博士論文（要約）

Migration behavior of DNA under electric field in homogeneous polymer network

(均一高分子網目構造内における DNA の電気泳動挙動)

李　响

Introduction

The dynamics of polymer chains in polymer networks are important in various applications, including gel electrophoresis, size exclusion chromatography, and cell culture. One of the oldest applications of polymer chain dynamics is gel electrophoresis, which has been widely used in the life sciences for the size separation of uniformly charged polymer chains (e.g., DNA, proteins). During electrophoresis, the interaction between the polymer network and the charged polymer chains is the key factor in differentiating the electrophoretic mobility (μ) of the charged polymer chains according to length.[1]

As for solid particles, the Ogston model has been used to explain the migration mechanism. The Ogston model treats the network as an assembly of randomly distributed pores formed with long stiff fibers, and estimates the probability that a particle can pass through the pores. The prediction of the Ogston model is $\mu = \mu_{\text{free}} \exp(-\phi R^2)$, where μ_{free} is electrophoretic mobility in free solution (no polymer networks), R is a characteristic size of the particle, and ϕ is polymer volume fraction. While the validity of the Ogston model was confirmed in the fibrous polymer network where the macroscopic pores exist (e.g. Agarose gel), this model seems invalid in flexible polymer networks such as polyacrylamide gel and semidilute polymer solutions, where polymer chains have excluded volume and fill the space.

Contrary to solid particles, for flexible DNA chains, Southern et al. experimentally discovered the relationship $\mu \sim n^{-\gamma}$, where $\gamma = 1$ in polymer networks under the influence of a moderate electric field, and n is the number of base pairs. The reptation model has been applied to explain this relationship. For gel electrophoresis, the reptation model predicts a relationship $\mu \sim n^{-2(1-\nu)}$, where ν is the scaling parameter (ν is 0.5 for an ideal chain and 0.6 for a real chain). The prediction of the reptation model for ideal chains corresponds well to the results of Southern et al. However, numerous experimental results with $\gamma \neq 1$ have been reported after the establishment of the reptation model for gel electrophoresis. The quantitative explanations for these results have never been accomplished. The mechanisms governing the migration behavior of charged chains,

which are relevant to both applied and fundamental polymer physics, remain unclear.

To fully understand migration behavior in polymer networks, a systematic study based on experiment is required. However, this kind of trials has been hindered by the heterogeneity of conventional polymer gels, which results in uncontrolled structural parameters, such as the polymer volume fraction (ϕ) and the degree of strand polymerization between cross-links (N). We recently fabricated a homogeneous gel system (Tetra-PEG gels) through the A−B type cross-link coupling of two mutually reactive tetra-arm poly(ethylene glycol) units.[2] We can precisely control ϕ and N by tuning the amount of prepolymer and the molecular weight of the prepolymer, respectively. In this presentation, we report a systematic study of the electrophoretic migration behavior of double-stranded DNA (dsDNA) in polymer networks with controlled network structures.

Results and Discussion

We performed capillary electrophoresis of dsDNA ($20 < n < 8000$ bp) in PEG solutions with various ϕ and in Tetra-PEG gels with various ϕ and N. μ was a decreasing function of n. The increase in ϕ and decrease in N led to the decrease in μ. These results indicate that the larger the dsDNA and the denser the network, the more interaction dsDNA feels during the migration. As for dsDNA with coil-like structure ($n >> 150$ bp), μ appeared to be a power law function of n as $\mu \sim n^{-\gamma}$, with $0.36 < \gamma < 1.46$. The variance in the exponent indicates that the simple reptation concept cannot explain the migration behavior of dsDNA in polymer networks under our experimental condition.

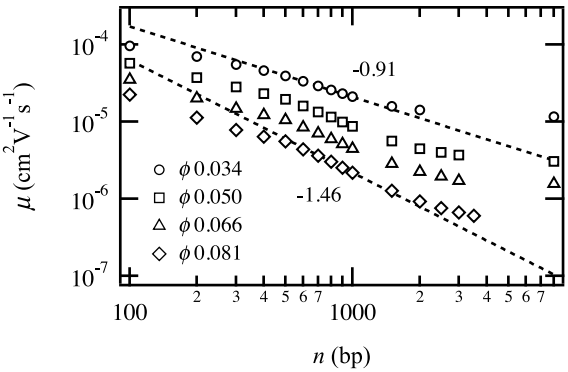

Figure 1. Double-logarithmic plots of μ as a function of n in Tetra- PEG gels with N = 117 and varied ϕ at E = 50 V/cm. The dashed lines are the guides showing the steepest power law slopes.

Linear relationships between logμ and ϕ were clearly observed, indicating that μ is an exponential function of ϕ, as $\mu = \mu_0 \exp(-B\phi)$, where μ_0 and B are functions independent of ϕ. Similar exponential relationships were observed in previous studies for the electrophoretic migration and diffusion of proteins and dsDNA in both gels and polymer solutions. The form of this equation may thus be universal for the dynamics of substances in polymer networks.

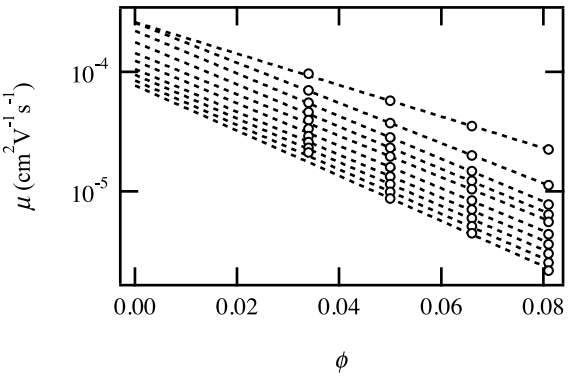

Figure 2. Semilogarithmic plots of μ as a function of ϕ in Tetra-PEG gels. dsDNA with different sizes (n: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 bp) are displayed from top to bottom in each graph. The original data are the same as in Figure 1. The dotted lines illustrate the fitting curves of eq 1

We estimated the values of μ_0 and B from the data. A clear difference was observed between the PEG solution and Tetra-PEG gels; μ_0 scales as $\mu_0 \sim n^{-0.17}$ in the PEG solution, while the scaling relationship changed from $\mu_0 \sim n^{-0.17}$ to $\mu_0 \sim n^{-0.81}$ in the Tetra-PEG gels, with a crossover at approximately n = 200 − 300 that is close to the persistence length of dsDNA (n_p ~ 150 bp). The small exponent -0.17 is similar to the prediction of the Rouse model ($\mu \sim n^0$), and the big exponent -0.81 corresponds to the reptation model for real chains ($\mu \sim n^{-0.8}$). As for B, two different

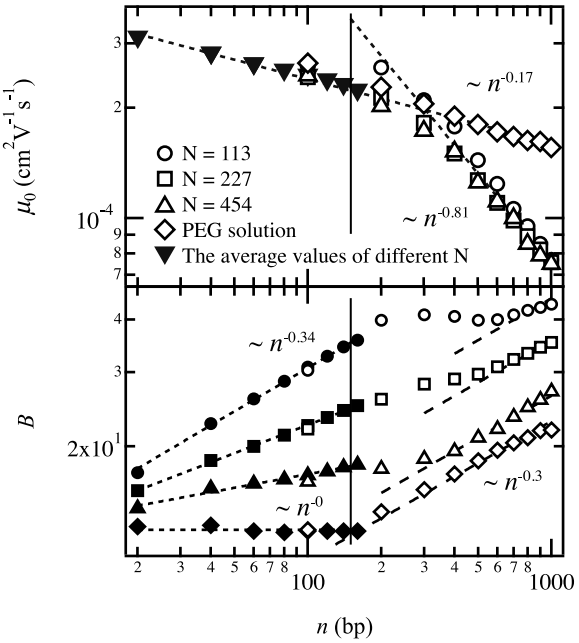

Figure 3. Double-logarithmic plots of μ_0 and B as a function of n. The filled symboles are the data in our previous paper. The dotted curves represent the fitting curves with power law function. The dashed lines represent the guide line $B \sim n^{0.3}$.

power law relationships ($B = \alpha n^{\beta}$) were observed with a crossover at approximately n_p. In the range $n < n_p$, β increased (0-0.34) with decreasing N, while β in the range $n > n_p$ was a universal value ($\sim$ 0.3) regardless of N. The form of $\exp(-B\phi)$ reminds us of the entropic trapping model, which consider the entropic barrier during the migration and predicts as $\mu \sim \exp(\Delta S / k_B)$, where ΔS denotes the entropy loss during the migration and is a function of n and network confinements, and k_B represents the Boltzmann constant. In this viewpoint, the drastic change in the term B around n_p may represent the conformation change of dsDNA from a rod to a coil.

Conclusion

Our results indicate that the μ can be expressed as the product of a power law function and an exponential function of n, which differs from any of the existing models. The migration behavior of dsDNA in polymer networks may be governed by the two different mechanisms simultaneously: basic migration (Rouse or reptation) and entropy loss during the migration. [3,4]

References

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