Photoinduced Release of Oligonucleotide-conjugated Silica-coated Gold Nanorods Accompanied by Moderate Morphological Changes

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Near-infrared pulsed-laser irradiation of silica-coated gold nanorods loaded with single-stranded oligonucleotides induced the release of the oligonucleotides without changing the morphology of the gold nanorods into spherical particles. Silica layers on the gold nanorods were advantageous to realize the efficient release of the oligonucleotides.

The controlled release of functional materials from molecular assemblies has been a hot research target in the design of sophisticated drug carriers.[1–4] Photoirradiation is a potential way to control the release of drugs from molecular assemblies. Various kinds of photosensitizers have been used to trigger structural or chemical changes of these carriers.[5,6] Gold nanoparticles are a possible candidate for the photosensitizer, because they present distinctive surface plasmon (SP) bands. The SP bands absorb photons and convert the photon energy into heat. This heat can then be used to trigger the release of various functional materials.[6,7] In addition, gold nanoparticles are highly advantageous in practical applications owing to their chemical stability and ready surface modification with thiol derivatives.[8] Previous works have indicated that gold nanoparticles have the potential to act as both a photosensitizer and a carrier for targeting tumors.[9]

Gold nanorods are rod-shaped nanoparticles that are prepared in a micellar solution of hexadecyltrimethylammonium bromide (CTAB).[10–12] Gold nanorods show two SP bands in the visible and near-infrared (near-IR) regions that are assignable to the transverse and longitudinal modes of SP oscillations, respectively.[11,12] Thus, near-IR irradiation of the gold nanorods can trigger various photoreactions resulting in the release of functional materials. Because near-IR light does not affect the majority of biomolecules, the gold nanorods are expected to be a photofunctional drug/gene carrier that is applicable in vivo.[13,14]

Previously, we reported the photoinduced release of plasmid DNA from conjugates of gold nanorods and plasmid DNA.[15,16] In these works, we prepared lipid-passivated gold nanorods that presented a positive zeta potential and then conjugated these with plasmid DNA through electrostatic interaction. The release efficiency of DNA from the conjugates was estimated to be 1.2%; this value corresponded to only one plasmid DNA which was released from about 700 gold nanorods.[16] In this work, silica layers were employed as passivating layers on the gold nanorods, and single-stranded oligonucleotides (20 bases) were chemically modified on the silica surface. The controlled release of the oligonucleotides by pulsed-laser irradiation was investigated.

Gold nanorods were obtained from a joint research project between Mitsubishi Materials Corp. and Dai-Nippon Toryo Co., Ltd. ($44 \pm 8 \times 10 \pm 1$ nm). The 5′-thiol-terminated oligonucleotide (20 bases: 5′-GGAGGGAAATCCCTTCAA-GG-3′) was obtained from Hokkaido System Science Co., Ltd. Thiol-terminated poly(ethylene glycol) (PEG-SH, MW: 2000) was obtained from NOF Corp. All other chemicals were commercially available and used without further purification.

Gold nanorods with silica shells (silica nanorods) were prepared as reported.[17] Conjugates of the silica nanorods and the oligonucleotides were prepared as below (see Supporting Information).[18] 3-Aminopropyltriethoxysilane in 2-propanol (10 μL, 10 wt %) was added to a silica-nanorod solution (10 mL, 1.52 mM (Au atoms)), and the combined solution was stirred overnight. The resultant solution formed precipitates of gold nanorods that had amine groups on their surfaces. The precipitates were washed twice with water, and 0.5 mL of phosphate buffered saline (PBS, pH 7.4) was added. A solution of succinimidyl[(*N*-maleimidopropionamido)dodecaethyleneglycol] ester solution (0.5 mL, 5 mg/mL, in PBS), which acted as "linker" molecules between the amine-modified gold nanorods and the thiol-terminated oligonucleotide, was then added to the nanorod solution. Incubation for 1 h with intermittent sonication gave precipitated gold nanorods. The supernatant was removed and the precipitates were dispersed in 0.4 mL of PBS, to which an oligonucleotide solution (0.1 mL, 1 mg/mL, in PBS) was added. After 1 h of incubation with intermittent sonication, a PEG-SH solution (0.5 mL, 0.5 mg/mL, in PBS) was added. After further 1 h of incubation, the precipitates were collected and washed twice with PBS. The final product was dispersed in 0.5 mL of PBS. Pulsed-laser light (≈10 ns, 870 nm, 16 mJ/pulse, 10 Hz, ≈3 mmϕ) from a Q-switched Cr-LiSAF laser (Indeco) was used to irradiate a nanorod solution (20 μL) in a plastic tube for 120 s. All experiments were performed at room temperature.

Figure 1 shows transmission electron microscopic (TEM) images of the silica-coated nanorods (A) and their oligonucleotide conjugates (B). These images indicated that the modification of the oligonucleotides decreased the thickness of the silica layers; the sonication probably induced fragmentation of the silica layers, because they were not sintered to form SiO_2 networks. Figure 2 shows the extinction spectra of the conjugates

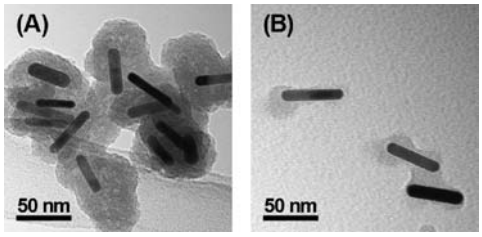

Figure 1. TEM images of silica nanorods (A) and of the conjugates of oligonucleotides and silica nanorods (B).

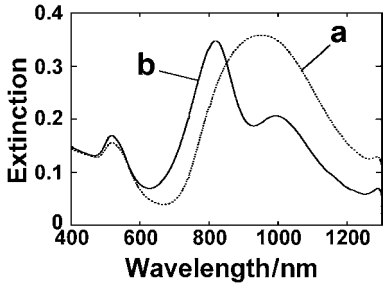

Figure 2. Extinction spectra of the conjugates before (a) and after (b) laser irradiation.

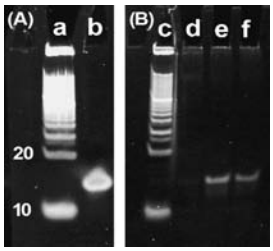

Figure 3. Gel electrophoresis images of single-stranded oligonucleotides (A) and their conjugates (B). (a), (c): DNA ladder markers (10–330 base pairs), (b): oligonucleotides, the conjugates before (d) and after (e), (f) laser irradiation.

and the silica nanorods before (a) and after (b) laser irradiation. The spectra indicate that the longitudinal SP bands in the near-IR regions were retained even after the laser irradiation, although the peak positions were shifted to the shorter wavelength regions. Thus, the laser irradiation did not transform the nanorods into spherical particles, but probably generated the ϕ-shape of the shorter gold nanorods.[11] A much smaller irradiation energy (16 mJ/pulse) than that used in the previous work (160 mJ/pulse)[16] contributed to the retention of the longitudinal SP band. Figure 3 shows poly(acrylamide) gel electrophoresis patterns of the oligonucleotides (A) and their conjugates before and after laser irradiation (B). Lanes (a) and (c) indicates patterns of DNA ladder markers (10–330 base pairs). The oligonucleotides present a band between the markers of 10 and 20 bases (lane (b)). The conjugates showed no band in lane (d); which indicated oligonucleotides, which were attached on the conjugates, were not dissociated from the conjugates during the electrophoresis. Lanes (e) and (f) show electrophoresis patterns of the laser-irradiated conjugates. It is clear that the oligonucleotides were released from the conjugates without changing their mobilities in the gel. The release efficiency was estimated by extinction of the laser-irradiated solution after centrifugation to precipitate the conjugates. The result indicated that 4.6 ng/μL of the oligonucleotides was released from 1.43 mM of Au atoms. That is, 20 oligonucleotides were released from a gold nanorod (400 bases/nanorod).

In the previous work,[15,16] plasmid DNA was attached onto gold nanorods through electrostatic interaction. Using intense laser light, it was possible to transform the gold nanorods into spherical particles, thereby affording an indispensable means to release the plasmid DNA from the gold nanorods. In addition, it was found that one plasmid DNA could be released from about 700 gold nanorods, which corresponded to 20 bases/nanorod (plasmid DNA consists of 7000 base pairs).[16] This number was much smaller than the number of bases released from a gold nanorod (400 bases/nanorod). Thus, it was shown that the silica layer is a preferable material to attach nucleotides to gold nanorods and to release them by near-IR laser irradiation without serious damage to the oligonucleotide. In this work, moderate laser irradiation, which did not transform the gold nanorods into spherical particles, was effective to release oligonucleotides from the nanorods. If the oligonucleotides were conjugated with gold nanorods through electrostatic interaction, the pulsed-laser irradiation would release the oligonucleotides (see Supporting Information).[18] It is probable that the degradable silica layer would contribute to the release processes. The heat jump given by the pulse-laser irradiation onto the gold nanorods would trigger the release of the oligonucleotides from the silica layers. It should be noted that the released oligonucleotides retained their structures, as shown in the gel electrophoresis patterns. Small SiO_2 clusters may be retained at the 5′-terminal of the released DNA, but molecular weights of the clusters must be much smaller than that of the oligonucleotides (MW: 6629).

At the present stage of this work, single-stranded oligonucleotides have been used; however, many kinds of biorelated materials can be fixed onto silica surfaces; that is, double-stranded DNA, polypeptides, and sugars will be applicable as functional molecules. This is the fist example describing a new strategy for preparing photoresponsive gene/drug carriers.

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18 Supporting Information is available electronically on the CSJ-journal Web site, http://www.csj.jp/journals/chem-lett/.

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