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Sequential Release of Single-stranded DNAs from Gold Nanorods Triggered by Near-infrared Light Irradiation

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Gold nanorods were modified with two types of double-stranded DNAs (dsDNA) with different melting temperatures. Irradiated by near-infrared laser light, the modified gold nanorods were heated, and single-stranded DNAs were released faster from the dsDNA with the lower melting temperature. Such combinations of different kinds of dsDNA allow us to construct a two-step release system of different types of oligonucleotides.

Functional oligonucleotides, e.g., small interfering RNA (siRNA) and antisense and decoy oligonucleotides are expected as drugs that control gene expression and protein function. Controlled release systems for such oligonucleotides are essential for therapeutic application. External stimuli and the microenvironment of the affected part can be used as triggers for the controlled release system. Ultrasound, radio waves, and light have been used as external stimuli.[1–5] The acidic environments in tumor tissue and inside of endocytotic vesicles in cells have attracted attention as triggers of controlled release.[6] Proteases, such as matrix metalloproteinases that are overexpressed in tumor tissue, can also act as a trigger.[7]

Gold nanorods are rod-shaped gold nanoparticles and have an absorption band in the near-infrared region[8] attributed to longitudinal surface plasmon resonance. Near-infrared light can deeply penetrate the human body because the near-infrared light region is located between strong absorption bands of water and hemoproteins. Gold nanorods can convert absorbed light energy to heat energy by the photothermal effect. Because of these unique properties, gold nanorods are expected to be developed as thermal converters for photothermal therapy.[9,10] Previously, we constructed a controlled release system of oligonucleotide from gold nanorods that responded to near-infrared laser irradiation.[11] In this system, we used double-stranded DNA (dsDNA)-modified gold nanorods. When the modified nanorods were illuminated with near-infrared laser light, they were heated, and single-stranded DNA (ssDNA) was released. In this study, to elucidate how the melting temperature of dsDNA affects the release efficiency of ssDNA, gold nanorods were modified with two kinds of dsDNAs with different melting temperatures. The release efficiencies of the ssDNAs from the gold surface were evaluated.

dsDNA-modified gold nanorods were prepared as described in our previous report.[11] Briefly, thiol-terminated poly(ethylene glycol) (m-PEG_{20000}-SH) was modified on the surface of gold nanorods via Au–S bonds. The PEG-modified gold nanorods were coated with a silica layer using the Stöber method[12,13] (silica-coated gold nanorods). To introduce primary and secondary amino groups to the surface, branched-poly(ethyleneimine) (PEI) was coated on the surface of the silica-coated gold nanorods (PEI-coated gold nanorods) via electrostatic interaction between the negative charge of the silica layer and the positive charge of PEI. Finally, two types of dsDNAs with different melting temperatures ($dsDNA_{Tm61}$ and $dsDNA_{Tm80}$, see Supporting Information[16]) were modified to the surface of the PEI-coated gold nanorods (dsDNA-modified gold nanorods) using a crosslinker (Mal–PEG_{12}–NHS, see Supporting Information). Each modification was confirmed by changes in the ζ potential of the gold nanorods (see Supporting Information). Figure 1 shows the absorption spectrum of the dsDNA-modified gold nanorods. After modification with $dsDNA_{Tm61}$, no change in the longitudinal surface plasmon bands in the near-infrared region was observed, indicating that the dsDNA-modified gold nanorods were well dispersed in the aqueous solution. Gold nanorods modified with $dsDNA_{Tm80}$ showed the same spectrum and ζ potential as those modified with $dsDNA_{Tm61}$ (data not shown).

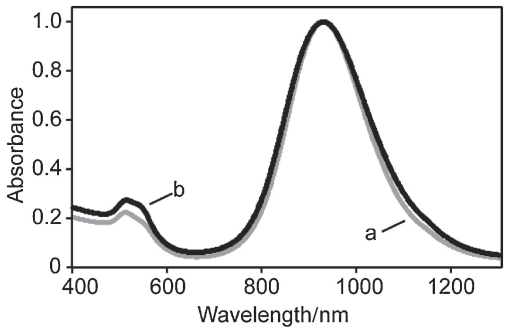

Figure 1. Absorption spectra of hexadecyltrimethylammonium bromide-stabilized gold nanorods (a) and $dsDNA_{Tm61}$-modified gold nanorods (b).

Next, the release of ssDNA triggered by the photothermal effect was investigated. To evaluate the release of ssDNA, the fluorescence intensity of Cy3 attached at the 5′-end of ssDNA (released chain) was measured. The fluorescence of Cy3 is quenched by gold nanorods: a phenomenon known as nanometal surface energy transfer. This phenomenon has been studied as a quenching property of gold nanoparticles.[14,15] Therefore, release of ssDNA can be evaluated using the increase of the fluorescence intensity of Cy3. In this experiment, an excess of free complementary ssDNA was added to the solution to avoid rehybridizing of the released ssDNA back to the anchored ssDNA on the gold nanorods. First, we examined the release of the ssDNAs induced by the photothermal effect of the gold nanorods as a function of laser exposure time. dsDNA-modified gold nanorod solutions [0.5 mM (Au atoms)] were irradiated by a near-infrared continuous-wave (CW) laser (wavelength

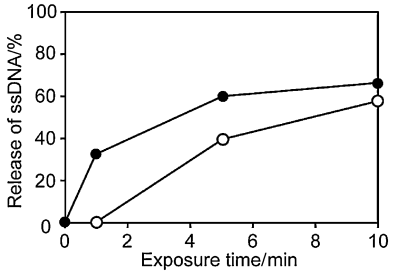

Figure 2. Release of ssDNAs from dsDNA-modified gold nanorods as a function of exposure time with near-infrared laser light. *Closed circles* and *open circles* indicate $dsDNA_{Tm61}$-modified and $dsDNA_{Tm80}$-modified gold nanorods, respectively.

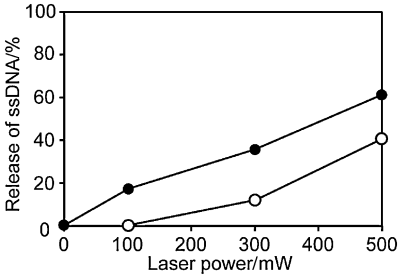

Figure 3. Release of ssDNAs from dsDNA-modified gold nanorods as a function of laser power. *Closed circles* and *open circles* indicate $dsDNA_{Tm61}$-modified and $dsDNA_{Tm80}$-modified gold nanorods, respectively.

807 nm, beam diameter 5.5 mm) at 500 mW at various laser exposure times (0, 1, 5, and 10 min). Releases of ssDNAs from gold nanorods modified by $dsDNA_{Tm61}$ and nanorods modified by $dsDNA_{Tm80}$ were dependent on the laser exposure time (Figure 2). The $dsDNA_{Tm61}$-modified gold nanorods showed faster release of ssDNA than the $dsDNA_{Tm80}$-modified gold nanorods did. However, after 10 min, ssDNA releases had reached similar levels.

The temperatures of the laser-irradiated solutions were simultaneously measured using a radiation thermometer (see Figure S1 in Supporting Information[16]). The temperatures of the solutions increased for 5 min and then leveled out at 60 °C. The melting temperatures of the two types of dsDNAs were 61 and 80 °C (Figure S2[16]). Therefore, the release of ssDNAs from both types of dsDNA-modified gold nanorods was induced at a temperature below the melting temperature of the dsDNA. The local temperature limited at the gold surface would be a key factor for ssDNA release, as we previously suggested.[11] This idea is also supported by a report of Barhoumi et al.; that is, release of ssDNA from gold nanoshells by photothermal effect was faster than that by simple heating without light irradiation even at the same solution temperature.[3]

Next, we examined release of ssDNAs from the dsDNA-modified gold nanorods as a function of laser power. Solutions of the dsDNA-modified gold nanorods [0.5 mM (Au atoms)] were irradiated using a near-infrared CW laser for 5 min at several laser powers (100, 300, and 500 mW). The releases of both types of ssDNAs were dependent on the laser power (Figure 3). The $dsDNA_{Tm61}$-modified gold nanorods once again exhibited faster release of ssDNA than the $dsDNA_{Tm80}$-modified gold nanorods did.

In this study, we showed that gold nanorods modified with dsDNA with a lower melting temperature released ssDNAs from the gold surface faster than those modified with the dsDNA with a higher melting temperature. Consequently, a two-step release system for oligonucleotides could be developed based on this difference in release behavior. Although the difference of the releases observed in this study was not so marked, it should be possible to improve it by optimizing each factor, i.e., the melting temperature of dsDNAs, the laser-light exposure time, and the laser power. If different kinds of gold nanorods having different absorption bands were used,[4] a further multidimensional selective release system could be established that could be controlled by different wavelengths of laser light. The oligonucleotides used in this study could be replaced by functional nucleic acid molecules, e.g., siRNA, antisense, and decoy oligonucleotides. Anticancer drugs such as doxorubicin could also be attached to the terminal of the oligonucleotides. The combination of several kinds of dsDNAs and gold nanorods should provide functional and multidimensional controlled delivery systems for drugs.

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16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

