Smart PEGylated Gold Nanoparticles for the Cytoplasmic Delivery of siRNA to Induce Enhanced Gene Silencing

Motoi Oishi,[1,2] Junpei Nakaogami,[2] Takehiko Ishii,[2,†] and Yukio Nagasaki*[1,2]
[1]*Tsukuba Research Center for Interdisciplinary Materials Science (TIMS), University of Tsukuba, 1-1-1 Ten-noudai, Tsukuba 305-8573*
[2]*Graduate School of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Ten-noudai, Tsukuba 305-8573*

(Received July 3, 2006; CL-060747; E-mail: nagasaki@nagalabo.jp)

A novel cytoplasmic delivery system of siRNA was developed by complexation between poly(ethylene glycol)-*block*-poly(2-(*N*,*N*-dimethylamino)ethyl methacrylate) copolymer (PEG–PAMA) and gold nanoparticles (GNPs), followed by the immobilization of siRNA bearing a thiol group (SH-siRNA). The PEGylated GNPs containing SH-siRNA showed a significant RNAi activity in HuH-7 cells.

Small interfering RNAs (siRNAs)[1] have attracted much attention as a new class of nucleic acid medicines, because they can be used to target mRNA for sequence-specific gene silencing via an RNA interference (RNAi) process in the cytoplasm. Nevertheless, the therapeutic value of siRNAs under in vivo conditions is still controversial due to their low stability against enzymatic degradation, low permeability across the cell membrane, and preferential liver and renal clearance.[2] A major key to the success of the cytoplasmic delivery of siRNAs is the development of effective carrier systems which achieve the modulated disposition in the body as well as a smooth release of siRNA in response to intracellular chemical stimuli such as pH,[3] ions,[4] and glutathione.[5]

Worth noticing in this regard is a new class of PEGylated gold nanoparticles (GNPs)[6] constracted through the self assembly of heterobifunctional poly(ethylene glycol) bearing a thiol group at the ω-end and a reactive acetal group (ligand installation moiety) at the α-end.[7] Ligand-installed, PEGylated GNPs have been found to exhibit excellent stability under physiological conditions, minimal interaction with biomacromolecules, and specific molecular recognition due to the steric stabilization of tethered PEG chains surrounding the GNPs through the thiol-interactions.[6] Furthermore, the thiol–Au interaction often causes an exchange reaction between R-SH/Au and thiol-containing compounds such as dithiothreitol (DTT) and glutathione, leading to the efficient dissociation of R-SH from the gold surface.[8] The concentrations of glutathione, which is an abundant thiol-containing compound in most cells, are in a millimolar range (1–10 mM) in cytoplasm, whereas those in the blood are in the micromolar range (2 μM),[9] indicating that R-SH/GNPs might be stable in the blood stream but show the glutathione-mediated release of R-SH in the cytoplasm.

A unique finding, which we would like to communicate here, is the enhancement of RNAi activity in cultured hepatoma cells by glutathione-sensitive PEGylated GNPs composed of poly(ethylene glycol)-*block*-poly(2-(*N*,*N*-dimethylamino)ethyl methacrylate) copolymer (PEG–PAMA)[10] and siRNA bearing a thiol group at 5′-end of the sense chain (SH-siRNA). Note that the PEGylated GNPs formed from PEG–PAMA copolymer showed excellent stability under physiological conditions even in the presence of high concentrations of thiol compounds, due to the multivalent coordination and electrostatic interaction between the negatively charged gold surface and the tertiary amino groups of the PAMA segment,[11] suggesting that the smart PEGylated GNPs composed of PEG–PAMA and SH-siRNA facilitate the specific glutathione-mediated release of siRNA in the cytoplasm.

Our strategy of constructing glutathione-sensitive PEGylated GNPs is based on the complexation between PEG–PAMA (PEG: $M_n = 5000$, PAMA: $M_n = 7500$, $DP_{PAMA} = 48$, $M_w/M_n = 1.6$) and commercially available GNPa with a size of 15 nm, followed by the immobilization of SH-siRNA. To estimate the average number of siRNA molecules per PEGylated GNP, various concentrations of fluorescein isothiocyanate-labeled SH-dsDNA (FITC-SH-dsDNA) were added to the PEGylated GNPs, followed by the ultrafiltration to remove the free FITC-SH-dsDNA molecules. Since GNPs are well known to serve as a fluorescence quencher,[12] the PEGylated GNPs containing FITC-SH-dsDNA were treated with cyan etching to ionize the GNPs; then the average number of dsDNA molecules per PEGylated GNP was estimated from the fluorescence intensity and the concentrations of gold ions. As can be seen in Figure 1a, the average number of FITC-SH-dsDNA molecules increased with increasing feed ratios, and reached to plateau value of almost 45 molecules per PEGylated GNP (0.06 molecules/nm^2) at feed ratios higher than 500. It should be noted that the use of the FITC-SH-dsDNA enabled a far more effective immobilization of dsDNA onto the PEGylated GNPs than the FITC-dsDNA without the thiol group. It is most likely that the immobilization of FITC-dsDNA molecules onto PEGylated GNPs

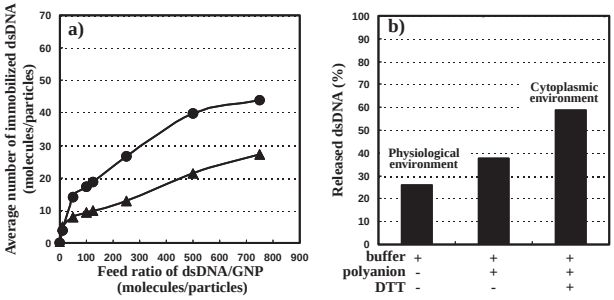

Figure 1. (a) Average number of immobilized FITC-SH-dsDNA molecules (circle) and FITC-dsDNA molecules (triangle) per PEGylated GNP at various feed ratios. (b) Release of the FITC-SH-dsDNA from the PEGylated GNPs. Samples in 10 mM Tris-HCl buffer, pH 7.4, with 0.15 M NaCl (physiological environment), in buffer with an excess amount of polyanions (sodium dextran sulfate), and in buffer with an excess amount of polyanions and DTT (10 mM) were incubated at 37 °C for 20 h.

occurs mainly through an electrostatic interaction between the negatively charged FITC-dsDNA and the cationic surface of the GNPs, leading to the lying orientation of FITC-dsDNA on the surface of the GNPs. On the contrary, the FITC-SH-dsDNA molecules were immobilized onto the PEGylated GNPs predominantly through the thiol–Au interaction, leading to the mainly standing and partially lying orientations of the FITC-SH-dsDNA on the surface of the GNPs. To confirm the DTT-sensitivity of the PEGylated GNPs containing FITC-SH-dsDNA, the PEGylated GNPs solution was incubated under physiological environment or in a cytoplasmic environment for 20 h at 37 °C. As can be seen in Figure 1b, only a 25% release of the FITC-SH-dsDNA was observed under physiological environment. Furthermore, a slight increase in the amount of released FITC-SH-dsDNA (10%) was observed even under physiological environment in the presence of an excess amount of the polyanions, suggesting that the FITC-SH-dsDNA molecules were obviously immobilized through the thiol–Au interaction. On the other hand, a 60% release of the FITC-SH-dsDNA was observed in the cytoplasmic environment, where an abundance of glutathione and polyanions (RNA and anionic carbohydrates) exists,[13] indicating that the effective release of the FITC-SH-dsDNA from the PEGylated GNPs is due to two exchange reactions, one with DTT and other with the counter polyanion.

To evaluate the RNAi activity (gene inhibition effect) of the smart PEGylated GNPs containing SH-siRNA, we carried out a dual luciferase reporter assay in HuH-7 cells (human hepatoma cells) in the presence of 10% fetal bovine serum, as shown in Figure 2. Almost no RNAi activity was observed for the free siRNA even at a siRNA concentration of 100 nM. The lack of RNAi activity for the free siRNA may be ascribed to the low tolerance against enzymatic attack. Both the PEGylated GNPs containing siRNA (without the thiol group) and the SH-siRNA revealed RNAi activity and, in particular, the PEGylated GNPs containing SH-siRNA achieved a far more effective RNAi activity (65% inhibition) than the PEGylated GNPs containing siRNA (25% inhibition). In addition, the RNAi activity of the PEGylated GNPs containing SH-siRNA at a siRNA concentration of 50 nM (47% inhibition) was found to be higher than that of the PEGylated GNPs containing siRNA even at siRNA concentration of 100 nM (25% inhibition), strongly suggesting that the pronounced RNAi activity of the PEGylated GNPs containing SH-siRNA is not due to the difference in the amount of siRNA molecules immobilized onto the PEGylated GNPs. Thus, the PEGylated GNPs containing SH-siRNA engage in the thiol–Au interaction, which exchanges SH-siRNA with glutathione in the cytoplasm; in other words, the efficient release of siRNA from the PEGylated GNPs occurred synchronously with the increase in the glutathione concentrations, leading to the enhancement of RNAi activity. On the other hand, the PEGylated GNPs containing siRNA, engaging only in electrostatic interaction, which is a weak form of interaction under extremely diluted conditions, led to the dissociation of the siRNA in the medium. Note that the RNAi activity of PEGylated GNPs containing SH-siRNA is remarkably similar to that of OligofectAMINE (cationic liposome) (60% inhibition). Cytotoxicity and the inhibition of the renilla luciferase expression were not observed at all, suggesting that the inhibition of firefly luciferase expression observed here indeed occurred through a sequence-specific RNAi effect.

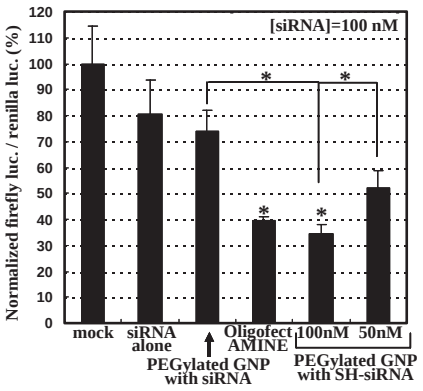

Figure 2. RNAi activity against the firefly luciferase gene expression in cultured HuH-7 cells. The normalized ratios between the firefly luciferase activity (firefly luc.) and the renilla luciferase activity (renilla luc.) are shown along the vertical of the figure. The indicated siRNA concentrations are as the feed concentrations of siRNA (not immobilized siRNA concentration). The plotted data are averages of triplicate experiments ± SD. Data points marked with asterisks are statically significant compared with the mock data (buffer-treated cells) ($P^* < 0.05$).

In conclusion, these results highlight the substantial value of the smart PEGylated GNP system with detachable siRNA for the cytoplasmic delivery of siRNA.

References

† Present address: Department of Bioengineering, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033.

1 S. M. Elbashir, J. Harborth, W. Lendeckel, A. Yalcin, K. Weber, T. Tuschl, *Nature* **2001**, *411*, 494.

2 D. Braasch, Z. Paroo, A. Constantiescu, G. Ren, O. K. Öz, R. P. Mason, D. R. Corey, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1139.

3 M. Oishi, Y. Nagasaki, K. Itaka, N. Nishiyama, K. Kataoka, *J. Am. Chem. Soc.* **2005**, *127*, 1624.

4 a) Y. Kakizawa, S. Furukawa, K. Kataoka, *J. Controlled Release* **2004**, *97*, 345. b) Y. Kakizawa, S. Furukawa, A. Ishii, K. Kataoka, *J. Controlled Release* **2006**, *111*, 368.

5 A. Muratovska, M. R. Eccles, *FEBS Lett.* **2004**, *558*, 63.

6 H. Otsuka, Y. Akiyama, Y. Nagasaki, K. Kataoka, *J. Am. Chem. Soc.* **2001**, *123*, 8226.

7 Y. Akiyama, H. Otsuka, Y. Nagasaki, M. Kato, K. Kataoka, *Bioconjugate Chem.* **2000**, *11*, 947.

8 a) A. Verma, J. M. Simard, J. W. Worrall, V. M. Rotello, *J. Am. Chem. Soc.* **2004**, *126*, 13987. b) R. Hong, G. Han, J. M. Fermandez, B. Kim, N. S. Forbes, V. M. Rotello, *J. Am. Chem. Soc.* **2006**, *128*, 1078.

9 A. Meister, M. E. Anderson, *Annu. Rev. Biochem.* **1983**, *52*, 711.

10 K. Kataoka, A. Harada, D. Wakebayashi, Y. Nagasaki, *Macromolecules* **1999**, *32*, 6892.

11 T. Ishii, H. Otsuka, K. Kataoka, Y. Nagasaki, *Langmuir* **2004**, *20*, 561.

12 B. Dubertret, M. Calame, A. J. Libchaber, *Nat. Biotechnol.* **2001**, *19*, 365.

13 P. Erbacher, A. C. Roche, M. Monsigny, P. Midoux, *Bioconjugate Chem.* **1995**, *6*, 401.

Published on the web (Advance View) August 12, 2006; doi:10.1246/cl.2006.1046