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Determination of histamine and histidine by capillary zone electrophoresis with pre-column naphthalene-2,3-dicarboxaldehyde derivatization and fluorescence detection

Li-Yao Zhang, Meng-Xiang Sun*

Key Laboratory of MOE for Plant Development Biology, College of Life Science, Wuhan University, Wuhan 430072, China

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Abstract

A rapid and sensitive method was developed for the simultaneous determination of histamine and histidine by capillary zone electrophoresis with lamp-induced fluorescence detection. A fluoregenic derivatization reagent, naphthalene-2,3-dicarboxaldehyde (NDA) was successfully applied to label the histamine and histidine respectively. The derivatization conditions and separation parameters including pH and concentration of electrolyte and sample injection were optimized in detail. The optimal derivatization reaction was performed with 1.0 mM NDA, 20 mM NaCN, and 20 mM borate buffer, pH 9.1 for 15 min. The separation of NDA-tagged histamine and histidine could be achieved in less than 200 s with 40 mM phosphate buffer (pH 5.8) as the running buffer. The detection limits for histamine and histidine were 5.5×10^{-9} and 3.8×10^{-9} M, respectively (S/N = 3). The relative standard derivations for migration time and peak height of derivatives were less than 1.5 and 5.0%, respectively. The method was successfully applied to the analysis of histamine and histidine in the P815 mastocytoma cells and the beer samples.

Keywords: Derivatization, electrophoresis; Histamine; Histidine; Naphthalene dicarboxaldehyde; Amino acids; Amines

1. Introduction

As an important neurotransmitter and a typical biogenic amine, histamine can be found in nervous and immune organizations, a variety of foods and beverages, and synthesized in vivo from its precursor amino acid histidine by the decarboxylation reaction. It was also found that ingestion of sufficiently high levels of histamine could result in symptoms of intoxication, such as headache and nausea, etc. [1].

Considering the potential harmful effect of histamine and to develop nervous and immune research, it is needed to develop sensitive methods for the analysis of histamine. In the past years, many analytical methods have been exploited to characterize histamine, such as high-performance liquid chromatography (HPLC) [2–5] and gas chromatography methods [6,7], etc.

Capillary electrophoresis (CE) has been a powerful method for years in the field of analytical chemistry to analyze biological substance ranging from amino acid to protein and DNA with many advantages, such as the high separation speed and efficiency, the relatively simple instrumentation and very low running costs. Some reports [1,8–10] have demonstrated the usefulness of CE as a method to identify the histamine in food samples coupled with direct or indirect ultraviolet detection. Oguri et al. [11–13] described a CE method with on-line mode in-capillary derivatization with *o*-phthalaldehyde (OPA) and *N*-acetylcysteine for the analysis of histamine in foods and mast cells. Some other fluorescence derivatization reagent such as fluorescein isothiocyanate (FITC) [14] and 3-(2-furoyl)quinoline-2-carboxaldehyde (FQ) [15] have been used for histamine analysis by CE and fluorescence detection. CE and amperometric detection methods also have been exploited for the analysis of histamine [16,17].

Naphthalene-2,3-dicarboxaldehyde (NDA) derivatization reagent has been developed for the amino acid analysis by liquid chromatography [18–20] and CE [21–24] with fluorescence or electrochemical detection. The reactions of NDA reagent with primary amine in the presence of cyanide are shown in Fig. 1. The derivatization procedure is

* Corresponding author. Tel.: +86-27-87682378; fax: +86-27-87646010.
E-mail address: mxsun@whu.edu.cn (M.-X. Sun).

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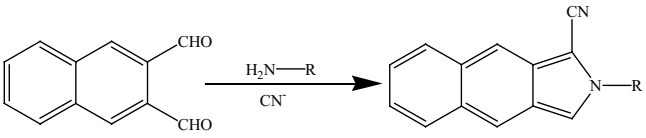

Fig. 1. Derivatization reaction scheme of NDA with amines in the presence of cyanide.

simple and quick, and the derivatives are very stable. However, up to now there has been no study on the determination of histamine with NDA derivatization by the capillary electrophoresis.

In this work, we describe a simple and sensitive capillary zone electrophoresis (CZE) with lamp-induced fluorescence detection method for the simultaneous analysis of histamine and histidine. NDA was used as the fluorescence derivatization reagent. The whole separation was within 200 s. The detection limits of 10^{-9} M for histamine and histidine were obtained. The method has been used for the characterization and measurement of histamine and histidine in the P815 mastocytoma cells and the beer samples.

2. Experimental

2.1. Apparatus

CE was carried out on a laboratory-built system based on an upright fluorescence microscope (Olympus, Japan), a photo-multiplier tube (PMT), a ±30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) and a uncoated fused-silica capillary of 45 cm (34 cm to the detector window) × 50 μm i.d. × 365 μm o.d. (Yongnian Optical Conductive Fiber Plant, China). A 100 W high-pressure mercury lamp was used as the excitation radiation. The optical sub-system in the microscope consisted of a 40× objective, a NIB excitation cube including an excitation filter (EX 400–490 nm), a dichroic mirror (DM 510 nm) and a barrier filter (BA 515 nm). The signal from the PMT was monitored using photon-counting device (Beijing Bingsong Photon Technological Corp., China) and collected by a computer (Inter PIII550) with photon-counting software, and processed with Origin software packages.

2.2. Chemicals

Histamine hydrochloride (histamine·HCl) and histidine (His) purchased from Sigma (St. Louis. MO, USA) were prepared at a concentration of 1.0×10^{-2} M in pure water, diluted to the desired concentration and stored in a refrigerator. NDA was obtained from Aldrich. 1.0×10^{-2} M NDA stock solution was prepared in methanol and diluted to the desired concentration in methanol, and stored in refrigerator. Mast cell ringer (MCR) buffer was used as the cell suspension buffer, which was composed of 140 mM NaCl, 2.5 mM KCl, 5.0 mM $MgCl_2$, 2.0 mM $CaCl_2$, 5.0 mM glucose and 10 mM 4-(2-hydroxyethyl)piperazineethanesulfonic acid (HEPES), pH 7.4. Other chemical reagents were of analytical grade and used without further purification. Carrier electrolyte for capillary electrophoresis was prepared daily, and then filtered through a 0.22 μm membrane prior to use.

2.3. Drivatization procedure

Thirty μl of a mixed histamine·HCl and His solutions, 10 μl of 2.0×10^{-2} M borate buffer (pH 9.1), 10 μl of 2.0×10^{-2} M cyanide, and 20 μl of 1.0×10^{-3} M NDA solution was added sequentially and thoroughly mixed. The resulting solution was allowed to stand for 15 min at room temperature prior to injection. The particular order of reagent addition is important in minimizing possible benzoin condensation reactions [20].

2.4. Capillary electrophoresis

A new capillary should be pre-treated with 1.0 M NaOH, water for 30 min sequentially. Each day before analysis, the capillary was rinsed with 0.1 M NaOH, water for 10 min and preconditioned with running buffer for 15 min at room temperature.

The electrophoresis buffer consisted of 40 mM sodium dihydrogenphosphate and adjusted to pH 5.8 with H_3PO_4. Sample injection was performed by hydrodynamic mode with sampling height at 9 cm for 30 s. Separations were carried out at a constant voltage of 18 kV.

2.5. Sample preparation

The P815 mastocytoma cells were obtained from 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, roughly 10^5 P815 cell was washed three times by MCR solution and resuspended in 0.5 ml of water. The cells were then stored in refrigerator at 0 °C for 5 min to be lysed. The suspension was centrifuged at 5000 rpm for 10 min. The supernatant was derived by NDA as the above derivatization procedure. Samples of beers were purchased from local supermarket. In order to make the concentration of histidine in linear calibration range and decrease the influence of other amino acid on derivatization reaction, the real beer was diluted with water in this case.

3. Results and discussion

3.1. Choice of derivatization conditions

Histamine is basic, and the amino group of histamine is protonated in acid or neutral buffers. Protonation of amino

group can inhibit the nucleophilic addition reaction. The effect of the pH value of the derivatization buffer in the range of 4.0–11.0 was investigated. It was found that the derivatization efficiency increased while the pH value was varied from 4.0 to 9.0, and decreased when the pH value was above 9.5. Also according to the previous report about the derivatization of amino acid [18], 20 mM borate buffer (pH 9.1) was chosen as the derivatization medium in this work.

We also compared the effect of reaction time on the derivatization efficiency. It was found that the reaction of NDA with histamine was very quick, the reaction completed in about 3 min. However, the reaction of NDA with histidine was lower, the derivatization efficiency enhanced with the time in the 30 min, but when the time was over 15 min, the derivatization efficiency enhanced slightly. Consequently, 15 min was chosen as the suitable derivatization time. The reason for the lower reaction rate of NDA with histidine compared with histamine may be that the space hinders of histidine is bigger than that of histamine. The effect of concentration of NDA and NaCN were also investigated. As reported in previous studies on derivatization of amino acid [18], when NDA and CN^- were in excess, the reaction rate was unchanged with the increase of concentration. Also it was reported that the optimal ratio between cyanide and NDA was at least 10 [25]. In summary, in this work the derivatization reaction was successfully carried out with 1.0 mM NDA, 20 mM NaCN, and 20 mM borate buffer, pH 9.1 for 15 min, the final ratio of $[CN^-]/[NDA]$ was 10. The stability of derivatization products was examined in the 2 h. It was found that the histamine derivatized product was stable within 30 min, but when the time was beyond 30 min, the product began to degrade as the peak signal began to decrease; the histidine derivatized product was very stable within 2 h. The better stability of histidine derivatized product than histamine derivatized product was probably due to the its better hydrophilic property.

3.2. Fluorescence characteristics of derivatized product

The excitation and emission spectra of the derivatized products were examined. And the effect of pH of medium on the excitation and emission spectra (Fig. 2) of the derivatized product was also studied. It can be seen that the excitation spectrum shows two maxima in the visible region at 417 and 440 nm whereas the emission spectrum exhibits maxima at 460 and 480 nm, which was similar to some other amines–NDA derivatives [26]. As indicated in Fig. 2A, the effect of pH of medium revealed that the fluorescence intensity of histamine–NDA derivative depends on the pH of medium, the fluorescence intensity decreases with the increase of pH value. Also from the Fig. 2B, it can be seen that the fluorescence intensity of histidine–NDA derivative was unchanged in the pH range of 3.2–8.0.

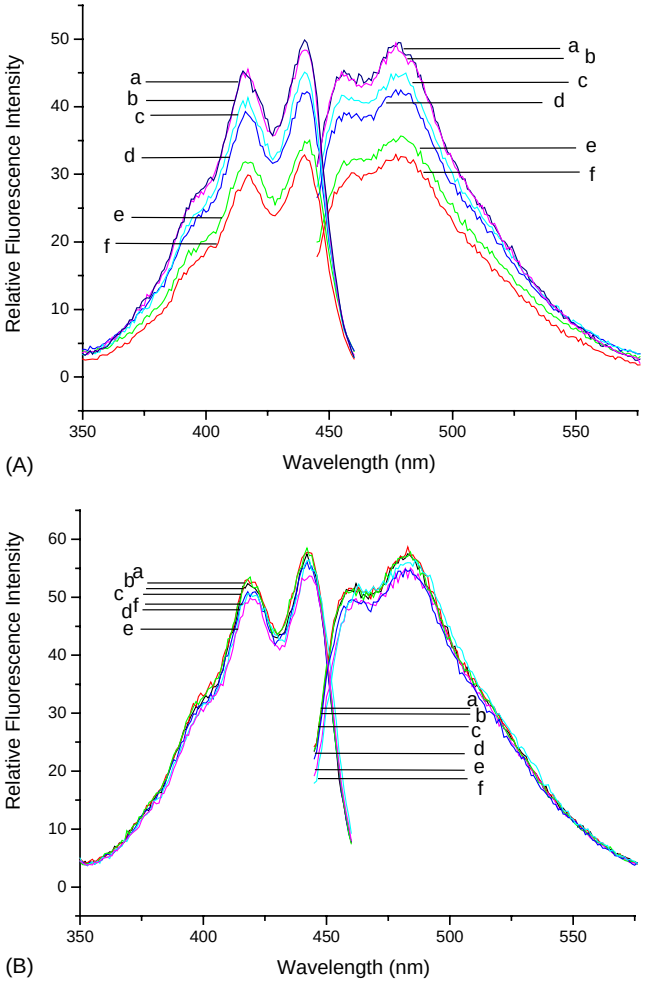

Fig. 2. Excitation and emission spectra of histamine–NDA (A) and histidine–NDA derivatives (B) in the different pH medium: (a) 3.2, (b) 4.0, (c) 5.0, (d) 5.8, (e) 7.0, (f) 8.0, 40 mM PBS.

3.3. Optimization of CE separation parameters

The composition of the running buffer is an important factor for the separation and detection in the capillary zone electrophoresis. Tris(hydroxymethyl)aminomethane (Tris), a common running buffer was examined, it was found that Tris could react with NDA in the presence of cyanide and the product was with fluorescence, so phosphate buffer solution (PBS)was used as the running buffer in this work. Since the NDA-tagged histamine is protonated in the acid solutions (dissociation constant of imidazole in histamine are pK_a 6.14 [27]), and the NDA-tagged histidine is zwitterions, the separation of NDA-labeled histamine and histidine could be expected to be carried out by capillary zone electrophoresis. It is well known that acidity of the running buffer play a key role in CE because of its effect on the electroosmotic flow as well as the over-all charges of the analytes. Fig. 3 shows the effect of pH of running buffer on the separation of NDA-labeled histamine and histidine. From the electropherograms, it can be seen that there is a

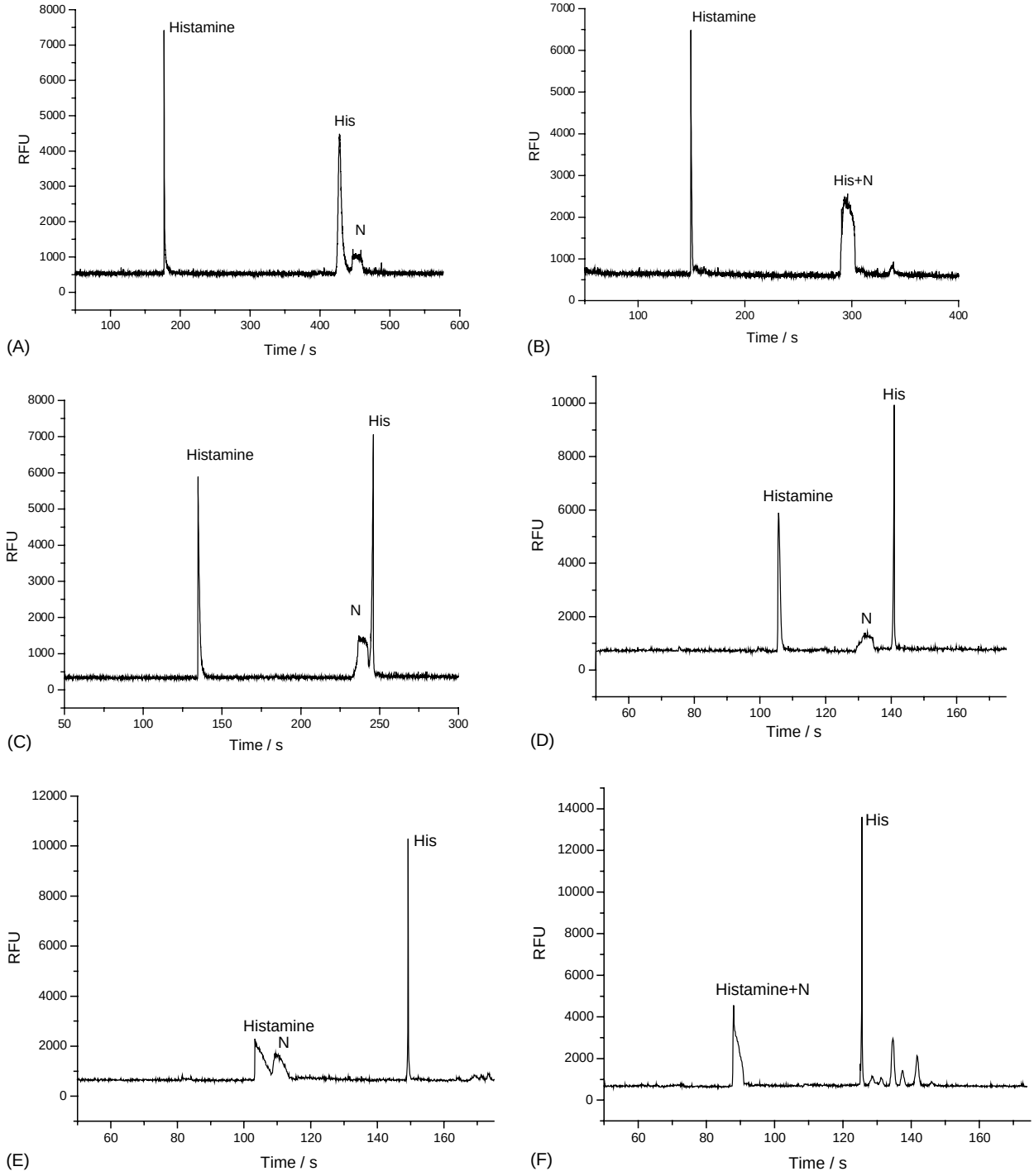

Fig. 3. Electropherograms of NDA-labeled histamine and histidine in running electrolytes of different pH values: (A) 3.2, (B) 4.0, (C) 5.0, (D) 5.8, (E) 7.0, (F) 8.0, 10 mM PBS. N, neutral.

nonanalyte peak under all pH conditions in addition to the peak of histamine and histidine. The same phenomenon was observed by Gilman and Ewing [22] and Lillard et al. [28], in which Tris or borate running buffer was used. These authors attributed the peak to a neutral impurity or believed that the peak was caused by formation of fluorescent benzoin condensation products or other side products of NDA upon exposure to aqueous buffer. In this system, we also found that the peak was present when Tris, borate or phosphate running buffers were used respectively. Also it was found that the peak was observed in the background analysis and was confirmed to migrate along with neutral label in the CZE. In this case, NDA-labeled phenylethylamine was used as the neutral label. So the separation of NDA-labeled histamine and histidine with the neutral peak is essential. As shown in Fig. 3, it can be seen that the separation of NDA-labeled

histamine and histidine with the neutral peak is difficult by CZE when the pH was either beyond 7.0 or between 4.0 and 5.0. This may be due to the decrease of the positive charges of the histamine and the decrease of the negative charges of histidine. And when the pH is further reduced to 3.2, the migration time increased and the peak efficiency of histidine was reduced greatly. Therefore, pH 5.8 is considered suitable for the simultaneous separation of histamine and histidine in this work. The numbers of theoretical plates of His in Fig. 3A, C, D, E and F were calculated to be 4.0×10^4, 9.2×10^5, 2.0×10^6, 9.6×10^6, 5.7×10^6, respectively. The numbers of theoretical plates of histamine in Fig. 3A, B, C, D, E were 1.4×10^6, 2.8×10^5, 1.5×10^5, 1.0×10^5, 5.6×10^3, respectively, using the formula $N = 5.545(l/w_{1/2})^2$, where l is the distance of the peak from the start on the record and $w_{1/2}$ is the width at half height [29]. It can be seen that the number of plates of His increases with the increase of pH value whereas histamine plate number decreases with the increase of pH value. The plate number of His in Fig. 3B and that of histamine in Fig. 3F were not calculated, as they co-migrated with the neural. It was noted that in Fig. 3F, there are several unknown peaks after the His peak. We have tried hard to eliminate these peaks, but unsuccessfully. These peaks might be impurities from the reagents.

The influence of the concentration of phosphate running buffer at pH 5.8 was examined at 5, 10, 20, 40 and 60 mM. It was found that the migration order of histamine, neutral peak, histidine remains unchanged, and the separation is much improved with increasing the concentration of the buffer, and the sensitivity of histamine and histidine and the migration time increased with increasing buffer concentration. Considering both optimal sensitivity and short analysis time, 40 mM phosphate buffer was chosen as the optimized concentration for this system.

As for sample injection, the electrokinetic mode gave better resolution of separation than the hydrodynamic mode. But sample discrimination occurred when the electrokinetic mode was used. Thus, gravity introduction of sample was used in this case. In order to avoid overloading effect the injection amount was optimized. When the sampling height was fixed at 9 cm, it was found that the peak height of histamine and histidine increased with increasing the sampling time simultaneously. When the injection time was more than 35 s, the peak height of histamine levels off and the peak efficiency of histamine decreased. Thus, the injection time of 30 s was selected as optimal condition.

3.4. Typical electropherogram, separation efficiency, reproducibility, linearity, and detection limit of histamine and histidine

Fig. 4A shows the separation of NDA labeled histamine and histidine by CZE under the optimum conditions. The separations were carried out within 200 s. The excellent separation efficiency was obtained with this system. The numbers of theoretical plates of histamine and His were

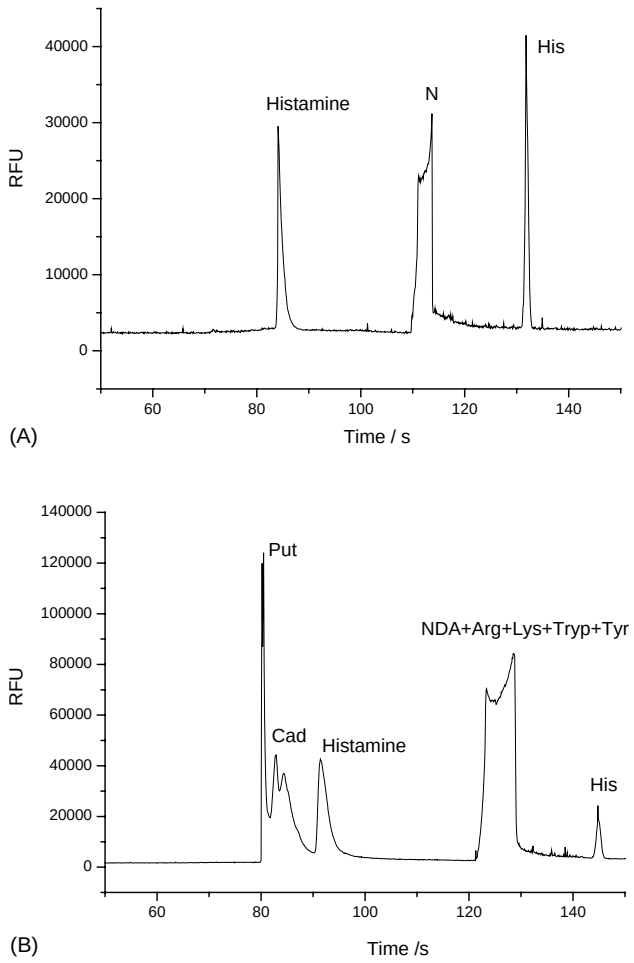

Fig. 4. (A) Electropherogram of a mixture of histamine and histidine (5.0×10^{-7} M) derivatives under the optimum conditions. Capillary, 45 cm × 50 μm i.d.; hydrodynamic injection, 9 cm (height) for 30 s; separation voltage, 18 kV; electrophoresis buffer, 40 mM, pH 5.8 PBS. (B) Illustration of the selectivity of the method towards histamine and His. A solution consisting of 18 amino acids, 4 biogenic amines, and His, histamine, His was analyzed by the presented method.

calculated to be 6.7×10^4, 3.2×10^5, respectively. In order to ensure the reproducibility of this method, the capillary must be washed by the running buffer, which can eliminate the residual of the last injection and the absorption of hydrophobic NDA onto the capillary wall, which would reduce the electroosmotic flow. The reproducibility was evaluated by repeating derivatization and injection ($n = 5$) of histamine and His standard solution at concentration of 5×10^{-7} M under the optimum conditions. The relative standard deviation (R.S.D.) of peak height was 4.5% for histamine, and 4.8% for His. The migration time R.S.D.s for histamine and His were 1.1 and 1.3%, respectively. Using peak height, linearity calibration curves were obtained for histamine ($R = 0.9996$) and His ($R = 0.9992$) over the concentration range of 1.0×10^{-8} to 5.0×10^{-6} M. The detection limits, obtained from signal-to-noise level at 3, are 5.5×10^{-9} M for histamine, and 3.8×10^{-9} M for

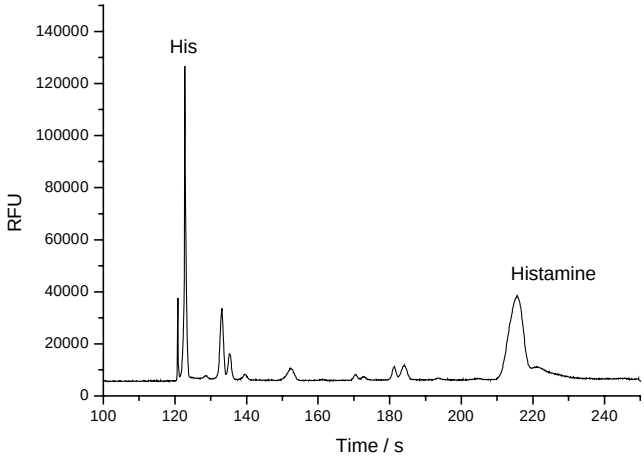

Fig. 5. Electropherogram of NDA-labeled histamine and histidine by MEKC. Capillary, 45 cm × 50 μm i.d.; hydrodynamic injection, 9 cm (height) for 30 s; separation voltage, 22 kV; electrophoresis buffer, 20 mM borate buffer containing 20 mM SDS, pH 9.2.

histidine, which is equivalent to or better than other CE methods without using laser as the excitation radiation.

3.5. Selectivity of the method

A mixture of 18 amino acids and some other biogenic amine such as tryptamine, tyramine, putrescine and cadaverine, and histamine, His was analyzed to demonstrate the selectivity of the method towards histamine and histidine. The corresponding electropherogram is shown in Fig. 4B. As expected, only positive-charge amino acid (lysine and argenine) and biogenic amine derivatives were detected within the separation time window of 200 s. Furthermore, none of these amino acid and amine derivatives peak overlapped with the histamine and histidine derivative peaks. This demonstrates the good selectivity of the method, and its suitability for biological sample analysis. The number of theoretical plates of histamine and His was calculated to be 1.2×10^4, 1.8×10^5, respectively.

Since micellar electrokinetic chromatography (MEKC) is an important and effective separation mode in CE, and have been applied to the analysis of many chemical and biological reagents in different samples [30,31], we further tried to separate NDA-labeled histamine and histidine by MEKC with sodium dodecyl sulphate (SDS) as the surfactant and the results were shown in Fig. 5. It can be seen that the separation efficiency of labeled histamine in our case was lower than that by CZE, which might be due to more obvious diffusion effect caused by the longer migration time of NDA-labeled histamine in MEKC.

3.6. Application

P815 mastocytoma cells is a mouse mastocytoma tumor cell, and has been used as the model of tumor immunology

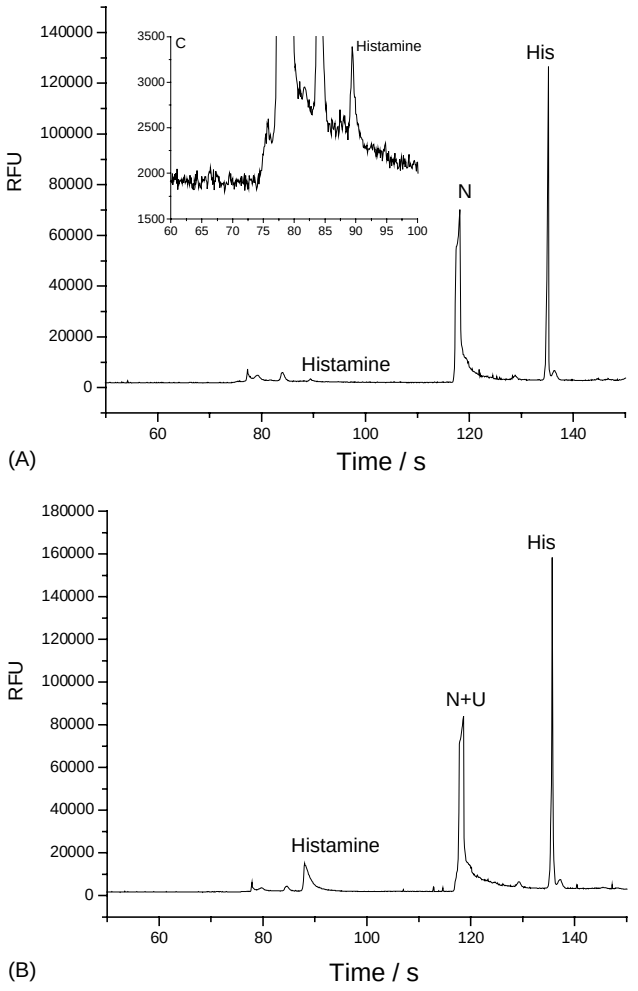

Fig. 6. Electropherograms of NDA-labeled histamine and His analysis in P815 mast cells. (A) Whole electropherogram; (B) electropherogram obtained by a standard addition method; (C) electropherogram intercepted from (A). N, neutral; U, unknown.

[32,33]. The developed method was applied to the determination of histamine and histidine in P815 mastocytoma cells. Fig. 6A shows the electropherogram of a 10^5 P815 cell lysate analysis. Fig. 6C exhibits the electropherogram intercepted from the corresponding part of Fig. 6A. The number of theoretical plates of histamine and His in Fig. 6A was calculated to be 7.5×10^4, 1.3×10^6, respectively. Furthermore, we have successfully applied this method to the analysis of the histamine and histidine in beer. Fig. 7A shows the electropherogram of a beer sample analysis. The numbers of theoretical plates of histamine and His in Fig. 7A were calculated to be 3.9×10^4 and 3.8×10^5, respectively. To further identify the peak of histamine and histidine, a standard addition approach was employed as shown in Figs. 6B and 7B. The analytical results of the P815 cell lysate and the beer sample were summarized in Table 1. The average amount of histamine and histidine in single P815 cell is estimated to be 90 amol and 8.0 fmol, which is lower than that of reported in individual normal mast cells [34]. The

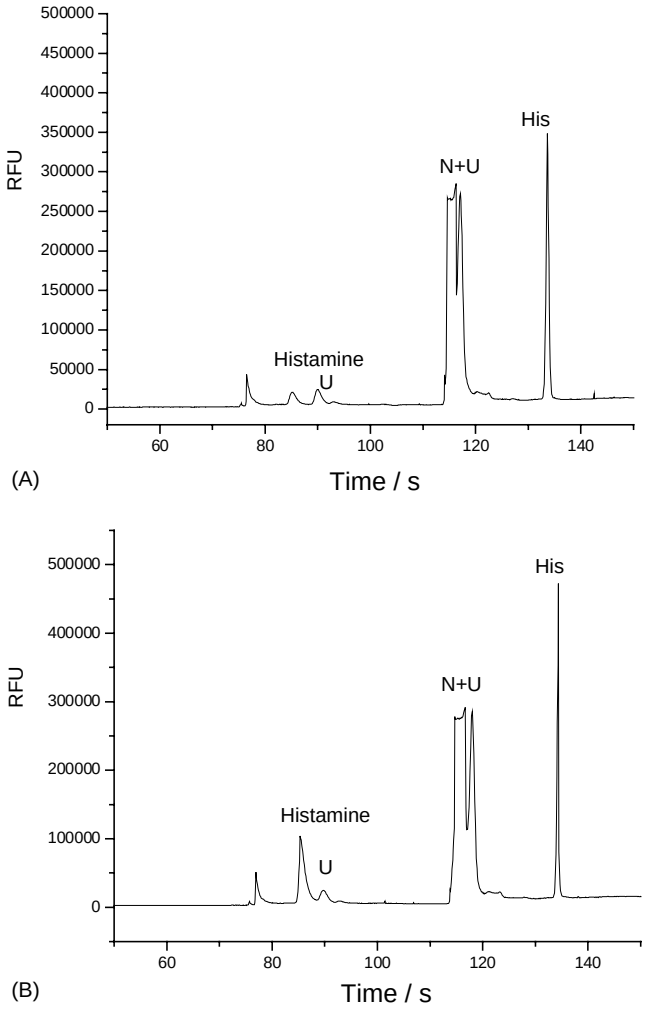

Fig. 7. Electropherograms of NDA-labeled histamine and His analysis in beer sample. (A) Whole electropherogram; (B) electropherogram obtained by a standard addition method. N, neutral; U, unknown.

reason may be that P815 cells used here are tumor cells, and the phenomena are similar to the case described by Tong, in which the insulin content in tumor cell are 0.5–2% of normal cell [35]. The contents of histamine and histidine in a beer were calculated to be 2.7×10^{-6} and 4.4×10^{-5} M, and the results were consistent with the previous reports about the histamine analysis and amino acid analysis in the beer [16,24].

Table 1
Analytical results of samples by the proposed method (μmol/l)

Analyte	Samples	Content	Added	Total amount	Recovery (%)
Histamine	Cell lysate	0.018	0.40	0.41	98.1
	Beer[a]	0.27	1.60	1.81	96.8
Histidine	Cell lysate	1.60	0.50	1.98	94.3
	Beer[a]	4.43	2.00	6.02	93.6

[a] The concentration of the beer sample is 10% of the real commercial beer.

4. Conclusion

A rapid CZE with lamp-induced fluorescence detection method has been developed for the simultaneous determination of histamine and histidine with NDA as the fluoregenic derivatization reagent. Optimum separation was obtained using 40 mM phosphate buffer pH 5.8. A detection limit of 10^{-9} M was obtained for histamine and histidine. It is believed that the sensitivity of the method could be further improved by using the helium-cadmium laser for fluorescence detection. This method was demonstrated by the histamine and histidine analysis in the P815 mastocytoma cells and the beer samples.

Acknowledgements

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