The hierarchical structure of chromatin
— Nucleosomal array reconstitution with ring and linear DNA —

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真核生物で見られる階層的な折りたたみの基本構造としてヌクレオソーム (長さ 50 nm の DNA がコアヒストンと呼ばれる直径 7 nm のタンパクに巻きついた構造) が知られている。DNA の長さ、ねじれ、大域的な構造（環状等）がヌクレオソーム形成に与える影響は未だ十分に理解されていない。本研究では DNA の両端がつながっている環状構造がヌクレオソーム形成に与える影響を明らかにするために、全長 1 マイクロメートルの線状・環状 DNA を用いて再構成を行い、その効率とヌクレオソーム間相互作用の検討を行った。その結果、環状の DNA を用いた場合の方が環状構造に由来して再構成の効率が高くなることが明らかになった。

1 Introduction

DNA composes nucleosome with core histone protein in the folding process of eucaryotes. DNA wrapps around core histone 1.7 times and this complex is called nucleosome. DNA-histone complexes (nucleosomes) play important roles in compacting DNA and regulating its genetic function. On the other hand, DNA is often found in circular form. In circular DNA, we recongnize two possible contributions to the stochastic mechanics of the formation of nucleosomes: internal twisting rigidity and ring architecture. While circular DNA is sometimes discussed in association with twisting rigidity, little is known for the ring architecture itself on the complexation of DNA with proteins. In the present study, we focused on the effect of ring architecture on the formation of nucleosomes. Linear and fully relaxed circular DNA(ring DNA) was used to reconstitute nucleosomes and the efficiency of the formation of nucleosomes were compared in association with its global architecture.

2 Results and Discussions

Ring and linear DNA were prepared and observed by atomic force microscope(AFM) to confirm their form. Nucleosomal arrays were reconstituted from these two types of DNA and core histones by salt dialysis as written in reference [1]. Reconstituted nucleosomal arrays were observed by AFM and appearance of beads-on-a-string structures were recognized, where the beads is nucleosomes and the string is DNA in the AFM images. The positions of nucleosomal array segments were digitized and analyzed. We observed end-to-end distance of linear nucleosomal arrays. The average end-to-end distance is decreased as the number of nucleosomes in a nucleosomal array increased. This suggests that the effective contour length of nucleosomal array decreased depending on the number of nucleosomes. We counted the number of nucleosomes in a nucleosomal array and summarize the result in Figure 1 as histograms; ca. 100 DNA molecules were counted for each fixed condition. The number of nucleosomes per ring DNA chain was 6.5 $\pm$ 2.4 and the number of nucleosomes per linear DNA chain was 4.5 $\pm$ 2.0. Ring DNA was more

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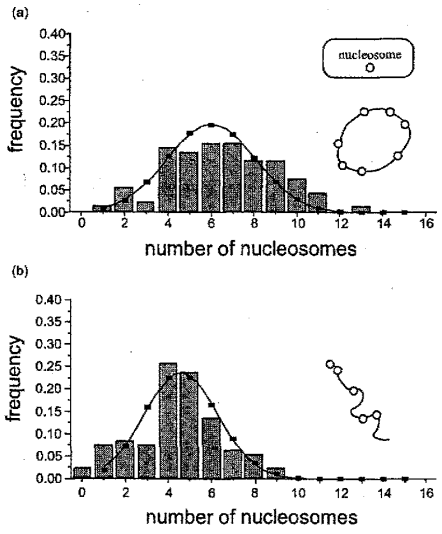

Figure 1: The probability distribution of the number of nucleosomes (a)on ring DNA and (b) linear DNA. The columns show experimental count data. The dots and lines show the distribution calculated from free energy. In this plot, we used the following values; $a = 5.02$, $b(linear) = -0.02$, $b(ring) = -0.055$, $L_0 = 1000$, $l_p = 50$, $d = 50$.

efficient at forming nucleosomes than linear DNA.
To understand this trend, we consider the free energy of single nucleosomal array with n nucleosomes. It includes change in free energy arising from formation of nuleosomes, free energy of configurational entropy due to nucleosome sliding, interaction between nucleosomes and free energy of the probability of ring closure and can be written as (in k_BT unit);

$$f_{linear} = an - n\ln(l) + \ln 2 + n\ln(n) - n + bn(n-1) \tag{1}$$

for linear nucleosomal array,

$$f_{ring} = an - (n-1)\ln(l) + n\ln(n) - n + bn(n-1) + \frac{11l_p}{4l} + \frac{107l_p^2}{30l^2} + 1.5\ln(\frac{\pi l}{3l_p}) \tag{2}$$

for ring nucleosomal array, where a is a constant to represent the chemical potential, b is a coefficient of interaction strength and expectation of inteacting state, l_p is persistence length of DNA chain, and l is contour length of DNA chain. The trend observed in the experiments is reproduced as shown in Figure 1. This suggest that the ring architecture is a factor to promote the formation of nucleosomes in terms of the probability of ring-closure and interaction between nucleosomes. We introduce here the interaction between nucleosome as three-dimensional interaction which is affected by the grobal architecture of a nucleosomal array chain.

References

[1] K. hizume, S. Araki, K. Yoshikawa and K. Takeyasu, Nucleic Acids Res. **35** (2007), 2787.