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Determination of purine and pyrimidine bases in DNA by micellar electrokinetic capillary chromatography with electrochemical detection

Gang Chen[a,*], Xiaohui Han[b], Luyan Zhang[a], Jiannong Ye[c]

[a]*Department of Chemistry, Fudan University, Shanghai 200433, China*
[b]*Department of Immunology, Fudan University, Shanghai 200032, China*
[c]*Department of Chemistry, East China Normal University, Shanghai 200062, China*

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Abstract

A method based on micellar electrokinetic capillary chromatography with electrochemical detection was developed for the determination of cytosine, 5-methylcytosine (5-MC), thymine, adenine, and guanine in the hydrolysates of DNA. The working electrode was fabricated in a novel self-positioning carbon disc electrode system that can align the capillary outlet with the working electrode without a three-dimensional micromanipulator. The five analytes could be well separated within 10 min in a 40 cm length capillary at a separation voltage of 9 kV in a 40 mmol/l borate buffer (pH 10.0) containing 100 mmol/l sodium dodecyl sulfate. Good linearity was observed between peak current and concentration of bases over three orders of magnitude with the detection limits ($S/N=3$) ranging from $1.28\cdot10^{-6}$ to $5.02\cdot10^{-6}$ mol/l. This proposed method demonstrated long-term stability and reproducibility with relative standard deviations of less than 5% for both migration time and peak current ($n=7$). It has been successfully applied to determine bases including 5-MC in the hydrolysates of fish sperm DNA, calf thymus DNA, and DNA isolated from spleen cells of female mice.

Keywords: Instrumentation; Electrochemical detection; Detection, electrochromatography; Electrochromatography; Purines; Pyrimidines; Methylcytosine; Adenine; Thymine; Guanine; Cytosine; DNA

1. Introduction

Deoxyribonucleic acid (DNA) is an important substance that plays a crucial role in the storage of genetic information and in protein biosynthesis. DNA backbone is a copolymer of phosphoric acid and 2-deoxyribose, with one of the four bases, adenine (A), guanine (G), cytosine, and thymine (T), linked to C-1 of each pentose unit. DNA occurs in the nuclei of all cells. It carries the information necessary for the exact duplication of the cell and for the construction of the entire organism in the precise sequence of purine and pyrimidine bases along its phosphodiester backbone [1]. Except for the four bases mentioned above, it also contains some modified bases such as 5-methylcytosine (5-MC), xanthine, hypoxanthine, etc.

Methylation of cytosines adjacent to guanines

*Corresponding author. Tel.: +86-21-6466-1130; fax: +86-21-6257-6217.

E-mail address: sgangchen@hotmail.com (G. Chen).

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(CpG) sequences in DNA occurs mainly in higher eukaryotes. The product of methylation of cytosine is 5-MC. In the human genome, as many as 70% to 80% of cytidines in CpG sequences may be methylated [2]. Changes in the extent of methylation have been observed in cell differentiation [3,4], X-chromosome inactivation [5,6], imprinting [7], and certain disease states such as cancer [8–12]. Hence, to establish an accurate analytical method for the simultaneous determination of 5-MC and other bases such as cytosine, T, A, and G in DNA would be useful for the investigations in molecular biology and other life sciences.

To our knowledge, liquid chromatography (LC) [13–15], gas chromatography (GC) [16,17], and thin-layer chromatography (TLC) [18] have been employed for the determination of 5-MC in DNA. Cunningham et al. have determined 5-MC in DNA by micellar electrokinetic capillary chromatography (MECC) with ultraviolet (UV) detection [19]. The most widely used technique in the analysis of bases in DNA is LC [15,20,21]. Nakahara et al. have determined A, T, cytosine, and G in DNA by LC with electrochemical detection (ED) [20]. Jin et al. have employed capillary zone electrophoresis (CZE) to the determination of A and G in the hydrolysis products of yeast RNA and calf thymus DNA by end-column amperometric detection with a carbon fiber micro-disc array electrode [22].

Nowadays, the application of capillary electrophoresis (CE) for the separation of a variety of biological samples has become increasingly widespread because of its minimal sample volume requirement, short analysis time, and high separation efficiency. The two commonly used CE modes are CZE and MECC. CZE can provide highly efficient separation for ionic solutes. In order to extend the advantages of CZE to neutral compounds, MECC was first introduced by Terabe et al. [23]. MECC employs buffers to which surfactants have been added at concentrations above their critical micellar concentrations (CMCs). Separation of nonionic solutes is based on their different distribution coefficients between the aqueous phase and the micellar phase [24].

For CE, ED has become one of the most attractive detection methods because it owns high selectivity and sensitivity for electroactive species and can be coupled with capillary to monitor the extremely small zone volumes of analytes at low cost [25,26]. To our knowledge, the two commonly used ED modes are off-column [27–30] and end-column [31–34]. For the off-column mode, a conductive junction between the separation and detection capillary is used to isolate the separation voltage from the ED system. For the end-column detection, a micro-electrode is place directly at the outlet of separation capillary without any junction. Although both ED modes work well, they have not been applicable to routine analysis because it is difficult to produce such sophisticated electrochemical cell reliably and inexpensively. One of the most major limitations that have held back the routine application of CE–ED is the precise alignment between the separation capillary and the working electrode [31]. In most cases, a micromanipulator has to be employed for positioning the working electrode with the aid of a microscope or a magnifier. If the working electrode needs to be polished or changed, the entire system has to be refabricated. It is rather time-consuming and requires special skilled personnel to operate the system. For the end-column detection, it is interesting to develop a simple and convenient device to simplify the alignment procedures, to improve the alignment reproducibility, and to eliminate the noises from mechanical vibrations.

In this study, a self-positioning electrode system has been fabricated for the precise alignment of the working electrode with the outlet of capillary without a manipulator. It has been coupled with MECC as an end-column detector for the determination of cytosine, 5-MC, T, A, and G in the hydrolysates of fish sperm DNA, calf thymus DNA, and DNA isolated from spleen cells of mice. This method is simple, sensitive, and efficient, providing a convenient technique for the determination of bases including 5-MC in DNA.

2. Experimental

2.1. Apparatus

The CE system has been described previously [35,36]. A ±30 kV high-voltage d.c. power supply (Shanghai Institute of Nuclear Research, China)

provided a voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential and the outlet of capillary was maintained at ground. The separations were proceeded in a fused-silica capillary (40 cm×25 μm I.D.×360 μm O.D., Polymicro Technologies, Phoenix, AZ, USA). In order to avoid contact between the operator and the high voltage and assure the safety of the CE–ED system, the entire capillary, the buffer reservoirs for CE, and all electrodes were enclosed in a Plexiglas box with a safety switch wired to terminate the high-voltage output whenever the box was opened.

End-column amperometric detection employed in this work was performed using the configuration illustrated in Fig. 1. Fig. 1A shows the elevation view of the novel self-positioning carbon disc working electrode system. A rectangular hole (10 mm×5 mm) was machined through the center of a Plexiglas block (20 mm×20 mm×5 mm). The hole not only allows for the diffusion of electrolyte solution away from the detection point, but also provides convenience for polishing the surface of working electrode. Two 400 μm diameter bores were drilled carefully through the block in a line for allocation of working electrode and capillary, respectively. A disc-type carbon working electrode is used in this experiment. To fabricate the disc electrode, a piece of carbon rod (300 μm diameter, 9 mm length, rotring, hi-Polymer, Germany) with one end linked conductively with a piece of copper magnet wire (100 μm diameter, 10 cm length) with the side covered by a layer of insulator coating was inserted into one bore in the block. Poly methyl methacrylate solution (5%, w/w) in chloroform was then applied to both ends of the bore to secure and seal the carbon rod in place and to insulate the connection between the carbon rod and the copper lead. The carbon rod (ca. 1 mm length) protruding in the hole was coated with epoxy. After the epoxy cured, the sealed end of the carbon rod in the hole was filed to form a disc electrode. Another bore in the block can guide the outlet of capillary to the surface of the carbon disc electrode. The self-positioning working electrode system makes it possible to align the detection electrode with the capillary outlet without a three-dimensional micromanipulator. A rubber chip with a self-sealing bore at its center was adhered on the top of the block to immobilize capillary.

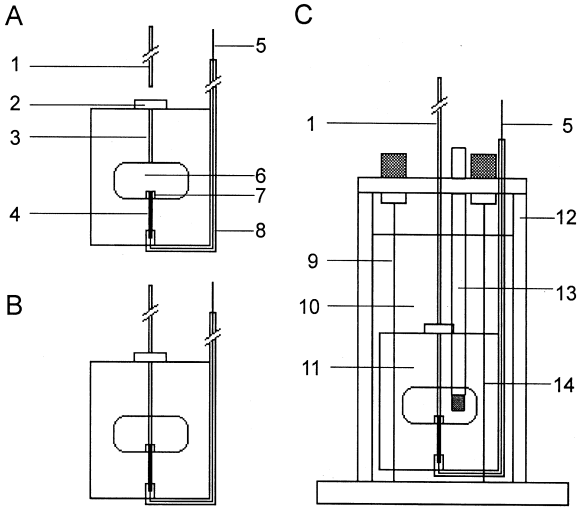

Fig. 1. Schematic diagrams of the self-positioning disc working electrode system without (A) and with (B) the capillary inserted and (C) the elevation view of electrochemical cell. (1) Separation capillary; (2) rubber chip; (3) 400 μm diameter bore; (4) 300 μm diameter carbon rod; (5) copper magnet wire; (6) hole; (7) epoxy; (8) polyvinyl chloride tube; (9) platinum auxiliary electrode; (10) electrolyte solution; (11) self-positioning working electrode system; (12) electrolyte reservoir; (13) reference electrode; (14) grounded platinum electrode.

In addition, a Plexiglas cell with inner dimension of 2.2 cm width, 2.4 cm length, and 5 cm height, was fabricated to accommodate a platinum ground electrode for CE, a platinum wire auxiliary electrode, a saturated calomel electrode (SCE), and the self-positioning working electrode system which were all immersed in the electrolyte solution contained in the cell. Fig. 1C illustrates the elevation view of the electrochemical cell configuration. A BAS LC-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA) was used to provide a constant potential to the working electrode and measure the output current in combination with the three-electrode electrochemical cell. The filter of the detector was set at 0.1 Hz. Before use, the carbon disc electrode was successively polished with emery paper and alumina powder, sonicated in doubly distilled water, and finally the outlet of the capillary was positioned carefully opposite the surface of carbon disc electrode through the guide bore in the Plexiglas block and arranged in a wall-jet configuration as shown in Fig. 1B [37]. The gap distance

between the disc electrode and the capillary outlet was adjusted to 25 μm approximately by comparison with the bore (25 μm diameter) in the capillary while being viewed under a microscope. The electropherograms were recorded using a LKB·REC 1 chart record (Pharmacia, Sweden). An YS 38-1000 220 V alternate constant-voltage power supply (Shanghai Instrumental Transformer Factory, Shanghai, China) was employed to suppress the voltage fluctuation of the power line. The whole system was assembled in a 10 m^2 Faraday room that was air-conditioned at 20 °C in order to minimize the effects of external noise sources.

MECC was performed in a 40 mmol/l borate buffer (BB, pH 10.0) containing 100 mmol/l sodium dodecyl sulfate (SDS) used as electrophoretic medium at the separation voltage of 9 kV. The potential applied to the working electrode was 1.05 V (versus SCE). Samples were injected electrokinetically at 9 kV for 8 s.

2.2. Reagent and solutions

Cytosine, 5-MC, T, A, G, hypoxanthine, xanthine, and proteinase K (EC 3.4.21.64, from *Tritirachium album*, 10–20 units per mg protein) were all obtained from Sigma (St. Louis, MO, USA) and used without further purification. Fish-sperm DNA and concanavalin A (from jack bean, Con A) were purchased from Shanghai Dongfeng Biochemicals Reagent Co. (Shanghai, China). Calf thymus DNA was obtained from Beijing Baitai Biotechnology (Beijing, China). Other chemicals were of analytical grade. All aqueous solutions used in this work were made up in doubly distilled water.

Stock solutions of 5-MC, A, and xanthine ($1.0\cdot10^{-2}$ mol/l) were prepared in doubly distilled water. cytosine and T were dissolved in 0.05 mol/l HCl aqueous solution to reach a final concentration of $1.0\cdot10^{-2}$ mol/l, respectively. Stock solutions of G, hypoxanthine, and uric acid ($1.0\cdot10^{-2}$ mol/l) were made in 0.05 mol/l NaOH aqueous solution. All stock solutions were stored in a 4 °C refrigerator. Samples were made by diluting stock solutions with appropriate amount of the electrophoretic medium just prior to use.

2.3. Sample preparation

Spleen tissue was taken aseptically from female BALB/c mice (4–6 weeks old, Experimental Animal Center in Chinese Academy of Sciences, Shanghai, China) and was subsequently ground to prepare individual normal spleen cells after a series of processes [38]. The active spleen cells were obtained by culturing $2.0\cdot10^{6}$ ml^{-1} normal cells in RPMI 1640 culture solution (complete, Gibco, USA) containing 5 μg/ml Con A in a 5% CO_2 culture box at 37 °C for 72 h [38]. DNA was isolated from about 10^{7} normal or activated spleen cells according to the procedure in the literature [39]. Briefly, the cells were resuspended in 0.01 mol/l phosphate-buffered saline (PBS, pH 7.2) and lysed in a solution (pH 8.0) containing 10 mmol/l ethylenediaminetetraacetic acid (EDTA), 0.4% (w/v) SDS, 15 mmol/l NaCl, 10 mmol/l trihydroxymethylaminomethane chloride (Tris-Cl), and 100 μg/ml proteinase K, at 37 °C for 24 h. DNA was extracted by phenol and chloroform after RNA and protein were removed. Purified DNA was precipitated with ethanol and allowed to air-dry. It was subsequently dissolved in appropriate amount of doubly distilled water to reach the final concentration of about 220 μg/ml for both normal DNA and active DNA. The DNA concentration was determined spectrophotometrically using the known absorption coefficient of 20 $(mg/ml)^{-1}$ cm^{-1} at 260 nm [20].

Accurate amounts of fish sperm DNA (19.93 mg) and calf thymus DNA (5.11 mg) were hydrolyzed in 0.5 ml of 88% (w/w) formic acid at 170 °C for 30 min in two glass tubes (15 cm×4 mm O.D.×2.5 mm I.D.) with two ends sealed according to the procedure reported in the literatures [19,20]. DNA solutions (0.5 ml, about 220 μg/ml) from normal and active mouse spleen cells were transferred to two glass tubes with one end sealed and then lyophilized, respectively. The residues were also hydrolyzed in 0.5 ml of 88% (w/w) formic acid as the procedure mentioned above. When the hydrolysates were lyophilized, the lyophilized residues of fish sperm DNA, calf thymus DNA, and DNA isolated from the normal spleen cells and the active spleen cells were dissolved in 10.0, 2.0, and 0.2 ml of the electrophoretic medium for analysis, respectively. Peak

identification was performed by standard addition method. Moreover, sample solutions of fish sperm DNA and calf thymus DNA, standard solutions, and electrophoretic mediums were all filtered through a syringe cellulose acetate filter (0.22 μm) prior to injection for analysis.

3. Results and discussion

3.1. Characteristics of the self-positioning electrode system

For end-column amperometric detection for CE, precise alignment and stabilization of the working are highly required. A micromanipulator was usually employed for the alignment of working electrode with the aid of a microscope or a magnifier [32,35–37]. This detection scheme often necessitates time-consuming alignment procedures. Poor alignment will result in marked changes in the detection currents and the deterioration of detection limit due to mechanic vibrations.

In this work, a self-positioning carbon disc electrode system has been fabricated for the precise alignment of working electrode with capillary outlet without a micromanipulator. It is characterized by its advantages of simple design and construction, reduced alignment time and low cost. As the working electrode and capillary are both secured in the same support that is immersed in the electrolyte solution, the noises from mechanical vibration and some other external movements which will disturb the electrode-capillary alignment are eliminated. This will lower the noise level so that the sensitivity and reproducibility of electrochemical detection are improved. In addition, good alignment for working electrode and capillary can be easily achieved in a wall-jet configuration because the inner diameter of capillary to the diameter of the carbon disc working electrode is in a ratio of 1:12. The gap distance between the disc working electrode and the outlet of capillary can be adjusted under a microscope. Although it is difficult to measure the distance accurately, the gap distance can be estimated on the basis of the inner diameter of the capillary used. In this experiment, the distance was about 25 μm in considering the peak current, peak broadening, and background noise from the separation voltage. The rubber chip with a self-sealing bore at its center on the top of the Plexiglas block cannot only immobilize the capillary, but also make it possible to leave an effective gap distance by pushing the capillary gently to the disc working electrode until a soft contact is attained due to the native elasticity of rubber. In order to demonstrate the feasibility of the self-positioning electrode system, it has been coupled with MECC for the determination of the bases in DNA as follows.

3.2. Hydrodynamic voltammograms (HDVs)

Fig. 2 shows the HDVs for cytosine, 5-MC, T, A, and G at the carbon disc working electrode. It is obvious that purine bases can be oxidized at lower potentials than pyrimidine bases. In addition, 5-MC and T were oxidized more efficiently than cytosine. As shown in Fig. 2, the peak current increases rapidly at a potential of 0.5 V for G, 0.7 V for A, and 0.9 V for cytosine, 5-MC and T, respectively. When the applied potential exceeds 0.8 V for G, 1.0 V for A, 5-MC and T, the peak current increases much

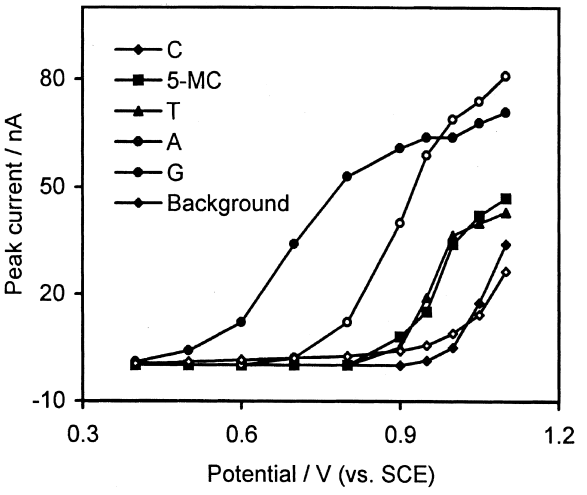

Fig. 2. Hydrodynamic voltammograms (HDVs, A) for $5.0 \cdot 10^{-4}$ mol/l of cytosine, 5-methylcytosine, thymine, adenine, and guanine in MECC. (A) Fused-silica capillary: 40 cm×25 μm I.D.; working electrode: 300 μm diameter carbon disc electrode; electrophoretic medium: 40 mmol/l BB (pH 10.0) containing 100 mmol/l SDS; separation voltage: 9 kV; electrokinetic injection: 8 s (at 9 kV). C=Cytosine.

more slowly. The oxidation current for cytosine did not reach the limiting current plateau in the investigated potential range. In this work, 1.05 V (versus SCE) was finally chosen as the working potential so that all bases could be detected sensitively. Although an applied potential greater than +1.05 V (versus SCE) results in higher peak currents, both the baseline noise and the background current increase substantially, because of solvent oxidation. The high background current leads to an unstable baseline, which is a disadvantage for sensitive and stable detection. As the working electrode was positioned opposite the capillary outlet that was maintained at ground, the baseline current decreased due to the slight negative shift of working potential when the high separation voltage (9 kV) was applied.

3.3. Optimum conditions for separation

MECC was run by employing a fused-silica capillary (40 cm×25 μm I.D.×360 μm O.D.) at a separation voltage of 9 kV. The optimal running buffer, in terms of resolution, peak current, analysis time, and buffer capacity, was 40 mmol/l BB (pH 10.0) containing 100 mmol/l SDS. Sample solutions were introduced into the capillary by electrokinetic injection at 9 kV for 8 s. Under the optimum conditions mentioned above, the typical electropherogram for a standard mixture solutions of $5.0 \cdot 10^{-4}$ mol/l of cytosine, 5-MC, T, A, and G is shown in Fig. 3A. All five analytes can be well separated within 10 min.

3.4. Reproducibility, linearity and detection limit

The reproducibility of the peak current and migration time of the five bases in this experiment was determined by repeated ($n=7$) injection of a standard mixture solution of $5.0 \cdot 10^{-4}$ mol/l of cytosine, 5-MC, T, A, and G in the electrophoretic medium under the optimum conditions every 30 min. The relative standard deviations (RSDs) for peak current and migration time are 4.43 and 1.70% for cytosine, 4.83 and 1.64% for 5-MC, 2.81 and 1.57% for T, 3.71 and 1.48% for A, 1.77 and 1.32% for G, respectively. The reproducibility exhibited in the present study shows that it is feasible to determine

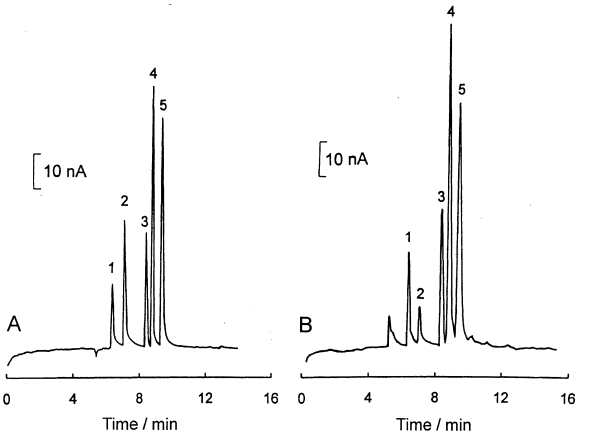

Fig. 3. (A) Typical electropherogram of a standard mixture solution of $5.0 \cdot 10^{-4}$ mol/l of cytosine, 5-methylcytosine, thymine, adenine and guanine and (B) typical electropherogram of the diluted hydrolysates of fish sperm DNA under the optimum conditions. Working potential: +1.05 V (versus SCE); other conditions as in Fig. 2. Peaks: 1=cytosine, 2=5-methylcytosine, 3=thymine, 4=adenine, 5=guanine.

purine and pyrimidine bases by MECC coupled with the self-positioning carbon disc electrode system.

A series of the standard mixture solutions of cytosine, 5-MC, T, A, and G were tested to determine the linear range of electrochemical detection. The results of regression analysis on calibration curves and detection limits are presented in Table 1. Determination limits are evaluated on the basis of a single-to-noise ratio of 3. The calibration curves exhibit excellent linear behavior over the concentration range of three orders of magnitude for all investigated compounds with the detection limits ranging from $1.28 \cdot 10^{-6}$ to $5.02 \cdot 10^{-6}$ mol/l, and the correlation coefficients are all around 0.999.

3.5. Application

Under the optimum conditions, the determination of cytosine, 5-MC, T, A, and G in the hydrolysates of fish sperm DNA, calf thymus DNA, and DNA isolated from the normal spleen cells and the activated spleen cells of mice was carried out according to the procedures described above. The typical electropherograms of samples are shown in Fig. 3B, Fig. 4, and Fig. 5, respectively. 5-MC was found

Table 1
The results of regression analysis on calibration curves and the detection limits[a]

Compound	Regression equation, $y=a+bx$[b]	Correlation coefficient	Linear range ($\cdot 10^{-3}$ mol/l)	Detection limit[c] ($\cdot 10^{-6}$ mol/l)
Cytosine	$y=-0.1585+35.882x$	0.9996	0.0075–5.0	5.02
5-Methylcytosine	$y=-0.2568+83.013x$	0.9984	0.0050–5.0	2.17
Thymine	$y=-0.1773+79.129x$	0.9990	0.0050–2.5	2.28
Adenine	$y=0.1439+140.38x$	0.9993	0.0020–2.5	1.28
Guanine	$y=0.1707+134.98x$	0.9989	0.0020–2.5	1.33

[a] Working potential is 1.05 V (versus SCE). Other conditions as in Fig. 2.
[b] Where y and x are the peak current (nA) and concentration of the analyte ($\cdot 10^{-3}$ mol/l), respectively.
[c] The detection limits corresponding to concentrations giving signal-to-noise ratio of 3.

presented in all four investigated kinds of DNA. The assay results are summarized in Table 2.

The methylation extent of cytosine in DNA was represented by the percentage of cytosine in the form of 5-MC that was calculated by the formula:

$$\%\ 5\text{-MC} = n_{5\text{-MC}}/(n_{5\text{-MC}} + n_{C}) \cdot 100 \quad (1)$$

where % 5-MC is the mole percentage of cytosine in the form of 5-MC, $n_{5\text{-MC}}$ and n_{C} are the molal quantities of 5-MC and cytosine, respectively. The assay results of % 5-MC ratios for various DNAs are presented in Table 3. The values for fish sperm DNA and calf thymus DNA were similar to the data in a literature [40]. As the methylation extent of DNA isolated from the spleen cells activated by Con A is

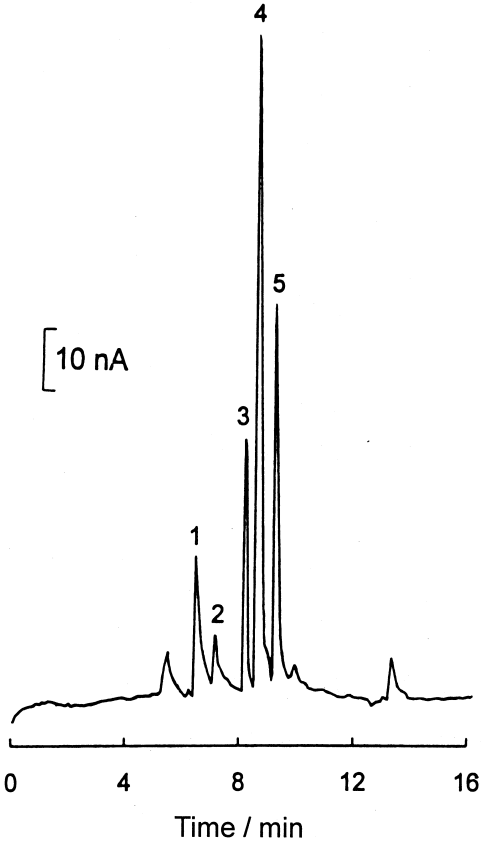

Fig. 4. Typical electropherogram of the diluted hydrolysates of calf thymus DNA under the optimum conditions. Working potential: +1.05 V (versus SCE); other conditions as in Fig. 2.

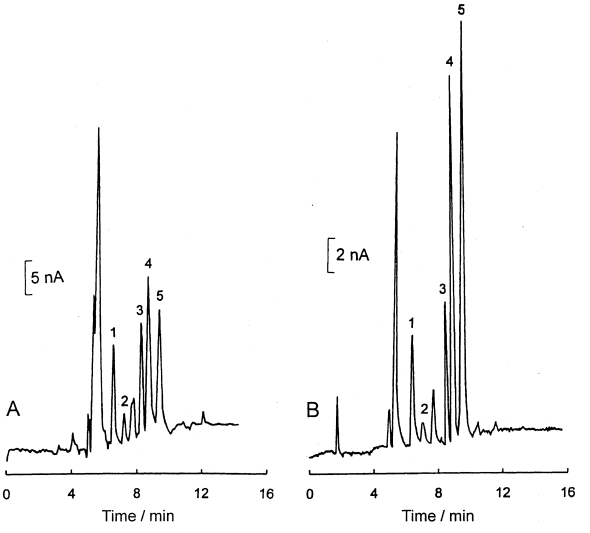

Fig. 5. Typical electropherogram for the diluted hydrolysates of DNA isolated from (A) normal and (B) activated spleen cells under the optimum conditions. Working potential: +1.05 V (versus SCE); other conditions as in Fig. 2.

Table 2
Assay results of the bases in DNA ($n=3$, mg/g)[a]

Sample	Cytosine	5-MC	Thymine	Adenine	Guanine
Calf thymus DNA	29.29 (4.31)[b]	2.871 (4.36)	23.39 (1.33)	37.93 (1.52)	27.57 (1.66)
Fish sperm DNA	35.21 (4.59)	3.706 (3.57)	31.46 (5.13)	42.12 (1.69)	33.94 (3.35)
Normal cell DNA	94.99 (3.70)	10.30 (1.68)	66.41(0.79)	48.50 (1.30)	48.41 (1.64)
Activated cell DNA	84.44 (3.33)	6.820 (4.62)	51.92 (2.13)	37.75 (1.77)	45.53 (1.29)

[a] Working potential is 1.05 V (versus SCE). Other conditions as in Fig. 2.
[b] The data in parentheses refer to the RSDs.

Table 3
Comparison of the methylation extents analyzed by MECC with literature values [33]

Sample	$n_{5\text{-MC}}/n_C$ (literature value) (%)	$n_{5\text{-MC}}/n_C$ (present method) (%)
Calf thymus DNA	5.24–8.96	8.03
Fish sperm DNA	8.74	8.57
Normal cell DNA	NA[a]	8.81
Activated cell DNA	NA	6.71

[a] Not applicable.

lower than that of DNA isolated from the normal spleen cells, it can be preliminarily concluded that the decrease of methylation degree in DNA is related with the activation of cells (containing the activated DNA) by Con A. More importantly, it has been demonstrated that immunization of syngeneic mice with the active DNA can induce them to occur systemic lupus erythematosus (SLE). However, the DNA from normal cell has no this function [38]. So, the methylation level of cytosine in DNA may play an interesting, even important role in the pathogenic mechanism of SLE.

Accurate amounts of cytosine, 5-MC, T, A, and G were added to the diluted hydrolysates of fish sperm DNA in separation medium, and the recovery values were obtained using their peak currents from the calibration curve under the same conditions. The average recoveries and RSDs for cytosine, 5-MC, T, A, and G are listed in Table 4. The results indicate that this method is accurate and rugged for all analytes.

The interferences of 2-deoxyribose and glucose were not found for they could not be oxidized under the optimal conditions. In order to determine the interference of some modified bases such as hypoxanthine and xanthine in DNA, a standard mixture solution containing $5.0\cdot10^{-4}$ mol/l of each interferences was injected under the same conditions. Al-

Table 4
Determination results of recovery for this method ($n=3$)[a]

Compound	Original amount ($\cdot10^{-3}$ mol/l)	Added amount ($\cdot10^{-3}$ mol/l)	Found amount ($\cdot10^{-3}$ mol/l)	Recovery (%)	RSD (%)
Cytosine	0.6317	0.5	1.1382	101.30	3.33
5-MC	0.05925	0.5	0.5532	98.79	2.85
Thymine	0.4971	0.5	0.9883	98.24	3.19
Adenine	0.6212	0.5	1.1046	96.68	4.34
Guanine	0.4470	0.5	0.9343	97.46	5.66

[a] Working potential is 1.05 V (versus SCE). Other conditions as in Fig. 2.

though hypoxanthine and xanthine could also be detected electrochemically under the optimum conditions, they did not interfere with the determination of the five investigated analytes as the migration times of hypoxanthine and xanthine are 11.75 and 12.56 min, respectively, which are longer than those of all analytes.

4. Conclusion

It has been demonstrated that the novel self-positioning detection electrode system facilitated the alignment of working electrode with the capillary outlet. The primary advantages of the capillary-electrode holder is its simple design and construction which make the present setup very attractive for the routine analysis of electroactive species by CE with electrochemical detection. The self-positioning carbon disc electrode system has been successfully coupled with MECC for the determination of cytosine, 5-MC, T, A, and G in real DNA samples. This technique provides a convenient and sensitive analytical method for determining the methylation extent of cytosine in bulk DNA that is important for some investigations in molecular biology, pathology, clinical chemistry, and other life sciences. It is can be concluded that MECC–ED is an alternative and competitive technique for the determination of base contents in DNA due to its special attributes such as high resolution and sensitivity, low operation expense, minimal sample volume requirement, etc.

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