LABEL-FREE DETECTION OF REAL-TIME DNA AMPLIFICATION USING NANOWALL ARRAY STRUCTURES

Kensuke Ogawa[1,2], Takao Yasui[1,2], Noritada Kaji[1,2], Yukihiro Okamoto[2], Manabu Tokeshi[2,3], Yasuhiro Horiike[4], Mats Nilsson[5] and Yoshinobu Baba[1,2,6]

[1]Department of Applied Chemistry, Nagoya University, Japan,
[2]FIRST Research Center for Innovative Nanobiodevices, Nagoya University, Japan,
[3]Division of Biotechnology and Macromolecular Chemistry, Hokkaido University, Japan
[4]National Institute for Materials Science, Japan,
[5]Department of Immunology, Genetics and Pathology, Uppsala University, Sweden,
[6]Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan

ABSTRACT

We performed label-free detection of real-time DNA amplification inside nanowall array structures, which have never realized in other techniques. The label-free and real-time detection of linear amplification of DNA molecules by circle-to-circle amplification (C2CA) was achieved because our label-free detection system could recognize the length of DNA molecules.

KEYWORDS

Label-free detection, Nanostructures, Biomolecules, Diffracted light, DNA amplification.

INTRODUCTION

Currently, diverse label-free detection techniques have been developed. These methods can achieve high sensitivity under a condition of surface immobilization of specific molecules to capture target ones; however surface immobilization of specific molecules before use is a major procedure and it is hard to combine the amplification and the time-course detection parts in parallel. To overcome these disadvantages, previously, we developed a new method for label-free detection of biomolecules by using the nanowall array chips, which based on the detection of diffracted light by nanostructures [1]. In this work, we demonstrated label-free detection of real-time DNA amplification.

EXPERIMENT

Our detection system is based on the diffraction of the light. Briefly, the experimental setup mainly consists of a laser, a ND filter, a light chopper, an objective lens, a nanowall array chip, a photodiode, and a lock-in amplifier, as shown in Figure 1(a). We consider nanowall array structures as the diffractive grating and utilize them to diffract 532 nm laser beam. Figure 1(b) shows the image of the detection site. As the samples amplified inside the point of focused laser, the intensity of the diffracted laser beam is slightly changed, and therefore, we can attain the label-free detection of amplified DNA molecule.

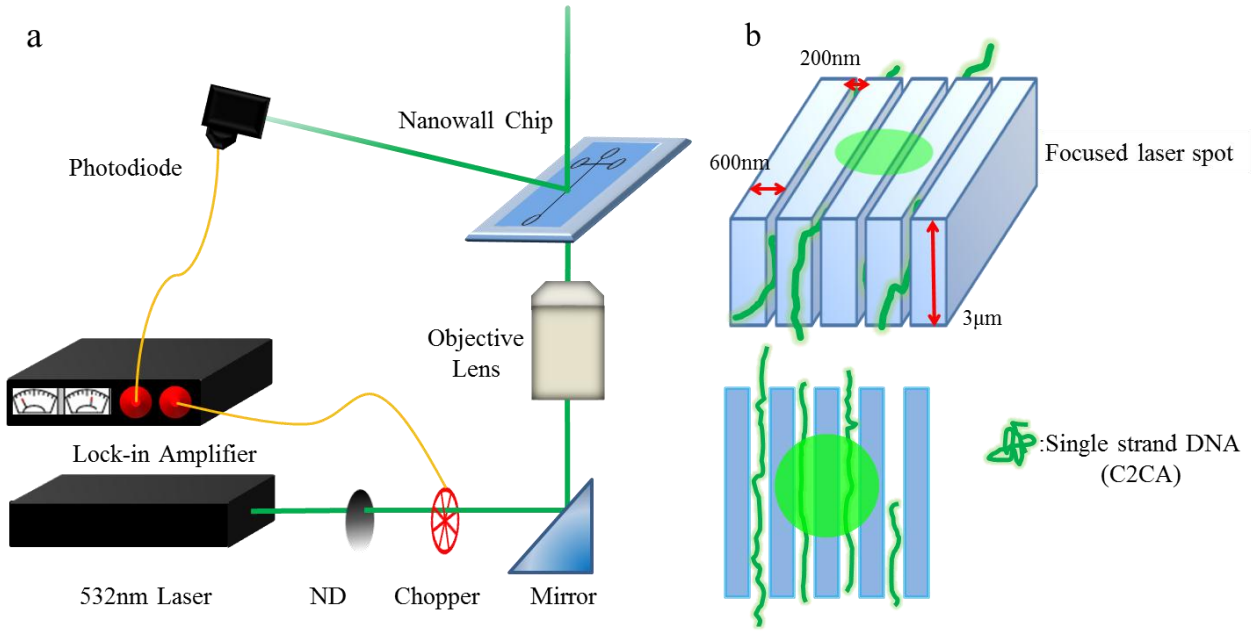

Figure 1. (a) Schematic of the system for label-free detection of real-time circle-to-circle amplification (C2CA). It consists of a 532 nm laser, a ND filter, a chopper, a nanowall array chip, a photodiode, and a lock-in amplifier. (b) The label-free signal is derived as DNA molecules amplified inside the focused laser spot.

In this experiment, DNA amplification method was based on C2CA method [2]. DNA circles were not formed in a target-specific padlock probe ligation reaction. DNA circles were prepared by ligation of padlock probes. We used the DNA sequences of target gene sequences for tubercle bacilli (TS), padlock probes (PP), capture oligonucleotide (CO), and replication oligonucleotide (RO). The padlock probes were phosphorylated before use in the rolling-circle amplification.

In Figure 2, we showed the C2CA mechanism; firstly, rolling-circle amplification (RCA) of a circular DNA molecule; secondly, forming double-stranded substrates for restriction digestion after adding restriction oligonucleotides; thirdly, digested fragments was ligated each ends, leading to DNA circles; finally, the circular DNA molecule is amplified again (2nd RCA). We performed real-time detection of RCA products using two strand-specific molecular beacon (MB) probes.

On the other hand, DNA amplification was detected using new label-free detection system. First, we prepared the samples using C2CA from 1st to 3rd step. And then, we introduced them by capillary force and carried out DNA amplification (2nd RCA) at room temperature. To get the effective signal change, we set the photodiode to get the local maximum signal. A light chopper modulated the incident laser with a modulation frequency at 1013 Hz. The time constant of the lock-in amplifier was set to 30 seconds and data was measured at intervals of 30 seconds.

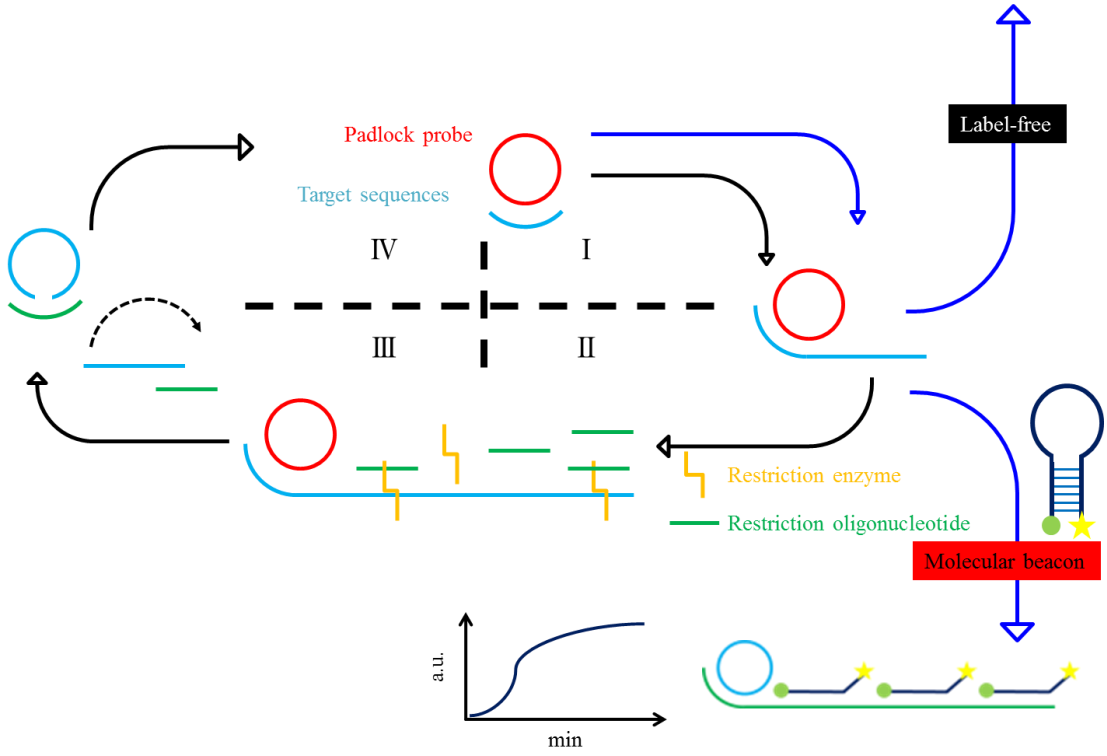

Figure 2. C2CA consisted of four steps; 1st step is rolling-circle amplification of the DNA circles (1st RCA); 2nd step is digestion of amplified DNA molecules; 3rd step is ligation of digested molecules; 4th step is rolling-circle amplification (2nd RCA).

RESULTS AND DISCUSSION

To elucidate a relationship between label-free signal change and photonic band gap, we demonstrated angular dependence of transmittance at 532nm which was laser wavelength. We could obtain the peak top of around 40° (Figure 3(a)). This was good agreement with the diffraction angle obtained by calculation. So we were measured wavelength dependence of transmittance at 40°, and investigated the relationship between signal change and the photonic band gap. We could not observe the photonic band gap peak at 532nm as shown in Figure 3(b). In our experimental setup, the photonic band gap did not affect on any signal changes.

Next, we could confirm that real-time DNA amplification by fluorescent signals in bulk using MB probes (Figure 4(a)). Since we could observe fluorescence intensity derived from DNA amplification by C2CA, we performed DNA amplification inside the nanowall array structures. Label-free detection of real-time DNA amplification was achieved as shown in Figure 4(b). Obviously, normalized intensity in positive samples (1 fmol) could make a difference from that in negative samples. The normalized intensity in the negative samples would be due to absorption of the C2CA reaction mixtures on the nanowall structures.

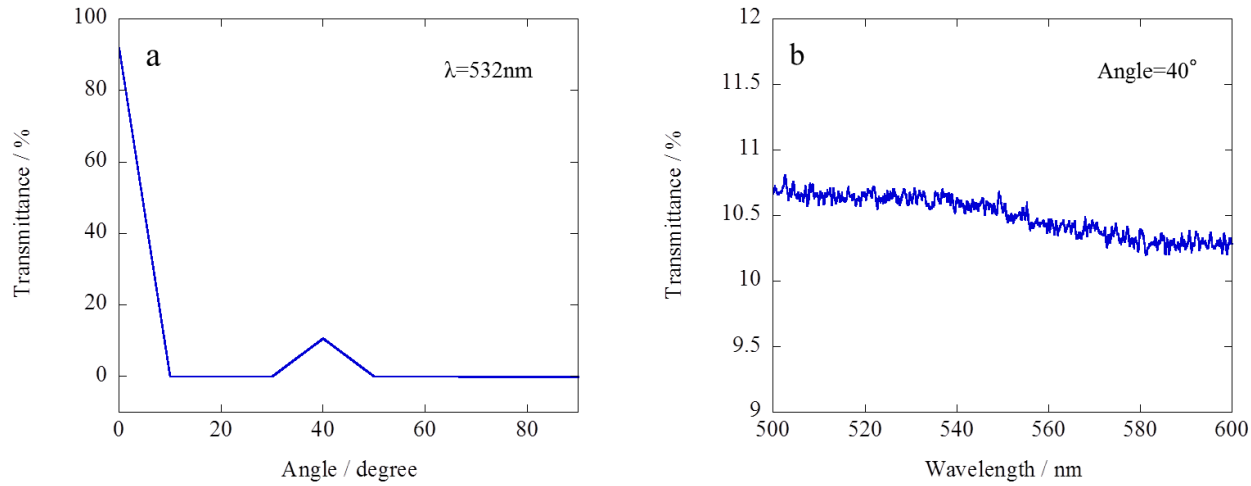

Figure 3. (a) Angular dependence on transmittance at 532nm. (b) Wavelength dependence on transmittance at 40 °

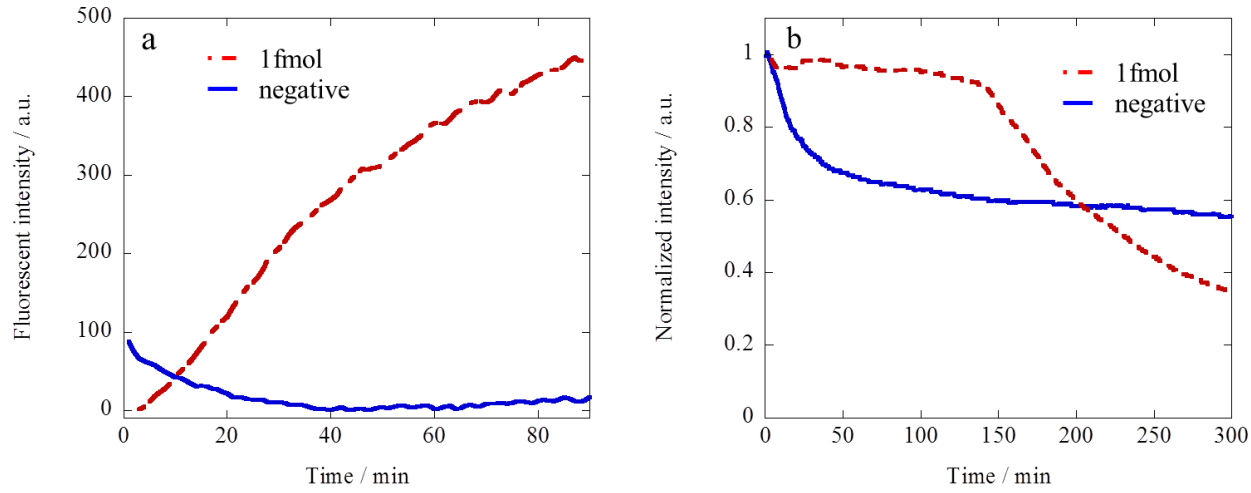

Figure 4. (a) Real-time analysis of DNA amplification using molecular beacons. As the DNA molecules amplified, the number of combined molecular beacons increased, resulting in higher fluorescence intensities. (b) Label-free detection of real-time DNA amplification.

CONCLUSIONS

We developed label-free detection of real-time DNA amplification by using our new method. In this experiment, we were carried out DNA amplification at room temperature and achieved label-free detection of C2CA products. It would be possible to carry out single DNA amplification detection in a single chip with an optimization of the optical system.

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CONTACT

Kensuke Ogawa, tel: +81-52-789-3560; ogawa.kensuke@c.mbox.nagoya-u.ac.jp