Single Macromolecules: Hierarchic Thermodynamics, Irreversibility and Biological Function

KENICHI YOSHIKAWA

*Department of Physics, Kyoto University, Kyoto 606-8503, Japan
(e-mail: yoshikaw@scphys.kyoto-u.ac.jp)*

Abstract. Single homo-polymers show two different paths in the folding transition; a liquid-like spherical globule is generated for flexible polymers, whereas a rich variety of ordered structures are formed for semi-flexible polymers. This unique characteristic is discussed in relation to its biological significance.

Key words: giant DNA, coil-globule transition, DNA condensation, genetic switching, genetic regulation

1. Introduction

Living organisms maintain their lives through the use of a rich variety of macromolecules. It is generally thought that very complicated networks comprised of many specific key-lock-type interactions between these macromolecules play a central role in life [1]. Thus, a precise understanding of the structural properties of macromolecules is essential for clarifying the intrinsic mechanism of life.

Most textbooks on macromolecules and polymers explain the change in the conformation of a single linear homo-polymer in terms of a coil-globule transition; the coil state exhibits a characteristic size of $R \sim N^{3/5}$ and the globule is $R \sim N^{1/3}$, where N is the number of segments [2, 3]. The coil-globule transition has been regarded as always continuous, due to the significant effect of fluctuation; thus, the central limit theorem is considered to break down at the level of single polymer chains. In contrast to this common description of the coil-globule transition, it has recently become clear that a single polymer chain undergoes a large discrete transition if the chain is sufficiently stiff [4–6]. The present paper describes this unique characteristic of single macromolecules in relation to its biological significance.

2. Hierarchical Thermodynamics

Recent single-chain observations on giant DNA have indicated that the folding transition between elongated coil and folded compact states is discrete. Detailed

theoretical analyses of single polymer chains have shown that, in general, the folding transition of semi-flexible polymers is discrete at the level of single chains, whereas the transition seems continuous for the ensemble [4, 7, 8]. Such a seemingly continuous nature of the transition corresponds well to the current reports on DNA condensation [9]. The most important physical parameter that determines the nature of the folding transition is chain stiffness, which is the ratio of persistence length to the width of the chain. For a polymer chain with small stiffness, the coil-globule transition resembles the transition between a gas and liquid, i.e., disorder-disorder transition, which corresponds to the current textbook description. In contrast, for a polymer above a certain stiffness, the transition is similar to that between a gas and solid/crystal, i.e., disorder-order transition. It is important to note that various kinds of nano-ordered compact structures can be generated spontaneously from the folding transition of a single semi-flexible polymer chain [5–8] (Figure 1).

Let us briefly discuss the effect of stiffness on the folding transition. We consider a polymer chain with Kuhn length l , thickness a , and contour length L , where the number of segments $N = L/l$ [2]. A single segment can be regarded as a linear train of beads with a diameter of a . The total number of beads is $N' = L/a$. In this system, the size of a polymer chain is

$$\langle R^2 \rangle = N' b'^2 \quad (1)$$

where $b' = (ab)^{1/2}$.

The second and third virial coefficients for the ensemble of beads in a single chain are $B' = a^3 \tau$ and $C' = a^6$, respectively. The free energy of

Figure 1. Schematic representation on the difference of the folding transition between flexible and semi-flexible polymers; the former gives a liquid-like globule and the latter gives an ordered crystalline state.

interaction is

$$F_{\text{int}}/k_B T \sim \frac{B' N^{1/2}}{b^3} \rho + \frac{C'}{b^6} \rho^2 \sim \frac{B N^{1/2}}{b^3} \rho + \frac{C}{b^6} \rho^2 \quad (2)$$

where $\rho \sim N/R^3$, $B = ab^2\tau$, and $C = a^3b^3$. By adding an elastic term, the total free energy is

$$F_{\text{total}}/k_B T \sim \rho^{-2/3} + \rho^{2/3} + \frac{a\tau N^{1/2}}{b^3} \rho + \frac{a^3}{b^3} \rho^2. \quad (3)$$

Mathematical analysis of this equation indicates the presence of double minima under the condition $b \gg a$, which indicates that a semi-flexible polymer undergoes a discrete transition at the level of individual polymer chains even with the effect of significant fluctuation in a “small system”. In addition, the second virial coefficient in the above equation reflects a single contact between an interacting pair of segments. Under actual conditions, the number of contacts between beads increases dramatically when the density of the segments approaches a critical high value. This effect can be renormalized in the free energy expression if the transition is interpreted in terms of competition between higher virial coefficients, for example, competition between the third and fourth terms, or even higher terms [6]. In summary, large discreteness in the folding transition is a general property of a single semi-flexible polymer and is actually observed in the compaction of giant DNA. The α -helix and β -sheet in proteins are rather stiff, which suggests that many single proteins may also show a discrete structural transition.

3. Effect of Charge

Semi-flexible polyelectrolytes, such as giant DNA, exhibit an on/off folding transition accompanied by complete charge neutralization [10]. However, a negative charge survives on the surface of the compact state, at least near the transition, indicating that the compact state is comparable to a charged colloid. When charge neutralization is incomplete or the packing of segments is rather loose, an intra-chain phase-segregated structure, or pearling, appears [11–20]. The nature of such intra-chain segregation is highly dependent on the effective volume in which the chain is encapsulated, since changes in the translational entropy of the counter ions dramatically affect the stability of the folding transition [6, 21].

4. Irreversibility in the Folding/Unfolding Transition

The discreteness of the conformational change implies that the transition is a first-order phase transition in terms of Landau's criterion and that the compact state shows polymorphism [2, 5, 6]. In general, single semi-flexible polymers show marked hysteresis in this transition. This means that the pathway of the transition in

the conformational or phase space is markedly different between the forward and backward processes. The molecular machinery in biological systems likely uses these characteristics to create a hysteresis loop in the force-response relationship [22].

5. How Do Macromolecules Work in Biological Systems?

The above unique characteristics of single macromolecules are likely associated with the mechanism of the self-regulation of genetic activity in living cells. The folding transition of giant DNA can be induced by environmental parameters, such as salt with small ions, ATP, RNA, etc. [23, 24]. It has been shown experimentally that changes in the concentrations of these chemical species regulate the relative stabilities of the elongated and folded states in DNA. Such an on/off nature of the transition is also encountered for chromatin, a DNA-histone complex. Since the folding transition of large DNA is rather robust with regard to external fluctuation, it can act as a regulatory mechanism for a large number of genes embedded on a rather large region of DNA. On the other hand, specific key-lock interactions may be suitable for fine-tuning the activity of individual genes. It is expected that life is maintained through hierarchic control, by combining specific key-lock interactions at a low level with large-scale regulation based on changes in higher-order structure [6] (Figure 2).

Figure 2. Fluorescence micrograph of individual plasmid DNA (40.8 kbp) together with the observation of transcribed RNA. On the right side of each column are shown the quasi-three dimensional representations. It is to be noted that transcription is completely inhibited by the folding transition into a tightly packed state [25].

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