Rhythmic Conformational Change in a Single Polymer Chain Induced by Laser Irradiation

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Abstract. We have investigated a rhythmic conformational change in a single polymer chain (T4DNA, 166 kbp, the contour length = 56 μm) between a folded state and a elongated state under thermodynamically open conditions using a focused continuons wave (cw) Nd:YAG laser beam (wavelength $\lambda = 1064\text{ nm}$), where the focused laser beam plays dual roles both of trapping of a polymer chain at the focus and making temperature gradient there. The oscillatory phenomenon is discuseed in terms of a limit-cycle oscillation with dissipation of photon energy.

Key words: dissipation structure, folding phase-transition in a single polymer chain, limit-cycle oscillation under thermal fluctuations, optical tweezers, thermodynamically open conditions, time-dependent Ginzburg-Landau equation (TDGL)

1. Introduction

Living matter on the earth maintain their lives under thermodynamically open conditions generated by exchange of material and energy with external environment [1, 2]. In whole process from birth to death, living matter exhibits numberless pattern formations and oscillatory phenomena such as circadian rhythm, heart beating and breath, i.e., spontaneous spatio-temporal pattern formation. Thus, life seems to be defined as matter showing spatio-temporal change. Therefore, investigation of behaviour in a molecular system under thermodynamically open conditions would be eventually important for understanding of essence of life. Relation to this, Belousov-Zhabotinsky reaction (so-called the BZ reaction), a reaction diffusion system which generates spatio-temporal pattern, has been studied experimentally and theoretically because it has been considered that the BZ reaction serves us a good model to understand essence of rhythmic phenomena in living matter [3–8]. Here, it should be noted that almost of them have never considered the size effect on chemical reaction diffusion system. Very recently, it has been shown that the BZ reaction in small system (system size $< 1\text{ mm}$) does not exhibit the pattern formation [9]. Since living matter also generates the spatio-temporal oscillation of the order of μm , oscillatory phenomenon in small scale using another system

might be helpful to understand the rhythm of the order of μm in living matter. From the standpoint of mesoscopic oscillatory phenomenon, we have proposed a working hypothesis of molecular machinery from a theoretical consideration [10], which predicts that a single polymer chain with a double-minimum free energy exhibits a rhythmic conformational change between a elongated coil and a folded compact state, i.e., between two stable states, under thermodynamically open conditions. We have already found that individual DNA molecules of the order of 100 kbp undergo an all-or-none-type transition, or the folding transition, between the elongated and folded states [11–13]. It has also been reported that a focused laser beam can trap directly a folded DNA chain [14, 15] and simultaneously make temperature gradient around the laser focus as a thermodynamically open condition [16]. Here, we report the rhythmic conformational change in a single DNA chain under thermodynamically open conditions using a focused cw Nd:YAG laser beam. Addition to this, we introduce briefly other novel oscillatory phenomena in laser fields.

2. Experiment

Bacteriophage T4DNA (166 kbp) was purchased from SIGMA. The fluorescence dye, 4',6-diamidino-2-phenylindole (DAPI), and potassium chloride (KCl) were obtained from Wako Pure Chemical. Poly(ethylene glycol) (PEG, average MW.4600) and heavy water (D_2O) were obtained from Aldrich. The sample was prepared as follows: PEG 100 mg/ml (pH 8.0), DAPI 0.6 μM , T4DNA 0.6 μM in nucleotides and KCl 0.52 M. The prepared sample solution was stood at ambient temperature of 23 ± 2 °C for 30 min before observation. Single-molecule observation and optical trap were performed using Nikon TE-300 microscope equipped with a SIT camera (Hamamatsu Photonics) and a cw Nd:YAG laser (Spectron SL902T, wavelength $\lambda = 1064$ nm).

3. Results and discussion

We designed our experimental conditions to generate the rhythmic conformational change in a single DNA chain under thermodynamically open conditions. First design is how to make thermodynamically open conditions. To accomplish it, we adopted a cw IR laser beam because an optical tweezers can trap a folded DNA chain and simultaneously induce local heating, or temperature gradient, around the laser focus. It has been that a cw IR laser beam induces local heating around the laser focus in aqueous solution and the rising temperature is estimated to be more than 14 °C [16]. Thus, we can put the folded DNA in the thermodynamically open condition. Second design is a competition of a first-order phase transition and the thermodynamically open condition. We designed a folding transition sensitive to temperature change. Here, to design the competition, it is necessary that a folded DNA chain is unfolded with rising temperature because temperature gradient is

Figure 1. Bimodal distributions of the long-axis length L of individual DNA chains at different temperatures. With permission, from Ref. [17].

induced at the laser focus [17]. We chose PEG and KCl as condensation agents in the folding transition because the folded DNA chain is unfolded with rising temperature under the existence of these chemicals [13]. Thus, we designed the experimental conditions.

We tried to find the temperature-sensitive folding transition. To generate the rhythmic change, the coexistence region is suitable because in the coexistence region the folded and unfolded DNA chains are very sensitive to temperature change. As a result, we found that there is the coexistence region at the solution including PEG = 100 mg/ml and KCl = 0.52 M at $23 \pm 2^\circ\text{C}$ [18].

Next, we investigated the effect of temperature of the folding transition in the sample. Figure 1 shows bimodal distributions of the long-axis length of individual DNA chains at different temperatures [17]. The probability of the folded state decreases with rising temperature, i.e., the folded state is not stable in higher temperature range and the unfolded state becomes stable. The changes in entropy ΔS and enthalpy ΔH are estimated to be $38 k_B$ and $1.6 \times 10^{-19} \text{ J/T4DNA-chain}$, respectively, where k_B is the Boltzmann constant [18].

Figure 2. (a) Time series of fluorescence images of the rhythmic conformational change in single DNA chain under irradiation of a cw laser beam in the PEG-KCl-H₂O solution. (b) Schematic representation of the rhythmic conformational change in a laser field. With permission, from Ref. [17].

Based on the experimental result of the temperature effect, we tried to generate the rhythmic conformational change in a single DNA chain under irradiation of laser beam [17]. Figure 2(a) shows a typical time series of fluorescence image of single DNA chain in rhythmic change around the laser focus. Figure 2(b) describes the schematic representation of the rhythmic conformational change in a laser field at laser power 150 mW. In Figure 2(a), bright spots correspond to the folded DNA chain (Figure 2(b), left), while blurred swollen images to the unfolded one (Figure 2(b), right). Thus, Figure 2(a) shows that the folded DNA is unfolded with a time interval of ca. 6 s under the thermodynamically open condition. To confirm whether the temperature gradient plays a significant role in the rhythmic conformational change, we performed same experiment using a aqueous solution including same composition except a substitution of D₂O for H₂O. It is well known that D₂O does not absorb the photon energy of the laser beam [16]. As a result, the disappearance of rhythmic change was observed [17]. Thus, we confirmed that the temperature gradient around the laser focus plays a significant role in the rhythmic conformational change.

Here, it is helpful to consider the characteristic scales in this rhythmic phenomenon. Firstly, the diameter of the local heating area is ca. $1\ \mu\text{m}$ and the rising temperature is more than $10\ ^\circ\text{C}$. Outside of the local heating area is kept at room temperature because thermal diffusion is very quick in the μm -size space, where the characteristic time scale of thermal diffusion is on the order of ms. Secondly, the characteristic long-axis length L of single DNA chain in the folded and unfolded states are ca. 0.5 and $2.5\ \mu\text{m}$, respectively. Under these situations, the folded DNA chain is exposed to the ‘hot’ environment and the unfolded DNA chain enters into the ‘cool’ ambient environment. Under these conditions, the rhythmic conformational change occurs in a laser field.

Next, we tried to analyze the rhythmic conformational change. Figure 3(a) shows the time traces of fluorescence intensity of single DNA chain at different laser powers and their Fourier transformations [17]. The appearance of peaks in the Fourier transformation should be noted. We confirmed experimentally that the rhythmic change is generated within suitable laser power range between ca. $150\ \text{mW}$ and ca. $250\ \text{mW}$, i.e., lower and higher thresholds, respectively. The Fourier transformations show obviously that the frequency of the rhythmic change is increased with increasing laser power, and it is not proportional to the laser power. These characteristics suggest that the rhythmic conformational change is a limit-cycle oscillation under thermal fluctuations. On the other hand, the oscillation is absent in D_2O solution [16].

Let us discuss briefly the mechanism of the rhythmic conformational change in terms of nonlinear dynamics. To discuss the mechanism clear, we consider a double-minimum free energy using an order parameter [19, 20], where the order parameter is the segment density of polymer chain. Using the order parameter, the free energy profile F of a first-order phase transition is represented as

$$F = \frac{1}{4}\eta^4 - \frac{1}{2}\eta^2 + \tau\eta \quad (1)$$

where η is the order parameter having two stable states at ± 1 . Each coefficient is determined arbitrarily to describe a double-minimum free energy. Here, τ is an environmental parameter related to the local temperature around DNA.

$$\tau = \alpha(T - T_c)/T_c \quad (2)$$

where T_c is the transition temperature in the folding transition and α is a positive constant. On the other hand, the order parameter η is written using the radius of gyration R as

$$\eta = -1 + 2(R^{-3} - R_{\text{unfold}}^{-3})/(R_{\text{fold}}^{-3} - R_{\text{unfold}}^{-3}) \quad (3)$$

where R_{unfold} and R_{fold} are the radii in the unfolded state ($\eta \approx -1$) and the folded state ($\eta \approx +1$), respectively. From the symmetry of free energy being considered, the temporal change in the order parameter (TDGL) with constant energy supply is described as

Figure 3. (a) Left: time trace of fluorescence intensity of single DNA chain at different laser powers. Right: their Fourier transformations, respectively. (b) Results of numerical simulation based on the coupled equations (4) and (5). The parameters are $\epsilon = 1.0$, $\beta = 1.5$, $\eta(t=0) = 0.4$, $\tau(t=0) = 0.4$ and $|\xi(t)|_{\max} = 10$, respectively, where $|\xi(t)|_{\max}$ is the amplitude of white noise in Equation (4). With permission, from Ref. [17].

$$\epsilon \frac{d\eta}{dt} \approx -\frac{\partial F}{\partial \eta} = -\eta^3 + \eta - \tau + \xi(t) \quad (4)$$

where ϵ is a positive constant and $\xi(t)$ is white noise. Let us consider a relationship of the order parameter with the local temperature here. In our experiment, the folded DNA chain ($\eta \approx +1$) trapped by an optical tweezers is exposed to ‘hot’ environment ($\frac{d\tau}{dt} > 0$) and it is unfolded. The unfolded DNA chain ($\eta \approx -1$) escapes from the trapping point with Brownian motion and it enters into ‘cool’ environment ($\frac{d\tau}{dt} < 0$). Then the unfolded DNA is folded and pulled into the

Figure 4. Schematic periodic growth and destruction of cluster of beads in a laser field [21]. (a) Formation of nucleus of cluster. Beads are pulled and trapped at the laser focus. (b) and (c) Growth of cluster. (d) Destruction of cluster. After that the cluster is formed again. Here, the diameter of beads is $0.2 \mu\text{m}$ and the period is 1–2 s.

trapping point. Therefore, the temporal change in the local heating is roughly given as

$$\frac{d\tau}{dt} = \beta\eta \quad (5)$$

where β is a positive constant. From Equations (4) and (5), the oscillation is constructed as shown in Figure 3(b). Thus, the oscillation is generated from ‘cross talk’ of the first-order phase transition with thermodynamically open conditions and is a limit-cycle oscillation under thermal fluctuations.

Figure 5. Schematic representation of lipid tubule with swinging motion in a bimodal trapping laser field [25]. When an end of the tubule is fixed steadily at the laser focus, the tubule is exposed to the local heating around the laser focus in an asymmetric manner under a trapping field. Then the gradient of surface tension is emerged. The gradient of surface tension causes the tilting motion of the tubule from the trapping field to other one. The gradient of surface tension is emerged again there and the tubule is swung to the initial trapping field. The contour length of lipid tubule is several tens μm and the diameter is several μm . The period is 1–25 s.

Besides of the oscillation of single DNA molecule, we have found various kinds of rhythmic phenomena under irradiation of laser beam. We have found that periodic growth and destruction are generated in the cluster of micrometer-sized plastic beads in a laser field [21]. Figure 4 shows a schematic representation of the periodic growth and destruction of beads in a laser field. Firstly, beads are pulled into the laser focus by a potential of laser field [22–24] and a cluster of beads is formed there. The size of the cluster is increased gradually. But, the cluster is smashed by light pressure at the critical size. After that beads are pulled into the focus and the cluster is formed again. Thus, the process from the clustering to destruction is repeated in a laser field. The competition between the attractive potential of an optical tweezers and light pressure plays a significant role in this phenomenon [24]. It is discussed in terms of a limit-cycle oscillation under the dissipation of beads in a laser field.

We have also found that a giant lipid tubule shows a rhythmic swinging motion in a laser field having a bimodal trapping field (see Figure 5) [25], where a focused laser beam is split into two laser beams by an adjustment of the optical system. When an end of the lipid tubule is trapped steadily at one stable point, the side of the tubule is trapped along one trapping field (one of the split beams). Then, the tubule is exposed to the local heating induced by the trapping field in an asymmetric manner. At last the gradient of the surface tension is emerged, causing the tilting motion of the tubule from one trapping field to another one in one direction (another of two split laser beams). After the tilting to other trapping field, the tubule is affected by asymmetric heating and the tubule tips to the initial trapping field in opposite direction. Thus, the rhythmic swinging motion is generated in the bimodal trapping field, where the end is fixed through the process. In this case, the competition between the bimodal trapping field and the asymmetric heating plays a significant role. This oscillatory phenomenon is discussed in terms of a limit-cycle oscillation of dissipation of photon energy.

In other experiment, the periodic growth and disappearance of a water droplet has been observed (see Figure 6) [26]. Firstly, a big water source is heated by a focused laser beam. Liquid water is evaporates gently and supersaturated vapor is generated. When a small partiele or crack on glass surface dust exist as a nucleus, then vapor is condensed to form a small water droplet at the nucleus and the droplet is grown. After that the growing droplet is disappeared by the absorption into the big water source because of contact. But since the air is still supersaturated, a small water droplet immediately appears at the nucleus. Thus, the periodic growth and disappearance of water droplet at the nucleus is repeated. This oscillatory phenomenon is also discussed in terms of dissipation of photon energy.

We have shown novel rhythmic phenomena in laser fields. They show that the molecular system generates spontaneously rhythm and motion under thermodynamically open conditions in mesoscopic scale. Further studies on oscillatory phenomena in mesoscopic system would serve a deeper understanding of an essence of life.

4. Conclusion

We have investigated the oscillatory phenomena in a single polymer chain under the irradiation of a cw Nd:YAG laser beam. In the H_2O -PEG-KCl solution, single DNA chain emerges the rhythmic conformational change, where the period is of the order of seconds and it depends on the laser power. On the other hand, the rhythmic change is absent in the D_2O -PEG-KCl solution. Based on the folding phase-transition and the 'cross talk' of single polymer chain with environment, we proposed the coupled equations as model of a limit-cycle oscillation in a single polymer chain under large thermal fluctuations. The observed oscillatory phenomenon is discussed in terms of a limit-cycle oscillation with dissipation of photon energy.

Figure 6. Schematic representation of periodic growth and disappearance of a small water droplet at nucleus [26]. (a) A big water source is heated and air is supersaturated. (b) A small water droplet appears at the nucleus and is grown. (c) The water droplet grown at the nucleus contacts with a big water source and it disappears by the absorption to the source. But, since the air is still supersaturated, the small droplet appears immediately at the nucleus. Thus, the process is repeated. The period is 1–3 s.

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