Dielectrophoretic Behavior of Single DNA in Planar and Capillary Quadrupole Microelectrodes

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Dielectrophoresis (DEP) of single DNA (about 40 kbp) labeled with a fluorophore was successfully observed in a planar quadrupole electrode cell whose working area was 65 μm in radius. The DEP behavior depended on frequency (*f*) of alternating current; positive DEP at lower *f* and negative DEP at higher *f*. The combination of the DEP force and a laminar flow in a capillary (82.5 μm in inner radius) with a quadrupole electrode controlled the elution of DNA from the capillary as a function of *f*.

Recently, electrophoresis has become a common method for the separation of DNA molecules whose size is smaller than 20 kbp (base pairs). On the other hand, there are only limited methods for the separation of larger DNA molecules. Dielectrophoresis (DEP) is a migration of a particle in nonuniform electric fields. Negative DEP is the migration to the region of smaller electric field strength, and positive DEP is the opposite migration. DEP is a promising method for the separation and characterization of various kinds of microparticles and biological samples. The behavior of DNA in nonuniform electric fields was observed,[1] and DEP field-flow fractionation (FFF) for DNA has been examined with a device of planar electrodes.[2] However, more studies on DEP for DNA should be performed. The purpose of this study is to show the theoretical treatment of the DEP behavior of single DNA molecules, and to demonstrate DEP-FFF for DNA with another type of device, i.e., capillary with quadrupole electrode. In a nonuniform electric field generated by the quadrupole electrode, the electric field strength is smallest at the center and largest at the electrode edge.[3] The capillary is considered to have some advantages: high efficiency and throughput due to the generation of a high gradient of square electric field strength ($\nabla|E|^2$) and a centro-symmetrical electric field, and easy calculation of the electric field strength.

A double-stranded DNA molecule of about 40 kbp has 14 μm in length and 1.0 nm in radius in the solid state, and it corresponds to a sphere of about 0.022 μm in radius (r_s) if the molecule is closely packed. The visualization of individual DNA molecules was made by staining with a fluorescent probe, 5,10,15,20-tetra(*N*-methylpyridinium-4-yl)porphine ($tmpyp^{4+}$). The aqueous porphyrin possessing positive charge was adopted because DNA had negative charge.

DNA was purchased from Wako (about 40 kbp; extracted from salmon spermary and purified). DNA sample solution was prepared just before the experiment, which contained 5.8 μg cm^{-3} DNA, 6.4×10^{-6} M (1 M = 1 mol dm^{-3}) $tmpyp^{4+}$, 8.3×10^{-6} M EDTA, 3.3×10^{-5} M HCl, and 4.1×10^{-5} M tris(hydroxymethyl)aminomethane. The conductivity and pH of the solution were 4.0×10^{-3} S m^{-1} and 6.5, respectively. The maximum wavelengths for excitation and emission of this solution were 422 nm and 670 nm, respectively. The DEP behavior of single DNA molecules was observed with a fluorescence microscope (BX60, OLYMPUS) whose excitation and emission wavelengths were set to 400 – 440 nm and 475 – 800 nm, respectively. All experiments were done in a thermostated room at 25 ± 1 °C.

An aliquot (3 mm^3) of the sample solution was dropped in the working area (65 μm in radius) of a planar DEP cell, shown in Figure 1.[4] An electrode and its opposite were wired to the same polarity of alternating current (ac) and the remaining two were wired to the other polarity. An applied voltage (U_{rms}, root mean square) and frequency (*f*) of ac were varied in the ranges of 1.2 – 7.1 V and 1.0 k – 1.0 MHz, respectively, with a function generator (FG-273, KENWOOD).

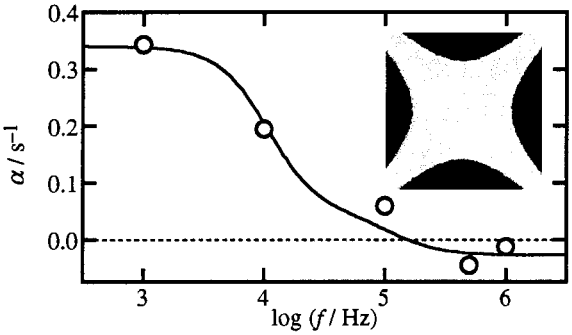

Figure 1. Effect of the ac frequency (*f*) on the DEP mobility coefficients (α) of single DNA in the planar quadrupole electrode cell (inscribed figure, black regions are electrode). U_{rms}, 3.54 V; σ_m, 4.0×10^{-3} S m^{-1}; pH, 6.5.

The DNA was observed as a sphere both with and without the nonuniform electric field under the fluorescence microscope. It is interesting that even single DNA molecules (about 40 kbp) show both positive and negative DEP depending on *f* of applied ac; positive DEP in lower *f* and negative DEP in higher *f*. This dependence is similar to that of polystyrene microparticles.[4]

The detailed method for the analysis of DEP was described elsewhere.[4,5] The distance from the quadrupole center to a DNA, *R*, can be expressed as a function of time, *t*: $\ln R = \alpha t + \ln R_0$, where R_0 is the *R* value at $t = 0$. The DEP mobility coefficient, α, can be obtained from the slope of the linear plots of ln *R* against *t*. In the present *f* range, α is expressed as:[5]

$$\alpha \approx \beta \frac{2 r_{DEP}{}^3 \varepsilon_m U_{rms}{}^2}{3 r_e \eta d^4} \left(\frac{\sigma_p - \sigma_m}{\sigma_p + 2\sigma_m} \right) \qquad (1),$$

where β is the practical efficiency of DEP force, r_{DEP} the effective DEP radius of DNA, ε_m the medium permittivity, σ_p and σ_m the conductivity of DNA and medium, respectively, r_e the radius of DNA, η the medium viscosity, and *d* the radius of working area of the quadrupole.

Figure 1 shows the plot of α against log *f*. The intrinsic σ_p of DNA was considered to be much less than σ_m, and thus the positive α was not predicted straightforward by eq 1. Our previous report concluded that a microparticle having surface charge showed a positive DEP in a lower *f* region due to dynamic move-

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ment of counter ions around the particle.[5] According to this model, r_{DEP} and σ_p can be expressed as:

$$r_{DEP} = \frac{r_{DEP,l} - r_e}{1 + \omega^2\tau^2} + r_e,\ \sigma_p = \frac{2\lambda_0}{r_{DEP}(1 + \omega^2\tau^2)} + \sigma_{p,h} \quad (2),$$

where $r_{DEP,l}$ is the limiting DEP radius at lower f ($\omega \to 0$), ω the angular frequency (= $2\pi f$), τ the relaxation time, λ_0 the limiting surface conductivity of DNA at lower f, $\sigma_{p,h}$ the limiting conductivity of DNA at higher f ($\omega \to \infty$) that should agree with the intrinsic conductivity of DNA. Since all the above parameters could not be determined simultaneously, β and $\sigma_{p,h}$ were fixed to unity[6] and 0, respectively. Furthermore, λ_0 of DNA could not be evaluated at this stage and thus a typical λ_0 value for microparticles, 9.1×10^{-8} S,[5] was employed. With these assumptions, $r_{DEP,l}$ = 0.73 ± 0.11 μm, τ = 11 ± 4 μs, r_e = 0.39 ± 0.18 μm were obtained by the least-squares method. The line in Figure 1 expresses the relationship of eqs 1 and 2 with the obtained parameters, and this line well reproduces the observed points. Furthermore, it was confirmed that linear relationships between α and U_{rms}^2 were obtained in the U_{rms} range of 1.2 – 7.1 V for both positive and negative DEP according to eq 1.

The positive and larger α of DNA can be explained in a similar way to the polystyrene microparticles whose surface has negative charge,[5] because DNA has a large number of negative phosphate groups. In the lower *f region*, the counter ions around the particle can be moved by a longer distance by the electric field and a larger dynamic ion cloud should be formed to produce a larger DEP force. The r_{DEP} (eq 2) is approximately equal to the sum of particle radius and dynamic ion cloud radius. Thus it was reasonable that the $r_{DEP,l}$ for DNA was smaller than that (= 2 – 3 μm) for the polystyrene microparticles of larger size (1.5 μm in radius).[5] Washizu et al. also suggested the enhancement of the polarizability or DEP force by the movement of counter ions around DNA and proteins.[2,7] In the meanwhile, the r_e value should agree with the intrinsic radius of DNA in aqueous solution but the obtained value, 0.39 ± 0.18 μm, was about 18 times larger than the r_s value (0.022 μm). The r_s is the value in the solid state, and the DNA form is changeable in aqueous solutions.[1] DNA is highly hydrated and has a large number of cations around it.[7] These facts can explain the r_e. The DNA size in solution may be measured by spectroscopy such as light scattering.

A capillary with a quadrupole electrode was fabricated with four platinum microwires (50 μm in radius) as shown in Figure 2.[8] The DEP capillary possessed a working bore of 82.5 μm in inner radius and 2.35 cm in length. The platinum electrodes were in electric contact with solution in the bore. Sample solutions were introduced to the DEP capillary with a microsyringe through a silica capillary that was connected at the entrance of the DEP capillary. The microsyringe was set to a syringe pump to supply the sample solution at a constant flow rate. The connection of platinum wires to the function generator was similar to that for the planar cell. DNA at the entrance and exit of the DEP capillary were observed with the fluorescence microscope.

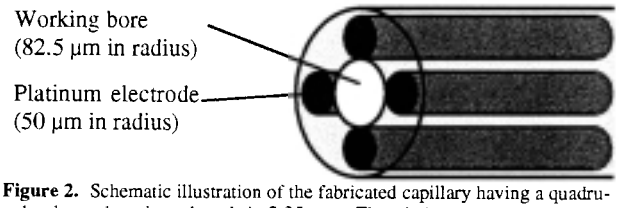

Figure 2. Schematic illustration of the fabricated capillary having a quadrupole electrode, whose length is 2.35 cm. The platinum electrodes are in electric contact with solution in the working bore.

When solvent is supplied to the DEP capillary with the pump, there generates a laminar flow in the capillary. The linear velocity in the laminar flow was found to be a function of R; the velocity was fastest at the capillary center and lowest at the capillary wall.[8] The combination of DEP force and the laminar flow in the capillary can work as FFF.

Without ac, the elution time of DNA at a flow rate of 5.0 $mm^3\ h^{-1}$ was in the range of 181 – 650 s, depending on the R value at the entrance.[8] To know the DEP effect simply, the number of DNA eluted from the capillary was counted in the time range of 240 – 270 s after applying ac. Without ac, the number of eluted DNA was 28 ± 1. As shown in Figure 3, the elution of DNA was scarcely observed at the lower f (1.0 kHz), and the number of eluted DNA increased with the increase in f. In the higher f region (100 k – 1.0 MHz), the number was almost the same as that without ac. In the lower *f region*, DNA showed positive DEP as shown in Figure 1 and it was carried slowly, and thus the number of DNA observed in the time range decreased.

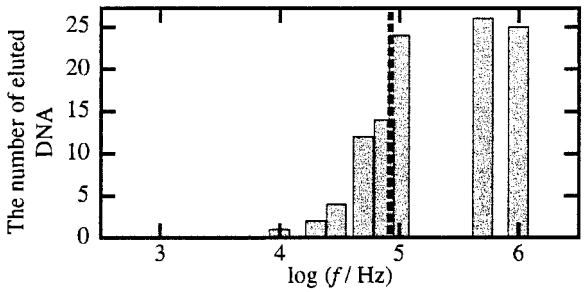

Figure 3. Elution profile of single DNA molecules from the DEP capillary. Flow rate, 5.0 $mm^3\ h^{-1}$; U_{rms}, 3.54 V. Without ac, 28 ± 1 DNA were eluted.

The present study showed that the DEP of even single DNA molecules was observed in the planar DEP cell and analyzed quantitatively. The cell will be useful for the direct observation of DEP and the characterization of various microparticles. The elution behavior of DNA from the DEP capillary was qualitatively explained from the results obtained by the planar cell. A multistage separation is performed in the DEP capillary, and thus it potentially distinguishes a little difference in DNA. Control of the elution from the capillary will be extended to the development of a new type of field-flow fractionation for the separation and characterization of various kinds of DNA.

References and Notes

1 M. Washizu and O. Kurosawa, *IEEE Trans. Ind. Appl.*, **26**, 1165 (1990).
2 M. Washizu, S. Suzuki, O. Kurosawa, T. Nishizaka, and T. Shinohara, *IEEE Trans. Ind. Appl.*, **30**, 835 (1994).
3 Y. Huang and R. Pethig, *Meas. Sci. Technol.*, **2**, 1142 (1991).
4 H. Watarai, T. Sakamoto, and S. Tsukahara, *Langmuir*, **13**, 2417 (1997).
5 S. Tsukahara, T. Sakamoto, and H. Watarai, *Langmuir*, **16**, 3866 (2000).
6 The β was a function of the relationship between the particle size and the electrode thickness, and it was assumed to be unity in the present study because the thickness (0.15 μm) was as large as the DNA size.
7 S. Suzuki, T. Yamanashi, S. Tazawa, O. Kurosawa, and M. Washizu, *IEEE Trans. Ind. Appl.*, **34**, 75 (1998).
8 S. Tsukahara, K. Yamanaka, and H. Watarai, *Anal. Chem.*, submitted.