Noradrenaline and dopamine are two of the most important monoamine neurotransmitters. They play important roles in learning and memory, and in regulation of cardiovascular activity and nervous system. Analysis of the monoamine neurotransmitters has conventionally used high performance liquid chromatography (HPLC) or gas chromatography. However, the determination of monoamine neurotransmitters in the biological matrix is very difficult because the compounds are usually present at very low concentrations, and many other compounds in biological matrix, which have also absorption in the low-wavelength region, interfere badly with the analysis of monoamine neurotransmitters. Cyanine derivative (Cy5) is a new fluorescence derivative reagent. The excitation and the emission wavelength of Cy5 are 649 and 670 nm, respectively. Thus diode lasers, which emit wavelengths from deep-red to infrared region, are chosen as the light source for laser-induced fluorescence (LIF). This is preferential for determining Cy5-labeled amine neurotransmitters, since almost all of the biological substances have no absorption band in the deep-red and infrared region, resulting in a remarkable decrease of background fluorescence noise and in an increase of detection sensitivity. Recently, LIF is widely used in the analysis of DNA sequence, oligonucleotides and proteins. But determination of Cy5-labeled monoamine neurotransmitters has not been reported. Capillary electrophoresis (CE) is a newly emerging technique for rapid and high-resolution separation of biomolecules. Recently, CE is widely used in the separation and determination of amine. The detection methods of amine in CE include UV-VIS, electrochemical detection and LIF. LIF is one of the most sensitive detection techniques in CE because of its low background signal and the small sample volume. In this paper, we report a rapid, sensitive method for the analysis of noradrenaline and dopamine in CE coupled diode LIF detection, in which Cy5 was chosen as the fluorescence derivatization reagent. The effects of buffer concentration, pH value, and reaction time on the derivatization reaction of monoamines were investigated.

Experimental

Reagents

Unless stated otherwise, all chemicals were of analytical-reagent grade (Beijing Reagent Corp., Beijing, China). Noradrenaline, dopamine and sodium dodecyl sulfate (SDS) were purchased from Sigma (St. Louis, MO, USA). Cy5 was obtained from Amersham Pharmacia Biotech, USA. Water was deionized and purified by a Milli-Q Millipore purification system (Millipore, Marlborough, MA, USA).

Apparatus

All analyses were performed on a Beckman P/ACE 5000 system equipped with a Beckman Laser Module 635 nm. This was used as an exciting light source (Beckman-Fullerton, CA, USA). Separations were performed in fused-silica capillaries (57 cm × 75 μm i.d.; Yong Nian Optic Fiber Factory, Hebei Province, China).

Derivatization procedure

Noradrenaline and dopamine were labeled with Cy5 as follows: Cy5 was dissolved in dimethylformamide. The solution with a concentration of 1.26 × 10^{-3} M was preserved in the dark at −20°C. It was diluted to the desired concentration before use. Then 20 μl of the Cy5 solution was added to 10 μl of 10 mM boric acid buffer (pH 8.5) containing noradrenaline and dopamine. The mixed solution was reacted for 90 min at room temperature in the dark; the solution was diluted by 70 μl of 10 mM boric acid buffer (pH 8.5) before analysis.

Results and Discussion

Effect of buffer on the derivatization reaction

The effect of different kinds of buffer on the derivatization reaction was investigated. Noradrenaline and dopamine were well derivatized with Cy5 in carbonate, phosphate and borate buffer. The best results were obtained with the carbonate buffer (pH 8.5) containing noradrenaline and dopamine. The mixed solution was reacted for 90 min at room temperature in the dark; the solution was diluted by 70 μl of 10 mM boric acid buffer (pH 8.5) before analysis.

Effect of buffer pH on the derivatization reaction

The effect of different buffer pH values on the derivatization reaction

Notes

Fluorescent Determination of Noradrenaline and Dopamine Derivatized with Cy5 in Capillary Electrophoresis

Dongming ZHANG, Min FU, Wanyun MA, and Dieyan CHEN

Key Laboratory of Atomic and Molecular Nanosciences of Education Ministry, Department of Physics, Tsinghua University, Beijing 100084, P. R. China

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reaction of amine was studied (Fig. 2). Noradrenaline and dopamine in salt form in the buffer with pH below 5 were not derivatized by Cy5. The derivatization efficiency increased with the accretion of buffer pH, reaching a maximum with the buffer with pH 8.5. Then the efficiency decreased with the increase in buffer pH, because noradrenaline and dopamine are easily oxidized in high alkaline condition. Therefore, the pH value of borate buffer was chosen at pH 8.5.

Effect of Cy5 concentration on derivatization reaction

Figure 3 shows the correlation between the increase in concentration of Cy5 and the increase in the fluorescence intensity of monoamine derivatives. The derivatization efficiency of amines was largely influenced by the concentration of Cy5. Though low concentration of Cy5 resulted in few interfering electrophoretic peaks, the reaction was incomplete and the detection sensitivity was very low. As the Cy5 concentration increased, the derivatization efficiency became better, and when the concentration of Cy5 exceeded by nearly 40 times that of the total concentration of analytes, the maximum efficiency was reached and the efficiency become stable.

Effect of reaction time on derivatization reaction

The derivatization reaction of primary amines using Cy5 is relatively quick compared to FITC and TRITC fluorescence reagents.13,14 The result showed the derivatization efficiencies for noradrenaline and dopamine increasing with time; after 90 min, a plateau was reached. Then the derivatization procedure tended to stabilize. The derivative remained stable in 6 h after equilibrium was reached; nearly the same results of CE were obtained. These data demonstrated that at least 90 min are required for Cy5 to react completely with an amine.

Separation condition

In our experiments, we investigated various kinds of separation buffers for separation of Cy5-labeled monoamines. The results show that borate buffer with SDS is a better resolution buffer to separate Cy5-labeled monoamines. The effects of various parameters such as pH value and concentration of buffer, concentration of SDS, applied voltage were optimized to achieve best separation, the highest sensitivity and the shortest analysis time. The optimum separation conditions to separate Cy5-labeled amines were found to be 125 mM boric acid buffer at pH 10.0 with 20 mM SDS. The applied voltage was 20 kV. Figure 4 shows a typical electropherogram of Cy5-labeled amine solution. The precision of the present method for noradrenaline, dopamine and 3,4-dihydroxybenzylamine was determined by analyzing the standard mixture seven times. The relative standard deviations (RSD) of the migration times of noradrenaline, dopamine and 3,4-dihydroxybenzylamine were 0.16%, 0.3%, and 0.33%, respectively. The RSDs of the peak areas were 4.47%, 1.87%, and 2.38%, respectively. The detection limits, which were calculated for $S/N = 3$, were $5.9 \times 10^{-9}$ M, $5.4 \times 10^{-9}$ M, and $2.7 \times 10^{-9}$ M for noradrenaline, dopamine and 3,4-dihydroxybenzylamine, respectively; the numbers of theoretical plates were 412130, 297696, and 416486, respectively. When $6.88 \times 10^{-7}$ M 3,4-dihydroxybenzylamine was used as an internal standard, the calibration curves of noradrenaline and dopamine showed good linearity in the concentration range from $9.30 \times 10^{-8}$ to $1.86 \times 10^{-6}$ M; the correlation coefficients of

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**Fig. 1** Effect of buffer concentration on amine derivatization.

**Fig. 2** Effect of buffer pH on amine derivatization.

**Fig. 3** Effect of Cy5 concentration on amine derivatization.

**Fig. 4** Electropherogram of amines: (1) Cy5, (2) decomposition products of Cy5, (3) noradrenaline, (4) 3,4-dihydroxybenzylamine, (5) dopamine.
noradrenaline and dopamine were both 0.995.

**Conclusion**

We have demonstrated that capillary electrophoresis coupled diode LIF with Cy5 monofunctional dye (Cy5) derivatization is an efficient and sensitive method to perform the analysis of trace monoamine neurotransmitters. Cy5 reacted with a primary amine such as noradrenaline and dopamine rapidly and the derivatization efficiency was high. Cy5 did not react well with a secondary amine, such as adrenaline, because of the steric hindrance. Compared with other amino-labeled fluorescence reagent, such as FITC and TRITC,13,14 Cy5 showed the higher reaction selectivity and shorter reaction time for primary amines. And the detection limits of Cy5-derivatized amines were the same as those of FITC-derivatized amines.13

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