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Effect of temperature on the separation of long DNA fragments in polymer solution

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Abstract

Electrophoresis of long DNA fragments in polymer solutions is still attractive when performed in short capillaries. Then the separations can be accomplished in minutes rather than hours as is usual in various slab electrophoresis techniques. In this paper we focused on the behavior of large DNA fragments in pulsed field capillary electrophoresis under various temperature conditions. The mobility dependence of fragments of λ-DNA single-cut mixture on various frequencies at three different temperatures showed that the antiresonance mobility minima are shifted to higher frequencies at higher temperatures. This interesting result is explained in terms of the geometration model of DNA motion.

Keywords: Temperature effects; DNA

1. Introduction

During the last 30 years electrophoresis in sieving media has continuously been used as almost the sole method for the separation of DNA fragments. In constant electric field electrophoresis the upper limit of separation of different lengths of DNA molecules is confined to the lengths of about 20–40 kilobase pairs (kbp) [1]. Longer fragments in constant field regime migrate with the same mobilities. A solution to the problem was proposed by Carle and Olson [2] and Schwartz and Cantor [3] who established pulsed field gel electrophoresis, a technique which uses alternating electric fields and which allows one to separate fragments as long as several Mbp [4].

A very concise explanation of the fact that large fragments can be separated in the alternating field, while in the steady field their mobilities are the same, lies in their size-dependent reorientation times. Naturally, more detailed explanation is much more complicated. All theoreticians attempting to understand and describe the complicated motion of the DNA fragments in the alternating field had to generalize present models of DNA migration in sieving media in the steady field. A nice criterion for examining the respective model validity was ability to explain the antiresonance, which is a minimum in the dependence of mobility on frequency of cyclically reversing field.

All models published so far were able to prove at least semi-quantitative agreements with experiments. Most of the theories employed classical tube models of DNA with reptation motion introducing additional

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modes. They were computer simulations [5,6] which first pointed out that transitions between relaxed and extended states are the key factors in explaining DNA motion in high alternating fields. Viovy [7,8] showed that considering length fluctuation or breathing could explain some phenomena occurring when opposite field of equal amplitude is applied. Zimm [9,10] proposed his lakes-straits model both for constant and inverting field conditions. He showed that for high fields and long chains the repeating conformation of fragment between Λ and V shapes can cause the antiresonance phenomenon. The period of the antiresonance is equal to 0.4 of time needed for the chain to traverse its own length in the constant field of the same intensity.

Another view on large DNA motion is considering it as a flexible chain with fixed distance between segments moving through the lattice of obstacles. Microscopic observation of fluorescently labeled DNA [11–14] showed that longer chains do not move within a tube but take up various configurations including kinks or hernias. The basic features of their motion can be adequately described by simplified models like the geometration model first introduced by Deutsch and Madden [15,16]. They proposed a motion consisting of several different configurations: a collapsed coil, a stretched chain sliding around an obstacle and a straight chain, which turn one to another. Deutsch and Reger also showed that the chain models should be expected especially in alternating electric field due to inherently nonequilibrium nature of the problem [17,18].

In our previous work [19] we derived an analytical description of the geometration in the steady electric field and also calculated peak dispersion due to geometration motion. While a lot of work of many authors has been done on the investigation of the influence on the field strength, pulsing protocols or concentration of the sieving media on migration of large DNA, nobody has tried to inspect the effect of elevated temperature. In the present paper we bring an experimental observation of the electromigration behavior of large DNA fragments in polymer solution at various temperatures. As many important parameters of the motion of the long chains are dependent on temperature, we believe that the experimental results obtained can help one to understand better the complicated motion of DNA in sieving media.

2. Materials and methods

Pulsed field capillary electrophoresis was performed in a laboratory-made apparatus with poly-(vinyl alcohol, PVA)-coated fused-silica capillary of 100 μm I.D.×375 μm O.D. and with an effective length L_D=5.5 cm and total capillary length L_t=10.8 cm. Electrodes in reservoirs were connected to the laboratory-made alternating power supply. This power supply is able to produce square wave pulses with variable voltage up to 10 kV and variable pulse times. In all experiments of this work the period of the forward pulse was two times as long as the period of the backward pulse, while voltage was the same in both directions. Detection was performed with a laboratory-made laser-induced fluorescence detector. Model DNA samples were stained with ethidium bromide and excited with He–Ne laser (Zeiss, Germany) at 543.5 nm. The fluorescence emission was collected by a microscope objective 40×0.65 (Oriel, USA) at 610 nm. A system of two filters, blocking filter 550 nm (Varian, USA) and a band-pass interference filter 610±10 nm (Oriel) was used to separate the 543.5 nm line of the laser beam. Fluorescence was detected with photomultiplier tube (R 647-01, Hamamatsu, Japan). Data were stored on computer via A/D converter interface (Apex, Prague, Czech Republic). The electrophoretic buffer was 50 m*M* Tris-*N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) (Sigma–Aldrich, USA) containing 0.5% (w/w) hydroxyethylcellulose (HEC), number-average molecular mass, M_n= 438 000 (Hercules, Rijswijk, The Netherlands) and 2 μg/ml ethidium bromide (EtBr). The DNA sample was Lambda DNA single-cut mixture, 15 fragments from 1.1 kbp up to 48.5 kbp (Sigma–Aldrich). Sample was injected electrokinetically. A 3-cm long laboratory-made heat exchanger, based on a solid–solid interface between the inner wall of the annular electric resistance heater and the outer wall of the separation capillary, enables precise and effective temperature control of the capillary. The temperature was controlled by an Omega CN76000 instrument (Omega, Stamford, CT, USA).

3. Results and discussion

It is supposed that the movement of the long DNA

fragments under the high field regime like in our case can be described by geometration motion [15,16,19]. The molecule is moving in the sieving matrix by changing between the coil, U shaped and stretched conformations. The U phase gradually stems from the former random coil conformation when DNA meets an obstacle. Then, two arms start to grow up with velocity proportional to the free solution mobility, m_0, and applied electric field strength. Finally, the DNA looks like a rope around the pulley with two arms of different lengths hooked around the obstacle. If the electric field was still applied in the forward direction, after a certain period the shorter arm would be drawn by the longer. The fragment of DNA would then unhook, completely stretch and start to collapse again. If, however, the direction of the field is changed at the moment when the tension in the chain appears, the U shape starts to collapse back to the previous random coil. The most important aspect for separating long DNA molecules in pulsed field regime is that the lifetime of the U conformation is proportional to the DNA size and inversely proportional to the fragment free velocity.

A series of experiments in the pulsed regime was performed in which pulsing frequency varied from 5 to 55 Hz maintaining the ratio of times of 2:1 for forward and backward direction ($t_f=2t_b$) and voltage of 3200 V in both directions. The field strength in both directions was then $E_f=E_b=3200/L_t=29\,630$ V/m. Temperature was held constant at 21, 30 and 40°C, respectively. One of the examples of separation of λ-DNA cut mixture fragments is shown in Fig. 1. Mobilities experimentally determined at respective frequencies and the three temperatures are shown in Figs. 2–4. A typical deep minimum, the antiresonance, is clearly seen especially for larger fragments. To determine a position of the minimum, i.e., the antiresonance frequency, f_a, the curves were fitted by the polynomial of the third order which provided the best correlation. The minimum was resolved by calculating the first derivative of the polynomial fit for each fragment.

The inverse value of the antiresonance frequency f_a can be interpreted as the antiresonance period $T_a=1/f_a$. Considering the geometration type of motion, T_a is the optimum time when a fragment oscillates in the pulsed field between two states when hooked around the obstacle: it swaps its conformation between the U shape and the coil. In other words, it is the time needed for arms to grow up to the approximately stretched state like a rope on the pulley and then collapse into coil during the backward pulse.

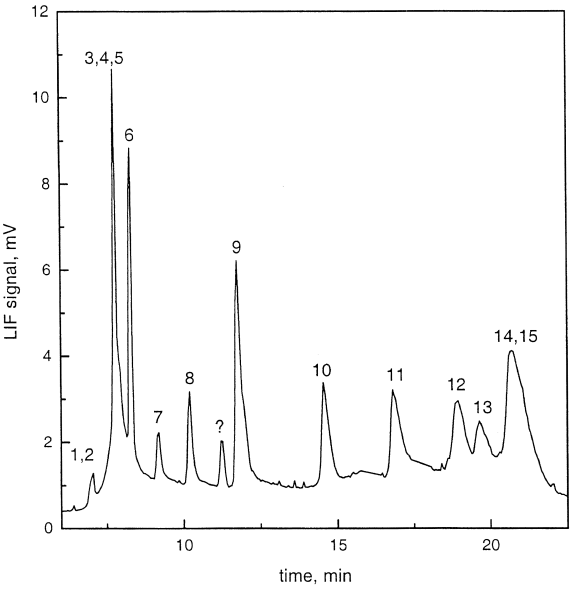

Fig. 1. Separation of λ-DNA cut mixture with pulsed field capillary electrophoresis at 30°C in 0.5% HEC, 50 m*M* Tris–TAPS, 2 μg/ml EtBr, coated capillary of 8 cm (5.5 cm to the detector)×100 μm I.D., $E_f=E_b=29\,630$ V/m and frequency 30 Hz. Injection electrokinetically, 2 kV, 8 s. DNA lengths are as follows: (1) 1.1 kbp, (2) 1.5 kbp, (3) 8.3 kbp, (4) 8.6 kbp, (5) 10.1 kbp, (6) 12.2 kbp, (7) 15 kbp, (8) 17.1 kbp, (?) unknown, (9) 19.4 kbp, (10) 22.6 kbp, (11) 24.8 kbp, (12) 29.9 kbp, (13) 33.5 kbp, (14) 38.4 kbp, (15) 48.5 kbp.

Fig. 5 shows the experimental dependence of the antiresonance period T_a on the molecular size for three different temperatures. Almost linear parts of the dependencies are evident for fragments longer than about 20 kbp, where the antiresonance period decreases with temperature and increases with molecular size. It should be in fact the size region where the geometration motion is supposed. These parts of the curves (the filled points starting with the 22.6 kbp fragment) were fitted with a linear regression to obtain the slope, which was $0.3556\cdot10^{-3}$ s/kbp, $0.3247\cdot10^{-3}$ s/kbp, and $0.2977\cdot10^{-3}$ s/kbp for 21, 30, and 40°C, respectively.

Considering the length of DNA chain per base pair to be 0.34 nm [20], the slope of the dependence of the antiresonance period on the DNA size expressed in kbp (Fig. 5) can be regarded as an increase of the

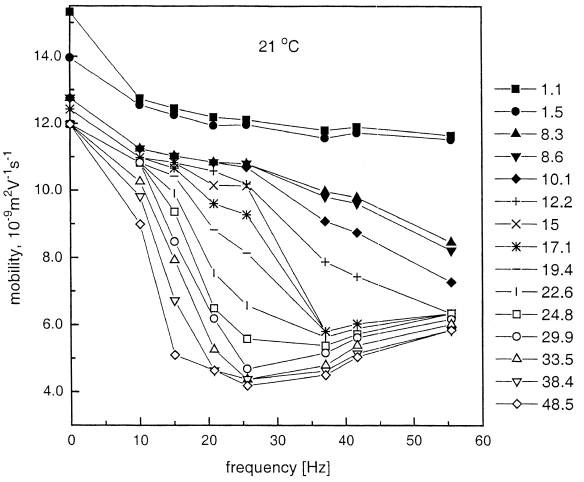

Fig. 2. Dependence of mobility on pulsing frequency for various fragments of λ-DNA single-cut mixture at a temperature of 21°C. The numbers at the right edge are lengths of fragments in kbp. Separation conditions: electrokinetic injection, $E_f=E_b=29\ 630$ V/m, $t_f=2t_b$, PVA coated fused-silica capillary of 100 μm I.D.×375 μm O.D., 50 m*M* Tris–TAPS containing 0.5% (w/w) hydroxyethylcellulose and 2 μg/ml ethidium bromide.

period when the chain length increases by 340 nm. This can be interpreted in velocity units, which gives 0.956, 1.047, and 1.142 mm/s for temperatures of 21, 30, and 40°C, respectively. Consequently, regarding the electric field strength of 29 630 V/m used in separations we can obtain a mobility for each temperature, which will be called the antiresonance mobility. Thus, we get the antiresonance mobilities $32.3\cdot10^{-9}$, $35.3\cdot10^{-9}$, and $38.5\cdot10^{-9}$ $m^2\ V^{-1}\ s^{-1}$ for the respective temperatures. Those values are very

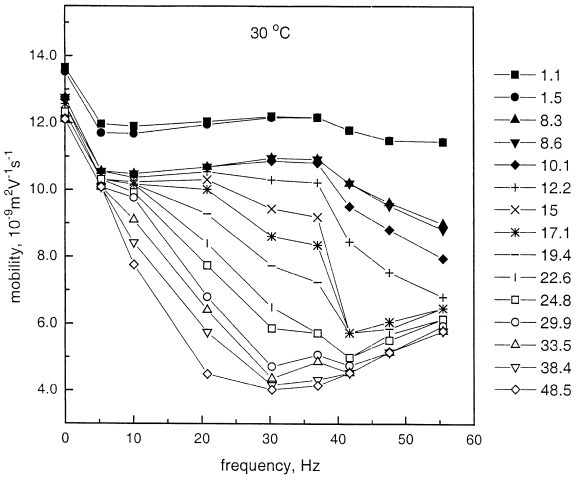

Fig. 3. Dependence of mobility on pulsing frequency for various fragments of λ-DNA single-cut mixture at a temperature of 30°C. Conditions as in Fig. 2.

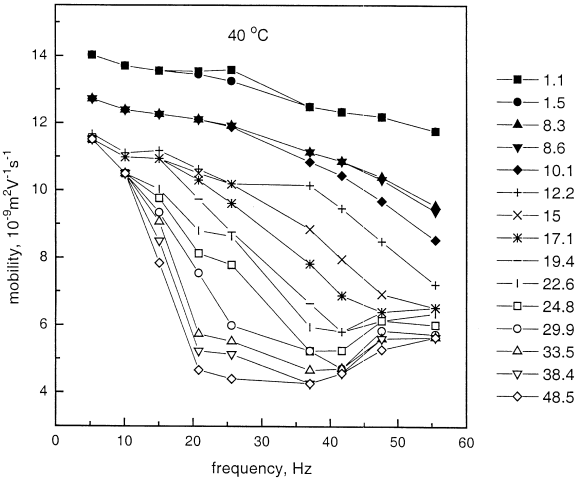

Fig. 4. Dependence of mobility on pulsing frequency for various fragments of λ-DNA single-cut mixture at a temperature of 40°C. Conditions as in Fig. 2.

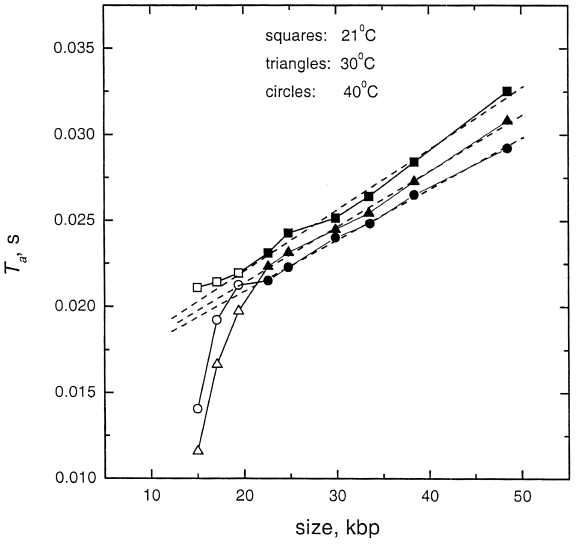

Fig. 5. Dependence of the antiresonance period on the size of the DNA fragments. Dashed lines are linear fits. Only the filled points were taken into account for linear regression (see text).

close to the free solution mobility m_0, $3.7–3.8 \cdot 10^{-8}$ $m^2\ V^{-1}\ s^{-1}$, determined in Ref. [21]. It is worth noting that the experimentally observed DNA mobility in the sieving medium for the strong constant field and large chains is about 1/3 of the free solution mobility m_0 [22], and the theoretical prediction gives 4/9 of the m_0 [19]. It then comes out that the mobilities derived from the antiresonance measurements are related to only one phase of the geometration motion: the oscillation between the U shape and the coil. All these findings strongly demonstrate that the geometration model of the movement of large DNA fragments is well founded.

This is further supported when considering the effect of temperature on the antiresonance frequency. A temperature dependence of the ionic mobility in a narrow temperature range for most of ions is about 2% per °C. This follows from the Walden rule [23] stating that the product of the ionic mobility and macroscopic viscosity of the solution is constant. This rule is evidently violated for the solutions of large molecules like polymers where the dependence of macroscopic viscosity on temperature can be quite different, and even, for the gel media, it reaches infinity. Therefore, the microviscosity of the solution which is caused rather by the small molecules of the solvent in the vicinity of the polymer chains should be considered. As the content of the polymer, which is HEC in our case, is only 0.5% (w/w) in the background electrolyte, it can be supposed that the microviscosity of the solvent and its dependence on temperature will be in the same extent as it is in the solution without polymer. Consequently, also the antiresonance mobility of DNA should depend on temperature in about the same way as the mobilities of small ions. The obtained values of the antiresonance mobilities $32.3 \cdot 10^{-9}$, $35.3 \cdot 10^{-9}$, and $38.5 \cdot 10^{-9}$ $m^2\ V^{-1}\ s^{-1}$ for 21, 30, and 40°C imply the linear dependence on temperature of 1.0% per °C. Those experimental results are at least in good qualitative agreement with the above presumptions.

The shift of the mobility minimum is convenient from a practical aspect. It is known that aggregations of the large DNA fragments in linear polymer solution are more pronounced at a lower frequency. Consequently, an aggregation can be suppressed at higher temperatures by application of a rather higher frequency.

4. Conclusion

Our experiments proved that pulsed-field capillary electrophoresis of large DNA fragments can be successfully performed in short capillaries without any loss of resolution. Thus, the analyses can be accomplished in minutes rather than in hours as usual in various techniques of pulsed field electrophoresis on slabs. The mobility dependence of fragments of λ-DNA single-cut mixture on various frequencies at three different temperatures showed that the antiresonance mobility minima are shifted to the higher frequencies at higher temperatures. This interesting result is explained in terms of the geometration model of DNA motion. Under the higher temperature, DNA molecules move faster in the free solution among the obstacles of a sieving medium. It follows that two identical molecules moving at different velocities will need different frequencies under pulsed field condition to periodically change conformation between U shape and collapsed state.

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