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Electromigration behavior of nucleic acids in capillary electrophoresis under pulsed-field conditions

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ABSTRACT

We have presented a study focused on the migration pattern of double-stranded DNA (dsDNA) and RNA under pulsed field conditions. By calculating the dependence of nucleic acid mobility on its molecular size in a double logarithm plot, we found that (I) dsDNA molecules proceeded by a sigmoidal migration regime which was probably related to Ogston sieving, transition regime, and reptation model. Furthermore, the transition regime disappeared if DNA was resolved in a higher molecular mass HEC. (II) The migration pattern of RNA was relevant to the denaturant used for separation. When RNA was denatured by acetic acid, its mobility parabolically declined with its molecular size. The mobility was linearly decreased with the molecular size if urea was employed as denaturant. (III) RNA may migrate by Ogston, reptation without orientation mechanism when denatured by urea, whereas these two models were not suitable for RNA if denatured by acetic acid. Even though the electrophoretic conditions of PFCE were varied, the sigmoidal, linear, parabolic migration patterns could still be observed. (IV) Under certain modulation depth, the migration time (T_m) of acetic acid decreased with the increase of average separation voltage (V_a), and when RNA denatured in 4.0 M urea, T_m showed a linear correlation with V_a. (V) The mobility of nucleic acids increased with the growth of artificial temperature in the capillary volume due to the decrease in the viscosity of the polymer. This is the first systematic and comparative research of high molecular mass nucleic acids in PFCE, which provides us deep insight into RNA and DNA migration behavior under pulsed electric field conditions.

1. Introduction

Capillary electrophoresis (CE) has been recognized as an attractive separation method because of its numerous advantages (e.g., low sample and electrolyte consumption, short analysis time, and high efficiency). Therefore, nucleic acid analysis by CE is now a powerful tool in many research fields, including polymerase chain reaction product analysis, DNA restriction fragments analysis, DNA fingerprinting and DNA sequencing [1–5]. However, at strong electric fields and/or large molecular weight, the velocity of an oriented DNA chains will segregate in aggregates, and thus DNA molecules of different size cannot be separated by direct current (DC) field CE [6,7,8]. For the first time, Carle et al. improved resolution between large DNA molecules by alternating fields in gel electrophoresis [9]. This technique was then used to confer size-dependent mobility by periodically changing the direction and magnitude of the applied field, essentially relaxing molecular elongation [10–12]. After that, Cohen and Karger further applied pulsed electric field to CE [13].

In pulsed field capillary electrophoresis (PFCE), the reorientation time of the DNA molecule is determined by the polymer concentration in background electrolyte, pulse frequency (time duration for the forward t_f and backward t_b), modulation depth (the separation voltage for the forward V_f and backward V_b), and DNA molecular size. They are the global electrophoretic parameters for DNA separations in PFCE. Our previous experiments showed that in square-wave PFCE ($t_f = t_b$, $V_f \neq V_b$), small DNA fragments were better separated with low polymer concentration and modulation depth, whereas larger fragments were better resolved with high polymer concentration and modulation depth [14]. Further research showed that in inversion field CE ($t_f \neq t_b$, $V_f = V_b$), both short and long DNA fragments could be simultaneously separated with high resolution [15]. Traditionally, RNA needs to be denatured to cleave the hydrogen bond in the secondary and tertiary structures prior to CE or slab gel electrophoresis. By adding acetic acid

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into the polymer, we developed an on-line PFCE technology to realize denaturing and separating RNA simultaneously in a capillary [16]. We have also analyzed the separation performance of RNA between 0.1 and 10.0 knt (kilo nucleotide) by PFCE with 4.0 M urea as denaturant [17].

The migration mechanisms of single and double strand DNA (dsDNA) in traditional constant field CE have been extensively studied in the past few years [18–21]. In semi-dilute solutions, Ogston model, reptation model, and their improved version were developed for the description of DNA movements in gels and polymer solution [19,22]. For short DNA with radius of gyration ($R_{g,DNA}$) smaller than the pore size (ξ_b), separation was deemed to relate to the classical Ogston sieving [23]. This theory assumed that the gel acted as a sieve with a distribution of pore sizes and the separation was regarded as a kind of filtration, driven by the electric field. For larger nucleic acids with $R_{g,DNA} > \xi_b$, the migration was supposed to proceed by reptation or segmental motion [24], in which the DNA was supposed to migrate in a snake-like fashion through a "tube" defined by the fibers (for a rigid mesh) or the "blobs" (for a flexible network) surrounding it. However, although PFCE was widely utilized for the separation of large DNA fragments, so far no research has yet been carried out on a detailed description of the migration mechanisms of nucleic acids under pulsed field conditions.

The relationship between nucleic acid size and its mobility exposes the migration behavior of RNA/DNA in the polymer, and this relationship is a key to optimize the separation and quantify the individual components in a mixture. Herein, for the first time, we present a systematic and detailed study on the motion of nucleic acids in hydroxyethyl cellulose (HEC) under pulsed field conditions. The main electrophoretic factors for PFCE were varied and the results were compared with existing electrophoresis theories in CE. In order to cleave the hydrogen bond in the secondary and tertiary structures, RNA was denatured by urea and acetic acid, respectively. Such a study is beneficial in elucidating nucleic acids migration information and achieving an optimal RNA/DNA separation conditions in PFCE.

2. Materials and methods

2.1. Chemicals

0.1 kbp (kilo base pairs) and 1.0 kbp- DNA ladders were purchased from Takara (Shiga, Japan). 0.1 and 0.2 knt- Perfect RNA™ Markers were received from Novagen (San Diego, USA). HEC with sizes of 250 k and 1300 k was bought from Polysciences (Warrington, PA, USA). Urea and acetic acid were obtained from Wako Pure Chemical Industries (Osaka, Japan). 10,000 × SYBR Green I and II were got from Invitrogen (Carlsbad, CA, USA). 10 × TBE (1 × TBE = 89 mM Tris/89 mM boric acid/2 mM EDTA, pH 8.4) buffer was bought from Bio-Rad (Hercules, CA, USA). 0.5 × TBE was prepared by mixing 10 × TBE and distilled water with a ratio 1:19, fluorescence dye (1 × SYBR Green I for DNA separation, 1 × SYBR Green II for RNA separation denatured by urea and 3 × SYBR Green II RNA separation denatured by acetic acid, respectively). 1 × or 3 × SYBR Green I/II was obtained by diluting the 10,000 × SYBR Green I/II to a final concentration of 1/10,000 or 3/10,000, respectively.

2.2. Pulsed-field capillary electrophoresis

A home-built PFCE system was described elsewhere [17]. The fused-silica capillaries (15 cm total length; 8 cm effective length; 75 μm i.d.; 365 μm o.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillaries were coated with polyacrylamide to suppress the electroosmotic flow [25,26]. The excitation wavelength from a mercury lamp was filtered to be 460–495 nm, which was the wavelength of the excitation maximum of the conjugate of SYBR Green II and the nucleic acid. The fluorescence emission was collected by a 60 × objective (PlanApo/IR, Olympus), and then was monitored by a photomultiplier tube (R928, Hamamatsu Photonics, Japan). A high-voltage power supply (MODEL 610E, TREK, Medina, NY, USA) was employed to drive the electrophoresis. The applied voltage and data collection were controlled by LabVIEW software (National Instrument, Austin, TX, USA). The entire detection system was enclosed in a dark box. Samples were electrokinetically introduced into the capillary. After each electro-separation, the capillary was flushed with sterilized water by pump (2XZ-1, Hede Laboratory Equipment Co., Ltd., Shanghai, China) for 1.0 min, and the air exhaust rate was 60 l per s. The viscosity of the HEC solution was measured by SNB-1 viscometer (Nirun Intelligent Technology Co., Ltd., Shanghai, China). All separations were performed at 26 °C in the super clean room controlled by central air conditioning.

3. Results and discussion

3.1. Nucleic acid separation

A series of experiments about dsDNA and RNA separation in semi-dilute polymer solutions were performed under different PFCE conditions, including polymer concentration, modulation depth and pulse frequency. Fig. 1 shows an example of electropherogram of nucleic acids separations in 0.5 × TBE buffer containing 0.5% HEC (250 k) with square-wave PFCE (100 V/cm average voltage, 167% modulation depth, 50 Hz of pulse frequency). DNA was stained with 1 × SYBR Green I, and the result was demonstrated in Fig. 1A. It reveals that PFCE yields better resolution for dsDNA fragments shorter than 7.0 kbp, only if nucleic acid chains between 0.6 and 1.0 kbp migrate together in the matrix, which resisted good resolution. The running buffer for RNA contained 4.0 M urea and 2.0 M acetic acid, respectively. Todorov et al. confirmed that if the urea concentration in the running buffer was above 4.0 M, RNA would be fully denatured and no secondary or tertiary conformations would be present [27,28]. So, prior to separation, RNA was dissolved in 4.0 M urea, heated at 65 °C for 5 min, and then cooled on ice for 3 min to denature. Afterwards RNA samples were introduced into the polymer with 4.0 M urea and 1 × SYBR Green II for separation (Fig. 1B). As described in our previous work [16,29], adding 2.0 M acetic acid into the HEC polymer can realize denaturing during separation, but weaken the fluorescent intensity of the SYBR Green II binding to RNA. Thus, we added 3 × SYBR Green II into the HEC polymer (with 2.0 M acetic acid) to increase the fluorescence signal and the result was demonstrated in Fig. 1C. Data on Fig. 1B and C demonstrate that PFCE yielded a very similar set of resolution for short RNA fragments (<3.0 knt). Moreover, RNA molecule moved faster in HEC with 4.0 M urea (Fig. 1B) than with 2.0 M acetic acid (Fig. 1C). In addition, when the nucleic acid was larger than 1.0 kbp/knt, dsDNA fragment moved faster than the corresponding size of RNA molecule, despite the separation conditions were nearly the same. We tentatively interpret this to the fact that the denaturant strengthens the viscosity of the HEC polymer.

Corresponding to the electropherograms in Fig. 1, Fig. 1D depicts the relative resolution of dsDNA/RNA by PFCE to the one by CE. It shows that the resolution of RNA nearly changes with the same tendency in acetic acid and urea, except in the case of large RNA fragment (>2.0 knt), where the resolution under PFCE seems to deteriorate with the growth of size. However, the resolution of dsDNA by PFCE was improved evidently if its size was above 2.0 kbp. This is possibly the persistence length of dsDNA (50 nm) is longer than RNA (1 nm), inducing large dsDNA fragments to easily aggregate under DC field.