Measurement of Electrophoretic Mobility of Dye-Labeled Large DNA Fragments in Agarose Gels by Movement of Fluorescence Pattern After Photobleaching

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Conventional gel electrophoresis has been a powerful analytical method for DNA separations. However, it has a limit for effective fractionation of large DNA fragments up to about 20 kilo base pairs (kbp). Recently, Schwartz and Cantor[1] introduced a pulsed-field gel (PFG) electrophoresis technique, which allows separation of DNA molecules up to 2 mega base pairs (Mbp). Modifications on the geometry of applied electric field and other considerations to this technique have further improved the size resolution limit to 5 ~ 10 Mbp.[2] The progress in PFG electrophoresis has been reviewed by Cantor et al.[3]

In PFG electrophoresis, the critical experimental parameter is the pulse width (i.e., the time an electric field is applied in one direction before it is switched off or to another direction) which sets an upper limit on the DNA size separation range. As the pulse width approaches the DNA reorientation time, the electrophoretic mobility changes sharply with molecular weight, and thus high resolution for DNA separation is achieved. Besides the pulse width, the separation resolution also depends on many other parameters, such as the field strength, the gel structure, the DNA conformation and effective charge, the geometry of the applied electric field, and the temperature. Therefore, if one could tune the parameters to an optimum condition, the resolution could be improved and even a higher separation limit could be achieved. As the smallest human chromosome is estimated to be 30 Mbp,[4] it appears that PFG electrophoresis could become one of the major analytical tools for the human genome project. However, the detailed fundamental mechanism of this very important new technique remains semiempirical at best. In order to provide a solid base for theoretical formulation of DNA dynamics in the gel network and for rational means to optimize the parameters in PFG electrophoresis, we need to understand how the DNA mobility is influenced by DNA chain conformation in the gel network in the presence of an electric field. Chu et al.[5] have studied orientation and stretching times of large DNA fragments in agarose gels by low-field electric birefringence. The low field with long pulse width electric birefringence measurements could provide information only on DNA conformation dynamics related to chain orientation and stretching.

In this communication, we report a new method for studying the electrophoretic mobility of labeled DNA chains in agarose gel by means of movement of fluorescence pattern after photobleaching (MOFPAP). The technique has been used to study two-dimensional lateral mobility of fluorescent particles in small regions of a single cell surface,[6] and lately, to study the translational and slow rotational diffusion of labeled specific molecules in solutions and on cell surfaces.[7] Electrophoretic mobilities of photochromically labeled ions have been measured by combining electrophoresis with holographic relaxation spectroscopy (HRS) or forced Rayleigh scattering (FRS).[8,9] However, the species of interest in HRS (or FRS) have to be photochromic or labeled with photochromic dyes whose lifetimes are longer than the relaxation time of interest.

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Unfortunately, for large DNA fragments, the lifetimes of most photochromic dyes are too short when compared with the time required to measure the slow DNA electrophoretic mobility in gels. Therefore, we have combined the periodic pattern fluorescence photobleaching[10] with the electric field induced phase modulation due to the electrophoretic drift of ethidium bromide (EB) labeled DNA molecules. It should be noted that for large DNA fragments in agarose gels, the self-diffusion is very slow when compared with the electrophoretic mobility even at very low electric field strengths. The signal we measure in MOFPAP is related to the bleached periodic pattern moving in and out of phase with the space-fixed periodic reading pattern. By using this method, we have succeeded in tracing the electrophoretic mobility of EB-labeled DNA molecules in the agarose gel network. The stationary electrophoretic mobilities were in agreement with those values obtained by conventional gel electrophoresis.[11] The changes in the initial mobilities, which we could measure by means of this new technique, indicate that conformational stretching and alignment processes of DNA molecules in the initial stages of an applied electric field have measurable effects on the DNA electrophoretic mobility.

Monodisperse λ-DNA (size = 48.5 kbp) was purchases from New England Biolabs. The stock solution was composed of 10 m*M* Tris buffer (pH 8.0), 1 m*M* EDTA, and 500 μg/mL DNA, and was stored at −20°C. The ultrapure electrophoresis grade agarose powder with low electroendosmosis was purchased from Bethesda Research Laboratories. The DNA molecules were labeled with a trace amount of EB (about 0.005% of base pairs per each DNA molecule were strained with EB molecules). For preparation of the DNA–EB/gel solution, a known amount of agarose powder was dissolved in a known volume of distilled deionized water by boiling in a microwave oven. The amount of water evaporated during boiling was corrected. Then the gel solution was transferred to an oven that was set at a constant temperature 55 ~ 60°C for equilibration. The DNA–EB solution was mixed with the gel solution at about 35°C, and then the mixture was pipetted into the sample cell for gelation. The DNA–EB/gel could be ready for experiment in about an hour. The final DNA concentration was about 15 μg/mL and two gel concentrations, 0.2 and 0.4%, were used. In the MOFPAP setup, a Ronchi ruling with a frequency of 100 lines per inch (purchased from the Edmund Scientific Company) was employed as the source of fringe pattern. A real image consisting of alternating bright and dark fringes of the Ronchi ruling was projected to the sample. A single laser beam (λ_0 = 488 nm) was used for both bleaching and, after appropriate attenuation, for observation of movement of the fluorescence periodic pattern using modulation detection.[12] The electric field strength was in the range of 1–4 V/cm. The measurements were performed at a temperature 23 ± 0.1°C.

Figure 1 shows a typical fluorescence intensity signal from a moving fluorescence periodic pattern for λ-DNA–EB in 0.2% agarose gel at a field strength of 2.9 V/cm. The electric field was applied right after photobleaching. The modulation of the bleached fluorescence periodic pattern is a cosine function with a basic frequency[12]

$$\omega = (2\pi/L)v \tag{1}$$

where L is the spatial period of the fringe pattern and v is the drift velocity. Thus the mobility $\mu = v/E$, with E being the applied electric field strength, could be calculated by measuring the period of the redistribution of fluorescence intensity. In Fig. 1, the time period in the phase modulation of the moving fluorescence pattern decreases and approaches a constant value about 120 s after 2 ~ 3 relatively longer periods. The stationary mobility turned out to be ~ 7.3×10^{-5} cm^2/s-V, in good agreement with the gel electrophoresis mobility[11] which represents a macroscopic net result after a long running time like 10 h with the electric field on. The mobility of λ-DNA in 0.4%

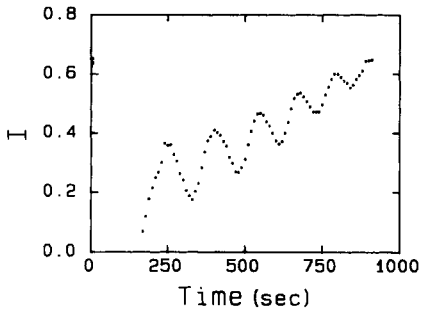

Fig. 1. Typical fluorescence intensity signal from a moving fluorescence periodic pattern of λ-DNA (size = 48.5 kbp) in 0.2% agarose gel at an electric field strength E of 2.9 V/cm. The net fluorescence intensity I is in arbitrary units. The DNA concentration C_{DNA} = 15 μg/mL in 0.3 mM Tris buffer and 0.03 mM EDTA. The stationary mobility $\mu = 7.3 \times 10^{-5}$ cm^2/s-V.

agarose gel is ~ 5.3×10^{-5} cm^2/s-V at the same field strength of 2.9 V/cm. The depth of modulation has reached ~ 60% instead of 100%, mainly because of imperfections in our periodic writing and reading patterns. In addition, the depth of modulation is decreased by a variety of factors, such as finite beam dimensions and nonuniform beam profiles. Here, we have about 8% uncertainty in determining the mobility, which could be improved. The orientation and stretching of DNA molecules along the field direction cause the initial nonstationary stage. Before the DNA molecules achieve a saturation alignment and stretching that are limited by the field strength and pulse duration, the orientation and the translational movement occur simultaneously. Therefore, movements of bleached fluorescence patterns could yield the net mobility of DNA molecules in gels at each stage (before, during, and after) of DNA orientation/stretching. Here, we refer to "after" as related to the field-free relaxation back to the DNA equilibrium conformation. Although intercalation of even a small amount of the EB dye into the base pairs of DNA molecules would stiffen the DNA chain, it does provide an appropriate demonstration. The DNA mobility can be studied as a function of EB concentration. Nonintercalating fluorescence dyes suitable for DNA labeling are also available. The orientation and stretching processes could be studied in more detail by changing the spacing and orientation of the fringe pattern, including the use of crossed laser beams instead of a Ronchi transmission ruling.

In conclusion, we have, for the first time, observed the electrophoretic mobility of large DNA fragments (e.g., λ-DNA) in agarose gel by using the MOFPAP technique. The DNA mobility in gels could be obtained in about 15 min with the present fringe period spacing (~ 254 μm) rather than the usual 10 h by conventional gel electrophoresis or PFG electrophoresis. More importantly, the orientation and stretching processes can be correlated with the time-dependent electrophoretic mobility. The new MOFPAP method should have great potential in revealing the DNA dynamics in gels. A detailed study of electrophoretic mobility and birefringence of DNA molecules in gels is underway.

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