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Pulsed-Laser Induced Fragmentation and Dissociation of DNA Immobilized on Gold Nanoparticles

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Plasmid DNA was immobilized on positively-charged gold nanoparticles (GNPs). Pulsed-laser irradiation induced fragmentation and dissociation of DNA from the GNP-DNA complexes. Dissociation of DNA without degradation was achieved when 80-mJ/pulse of laser light irradiated the GNP-DNA complexes.

Keywords: controlled release; gold nanorod; plasmid DNA

INTRODUCTION

Noble metal nanoparticles have been widely studied in recent years because of their novel photophysical and photochemical behaviors [1]. Since the gold nanoparticles (GNPs) show distinctive surface

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(SP) bands in the visible region (~520 nm), they are of particular interest for use in photoelectrochemical devices [2,3], photocatalysis [4], biological sensors [5,6], and so on. In general, dispersion of colloidal nanoparticles in a solution owes to repulsive interactions between the nanoparticle. A protective layer on the nanoparticles is usually provided the repulsive interaction. The protective layer also provides functionality to the GNPs in addition to preventing their aggregation. It has been known that self-assembled monolayers of organic compounds containing thio- or amino-functional groups provide functional protective layers [5–8]. For example, 3′- or 5′-(alkanethiol)-capped oligonucleotides could be used as a protective layer of GNPs [5,6]. It has been reported that hybidization of nucleotides triggers a drastic change in the color of colloidal gold solution [5,6].

Pulsed-laser irradiation of GNPs can also trigger photochemical reactions of GNPs [9–11]. A typical reaction induced by pulsed-laser irradiation is the fragmentation of GNPs into smaller particles [9,10]; on the contrary, Kamat and coworkers [11] and we [12] reported pulsed-laser-induced fusion of aggregated gold particles capped with organic molecules. Those results indicate that the characteristics of the protective layer affect the photoreactions of GNPs induced by pulsed-laser irradiation.

Recently, we prepared cationic GNPs that could form complexes with DNA [13]. In this paper, we have found photodissociation and photofragmentation of DNA from the GNP-DNA-complex, and especially strict adjustment of laser power has enabled exclusive photodissociation.

EXPERIMENTAL

Cationic GNPs were prepared according to the method reported in a previous article [14]. The method is based on $NaBH_4$ reduction of $HAuCl_4$ in the presence of 2-aminoethanthiol hydrochloride (AET). AET (180 μL, 213 mM) and polyethylenegrycolorthopyridyldisulfide (1 mL, 20 mM, PEG-OPSS, MW: 5000, Shearwater Polymers Inc.) were added to a $HAuCl_4$ aqueous solution (20 mL, 1.42 mM) were added in a vessel. Then, addition of 500 μL $NaBH_4$ solution (26.4 mM) to the above solution resulted in the formation of GNPs. The PEG-OPSS was effective for suppressing the aggregation. Dynamic light scattering measurements (Otsuka Electronics ELS-8000, He-Ne laser) indicated that the mean size of GNPs was 44 ± 5 nm. The zeta potential of the GNPs was $+22 \pm 3$ mV. Plasmid DNA (7 kbp) was prepared as described previously [15]. To a DNA solution (25 ng/μL), the GNP solution (10 μL) and 4 μL of TBE × 5 buffer

solutions were added. For the pulsed-laser irradiation, fundamental light from a Q-switched Nd:YAG Laser (1064 nm, 280 or 80 mJ/pulse, ~10 ns, beam diameter: ~6 mm, Continuum Surelite I) was used.

RESULTS AND DISCUSSION

Formation and photoreaction of GNP-DNA complexes were examined by measuring electrophoretic mobilities on an agarose gel. The visible image (A) and the fluorescence image (B) of the agarose gel after electrophoresis are shown in Figure 1. Lanes 1 and 2 are controls without laser irradiation: lane 1 is DNA in the absence of GNPs, and lane 2 is GNPs in the absence of DNA. In lanes 2, a faint smear of GNPs is observed at the position indicated by an arrowhead in Figure 1(A). This indicates that negatively charged GNPs are formed in the buffered solution. It is plausible that the anionic GNPs are formed by the adsorption of boric acid and/or EDTA onto the GNPs, where these anionic species are contained in the TBE buffer.

As shown in Figure 1(A), the GNPs mixed with DNA also migrate to the positive direction, irrespective of laser irradiation (lanes 4–10). The fluorescence image (Fig. 1(B)) shows that the DNA mixed with GNPs does not migrate to the position of the original DNA. It is noteworthy that most of DNA locates at the same position as that of the GNPs. This indicates that DNA is adsorbed on the GNPs, that

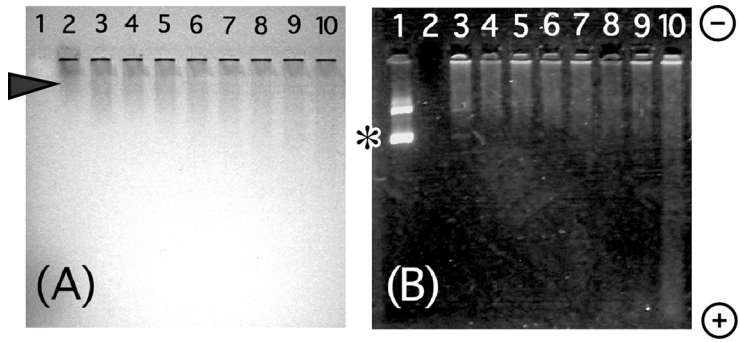

FIGURE 1 Visible image (A) and fluorescence image (B, stained with Cyber Green I) of the agarose gel (1% w/v) after electrophoresis. Lane 1 and 2 are controls without laser irradiation; lane 1 is DNA in the absence of GNPs, lane 2 is free GNPs in the absence of DNA. Lane 3 is GNP-DNA complex without laser irradiation. Lanes 4–10 are GNP-DNA complexes irradiated for 5, 10, 20, 25, 30, and 40 s, respectively. The arrow head on the left side of gel (B) indicates GNPs move to the positive direction. The asterisk of gel (B) indicates the position of the original plasmid DNA. Laser irradiation: 280 mJ/pulse.

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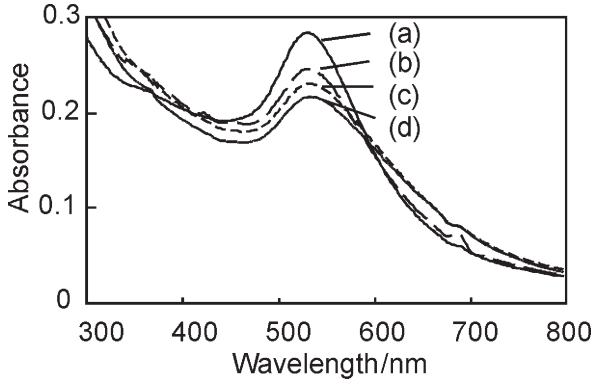

FIGURE 2 Absorption spectra of GNP-DNA complexes before (a) and after (b – d) laser irradiation. Irradiation times: (b) 20, (c) 30, (d) 40 s.

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is, DNA-GNP complexes are formed in the solution. Laser irradiation of up to 30 s induces no remarkable changes in the electrophoretic patterns (Fig. 1(B), lanes 3–9). Thus, the DNA is still adsorbed on the GNPs up to 30 s of irradiation. In contrast, after 40 s of laser irradiation, the solution contains a large amount of DNA that migrates faster than the original plasmid DNA (Fig. 1(B), lane 10). This indicates that fragmentation of DNA is initiated after 30 s of laser irradiation and the fragmented DNA is then dissociated from GNPs.

Absorption spectra of GNP-DNA complexes before (a) and after (b–d) laser irradiation are shown in Figure 2. The GNP-DNA complex shows the clear SP band at around 520 nm (a). This implies that the GNPs are covered with DNA without the formation of aggregates under our experimental conditions. Thirty (c) and 40 (d) seconds of laser irradiation broadens the SP band in the longer wavelength region (>600 nm). This spectral change suggests that the formation of GNP aggregates [12]. Then, laser-induced melting (fusion) and subsequent fragmentation (ablation) of the aggregates occurs by the intense laser light (280 mJ/pulse), which concomitantly induce fragmentation of the counterpart, DNA, of the GNP-DNA complexes.

Visible (A) and fluorescence (B) images of electrophoresis patterns of GNP-DNA complexes irradiated by a lower energy of laser light (80 mJ/pulse) are shown in Figure 3. The visible image (Fig. 3(A)) indicates that the GNPs are not fragmented, even after 6 min of laser irradiation (lane 8). Faint bands of original DNA can be seen in lane 2 of the fluorescence image (Fig. 3(B)), indicating that free DNA is

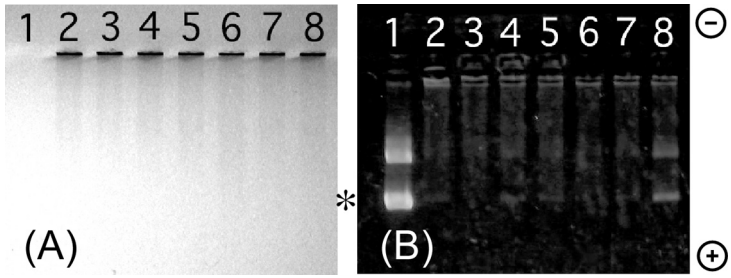

FIGURE 3 Visible image (A) and fluorescence image (B, stained with Cyber Green I) of the agarose gel (1% w/v) after electrophoresis. Lanes 1 and 2 are controls without laser irradiation; lane 1 is DNA in the absence of GNPs, lane 2 is free GNPs in the absence of DNA. Lane 3 is GNP-DNA complex without laser irradiation. Lanes 4–8 are GNP-DNA complex irradiated for 1, 2, 3, 4, 5, and 6 min, respectively. The asterisk on the left of gel (B) indicates the position of the original plasmid DNA.

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present in the solution prior to the laser irradiation. Laser irradiation induced dissociation of DNA from GNP-DNA complexes, which is confirmed by the increase in intensity of the original DNA bands (position of the asterisk in the figure) with increasing laser irradiation times. After 6 min of laser irradiation, distinctive DNA bands can be observed (lane 8). Because the bands can be observed at the same positions with those of the original DNA, we could confirm that the DNA was released from the GNP-DNA complexes by 80 mJ/pulse of laser light without appreciable degradation. Absorption spectra of the laser-irradiated GNP-DNA solution under present irradiation condition, 80 mJ/pulse of laser light induces no remarkable changes of GNPs themselves. These results show that moderate irradiation, which does not induce drastic photoreactions such as fragmentation, is favorable for the release of DNA from the GNP-DNA without degradation.

CONCLUSION

We have found pulsed-laser induced fragmentation and dissociation of DNA from the GNP-DNA complexes. Strict adjustment of laser power could realize a new type of photoreactions of GNPs that were release of DNA from the GNP-DNA complexes without appreciable degradation. Now, we are trying to examine the variation of biomaterials to be released from GNP complexes by the pulsed-laser induced photoreactions.

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