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Single-chain Characteristics in Giant DNA

— Larger Hydrodynamic Radius in Circular than that in Linear—

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環状高分子の広がり(慣性半径や末端間距離の2乗平均)が同じ全長の線状高分子のものより小さいことはよく知られている。しかし環状高分子の合成は難しく、実験的な検証が困難なため環状高分子に関しては不明な点が多い。一方天然高分子 DNA では生体内にウィルスのプラスミドなど環状 DNA が存在し、制限酵素を用いて一箇所切断することにより、容易に全長が単分散な環状および線状高分子を得ることができる。そこで本研究では106キロ塩基対(全長約36 μm)の線状および環状 DNA の流体力学的半径を蛍光顕微鏡を用いた単分子直接観察によって測定した。その結果、長鎖環状 DNA の流体力学的半径は線状のものよりも25%以上大きいことを見出した。

1 Introduction

The conformation of polymer chains in dilute solution is the subject of first chapter in polymer physics. The sizes of polymer, such as radius of gyration and hydrodynamic radius, are important fundamental characteristics because they can be examined with experimental methods. For an ideal circular chain, end-to-end distance L=0 by the definition, and the gyration radius R_g is deduced as [1]

$$< R_g^2 >_{circular} = b^2 N/12 = < R_g^2 >_{linear} /2.$$
 (1)

where b is the segment length and N is the number of segments. This indicates that the gyration radius in an ideal circular chain is smaller by a factor of $\sqrt{2}$ than that of linear chain of the same contour length. On the other hand, the hydrodynamic radius R_H in an ideal linear chain defined as the radius of hydrodynamically equivalent sphere is expected to be proportional and larger than the R_g as calculated from the Zimm model. Thus, it is expected that the hydrodynamic radius in circular chain is smaller than that in linear chain. As far as we know, experimental verification of such theoretical expectation has not been established yet, because it is difficult to synthesize monodisperse circular polymer. In this study, we measured hydrodynamic radius of linear or circular DNA with 106 kilo base pairs using fluorescence microscopy (FM). To examine hydrodynamic radius of giant DNA in dilute solution, FM is useful method because we can directly observe motions of individual molecules in dilute solution. It is found that, unexpextedly, the hydrodynamic radius of circular DNA is definitely larger than that of corresponding linear DNA.

2 Results and Discussions

Figure 1 (a) shows Brownian motion of individual DNA stained with fluorescence dye (DAPI) observed by FM as previously reported [2]. From the trails of the center of mass of the DNA molecule, we obtained the two-dimensional diffusion coefficient D. The R_H is calculated from

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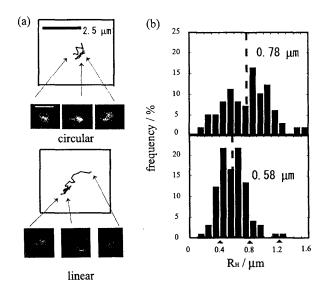


Figure 1: (a)Brownian motion trails as observed by FM. (b)the histograms of hydrodynamic radii.

D based on the Stokes-Einstein relation

$$R_H = k_B T / 6\pi \eta D,\tag{2}$$

where k_B is Boltzmann constant and η is the viscosity of the solvent. The results of analysis of Brownian motion are summarized in Fig. 1 (b). It is clear that R_H in circular form (0.78 \pm 0.30 μ m) is at least 25% larger than linear form (0.58 \pm 0.19 μ m). This results show opposite trend to the above mentioned expectation from theoretical discussions. On the other hand, from fluorescence micrograph, it is found that radius of gyration in circular chain is smaller than that in linear chain. Thus it becomes evident that hydrodynamic radius exhibits the opposite trend to the radius of gyration. Our experimental results indicate that the "sizes" of circular and linear polymer chains are the still unsolved problem in polymer physics.

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