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Triplet fraction buildup effect of the DNA–YOYO complex studied with fluorescence correlation spectroscopy

Masafumi Shimizu [a,*], Satoshi Sasaki [a], Masataka Kinjo [b]

[a] School of Bionics, Tokyo University of Technology, 1404-1 Katakura, Hachioji, Tokyo 192-0982, Japan
[b] Laboratory of Supramolecular Biophysics, Research Institute for Electronic Science, Hokkaido University, N12W6, Kita-ku, Sapporo, Hokkaido 060-0812, Japan

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Abstract

DNA fragments of various lengths and YOYO-1 iodide (YOYO) were mixed at various ratios, and fluorescence was measured using fluorescence correlation spectroscopy. The number of substantially emitting YOYO molecules binding to the DNA and the binding intervals between the YOYO molecules were estimated for DNA–YOYO complexes of various lengths. In the present study, we found an interesting phenomenon: triplet buildup. Because fluorophores that fall into the triplet state do not emit fluorescence, a part of the dark period can be recovered by emitting photons from other excited YOYO molecules in the same DNA strings in the confocal elements. The remaining dark period can be considered to be the total miss-emission rate. Estimates of the total miss-emission rate are important for calculation of the length and amount of DNA.
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Cyanine dyes such as YO-PRO-1 iodide and YOYO-1 iodide (YOYO) have unique characteristics: they have large molar absorptivity values and are virtually nonfluorescent in the free form but show very strong fluorescence when complexed with double-stranded DNA [1–17]. In our previous study [17], DNA fragments of various lengths were prepared and mixed with YOYO prior to fluorescence correlation spectroscopy (FCS)[1] measurements. FCS is known to be a powerful tool for investigating the dynamic properties of fluorescently labeled molecules. In FCS, the fluorescence intensity of molecules, which fluctuates due to variations in the number of the molecules in a confocally defined volume element, is recorded as photon counts and correlated in time [18–36]. Several dynamic properties, such as the number of fluorescent molecules in the volume element and the diffusion time of fluorescent molecules, can be determined. Furthermore, fluorescent properties, such as photon count rate, photon count rate per fluorescent molecule, and fractional population and decay time of the triplet state, can be determined simultaneously [21,30].

In our previous study [17], we focused on the diffusion time of the DNA–YOYO complex. Generally, fractional population and lifetime of triplet state distorts the evaluation of the number and diffusion rate of target molecules, and this is strongly affected by laser power used. However, we found that, interestingly, triplet buildup for the DNA–YOYO complex depends on both DNA length and the DNA/YOYO ratio without laser power change. In the present paper, we describe this phenomenon and attempt to explain the mechanism underlying it.

Materials and methods

Materials

Dimeric cyanine dye YOYO-1 iodide was purchased from Molecular Probes, USA. Sequence-grade oligodeoxy-

* Corresponding author. Fax: +81 0 42 637 2118.
E-mail address: shimizu@bs.teu.ac.jp (M. Shimizu).

[1] *Abbreviations used:* FCS, fluorescence correlation spectroscopy; DMSO, dimethyl sulfoxide.

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ribonucleotides were purchased from Date Concept, Japan. Unless otherwise mentioned, other chemicals were purchased from Wako Chemicals, Japan. All materials were of analytical grade and used without further purification.

Preparation of DNA fragments

Fifty-base pair DNA fragments were prepared by annealing an arbitrary sequence (5′-TCT ACT GGG ACG GAA CAG CTT TGA GGT GCG TGT TTG TGC CTG TCC TGG GA-3′) and its complementary single-stranded DNA oligonucleotide. The synthesized single-stranded DNA oligonucleotides were dissolved and mixed stoichiometrically in a dilution buffer (10 mM Tris, 1 mM EDTA, 50 mM NaCl, pH 7.4). The solutions were heated to 94 °C and gradually cooled to 4 °C over 7 h using a thermal cycler (PTC-200; MJ Research, USA). These DNA solutions were used without further purification.

For DNA fragments longer than 100 bp, the Forever 100-bp Ladder Personalize Kit (Seegene, Korea), which contains plasmids enabling us to amplify 100- to 1500-bp DNA fragments using PCR, was used. After PCR using Ex Taq polymerase (TaKaRa, Japan), the DNA samples were purified with the QIAquick PCR Purification Kit (Qiagen, USA). The concentrations of the DNA samples were determined using a UV spectrometer (Ultrospec 2100 pro; Amersham Biosciences, Sweden) using 1 OD_{260} corresponding 1 μg to 50 μg/ml of double-stranded DNA solution.

Preparation of DNA–YOYO complex solutions

Commercially available YOYO solution, which is supplied as a 1 mM solution in DMSO, was diluted further with DMSO (Nacalai Tesque, Japan) to prepare a stock solution of 20 μM YOYO. A working solution of 100 nM YOYO was freshly prepared immediately prior to use by dilution with DMSO.

The solutions of DNA fragments were freshly diluted to the desired concentrations prior to use with the aforementioned dilution buffer. Each DNA solution diluted with dilution buffer was mixed with a 5% volume of 100 nM YOYO DMSO solution, and the resulting solution was then vigorously mixed by vortexing.

FCS measurements

FCS measurements were performed by using a commercial combination system (ConfoCor 2, Carl Zeiss, Germany) equipped with a water-immersion microscope objective (40×, NA 1.2), and a 488-nm Ar^+ laser for excitation, for which the power was fixed at 150 μW. All FCS measurements were carried out using 20 μl of sample solution placed on a NEO microcover slip (25 × 55 mm, No. 1; Matsunami Glass, Japan) followed by 1-min wait for matching the influence of DNA adsorption on the glass surface. All spectroscopic measurements were carried out over a period of 20 s at room temperature (around 25 °C), and measurements were obtained five times. For calibration, an aqueous solution of 10 nM Rhodamine 6G (Aldrich, USA) was measured as a control before each data acquisition series.

Each correlation data set of obtained was fitted to the FCS autocorrelation function, taking into account the triplet state buildup of the fluorophores [21] and neglecting sample volume distortions,

$$G(\tau) = 1 + \frac{1}{N(1-T)}\left(1 - T + Te^{-\frac{\tau}{\tau_T}}\right)\left(1 + \frac{\tau}{\tau_D}\right)^{-1} \times \left[1 + \left(\frac{w_0}{z_0}\right)^2 \frac{\tau}{\tau_D}\right]^{-\frac{1}{2}} \quad (1)$$

where τ is the correlation time, N is the average number of fluorescent molecules in the detection volume, T and τ_T are the fractional population and decay time of the triplet state, respectively, τ_D is the characteristic diffusion time, and w_0 and z_0 are the distances from the center of the laser beam focus in the radial and axial directions, respectively. τ_D corresponds to the time taken for a fluorescent molecule to diffuse across the confocal volume element and is related to the translational diffusion constant D of the fluorescent species by $\tau_D = w_0^2/4D$.

Results and discussion

Fluctuation of photon count rate

The different lengths of dsDNA fragments were diluted to various concentrations, mixed with 5 nM YOYO, and subjected to FCS analysis with 488-nm Ar^+ laser for excitation, for which the power was fixed at 150 μW. In these measurements, DNA–YOYO complexes behave as fluorescent molecules because YOYO molecules in the free form are almost nonfluorescent [1–17]. Typical fluctuations of count rate are shown in Fig. 1. Because these measurements were carried out using the laser with its power weak enough, conspicuous decreases of count rate were not seen, suggesting that remarkable photobleachings were not occurred during these FCS measurements. These show remarkably large spikes for the 1500-bp (Figs. 1A and C) compared to the 50-bp DNA–YOYO complex (Figs. 1B and D). This indicates that one DNA–YOYO complex emits strongly because 1500 bp DNA can bind a large number of YOYO molecules compared to 50 bp DNA.

Binding of YOYO to DNA

Typical normalized correlation functions obtained for DNA–YOYO complexes are shown in Fig. 2. The correlation of the 1500-bp DNA–YOYO complex decreased more slowly than that of the 50-bp DNA–YOYO complex for DNA/YOYO ratios of both 10 (Fig. 2A) and 0.01 (Fig. 2B). These results suggest that the correlation time is longer and that the 1500-bp DNA–YOYO complex

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