Controlled release of plasmid DNA from gold nanorods induced by pulsed near-infrared light†

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Pulsed near-infrared laser irradiation induced release of plasmid DNA immobilized on gold nanorods without structural degradation, by selective excitation of longitudinal plasmon oscillation.

Gold nanoparticles (NPs) show strong surface plasmon (SP) bands in the visible region. Thus, interaction of NPs with visible light opens up various photophysical and photochemical applications of NPs.[1,2] Surface modifications of NPs have also been carried out for the use of photochemical applications. For example, irradiation of visible laser light onto aggregates of NPs induced dissociation of immobilized rhodamine dyes from the aggregates;[3] dissociation of the capping molecules was accompanied by the fusion of the NPs.

These photoinduced dissociations of functional molecules from the complexes can constitute a core technique for active drug- or gene-delivery systems.[4,5] In using such light-triggered systems, it is essential to minimize the damage of bio-functional materials, such as proteins, DNA and antibodies. From this viewpoint, the use of near-infrared (near-IR) light is quite attractive because most of those bio-functional materials and living organisms are transparent to near-IR light. Irradiation of near-IR light onto gold nanoshell-composites for tens of minutes achieved release of proteins from the composites.[5] However, the SP bands of gold nanostructures are usually larger in the visible region. In order to make the most of the SP band excitation with the near-IR light, a smart choice of a gold nanostructure is fundamentally important.

Gold nanorods (NRs) are rod-shaped nanoparticles having distinctive optical properties that depend on their shape.[6,7] Especially, they show two absorption bands that are assignable to transverse and longitudinal modes of SP oscillations; the former is located in the visible region at around 520 nm, while the latter is located in the near-IR region and has a substantially larger extinction coefficient compared with that of the former.[7] This unique optical property of NRs may open up fascinating applications of NRs. Early studies showed that irradiation of pulsed near-IR laser light effectively induced the reshaping of NRs into spherical nanoparticles.[8,9] In this paper, we report a novel approach to release plasmid DNA immobilized on NRs, triggered by the reshaping of NRs resulting from selective excitation of the longitudinal SP band by pulsed near-IR laser light.

Several approaches for the preparation of NRs have been carried out, such as electrochemical,[6] photochemical,[10] and seeding methods,[11] and a combined method using chemical reduction and photoirradiation.[12] In all methods, NRs are prepared in the presence of a large amount of hexadecyltrimethylammonium bromide (CTAB), which is essential for the generation and stabilization (dispersibility) of NRs in solution. However, a large amount of CTAB in the NR solution induces unwanted irreversible compaction of DNA.[13] In addition, CTAB is biologically hazardous. Nevertheless, removal of CTAB from the NR solution resulted in irreversible aggregation of NRs. Thus, some capping agents are essential to avoid aggregation of NRs. From these viewpoints, phosphatidylcholine (PC) modified NRs (PC-NRs) were employed in this work.

NRs, prepared according to a slight modification of our previous method,[12] were supplied from Mitsubishi Materials Co. Ltd. The initial NR solution [$\sim$1 mM (Au atoms)] contained 80 mM of CTAB. Some of the CTAB precipitated when the solution was kept in a refrigerator ($\sim$4 °C). The precipitated CTAB was removed using a membrane filter (pore size, 0.8 μm). Then, a chloroform solution of PC (from egg yolk, Nacalai Tesque, 10 mg mL^{-1}, 10 mL) was added to the above filtrate containing NRs (20 mL), in order to extract the CTAB into the chloroform phase and to replace some of the CTAB molecules adsorbed on the surface of the NRs with PC molecules. After repeating two more extraction procedures, the aqueous solution containing NRs was centrifuged to remove residual CTAB. Finally, the NR precipitates were dispersed again in an appropriate volume of pure water. The zeta potential of NRs was evaluated by ELS-8000 (Otsuka Electronics, He-Ne laser). The zeta potential of the resultant NRs was 15 $\pm$ 1 mV. This value originates from the CTAB molecules still adsorbed on the surface of the NRs, being substantially smaller than that of the initial NRs (67 $\pm$ 1 mV). This clearly indicates that some of the adsorbed cationic CTAB molecules are replaced with neutral PC molecules. Accordingly, we have obtained the NRs capped with CTAB and PC (PC-NRs) and used them for the present study. Plasmid DNA[14] (7 kbp, 0.025 mg mL^{-1}, 2 μL) was mixed with PC-NR solutions of various concentrations [0.5–1.5 mM (Au atoms), 8 μL]. After the addition of 4 μL of 5 × TBE buffer and 6 μL of pure water, the mixed solutions were irradiated by the fundamental light of a Q-switched Nd:YAG laser (Continuum Surelite I, 1064 nm, pulse duration: 5–7 ns, repetition rate: 10 Hz, beam diameter: $\sim$6 mm). The formation of the complexes of PC-NRs with DNA (PC-NR–DNA) was confirmed by electrophoretic mobility in an agarose gel. The photoinduced morphological changes of the PC-NR–DNA complexes were evaluated by absorption spectroscopy and transmission electron microscopic (TEM) observations.

† Electronic supplementary information (ESI) available: agarose gel electrophoresis of PC-NR–DNA complexes before and after 15 seconds of laser irradiation. See http://www.rsc.org/suppdata/cc/b5/b500337g/

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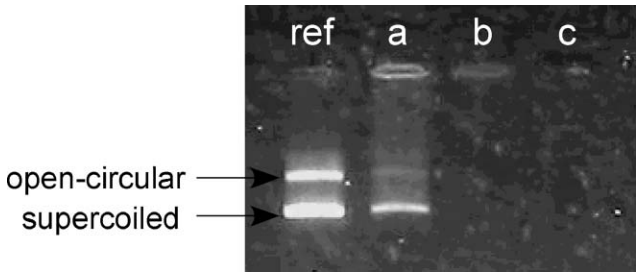

Fig. 1 Agarose gel (1% w/v) electrophoresis of plasmid DNA (0.05 μg) in the absence (ref) and the presence of PC-NRs (a–c). Concentrations of PC-NR were (a) 0.5, (b) 1, and (c) 1.5 mM (Au atoms). The plasmid DNA was stained with SYBR® Green I (Molecular Probes).

In Fig. 1, the electrophoretic patterns of the plasmid DNA (ref) and the mixed solutions of PC-NRs and plasmid DNA (a–c) are shown. Migrated DNA decreased with increasing concentration of the PC-NR solution. When the 0.5 mM PC-NR solution is added to the DNA solution (a), a relatively small amount of the supercoiled DNA is electrophoresed, and the open-circular DNA is scarcely migrated. Fluorescence around the well of lane (a) indicates that some of the plasmid DNA remains in the well even after electrophoresis. Additions of 1 (b) and 1.5 mM (c) PC-NR solutions completely suppressed the migration of the plasmid DNA, and decreased the fluorescence around the wells. This fluorescence decrease probably originates from compaction of the DNA that is in contact with the PC-NRs through electrostatic interactions.

Fig. 2 shows absorption spectra of the PC-NR solutions in the absence (a) and presence (b–e) of plasmid DNA. The PC-NR solution shows an intense absorption peak around 900 nm (a), while the PC-NR–DNA solution shows very broad SP band (b). This spectral change indicates the formation of small aggregates of the PC-NRs,[15] as can be supported by Fig. 1. Two minutes of pulsed-laser irradiation onto the mixed solutions remarkably reduced the SP bands in the near-IR region, and induced considerable blue-shifts of the SP bands (c–e). Upon 50 (c) and 100 mJ/pulse (d) of laser irradiation, the longitudinal SP band around 900 nm remarkably decreased, and a shoulder peak around 700 nm appeared. Upon 150 mJ/pulse of laser irradiation (e), the SP band in near-IR region completely disappeared, and the absorption spectrum was roughly the same as that of spherical gold nanoparticles. These spectral changes are assignable to the laser-induced reshaping of NRs into spherical particles.[8,9]

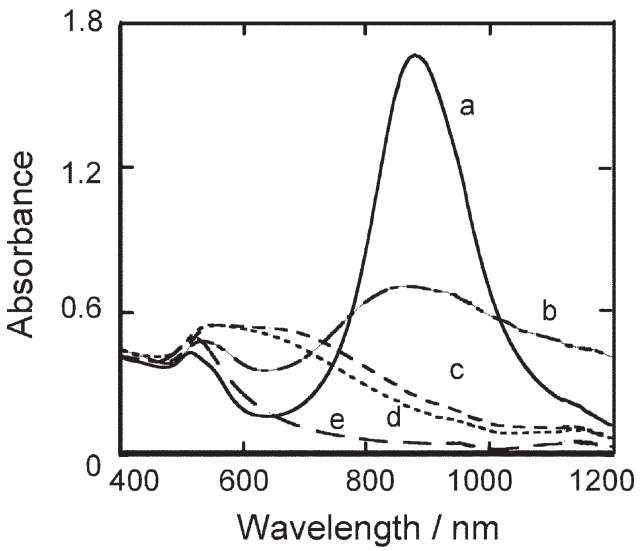

Fig. 2 Absorption spectra of the PC-NR solution (a), and the PC-NR–DNA solutions before (b) and after (c–e) laser irradiation (1064 nm, 10 Hz, 2 min). Laser intensities: (c) 50, (d) 100 and (e) 150 mJ/pulse. Plasmid DNA (0.025 mg mL^{-1}, 4 μL) was mixed with the PC-NR solution [1 mM (Au atoms), 16 μL], and 70 μL of pure water was added to the PC-NR–DNA solution.

Fig. 3 shows TEM images of PC-NRs before laser irradiation (a) and PC-NR–DNA complexes after laser irradiation (150 mJ/pulse, 2 min) (b). A rough estimation of the mean volume of NRs is 6.2×10^3 nm^3 (average length: 65 ± 5 nm, average width: 11 ± 1 nm, aspect ratio: 5.9). As can be recognized from the TEM image after laser irradiation (b), NRs were no longer present, but changed into spherical nanoparticles. The mean volume of the spherical particles was evaluated to be 11.5×10^3 nm^3. This indicates that some PC-NRs are fused into spherical particles by the laser irradiation. From these results, the spectral changes in Fig. 2 are assignable to the morphological changes of PC-NRs into larger spherical nanoparticles. The aggregation of PC-NRs by the addition of plasmid DNA must facilitate the laser-induced fusion of NRs.[2,3]

Gel electrophoretic patterns of PC-NR–DNA complexes before (a) and after (b–g) 2 min of the laser irradiation are shown in Fig. 4. Before laser irradiation (a), all plasmid DNA remains in the well because of the formation of PC-NR–DNA complexes. After 100–140 mJ/pulse of laser irradiation (b–d), no fluorescent bands of migrated DNA can be seen in the gel. When 160, 180, and 200 mJ/pulse of laser light are irradiated onto the PC-NR–DNA complexes respectively (lanes e–g), the fluorescent bands can be seen at the same position as that of the supercoiled DNA [lane of (ref)]. This indicates that the plasmid DNA is certainly released from the PC-NR–DNA complexes by the laser irradiation. Moreover, supercoiled DNA is dominantly released from the PC-NR–DNA complexes. When a higher energy of pulsed-laser light than the 200 mJ/pulse was irradiated onto the PC-NR–DNA complexes, migration of the supercoiled DNA could be observed after 15 seconds of laser irradiation (see ESI†). Thus, it is most likely that the release of plasmid DNA can be controlled by severely controlling the laser irradiation conditions (pulse energy and irradiation time).

The pulsed laser-induced morphological changes of the PC-NRs (reshaping and fusion) decrease the surface area of PC-NRs

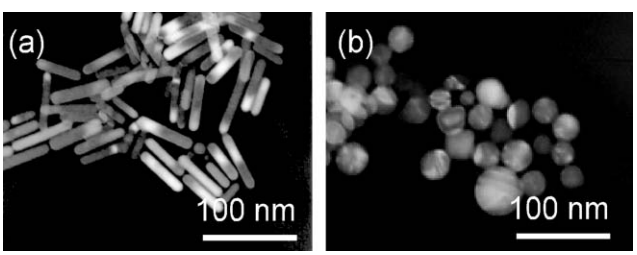

Fig. 3 TEM images of the PC-NRs before laser irradiation (a) and the PC-NR–DNA complexes after laser irradiation of 1064 nm light (150 mJ/pulse, 2 minutes) (b).

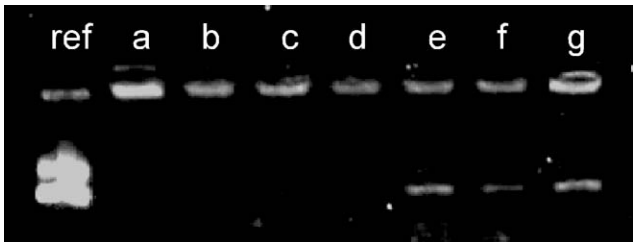

Fig. 4 Agarose gel electrophoresis of plasmid DNA (ref), and PC-NR–DNA complexes before (a) and after (b–g) laser irradiation. Plasmid DNA was mixed with 1 mM PC-NR solutions (8 μL) ("ref" is 0.05 μg of plasmid DNA without PC-NRs). Laser intensities: (a) 0, (b) 100, (c) 120, (d) 140, (e) 160, (f) 180 and (g) 200 mJ/pulse. PC-NR–DNA complexes were electrophoresed in the agarose gel (1% w/v) and were stained with SYBR® Green I.

available for binding plasmid DNA. Thus, it is clear that the morphological changes of PC-NRs trigger the release of DNA from PC-NR–DNA complexes. From Fig. 4, plasmid DNA seems to be released without any damage by laser irradiation, because it migrates to the same position of the original plasmid DNA. Thus, the near-IR laser irradiation onto the complexes has realized the selective release of the plasmid DNA without appreciable structural changes. In this study, only the tens of seconds of the near-IR laser irradiation enabled the release of plasmid DNA. This rapid release must be advantageous to minimize unwanted photochemical damages.

In conclusion, we have found that morphological changes of NRs induced by 1064 nm-laser irradiation could trigger the release of plasmid DNA from PC-NR–DNA complexes. It is expected that the photoinduced reshaping of NRs into spherical nanoparticles will be a key reaction for realizing a photo-triggered gene delivery system.

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Notes and references

1 P. V. Kamat, *J. Phys. Chem. B*, 2002, **106**, 7729.
2 H. Takahashi, Y. Niidome, T. Sato and S. Yamada, *Colloids Surf., A*, 2004, **247**, 105.
3 N. Chandrasekharan, P. V. Kamat, J. Hu and G. Jones, II, *J. Phys. Chem. B*, 2000, **104**, 11103.
4 N. K. Mai, M. Fujiwara and Y. Tanaka, *Nature*, 2003, **421**, 350.
5 S. R. Sershen, S. L. Westcott, N. J. Halas and J. L. West, *J. Biomed. Mater. Res.*, 2000, **51**, 293.
6 Y.-Y. Yu, S.-S. Chang, C.-L. Lee and C. R. C. Wang, *J. Phys. Chem. B*, 1997, **101**, 6661.
7 S. Link, M. B. Mohamed and M. A. El-Sayed, *J. Phys. Chem. B*, 1999, **103**, 3073.
8 S. Link, C. Burda, B. Nikoobakht and M. A. El-Sayed, *J. Phys. Chem. B*, 2000, **104**, 6152.
9 Y. Niidome, S. Urakawa, M. Kawahara and S. Yamada, *Jpn. J. Appl. Phys., Part 1*, 2003, **42**, 1749.
10 F. Kim, J. H. Song and P. Yang, *J. Am. Chem. Soc.*, 2002, **124**, 14316.
11 B. D. Busbee, S. O. Obare and C. J. Murphy, *Adv. Mater.*, 2003, **15**, 414.
12 Y. Niidome, K. Nishioka, H. Kawasaki and S. Yamada, *Chem. Commun.*, 2003, 2376.
13 S. M. Mel'nikov, V. G. Sergeyev and K. Yoshikawa, *J. Am. Chem. Soc.*, 1995, **117**, 2401.
14 M. Ohsaki, T. Okuda, A. Wada, T. Hirayama, T. Niidome and H. Aoyagi, *Bioconjugate Chem.*, 2002, **13**, 510.
15 B. Nikoobakht and M. A. El-Sayed, *J. Phys. Chem. A*, 2003, **107**, 3372.