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Determination of polyamines in serum by high-performance capillary zone electrophoresis with indirect ultraviolet detection

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Abstract

A method for determining polyamines in serum by capillary zone electrophoresis (CZE) with indirect ultraviolet detection was established. The concentrations of polyamines in the sera of six healthy adults were determined and the results were in accordance with those obtained previously by high-performance liquid chromatography (HPLC). However, the CZE method is superior to HPLC in that it has high sensitivity, small sample consumption and easy sample pretreatment.

1. Introduction

Polyamines, mainly putrescine, spermidine and spermine, exist in all living organs and play important roles in cell growth and differentiation. Since Russell [1] reported in 1971 that polyamine concentrations in the urine of some tumour patients were higher than normal, the relationship between polyamines and tumour status has aroused the interest of many researchers and many cases of different tumours with variations in polyamine concentrations have been reported [2,3], and appropriate evaluation of the relationship between polyamine levels and some diseases, including tumours, is still progressing. The main methods for polyamine detection have been high-performance liquid chromatography (HPLC) and thin-layer chromatography. However, both methods require that polyamines be derivatized or labelled before detection since they have no chromophore and cannot be detected with an ultraviolet detector. This makes the methods tedious and time consuming.

Capillary zone electrophoresis (CZE) has proved to be a powerful technique in separating charged biomolecules with high resolution [4,5], and indirect detection has become a simple and sensitive method for CZE [6,7]. CZE involving indirect detecting has not been reported in serum polyamine detection. Polyamines in serum can reflect most directly the body polyamine level. In this work, CZE with indirect detection was employed for detecting polyamines in serum and proved to have several advantages, such as high sensitivity, small sample consumption and easy sample pretreatment.

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2. Experimental

2.1. Equipment

A Bio-Focus 3000 and HPE-100 capillary electrophoresis system (Bio-Rad Labs., Richmond, CA, USA), UV detection and electrokinetic injection were used.

2.2. Pretreatment of capillary column

Capillary columns (Hebei Yongnian Photoconductive Fibre Factory, Hebei, China) of 50 mm I.D. were cleaned with 0.1 *M* HCl and 0.1 *M* NaOH in sequence and then coated with polyacrylamide. Coated columns with effective lengths of 35.2–40.2 cm were used

2.3. Reagents

Quinine sulfate monohydrate was purchased from Fisher Scientific (Fairlawn, NJ, USA). Putrescine (PU) and spermine (SPM) were purchased from Sigma (St. Louis, MO, USA) and spermidine (SPD) from Serva (Heidelberg, Germany).

2.4. Preparation of background electrolyte [8]

Quinine sulfate (392 mg) was dissolved in a mixture of 20 ml of 95% ethanol and 70 ml of deionized water, the pH was adjusted to 3.0 with 0.1 *M* HCl and the volume was then brought to 100 ml.

2.5. Pretreatment of serum

A 1-ml volume of acetone was added to 100 ml of serum and the denatured proteins were removed by centrifugation at 30 000 *g* for 10 min. The deproteinized serum was obtained after volatilizing acetone from the supernatant. SPD and SPM in serum were determined by adding SPD and SPM standards to the original deproteinized serum. As the PU peak was covered by the first unknown peak in the electropherogram of serum, the identification of PU was subject to interference. After diluting the serum tenfold, the diluted unknown substance and PU were satisfactorily separated. Hence the position of PU was determined in the electropherogram of the tenfold dilution of the original deproteinized serum. A volume of 72–75 ml of deproteinized serum was obtained after pretreatment of 100 ml of serum and the concentrations of SPD and SPM in the serum were converted into concentrations in 100 ml after obtaining the concentrations in the original 72–75 ml of deproteinized serum.

3. Results and discussion

3.1. Determination of polyamine standards

The electropherogram of the three polyamine standards is shown in Fig. 1. The relative peak positions of the three polyamine standards were in accordance with the expected result based on the consideration of the Z/M values, which are PU 0.0227, SPD 0.0206 and SPM 0.0197. Because of the absorption of quinine sulfate at 236 nm, the three polyamine peaks actually appeared upside down. For convenience of observation, the electropherograms in this paper have been reversed to that the sample peaks appear as if they were absorption peaks.

With injection conditions of 5 kV, 3 s and a separation voltage of 9 kV, the regressional equations for the polyamines were $y = 9.325 + 0.1511x$ for PU, $y = 22.30 + 0.9654x$ for SPD

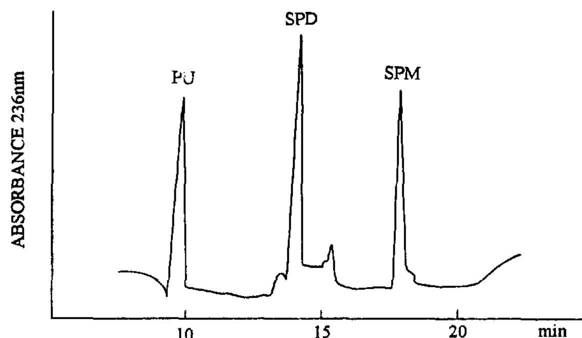

Fig. 1. Determination of polyamine standards by CZE with indirect UV detection. Load, 8 kV, 2 s; run, 8 kV; detection, 236 nm, 0.02 AU.

and $y = 79.94 + 2.830x$ for SPM, in which y is the peak height (mm) and x is the concentration (10^{-8} mol/l). The linear range of polyamine detection was investigated and a linear response over two orders of magnitude ($5 \cdot 10^{-8}$–$5 \cdot 10^{-6}$ mol/l) for each polyamine was obtained. The detection limit for the three polyamines was $1 \cdot 10^{-12}$ mol/l.

3.2. Precision

A mixture of polyamine standards was analysed five times repeatedly and the results expressed as average peak height ± R.S.D. were 12.2 mm ± 4.1% for PU ($4.39 \cdot 10^{-7}$ mol/l), 46.1 mm ± 2.3% for SPD ($2.21 \cdot 10^{-7}$ mol/l) and 113.3 mm ± 5.7% for SPM ($1.35 \cdot 10^{-7}$ mol/l).

3.3. Relative recovery of polyamines

A 20-ml volume of polyamine standard mixture (PU $3.51 \cdot 10^{-7}$, SPD $1.76 \cdot 10^{-7}$ and SPM $1.08 \cdot 10^{-7}$ mol/l) was added to 100 ml of serum and the sample was subjected to the entire pretreatment procedure as described above. The relative recovery was calculated using the following equation:

$$R_r = (C_{Srm+Std} - C_{Srm})/C_{Std}$$

where R_r = relative recovery, $C_{Srm+Std}$ = polyamine concentration in the serum sample with polyamine standards added, C_{Srm} = polyamine concentration in the serum and C_{Std} = concentration of polyamine standards. The PU concentration in serum was assumed to be zero. The average recoveries ±R.S.D. ($n = 5$) of the polyamines were PU 92.3 ± 10.2, SPD 94.3 ± 10.3 and SPM 99.7 ± 12.5%.

3.4. Determination of polyamines in serum

The three polyamines in serum were identified

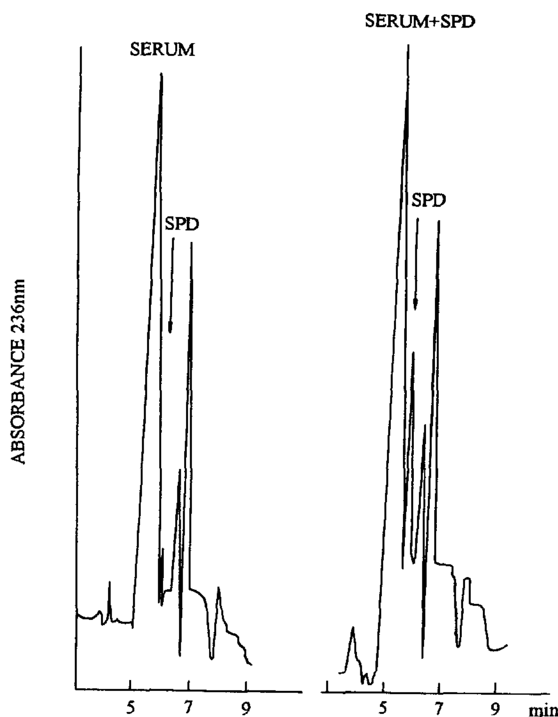

Fig. 2. Determination of spermidine in serum by CZE with indirect UV detection. Load, 5 kV, 3 s; run, 9 kV; detection, 236 nm, 0.005 AU.

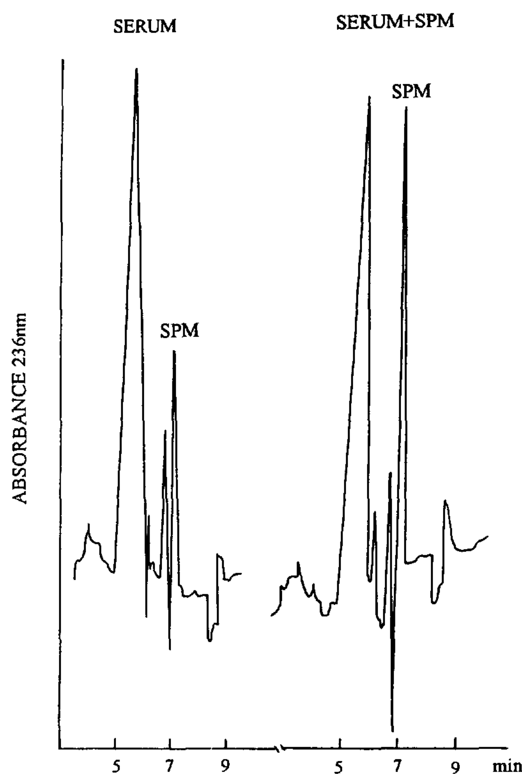

Fig. 3. Determination of spermidine in serum by CZE with indirect UV detection. Conditions as in Fig. 2.

Table 1
SPD and SPM concentrations (10^{-7} mol/l) in human sera

Polyamine	Sample						
	1	2	3	4	5	6	Average
SPD	1.11	1.01	1.15	1.22	1.21	1.22	1.15
SPM	2.12	1.39	1.61	1.58	1.09	1.06	1.48

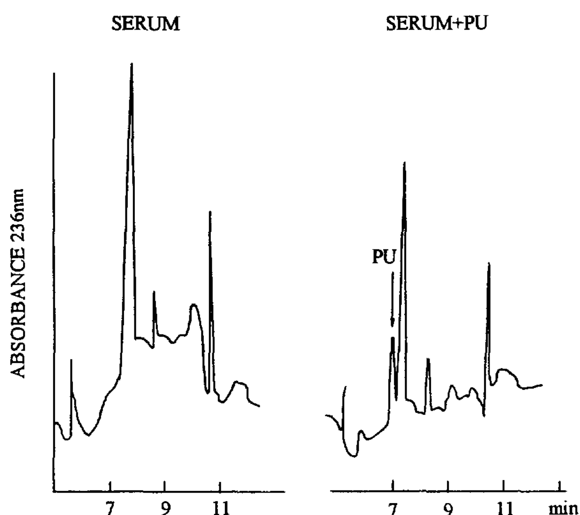

Fig. 4. Determination of putrescine in serum by CZE with indirect UV detection. Conditions as in Fig. 2.

by adding standards to serum as shown in Figs. 2–4. The relative positions of the three polyamines in the electropherograms were in accordance with those of the standard solution. SPD and SPM were detected in the original deproteinized serum. PU was not found in the serum, and after adding PU standard to the sample the PU peak and the unknown peak in the electropherogram overlapped. Subsequently PU standard was added to a tenfold dilution of the serum and the PU was well separated. Of the three polyamines, SPM was found to have the highest concentration in serum, then SPD, and PU was almost undetectable. The result was in accordance with previous reports on polyamine detection using other methods [2,4].

Blood erythrocytes have a great ability to absorb polyamines in blood plasma, especially PU and SPD. Although 80% of blood polyamines are in erythrocytes, there is still very little PU present in erythrocytes [9]. This might account for our not finding PU in serum. We only gave the position where PU should be located in the electropherogram if there were a sufficient concentration in the serum.

The concentrations of SPD and SPM in the sera of a group of healthy adults are shown in Table 1. The average concentrations of SPD and SPM were $1.15 \cdot 10^{-7}$ and $1.48 \cdot 10^{-7}$ mol/l, respectively, in agreement with reports of polyamine concentrations of many types of body cells within the submillimolar range. For example, Loser et al. [3] reported $2.86 \cdot 10^{-7}$ mol/l for SPD and $1.75 \cdot 10^{-7}$ mol/l for SPM in human serum, obtained using HPLC. Although only six subjects were included in our experiment, the average concentrations of serum polyamines were within the range of common acceptance.

4. Conclusion

A rapid, simple and sensitive method for determining polyamines in serum was developed. The sample consumption is small and the pretreatment is easy.

Acknowledgements

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