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Separation of purine and pyrimidine bases by capillary electrophoresis using β-cyclodextrin as an additive

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Abstract

Capillary electrophoresis was applied to separate purine and pyrimidine bases in the basis of their partial ionization in the alkaline buffer. The effects of buffer pH, buffer and β-cylclodextrin concentration were systematically investigated using a commercial capillary electrophoresis instrument with UV detector at 254 nm. We found that the resolutions of bases (especially for adenine and thymine) were significantly improved in the presence of β-cylclodextrin. The satisfactory separation of five bases such as cytosine, thymine, adenine, guanine and uracil were achieved by capillary electrophoresis using β-cylclodextrin as an additive. Under the optimal conditions, the linear range was from 2 to 200 μg/ml for bases ($R = 0.991$–0.997) and the detection limits were from 0.8 to 1.8 μg/ml ($S/N = 2$). The detection limit of 0.05 μg/ml ($S/N = 2$) for uracil was obtained by stacking injection mode. The assay was used to determine the deamination of cytosine to uracil by heating in the presence of sodium hydroxide. Our primarily results show that capillary electrophoresis is a very useful tool for determination of purine and pyrimidine bases and study on nucleic acids.

Keywords: Capillary electrophoresis; Purine; Pyrimidine; Deamination; β-Cylclodextrin

1. Introduction

Purine and pyrimidine bases are the building blocks in both DNA and RNA that play important roles in cell metabolism. Base changes in DNA may affect seriously the structure and function of products of gene expression—protein, which is considered to be the main causes of inherited diseases and most human cancers [1–3]. Many areas such as pharmacological studies, clinical diagnosis and DNA damage assay, need a quick, inexpensive and accurate method for determination of purine and pyrimidine bases [4–9]. High performance liquid chromatography (HPLC) is a commonly used method for analysis of purine and pyrimidine bases [10–12].

Capillary electrophoresis (CE) is a powerful alternative to HPLC for the separation of charged and polar compounds [13–16]. CE has been successfully used for analysis of nucleic acids and nucleotides because they are negatively charged in neutral pH buffer [17–20]. But only a few reports involved the separation of purine and pyrimidine bases [21–24]. Differing

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from nucleic acids, purines and pyrimidines are only partially ionized in the solution. On the other hand, due to their similar pK_a values, it is very difficult to achieve baseline separation of purines and pyrimidines by CE in free solution, especially for thymine and adenine. Certain efforts such as changes of pH and buffer types could not improve the resolution between thymine and adenine. Cyclodextrin (CD) has been extensively used as a chiral selector for separation of enantiomers and as an additive for improving separation of some cyclic compounds [25–29]. CDs are cyclic oligosaccharides with truncated cylindrical molecular shapes. They have particular names: α-CD, β-CD and γ-CD for those containing six, seven and eight glucopyranose units, respectively. Since the inside surfaces of the CD's cavity are hydrophobic, CDs tend to form inclusion complexes with certain compounds whose molecular size and structure match with CD's cavities by hydrophobic interaction. Since the cyclic size of thymine is smaller than that of adenine, we predict that there is certain difference in their interactions with CD. In this paper we will explore the possibility of improving the separation of purines and pyrimidines using β-CD as an additive. Furthermore, we try to develop a CE method to determine the deamination of thymine to uracil.

2. Experimental

2.1. Reagents and materials

Adenine (A), guanine (G), thymine (T), cytosine (C), uracil (U) and β-cyclodextrin were purchased from Sigma (St. Louis, MO, USA). Sodium tetraborate and sodium hydroxide were provided by Shanghai Reagents Co.(Shanghai, China). Double-distilled water was used for preparation of all aqueous solutions. The pHs of various borate solutions were adjusted by 1 M sodium hydroxide using a pH meter (Lezi Instrument Factory, Shanghai, China). Buffers were filtered with a membrane filter of 0.45 μm pore size prior to use.

Fused silica capillaries with 75 μm internal diameter (i.d.) and 365 μm outer diameter (o.d.) were from Yongnian Optical Fiber Factory (Yongnian, Hebei, China).

2.2. Deamination of cytosine into uracil

Several hundreds μl of 1 mg/ml cytosine sample solutions were heated at 100 °C from 5 to 60 min in the presence or absence of 6 mM sodium hydroxide. One hundred μl of the reaction products were taken out and analyzed by CE using 2 μg/ml thymine as an internal standard.

2.3. Capillary electrophoresis procedure

A Waters Quanta 4000E Capillary Electrophoresis system with a UV detector (Milfod, MA, USA) was used in the experiment. Caesar software (version 4.0, Princes Technologies, Emmn, The Netherlands) was used for data collection and processing.

A new capillary (43 cm total length, 36 cm effective length) was rinsed successively with 0.1 M sodium hydroxide, water and borate buffer. The temperature was set at 25 °C and the detection wavelength was at 254 nm. The samples were introduced by hydrostatic injection, and electrophoresis was performed at positive polarity under the conditions specified in the figure legends. The capillary was rinsed with fresh buffer between each run.

3. Results and discussion

The structures of five purine and pyrimidine bases to study are displayed in Fig. 1. At first, we wanted to develop a CE method for separation of bases by optimizing buffer pH and concentration and electrophoresis conditions. However, the separation of thymine and adenine was not achieved (partial data shown in Fig. 5A).

3.1. Buffer pH

Since purine and pyrimidine bases are partially ionized to negative charged ions in the alkaline solution, the buffer pH is an important factor affecting their separation by CE. Fig. 2 shows the effects of buffer pH on the migration times of purine and pyrimidine bases. Twenty mM sodium tetraborate solution containing 25 mM β-CD was used as running buffer, and the influences of pH range from 9.2 to 11.0 were investigated. In the Fig. 2, we examined that the migration

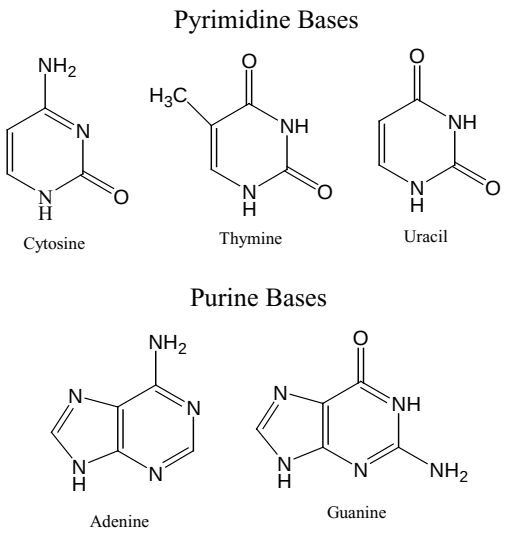

Fig. 1. Structures of pyrimidine and purine bases.

times of bases dramatically increased with buffer pH. This result was mainly attributed to an increase in the dissociation degrees of bases with an increase at buffer pH. The resolutions between bases were also considerably improved with buffer pH. However, when buffer pH was over 10.5, the resolutions were not further improved, and the poor reproducibility of migration time was examined. This phenomenon was due to the deterioration of the inside surface of the silica capillary in the high pH buffer.

3.2. β-CD concentration

Fig. 3 displays the effects of β-CD concentration on the separation of purine and pyrimidine bases. We found that the differences of migration time of thymine and adenine gradually increased with an increase in β-CD concentration. This result likely owed to the different interaction between thymine -β-CD and adenine-β-CD. The cyclic size of adenine possibly matched well with the cavity of β-CD and led to formation of the adenine-β-CD inclusion complex. From the results in Fig. 3, we expected that high concentration of β-CD was benefited for improvement of the separation of bases. However, the dissolution degree of β-CD limited further increasing in the concentration of β-CD.

3.3. Buffer concentration

Buffer concentration effects on the separation of purine and thymine bases are shown in Fig. 4. The

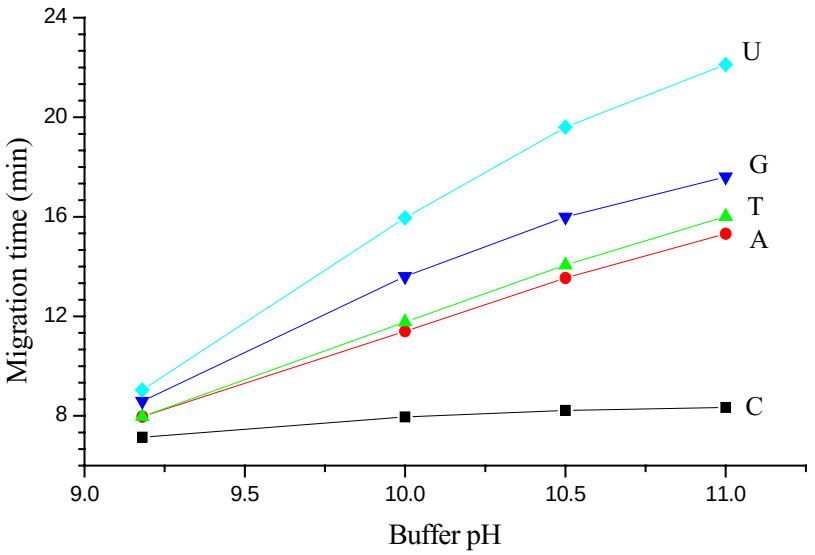

Fig. 2. Effects of buffer pH on the migration time of bases. The solutions of 20 mM sodium tetraborate including 25 mM β-CD were used as running buffer and their pH range was from 9.2 to 11.0. All sample concentrations were 50 μg/ml. Hydrostatic injections for 10 s were used, the temperature was 25 °C, and the applied voltage was 8 kV.

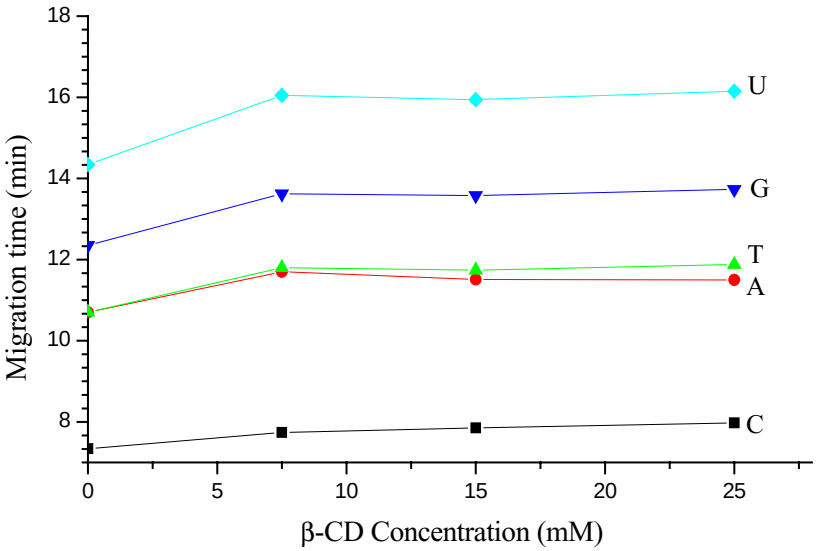

Fig. 3. Effects of β-CD concentration on the migration time of bases. The solutions of 15 mM sodium tetraborate, pH 10.5, were used as running buffer. Other conditions were as described in the caption to Fig. 2.

migration times of bases were gradually increased and the resolutions were improved with buffer concentrations. These effects were mainly attributed to the reduction of the electro-osmotic flow with the buffer concentration. Moreover, as the buffer concentration increased, the bases were stacking into narrower zones and formed sharper peaks, because of a marked difference in the effective electric field between the buffer and sample zone. But, the current in electrophoresis rapidly increased with buffer concentration, which

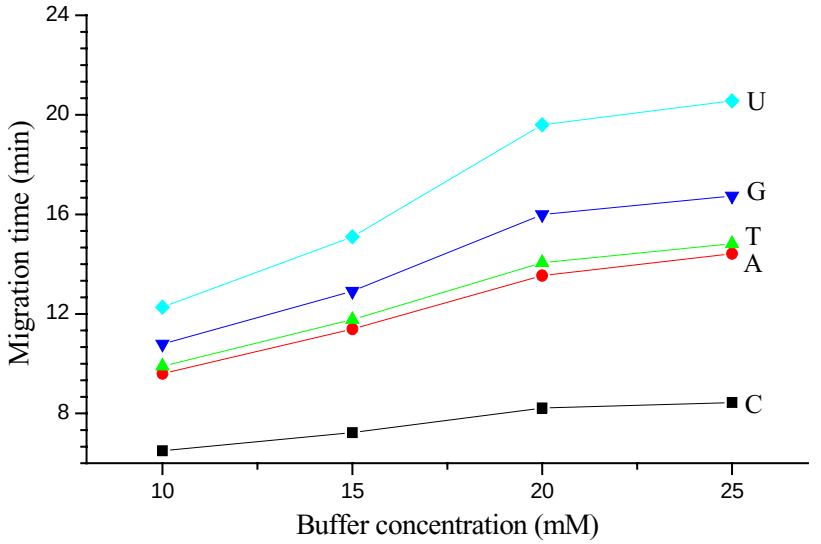

Fig. 4. Effects of buffer concentration on the migration time of bases. The solutions of sodium tetraborate including 25 mM β-CD (pH 10.5) were used as running buffer. The concentration of sodium tetraborate ranged from 5 mM to 25 mM. Other conditions were as described in the caption to Fig. 2.

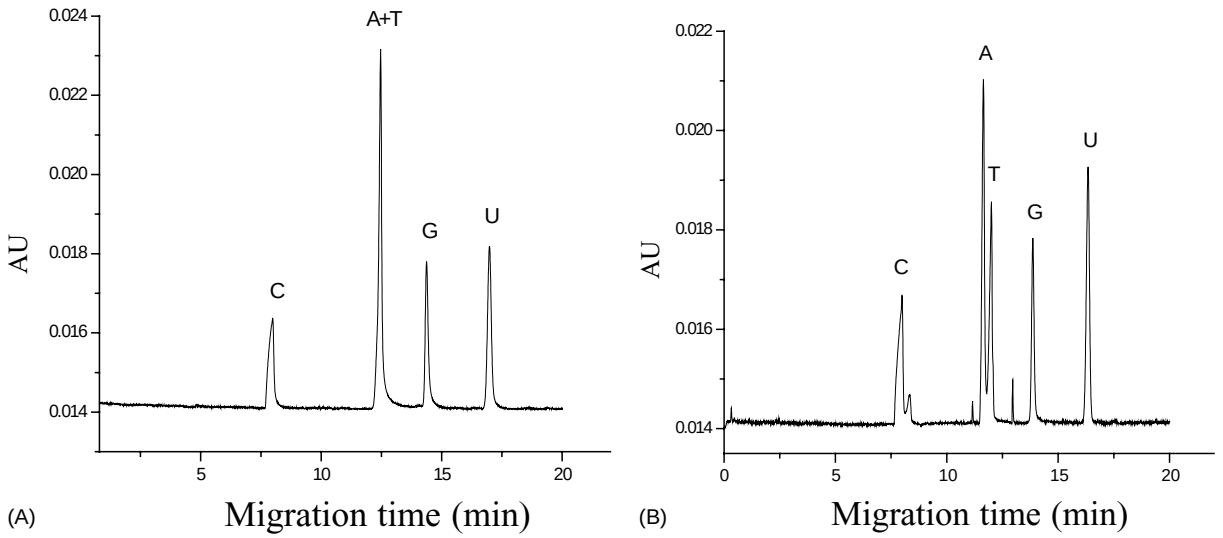

Fig. 5. Separation of purine and pyrimidine bases by CE. (A) 20 mM sodium tetraborate (pH 10.5) was used as running buffer; (B) 20 mM sodium tetraborate including 25 mM β-CD (pH 10.5) was used as running buffer. All sample concentrations were 100 μg/ml. Other conditions were same as described in the caption to Fig. 2.

resulted in Joule heating effect. When the buffer concentration was more than 25 mM, it was observed that Joule heating effect led to substantial broadening of the peaks and a decrease in the resolutions of bases (data not shown).

3.4. Separation of bases

Fig. 5 shows the separation of purine and pyrimidine base in the borate buffer with or without β-CD. In the borate buffer without β-CD, five bases were not completely separated, and the co-migration of adenine and thymine was observed (as shown in Fig. 5A). Notably, five bases were well separated in the presence of 25 mM β-CD (as shown in Fig. 5B). The data demonstrated that use of β-CD as an additive was of paramount importance to improve the separation of purine and pyrimidine bases by CE. This result likely was attributed to the interaction between thymine-β-CD and adenine-β-CD.

3.5. Detection limits, linearity and reproducibility

We measured the detection limits and linearity for purine and pyrimidine bases and the results obtained were shown in Table 1. These data illustrated that the wide linear ranges and low-detection limits for purine and pyrimidine bases were obtained by CE with UV detection. The relative standard deviations (R.S.D.) of migration times in one day and between days were less than 1.5% ($n = 5$) and 4.3% ($n = 5$), respectively, and the R.S.D. of peak areas in one day and between days were less than 2.4% ($n = 5$) and 5.2% ($n = 5$), respectively. These data showed that CE with UV detector

Table 1

Bases	Equations for calibration curves	Linear range (μg/ml)	R	Detection limit (μg/ml)
Adenine	$A = 0.055 + 7.53 \times 10^{-4}C$	2–200	0.996	0.8
Gaunine	$A = 0.038 + 5.60 \times 10^{-4}C$	2–200	0.996	1.2
Cytosin	$A = 0.067 + 4.16 \times 10^{-4}C$	2–200	0.991	1.5
Thymine	$A = 0.036 + 4.25 \times 10^{-4}C$	2–200	0.995	1.3
Uracil	$A = 0.031 + 5.01 \times 10^{-4}C$	2–200	0.997	1.8

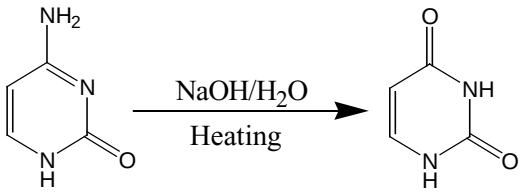

Fig. 6. Reaction of cytosine into uracil by heating in the presence and absence of sodium hydroxide.

had a good reproducibility. Stacking injection [30,31] of a large volume of samples markedly improved the concentration detection limits of bases. The concentration detection limit of 0.05 μg/ml ($S/N = 2$) for uracil was obtained at 50 s injection time when sample dissolved water.

3.6. Deamination of cytosine by heating and sodium hydroxide

The deamination of cytosine into uracil in DNA may occur in response to exposure to chemical mutagens such as hydrogen peroxide [32–34]. In addition, it also occurs spontaneously when DNA is exposed to high temperature and sodium hydroxide. Deamination reaction of cytosine into uracil was expressed in Fig. 6. In present study, we wanted to use CE technique to examine the temperature-dependence of the cytosine deamination in the presence or the absence of sodium hydroxide. In order to improve the precise of determination of uracil from deamination of cytosine, we employed thymine as an internal standard in CE analysis. The progressing of deamination of cytosine by heating is shown in Fig. 7 in the presence and absence of sodium hydroxide. The data demonstrated that sodium hydroxide significantly speeded up the deamination of cytosine into uracil. This preliminary result indicated that CE was a useful tool for study on the chemistry of nucleic acids.

4. Conclusions

We systematically investigated the effects of buffer pH, buffer concentration and β-CD on the CE separations of purine and pyrimidine bases, and demonstrated that separations of thymine and adenine was significantly improved by use of β-CD as an additive. Our data also illustrated that the CE with UV detection possessed wide linear ranges and low detection limits for purine and pyrimidine bases. Furthermore, we first demonstrated that CE technique was

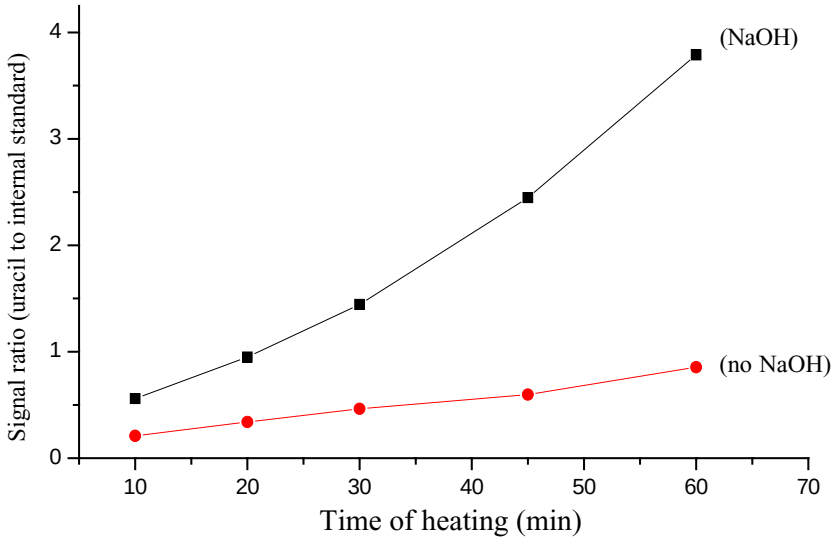

Fig. 7. Progressing of deamination of cytosine into uracil by heating in the presence and absence of sodium hydroxide. The reaction temperature was set in 100 °C. The solutions of 20 mM sodium tetraborate (pH 10.5) were used as running buffer. Two μg/ml of thymine was used as an internal standard. Other conditions were same as described in the caption to Fig. 2.

successfully applied to study on the deamination of cytosine.

Acknowledgements

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