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Indirect photometric detection of polyamines in biological samples separated by high-performance capillary electrophoresis

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ABSTRACT

A rapid separation of polyamines and some related amino acids in cultured tumor cells by high-performance capillary zone electrophoresis with indirect photometric detection is demonstrated. 60 cm × 75 μm I.D. fused-silica capillary was used for the separation and quinine sulfate was used as a background electrolyte (BGE). Several polyamines (putrescine, spermidine and spermine), amino acids (lysine, arginine, histidine) and simple cations (K^+, Na^+) were easily separated in less than 10 min. Using the indirect photometric detection method, femtomole amounts of polyamines extracted from the tumor cells were detected from nanoliter injection volumes, and the signal response was linear over two orders of magnitude.

INTRODUCTION

Polyamines such as putrescine, spermidine and spermine are small polycations that are essential for cell viability and are present in sub-millimolar concentrations in many tissues [1,2]. It is widely accepted that they have been implicated in a variety of cell functions involving DNA replication, gene expression, protein synthesis and cell surface receptor function [3,4]. Over-production of polyamines is toxic to cells and facilitates cell death by oxidative mechanisms [2]. Although many studies have been conducted on polyamines, methods for separation are largely confined to high-performance liquid chromatography [5–7] and thin-layer chromatography [8]. Both of these methods require that the polyamines be derivatized or labelled before detection [7,8]. Although several reports have demonstrated that the derivatization of amino acids and peptides with fluorescamine or dansyl chloride can be easy and fast (nanoseconds) [9–11], the derivatization and labelling procedures for polyamines are often tedious and time consuming [7,8].

High-performance capillary zone electrophoresis (HPCZE) has proved to be a powerful technique in the separation of charged biomolecules with very high resolution [12–14]. Indirect detection techniques have become sensitive and simple to use [15,16]. However, the separation of polyamines in biological samples by HPCZE has not yet been assessed. This paper presents a rapid method to separate and detect extracted polyamines from an established tumor cell line of the rat pheochromocytoma, PC12, using HPCZE and indirect photometric detection.

EXPERIMENTAL

Reagents

All chemicals were of analytical-reagent grade unless stated otherwise. Deionized water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

Quinine sulfate monohydrate was purchased

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from Fisher Scientific (Fairlawn, NJ, USA). Putrescine dihydrochloride, spermidine triphosphate, spermine diphosphate, L-arginine hydrochloride, L-histidine hydrochloride (monohydrate) and L-lysine monohydrochloride and cell culture materials were obtained from Sigma (St. Louis, MO, USA). HPLC-grade ethanol, hydroxypropylmethyl cellulose (HMC) (4000 cP at 25°C for a 2% solution) and all other inorganic chemicals were purchased from Aldrich (Milwaukee, WI, USA).

Equipment

The (Model 3850) HPCZE system with a UV detector was purchased from ISCO (Lincoln, NE, USA). A positive high voltage was applied to the capillary by maintaining the injection end at a positive high potential while the cathodic end was held at ground potential. Data were collected with a Datajet computing integrator (Spectra-Physics, Mountain View, CA, USA). The capillary columns (Polymicro Technologies, Phoenix, AZ, USA) were 60 cm (35 cm to the detection system) × 150 μm O.D. × 50 or 75 μm I.D. The polymer coating was burned off 25 cm from the cathodic end of the capillary to form the detection window.

Pretreatment of the capillary column

All new capillary columns were filled with 0.1 *M* sodium hydroxide solution for about 30 min to clean the column. The column was then washed with deionized water and background electrolyte (BGE). The capillary was ready for use thereafter.

Preparation of background electrolyte (BGE)

BGE 1. Quinine sulfate (313 mg) was dissolved in 20 ml of 95% ethanol with stirring for several minutes. About 70 ml of deionized water were added with stirring until all the quinine sulfate was dissolved (about 5 min). The concentration of quinine sulfate was 8 m*M* (20% ethanol) after bringing the volume to 100 ml in a volumetric flask, and the pH was 5.9. The BGE was vacuum degassed before use.

BGE 2. HMC was added to BGE 1 to a concentration of 0.5% (w/v) and stirred for 40 min until dissolved. The solution was vacuum degassed before use.

Growth of PC12 tumor cells

Rat pheochromocytoma (PC12) tumor cells were purchased from the American Type Culture Collection (Rockville, MD, USA) and were cultivated on RPMI medium supplemented with 5% fetal bovine serum, 10% heat-inactivated horse serum (30 min at 56°C), 100 units/ml of penicillin G and 100 μg/ml of streptomycin, and buffered with 0.2% (w/v) sodium hydrogencarbonate (pH 7.4). Cultures were propagated in a humidified growth chamber (Queue Systems, Parkersburg, WV, USA) with 10% carbon dioxide gassing at 37°C in 75-cm^2 flasks.

Preparation of tumor cell extract

PC12 cells ($4.7 \cdot 10^7$) were harvested by centrifugation at 1000 *g* for 15 min. The pellet was washed with 0.3 *M* sucrose. Deionized water (3 ml) was added to the pellet and the sample was held at 0–4°C for 2.5 h. Insoluble and membranous materials were removed by centrifugation at 60 000 *g* at 0°C for 30 min. The supernatant was analyzed by HPCZE to determine cytosolic polyamines. For total cellular polyamine analysis, the pellet was resuspended with the supernatant.

Electrophoresis

Samples were injected electrokinetically at 30 kV for 3 s, and the separation was carried out at 30 kV for 15 min. The wavelength of detection was et at 236 nm owing to the maximum absorption coefficient of quinine sulfate at this wavelength (ε = 34 900 l/mol · cm) [17]. Data were collected and processed by the Datajet integrator. For the convenience of integration, the polarity of the integrator was reversed, so that the sample peaks shown in the following electropherograms appear as if they are absorption peaks.

RESULTS AND DISCUSSION

Fig. 1 shows the separation of three polyamines and other commonly existing cations in the cell culture media and other biological samples, such as K^+, Na^+, L-lysine, L-arginine and L-histidine, with BGE 2. All these components were completely separated in less than 10 min. The effect of HMC in the BGE was twofold: it blocked the evaporation of ethanol and maintained a reproducible migration time for the sample peaks, and an improved resolution was obtained owing to the elimination of electroosmotic flow by the linear HMC polymers. L-

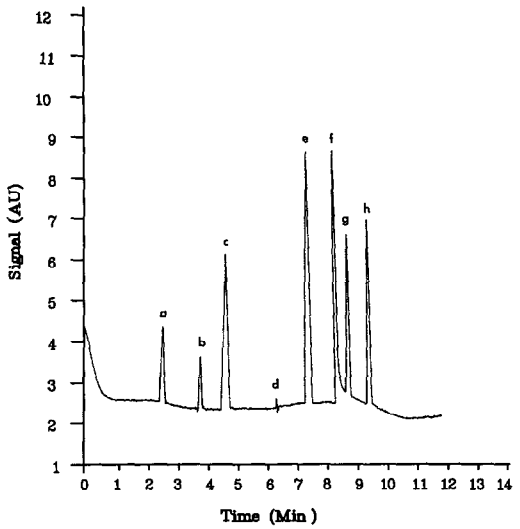

Fig. 1. Separation of three polyamine standards and other commonly co-existing cations in the cell culture media in BGE 2 by HPCZE with indirect photometric detection. A 3-s, 30-kV injection of 100 μM each was followed by electrophoresis at 30 kV on a 60 cm × 75 μm I.D. Pretreated column. Injection volume, 26.1 nl; detection wavelength, 236 nm. Peaks: a = K^+; b = Na^+; c = putrescine; d = L-histidine; e = spermidine; f = spermine; g = L-lysine; h = L-arginine.

Histidine gave a very small signal in BGE 2, as shown in Fig. 1. This is due to its absorption at 236 nm, which almost compensates for the displacement signal.

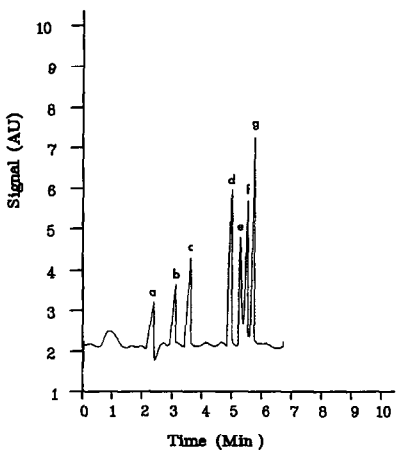

Fig. 2. Separation of polyamines and other cations using BGE 1 by HPCZE with indirect photometric detection. Peaks: a = K^+; b = Na^+; c = putrescine; d = spermidine; e = L-lysine; f = L-arginine; g = spermine. Electrophoresis conditions as in Fig. 1.

We also investigated the separation of the same components shown in Fig. 1 with BGE 1, and the results are shown in Fig. 2. It can be seen that the separation with BGE 1 is not as good as with BGE 2, the elution order of L-lysine and L-arginine changed from BGE 1 to BGE 2 and the L-histidine peak was missing. BGE 1 had another drawback in that the quinine sulfate crystallized very quickly in the open buffer reservoir. This was due to the evaporation of ethanol, which changed the BGE composition and decreased the solubility of quinine sulfate.

Fig. 3 shows the results of using BGE 2 to separate polyamines extracted from tumor cells. It is shown that femtomole amounts of polyamines can be easily detected with the indirect photometric detection technique. The amounts of polyamines extracted and injected on to the column are summarized in Table I. The unidentified peaks may be proteins in the cells, although this was not confirmed.

The linearity of polyamine detection was also investigated, an a linear response over two orders of magnitude ($1.0 \cdot 10^{-3} - 3 \cdot 10^{-6}$) for each poly-

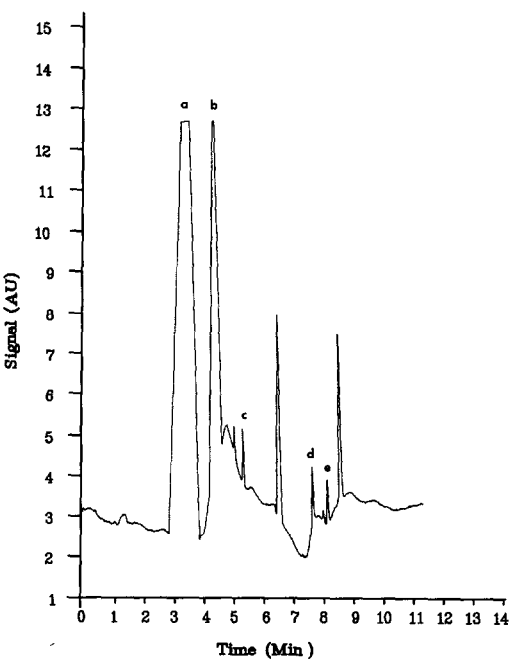

Fig. 3. Separation of polyamines in the tumor cell culture in BGE 2 by HPCZE with indirect photometric detection. Peaks: a = K^+; b = Na^+; c = putrescine; d = spermidine; e = spermine. Electrophoresis conditions as in Fig. 1.

TABLE I

POLYAMINE MEASUREMENTS IN PC12 CELL EXTRACTS

The injection volume per run was 37.7 nl and the aqueous extract was made from $35 \cdot 10^6$ cells.

Polyamine	Polyamine extracted (fmol/nl)	Total polyamine injected per run (fmol)	Amount per 10^6 cells (nmol)
Putrescine	13.6	513	0.39
Spermidine	25.2	952	0.72
Spermine	20.8	786	0.59

amine was obtained, which would be difficult using direct UV detection.

CONCLUSION

Indirect photometric detection of polyamines in tumor cells separated by HPCZE has been demonstrated. The method is rapid, simple to use and femtomole amounts of polyamines and other biological cations can be separated and detected. The method should be highly suitable for the separation and determination of polyamines from animals, plants, microorganisms and biological fluids from tumor-bearing patients or animals. Importantly, a simple aqueous extract was injected directly into the capillary column. A preliminary trichloroacetic acid (TCA) extraction of polyamines was found not to be necessary. Indeed, the supernatants of TCA extractions were found to reduce the polyamine signals in both biological samples and standards (data not shown).

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NOTE ADDED IN PROOF

The polyamine measurements given in Table I do not account for polyamines bound tightly to macromolecules such as DNA.

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