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Assay of Histamine in Single Mast Cells by Capillary Zone Electrophoresis with Electrochemical Detection

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Abstract: Capillary zone electrophoresis was employed for the analysis of histamine in single rat peritoneal mast cells using an amperometric detector. 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. A cell injector was constructed for easy injection of single cells. Histamine in single mast cells has been identified and quantified.

Keywords: Capillary electrophoresis, electrochemical detection, histamine, mast cell.

Histamine is one of the chemical mediators in connection with allergies. Only Pihel *et al.*[1] reported a method for detection of histamine in isolated mast cells by high-performance liquid chromatography with electrochemical detection. In this method, single cells were removed and transferred to 300-nL microvials. A part of the supernatant from individual vials was injected into the chromatography column. 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 (25 μm inside diameter) and then the contents in the single cell was separated and detected. The method is simple, sensitive and relatively easy. Rat peritoneal mast cells (RPMCs) were isolated from a female rat by the method described in Ref. 2. In order to inject single cells easily, a cell injector was constructed. The cell injector is showed in **Figure 1**. The separation capillary and the buffer reservoir were filled with electrophoresis buffer. The injection end of the separation capillary was gently inserted in the suspension of mast cell. The other end of the separation capillary was inserted into a buffer reservoir (not shown in **Figure 1**) with a cathode. 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.

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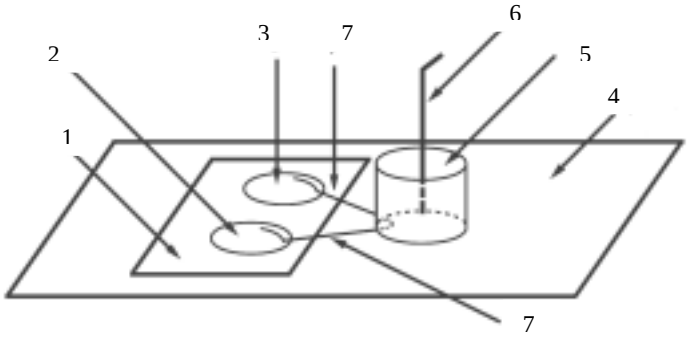
Since the anode for injecting single cells was not directly inserted in the cell suspension, the cell suspension was stable when a voltage for injecting single cells was applied. Then, the injection end of the separation capillary was taken out from the suspension of mast cell and was gently immersed in the lysis solution (0.02 mol/L NaOH). The electromigration injection of this solution at 2.0 kV for 5 s was carried out. After the cell was lysed, the separation capillary was carefully immersed to a reservoir containing electrophoresis buffer with an anode for electrophoretic separation. Then the electropherogram of the single cell was recorded. The other apparatus are the same as in Ref. 3.

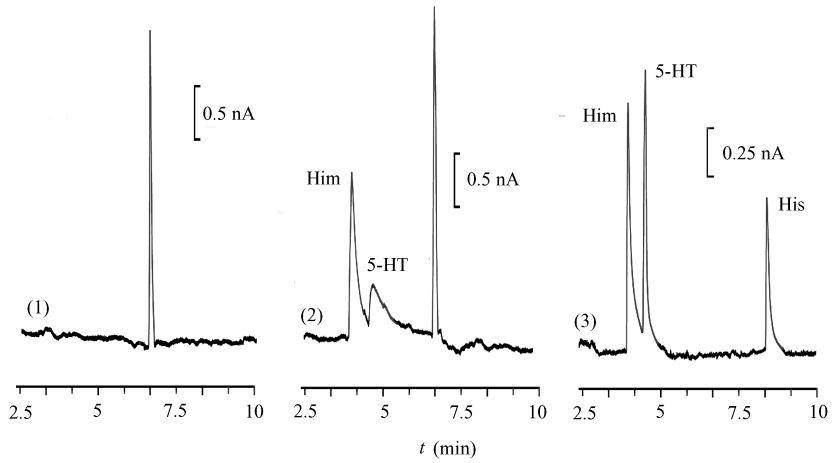
Figure 1 Schematic diagram of the cell injector.



(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.

The conditions of separating and detecting histamine were: 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 respectively, and 1.30 V versus SCE for the detection potential. **Figure 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, appeared in the electropherogram of the single cell (curve 2). By comparison with the electropherogram of the lysis solution shown in curve 1, the lysis solution should be responsible for the peak eluting at 400 s, because they have the same migration time and shape in both electropherograms. Comparison of curve 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. The external standardization was used for the quantification of histamine in an individual mast cell. The mean amount of histamine for nine cells is 95.8±49.9 fmol (mean±standard deviation), which is consistent with the literature value (64~114 fmol)[4,5].

Figure 2 Electropherograms of (1) the lysis solution), (2) a single mast cell and (3) the standard solution containing histamine (Him), serotonin (5-HT) and histidine (His).



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