EFFECT OF INTERMITTENT AND HIGH FIELD ON TRAPPING OF MEGABASE-SIZED DNA UNDER ASYMMETRIC PULSED FIELD IN NANOPOROUS STRUCTURES ON CHIP

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ABSTRACT

The trapping behavior of model molecules, 166 kbp and 1Mbp DNA under asymmetric pulsed field on silica particle array packed chips is studied using total internal reflectance microscopy. The influences of pore size, electric field, frequency and molecule size are studied by measuring the percentage of trapped molecules. We observed a much higher trapping voltage for both molecules in silica particle arrays, typically more than 100 V/cm, compared to results in gels (< 10 V/cm). The result indicates that particle packed nanoporous structures in microchip-based devices have the potential to dramatically reduce the run time for separation of megabase DNA.

KEYWORDS: Megabase D A, D A Trapping, Pulsed Field, Particle Arrays, Colloidal self-assembly

INTRODUCTION

Trapping of D A at low field strength is the biggest barrier to rapid separation of megabase D A, even when using pulsed field gel electrophoresis (PFGE), yet this technology is very useful in the first stages of chromosome and gene isolation. Asymmetric pulsed field electrophoresis (APFE) within nanoporous materials in microfluidic structures, such as in Figure 1b, offers a high-speed technique for separation of 1-100 kbp D A, but has not been applied to megabase D A. Here we present the first study of > 100 kbp D A trapping in crystalline nanoparticle arrays formed by colloidal self-assembly (CSA), used to create D A sieving matrices for APFE. Trapping, not Joule heating as for smaller D A, limits the voltage and speed at which separation can occur in these structures, just as in gels, however, the fields and speeds allowed are higher.

Several groups have studied the trapping of megabase D A for gel electrophoresis. Viovy and coworkers measured the \( E_{\text{critical}} \), defined as the field where 50% of the band is trapped, of large D A molecules (250 kbp to 1.7 Mbp) under PFGE [1]. Gurrieri and coworkers measured the \( E_{\text{critical}} \) under DC field in agarose gel electrophoresis [2]. They concluded that the field and size dependence of trapping indicated a critical molecular tension is required for trapping. Turmel and coworkers used high frequency modulated pulsed field to reduce trapping [3]. All these studies indicated that the \( E_{\text{critical}} \) for megabase D A is lower than 10 V/cm.

Figure 1: (a) Schematic of the intermittent asymmetric pulsed field. Sq6 indicates the primary pulse is divided by six secondary pulses. \( E_1 \) and \( E_2 \) are two pulsed fields in APFE. \( E_1 = 1.4 E_2 \). (b) Schematic of the chip design. The central chamber is filled with a colloidal self-assembled silica bed. (c) TIRFM image of 166 kbp T4 DNA molecules under the field. A trapped molecule is shown in the circle.
THEORY

The chip design is shown in Figure 1b, with a separation chamber in the middle and six surrounding reservoirs. We applied voltage sequences, Figure 1a, to the corresponding reservoirs to generate regular and intermittently modulated APF. The net fields $E_1$ and $E_2$ in the middle chamber drive separation. For regular APF, only the primary square pulses are applied. A secondary pulse is superimposed onto the primary pulse for intermittent modulation. DNA molecules move along the applied field direction, leading by one head, then reorienting when the other field is applied, switching head and tail.

To quantitatively study the trapping behavior on chip, we use total internal reflectance microscopy (TIRFM) to video capture images of molecular behavior under APFE, Figure 1c. The number of trapped molecules are counted and divided by the total number of molecules in the field of view. Videos are captured at multiple random locations in the bed for statistical analysis.

EXPERIMENTAL

DNA (166 kbp, Nippon Gene, 0.95 Mbp, *Saccharomyces cerevisiae*) is stained by YOYO-1 (Invitrogen), and observed by TIRFM (ikon), with the excitation of 488 nm and emission of 530 nm. The 0.95 Mbp DNA was isolated from *Saccharomyces cerevisiae*, yeast cell chromosome. The chip is fabricated with PDMS and glass slides, as described elsewhere [4], where the silica particles are packed by evaporation induced in-channel packing method. We avoided sedimentation in the reservoirs by placing the chip in a vertically rotating plate. Monodisperse silica particles of 0.7 and 1 µm diameter (Bangs Laboratories) are self-assembled on chip. The injection channel is free of packing for easy injection and less shearing of DNA.

RESULTS AND DISCUSSION

Figure 2a shows the results for 166 kbp DNA. The % trapping is plotted vs. field strength for 0.7 µm particles. Frequencies from 0.2 to 5 Hz were studied for both 1 and 0.7 µm particles. We define 50% trapping as the critical field, and 10% trapping as the effective field for separation. They are both more than 100 V/cm. The recommended separation field for 166 kbp for commercial gel is around 6 V/cm. Figure 2b shows the results for 0.9 Mbp DNA for 0.7 µm particles. 1 µm particles were also used, but are not shown here. Although there is some complexity in frequency and pore size, the effective separation field is over 100 V/cm, which is more than 10 times of that for gel.

The Slater group used intermittent field to detrap megabase DNA [3]. To further reduce the trapping for our chip, intermittent modulation is used as described. In Figure 3, results of ten secondary square pulse modulation for 0.2 and 0.5 Hz are compared. Control experiments without modulation are done right after the modulated experiments. As a result, the effective separation field is increased to 200 V/cm, almost double of the regular APFE results.

![Figure 2: (a) % Trapping of 166 kbp DNA in 0.7 µm silica beds as a function of field strength at different frequencies. (b) % Trapping of 0.9 Mbp DNA in 0.7 µm silica beds as a function of field strength at different frequencies.](image-url)
CONCLUSION

The effective separation field of megabase DNA obtained on our chip is higher than 100 V/cm for regular APF, and doubled by intermittent field modulation, which means the separation time may drop from days to hours. Trapping varies with frequency non-monotonically. Trapping does not change with pore size dramatically for megabase DNA. However, shearing at low frequency and extremely high field over 260 V/cm is a problem for the potential application of separation. But we can avoid most of the shearing by choosing an optimal frequency and field range, while still maintaining the high speed advantage.

ACKNOWLEDGEMENTS

The authors acknowledge the financial funding from Natural Sciences and Engineering Research Council of Canada and Defence Research and Development Canada and thank University of Alberta for the traveling and facilities support. We want to express the gratitude to Gareth Lambkin for his work in DNA isolation.

REFERENCES


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