Talanta 79 (2009) 963–966

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Millisecond analysis of double stranded DNA with fluorescent intercalator by micro-thermocontrol-device

Hideyuki F. Arata [a,b,*], Frederic Gillot [a,c,1], Dominique Collard [a,c], Hiroyuki Fujita [a]

[a] Institute of Industrial Science (IIS), The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8505, Japan
[b] Japan Society for the Promotion of Science (JSPS), Japan
[c] LIMMS-CNRS/IIS, France, Japan

ARTICLE INFO

Article history:
Received 18 February 2009
Received in revised form 15 April 2009
Accepted 16 April 2009
Available online 3 May 2009

Keywords:
Intercalator
DNA
Denaturation
Microheater
Millisecond

ABSTRACT

Study of interaction between DNA and intercalator at molecular level is important to understand the mechanisms of DNA replication and repair. A micro-fabricated local heating thermodevice was adapted to perform denaturation experiments of DNA with fluorescent intercalator on millisecond time scale. Response time of complete unzipping of double stranded DNA, 16 μm in length, was measured to be around 5 min by commercial thermocycler. Response time of quenching of double stranded DNA with fluorescent intercalator SYBR Green was measured to be 10 ms. Thus, quenching properties owing to strand unzipping and denaturation at base pair level were distinguished. This method has provided easy access to measure this parameter and may be a powerful methodology in analyzing biomolecules on millisecond time scale.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Aromatic molecules of planer structure are known to intercalate between base pairs of double stranded DNA (hereinafter dsDNA) [1]. These intercalators inhibit DNA replication and repair, thus playing a substantial role in controlling cancer development and genotoxicity [2]. Therefore, study of intercalation mechanism and its application has attracted great interests in genetics and medical researches. SYBR Green (hereinafter SG) is an intercalator which is commonly used to monitor dsDNA [3]. It shows strong fluorescent intensity when intercalated between base pairs of a dsDNA whereas it shows almost negligible fluorescence when a dsDNA is denatured and structurally unzipped [4]. A dsDNA starts separating into single strands at a temperature higher than 90 °C without any external mechanical force [5]; this phenomenon is commonly used in the first stage of the polymerase chain reaction (PCR) cycle. Fluorescent intercalators can verify unzipping of dsDNA into two single strand DNAs (hereinafter ssDNAs). This process takes a few seconds to a few minutes, mainly depending on length of DNA. However, dynamic properties of the quenching process have not been precisely studied. The main reason is due to the difficulties in observing phenomena at molecular level, such as DNA and SG interaction or denaturation at base pair level, which may happen on an extremely short time scale. Thus, the terminology "denaturation" has been used both as "unzipping of double strands" and "denaturation at base pair level". To analyze quenching dynamics on millisecond time scale is inevitable for further genetic researches.

Microfabrication technology has realized miniaturized devices of several kinds, such as sensors [6,7], reactors [8,9], and actuators [10]. In addition, these devices have fast response and high sensitivity due to the merits of miniaturization. Application of microdevices to chemical or biological experiments has realized integrating multiple functional units onto a small chip, so-called micrototal analysis systems (μTAS) [11]. μTAS allows rapid chemical or biological analysis with small amounts of samples [12,13]. Therefore, applications aiming at manipulations and characterization of biomaterials by microsystem have become one of the major trends in this field [14]. Some procedures which require rapid temperature switching, for example on-chip PCR [15,16], have been improved by miniaturization. This is because miniaturization enables rapid temperature control simultaneously with real-time observation owing to the small amount of volume that must be heated [17–21]. Thermal control by micro-thermodevice has various advantages, such as compactness and simple operation, to that of laser heating system [22,23] which requires manual operation and adjustments from one run to another.

A square-shaped micro-thermodevice for biological applications with side length of 20 μm was manufactured (Fig. 1(a)) [24] (Precise fabrication process and evaluation of the device will be reported elsewhere). This device consisted of a microheater and

* Corresponding author. Current address: Curie Institute, 11 rue P. et M. Curie, Paris, France.
E-mail address: arata@sanken.osaka-u.ac.jp (H.F. Arata).
1 Current address: Ecole Centrale de Lyon, France.

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.04.045

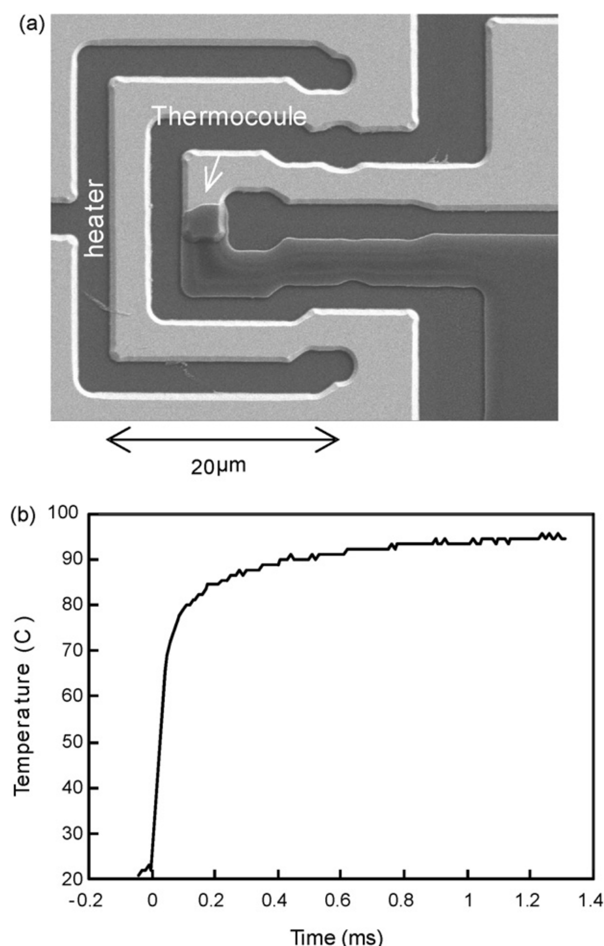

Fig. 1. Micro-thermocontrol-device of millisecond response time. (a) Scanning electron microscope image of the device. The micro heater was designed rectangle of 20 μm × 20 μm. The width of the heater is 2 μm. The thermosensor is a Pt–Cr thin film thermocouple (TFTC) with a hot junction area of 2 μm × 2 μm. (b) Step response of the micro-thermodevice. This device realized real-time temperature switching in about 1 ms under microscopic observation.

a micro-thermocouple integrated on a Si wafer covered with a 100 nm thick SiO_2 insulation layer. The platinum microheater was designed rectangle of 20 μm squared. The width of microheater was 2 μm. The thermosensor was a Pt–Cr thin film thermocouple (TFTC) with a hot junction area of 2 μm × 2 μm. The temperature calibration of the thermocouple can be carried out by either immersing the device into a hot water [18] or using 8CB liquid crystal that shows a distinctive phase change at 313 K [24]. This micro-thermodevice has realized real-time temperature switching under the microscope in 1 ms (Fig. 1(b)). Thermal characteristics and temperature distribution of a heater in the micrometer scale were investigated by numerical simulations previously [24,25]. We firstly used our micro-thermodevice of this design coupled with microcontainers to reveal the millisecond dynamics of fluorescent proteins denaturation [26].

In this report, this micro-thermodevice was used to measure the response time of fluorescent quenchings of dsDNAs/SG and free SG on millisecond time scale. Consequently, “unzipping of double strands” and “denaturation at base pair level” were clearly distinguished by measuring their response time. Thus, hereinafter, we use a term “denaturation” as denaturation at base pair level to distinguish from mechanical unzipping of dsDNA. Response time of unzipping was measured by commercial thermocycler on a time scale of minutes and denaturation was measured by micro-thermodevice on millisecond time scale.

2. Materials and methods

2.1. Double stranded DNA

λ-Phage DNA of 16 μm length (3010: TAKARA) was chosen for denaturation experiments. A relatively long dsDNA may take longer time for unzipping into ssDNAs compared to a shorter dsDNA because 2 strands require a certain time to be mechanically unzipped by thermal energy whereas melting of base pairs does not depend on the length of DNA. The melting temperature of this λDNA at which half of the base pairs break is 66 °C calculated from an established formula [27]. However, to unzip dsDNA completely and irreversibly, temperature higher than 90 °C is required [5].

2.2. Unzipping measurements on long time scale

To measure the response time for dsDNA to unzip completely and irreversibly, dsDNA/SG were exposed at high temperatures for various time spans. SYBR Green (SYBR Green 1 Nucleic Acid Gel Stain: Molecular Probes) diluted 10,000 times was mixed with a 4.6 pM solution of λDNA—a concentration low enough to prevent unzipped ssDNAs from reforming dsDNA—and sustained in TE buffer (Tris + EDTA, pH 7.4). The buffer containing dsDNA/SG was aliquot (5 μL in each tube) and was exposed at high temperatures on a thermocycler (Chill Heat CHT-102HT: Iwaki); 10 min at various temperatures and various time spans at 91 °C. Temperatures of the samples were measured by a commercial thermocouple immersed in the solution. Each tube was mounted on ice immediately after the heating to prevent from renaturation. The solution was poured onto a glass plate (36 mm × 24 mm: Matsunami) and a cover glass (18 mm × 18 mm: Matsunami) was mounted without any spacer; this created a flow cell of less than 5 μm in height to facilitate counting numbers of DNAs under the microscope. The sample was exposed by a mercury lamp through filters (WIG, ND6). Fluorescent quenching effect by the exposure was limited to 0.4% in 1 s which is negligible in our experiment. Images were captured by CCD camera (Cascade 2: Photometrix). Number of non-denatured dsDNA per microscopic view was counted manually in 12–16 buffer droplets in each condition.

2.3. Denaturation measurements on millisecond time scale

To observe quenching dynamics of dsDNA/SG, we mounted the micro-thermodevice on a fluorescent microscope (Olympus, BX51). dsDNAs dyed by SG were maintained in a TE buffer at the same condition as in the unzipping experiments. The solution was poured onto the micro-thermodevice and a cover glass (Matsunami 2 mm × 2 mm) was mounted onto the solution without any spacers. Images were captured by a high speed CCD camera (Cascade 2: Photometrix) with a frame rate of 10.5 ms per frame. The time courses of fluorescent intensities of each dsDNA were analyzed with commercial software (Metamorf). Analysis at single DNA molecule level allows us to measure the dynamics on short time scales. The response time of SG dye free in solution was measured by analyzing the intensity of fluorescent solution where no DNA exists.

To measure the delay of the optical systems (i.e. the microscope and the camera), we measured the step response by switching an LED on and off under the microscope with the same camera condition as in the experiment but with faster frame rate. The time response was measured to be less than 5 ms. We concluded that all the experimental data obtained at frame rate larger than this value were not affected by the delay of the optical setup.

3. Results and discussions

Fig. 2(a) shows the number of dsDNA per microscopic view after 10 min exposure at various temperatures. The number of

Download English Version:

https://daneshyari.com/en/article/10559660

Download Persian Version:

https://daneshyari.com/article/10559660

Daneshyari.com