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快 报

利用分子梳方法研究单个 DNA-YOYO-1 复合体的光漂白性质*

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摘 要: 利用分子梳方法结合荧光显微术对单个 DNA-YOYO-1 复合体的光漂白过程进行了研究,发现单个 DNA-YOYO-1 复合体的光漂白是一个随光照时间指数衰减的过程,并给出了 DNA 拓扑结构以及光照强度对光漂白过程的影响.

关键词: DNA; YOYO; 光漂白; 分子梳
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Investigating the Photobleaching Property of Single DNA-YOYO-1 Complex by Molecular Combing Method*

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Key words DNA, YOYO, Photobleaching, Molecular combing

The two dyes named YOYO and TOTO used as fluorescent probes in DNA experiments were reported in 1992[1]. When the cyanine dye YOYO is bound to DNA its fluorescence quantum yield can be 1000-fold higher than when it is free in solution[2]. Because of this YOYO dye is widely used not only for detection of DNA in electrophoresis[2-4] and visualization of DNA in fluorescence microscopy[5-7], but also for single molecular detection of DNA[8,9]. On the other hand, photobleaching of YOYO is significant limiting factor in fluorescence detectability when high-intensity illumination condition and long exposure times are necessary. The photobleaching phenomenon of YOYO was previously investigated in a bulk solution with fluorescence microscopy[10]. In the present work we experimentally study the bleaching process of a single DNA-YOYO-1 complex with a molecular together method combined with fluorescence microscopy[11]. This method allows us to detect the photobleaching process for a single DNA molecule with different topological shapes. Most recently (after the submission of this paper), the photobleaching processes of fluorescent molecules (both the well-defined ensemble and the single-molecule levels) have been studied extensively[12,13].

We used λ-DNA (48.5 kb) (Sino-American

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Biotechnology Company, China) in the experiment. Fluorescent dye, oxazole yellow dimer (YOYO-1) was purchased from Molecular Probes Company (USA). Bis-Tris buffer was purchased from Sigma Company (USA). All solutions were made with 18.2 MΩcm water from the Milli-Q Water Purification System (Millipore Corporation, France). λ-DNA was stained with YOYO-1 by mixing DNA sample with a specific volume of freshly prepared 0.1 mol/L dye solution (10 mmol/L Tris, 1 mmol/L EDTA buffer, pH = 8.0) and incubated in the dark for 30 min.

Quartz plates (21 × 42 mm^2) were immersed in hot NaOH (0.5 mol/L) for about 10 min, and then rinsed thoroughly in high-purity water (18.2 MΩcm). Quartz surfaces were rendered hydrophobic by coating the surfaces with PMMA. A droplet (0.2 – 0.3 mL) of PMMA (560F, Japan) in chloroform (10%) was applied to the center of a cleaned quartz surface, which was mounted horizontally on a spin-coating machine using a double-sided tape, and spread by spin-coating at 5500 revolutions per minute for 1 min. After spin-coating, the PMMA film was distributed evenly on the whole surface. The quartz plate was then baked at 145 ℃ for about 30 min and then stored at room temperature in a dust-free environment.

A droplet (1 – 2 μL) of the stained DNA solution was deposited onto a PMMA surface. With the drying of the droplet, DNA molecules originally bound to the surface with one extremity were extended and immobilized on the hydrophobic surface. The combed DNA molecules were observed by using an inverted epi-fluorescence microscope (IX-70, Olympus, Japan) with a 20 × objective. A 100 W mercury lamp was used in combination with a U-MWB excitation cube. The images were captured by a cooled CCD camera (CoolSNAP-HQ, Roper Scientific, USA). The CCD acquisition time was five seconds. All the images were stored in a computer. The fluorescent intensities of individual DNA-YOYO-1 complexes were analyzed with A MetaMorph software (Universal Imaging Corporation).

Figure 1 shows a typical image of the DNA-YOYO-1 complex. It can be seen that some DNA molecules are stretched to a straight line and some others keep coiled (bright spots). This phenomenon provides a means to measure the bleaching process of the stretched DNA and coiled DNA individually.

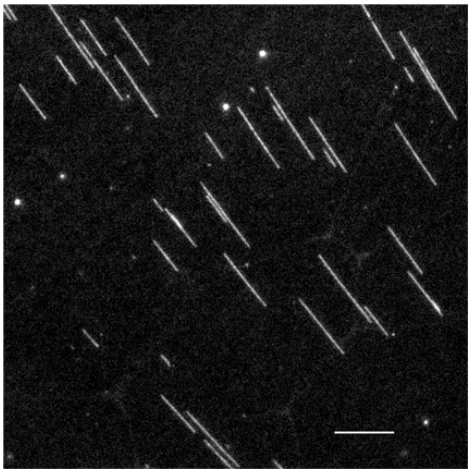

Fig. 1 Fluorescence image of the combed DNA-YOYO-1 complex
The inclined straight lines are stretched single DNA molecules. The bright spots are coiled DNA molecules. Bar = 20 μm. The ratio between DNA base pairs and dye molecules is bp/dye = 5. This ratio is fixed throughout this work.

Typical results of the relative fluorescence intensity versus time for stretched DNA and coiled DNA are shown in Fig. 2. An exponential decay function was used to fit the data.

$$y = A\exp(-t/\tau) + y_0$$

where y is intensity, A and y_0 are constants, t is time, τ is time contant. This appears to be a good technique for the bleaching process. Measurements have been made for many stretched DNA (straight lines in Fig. 1) and coiled DNA (bright spots in Fig. 1) molecules and similar decay curves were obtained (data not shown).

From the fitted parameters of Fig. 2 we can see that the time constant for the stretched DNA is shorter than that for the coiled DNA, (i. e. for a stretched DNA the bleaching is faster). Because the combing force could stretch DNA molecules to about 1.6 times their contour length[11], overstretching transition from B-form to S-form DNA occurred. As to the S-form structure, it was proposed that the overstretched DNA adopts a diameter-reduced double-stranded

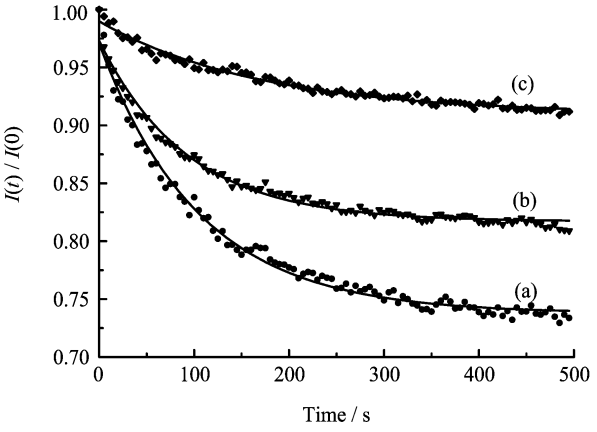

Fig. 2　Relative fluorescence intensity versus time
$I(0)$ is the fluorescence intensity at the beginning of illumination. Symbols are experimental data and curves are fittings. (a), (b) and (c) correspond to the coiled DNA, stretched DNA and stretched DNA with reduced illumination intensity (25% of (b)), respectively. The fitted parameter values are (a) $A = 0.233$, $\tau = 106$, $y_0 = 0.738$; (b) $A = 0.156$, $\tau = 92.7$, $y_0 = 0.817$; (c) $A = 0.0799$, $\tau = 171$, $y_0 = 0.910$.

structure[14], or ladderlike structure[15]. Our present results indicate that these DNA structures may increase the bleaching rate. Considering that the B-form to S-form transition is a counter deformation of DNA supercoiling, the tendency is consistent with the bleaching result in the bulk solution for supercoiled DNA[10]. Also obvious from Fig. 2, the illumination intensity affects the bleaching time constant significantly. With reduced illumination intensity, the bleaching rate slows.

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