotonin (5-HT) and histidine (His) (curve 3). Injection for curve1 and 3, 5.0 kV for 10 s. Concentration, 2.5×10^{-5} mol/l; electrophoresis buffer, 1.56×10^{-2} mol/l NaH_2PO_4–2.44×10^{-2} mol/l Na_2HPO_4 (pH 7.0); capillary, 25 μm I.D., 375 μm O.D., 46 cm length; separation voltage, 12.5 kV; detection potential, 1.30 V.

solution should be responsible for the peak eluting at 400 s, because they have the same migration time and shape in the both electropherograms. Comparison of curves 2 and 3 shows that the peaks eluting at 240 and 270 s should be histamine and serotonin, respectively, based on their migration times. However, serotonin could be detected only in few mast cells. Fig. 3 shows three typical electropherograms of the individual mast cells obtained.

The reproducible peak currents with the RSD of 3.2%, together with the large linear dynamic range for standard histamine (5×10^{-6} to 1×10^{-4} mol/l) made it suitable to use external calibration for the quantification of histamine in an individual mast cell.

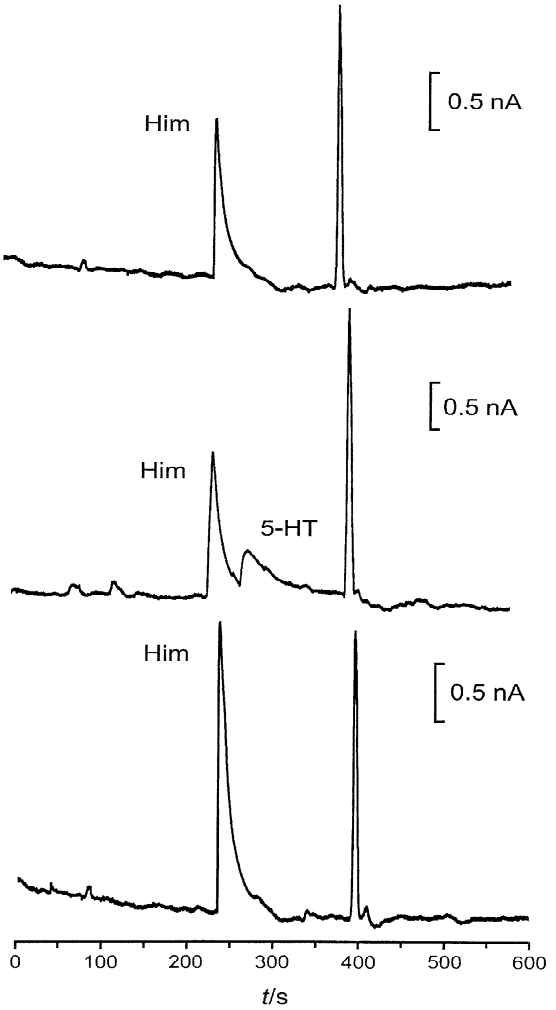

Fig. 3. Electropherograms of contents in three individual mast cells. Conditions as in Fig. 2.

Table 1
Migration time, peak current and number of theoretical plates of histamine in electropherograms and amount of histamine determined in nine individual rat peritoneal mast cells

Run no.	t_m (s)	i_p (nA)	Amount (fmol)	$N\times10^{-3}$
1	238	1.39	102	3.4
2	241	1.54	112	3.5
3	239	0.29	26.2	3.5
4	246	0.93	70.0	3.3
5	247	2.54	180	3.2
6	252	1.72	124	3.2
7	255	0.61	48.1	3.3
8	257	2.01	144	3.1
9	256	0.72	55.7	3.2

Table 1 shows the migration time, electrophoretic peak current, amount and number of theoretical plates of histamine determined in nine individual mast cells consecutively. The run numbers refer to the chronological order of the nine cells studied. There is a clear drift in migration time. Overall ζ-potential changes of the capillary wall, resulting from the adsorption of the substances in the cells on the surface of the capillary wall, were probably responsible for this. This phenomenon was also observed in previous work of analysis of single cells [17,18]. The number of theoretical plates decreases with increasing the run number in a small range. The amount of histamine in individual mast cells has no correlation with respect to the run number, which proves that the influence of the other substances in individual cells on the determination of histamine has been minimized. It can be found that amounts of histamine in single mast cells differ from cell to cell. As explained by Hogan and Yeung [23], the variations among cells result from the difference in actual cell volume from mean values, variability in membrane permeability, and intercellular histamine content and status, rather than measurement and sampling errors. The mean amount of histamine for nine cells is 96 fmol, which is consistent with the literature value (64–114 fmol) [24,25].

4. Concluding remarks

The results of this study show that capillary zone electrophoresis with amperometric detection at a

carbon fiber microdisk bundle electrode can be used to determine histamine in individual rat peritoneal mast cells. In this method, the whole cell is introduced into the injection end the separation capillary by electromigration, where the cell is lysed by 0.02 mol/l NaOH. Using this technique, the dilution of the contents in single cells can be minimized. No derivatization or handing of the intracellular fluid is needed, which means that quantification will be independent of derivatization reaction efficiency. Calibration curves can be used to quantitation. It should be possible to use this method for qualitative and quantitative analysis of the electroactive contents in single cells.The method is simple, sensitive and relatively easy.

Acknowledgements

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

References

[1] D. Egger, G. Reisbach, L. Hültner, J. Chromatogr. B 662 (1994) 103.
[2] S. Oguri, Y. Ohta, C. Suzuki, J. Chromatogr. B 736 (1999) 263.
[3] K. Pihel, S. Hsieh, J.W. Jorgenson, R.M. Wightman, Anal. Chem. 67 (1995) 4515.
[4] C.A. Monnig, R.T. Kennedy, Anal. Chem. 66 (1994) 280R.
[5] H.A. Bardelmeijer, H. Lingeman, C. de Ruiter, W.J.M. Underberg, J. Chromatogr. A 807 (1998) 3.
[6] A.G. Ewing, J.M. Mesaros, P.F. Gavin, Anal. Chem. 66 (1994) 527A.
[7] J.A. Jankowski, S. Tracht, J.V. Sweedler, Trends Anal. Chem. 14 (4) (1995) 170.
[8] E.S. Yeung, J. Chromatogr. A 830 (1999) 243.
[9] R.A. Wallingford, A.G. Ewing, Anal. Chem. 60 (1988) 1972.
[10] J.B. Chien, R.A. Wallingford, A.G. Ewing, J. Neurochem. 54 (1990) 633.
[11] T.M. Olefirowicz, A.G. Ewing, Anal. Chem. 62 (1990) 1872.
[12] T.M. Olefirowicz, A.G. Ewing, J. Neurosci. Methods 34 (1990) 11.
[13] T.M. Olefirowicz, A.G. Ewing, Chimica 45 (1991) 106.
[14] H.K. Kristensen, Y.Y. Lau, A.G. Ewing, J. Neurosci. Methods 51 (1994) 183.
[15] F.D. Swanek, G. Chen, A.G. Ewing, Anal. Chem. 68 (1996) 3912.
[16] S. Hu, D. Pang, Z. Wang, J. Cheng, Z. Li, Y. Fan, H. Hu, Chem. J. Chin. Univ. 17 (1996) 1207.
[17] W. Jin, W. Li, Q. Xu, Electrophoresis 21 (2000) 774.
[18] W. Jin, Q. Dong, X. Ye, D. Yu, Anal. Biochem. 285 (2000) 255.
[19] W. Jin, Q. Weng, J. Wu, Anal. Chim. Acta 342 (1997) 67.
[20] W. Jin, D. Yu, Q. Dong, X. Ye, J. Chromatogr. Sci. 38 (2000) 11.
[21] H. Yi, Z. Qian, Acta Anat. Sinica 29 (1998) 81.
[22] Q. Weng, W. Jin, Electrophoresis 22 (2001) 2797.
[23] B.L. Hogan, E.S. Yeung, Anal. Chem. 64 (1992) 2841.
[24] Z. Ma, K. Zhao, W. Qian, X. Zheng, Fenxi Huaxue (Chin. Anal. Chem.) 25 (1997) 750.
[25] Q. Xue, in: Physiological and Pathological Chemistry of Nervous System, Science Press, Beijing, 1992, p. 219.