J|A|C|S COMMUNICATIONS

Published on Web 03/03/2009

Light-Regulated Activation of Cellular Signaling by Gold Nanoparticles That Capture and Release Amines

Jun Nakanishi,*[,†,‡] Hidekazu Nakayama,[†,‡] Takahiro Shimizu,[§] Haruhisa Ishida,[§] Yukiko Kikuchi,[†] Kazuo Yamaguchi,[§] and Yasuhiro Horiike[†]

World Premier International Research Center Initiative, International Center for Materials Nanoarchitectonics, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan, PRESTO, Japan Science and Technology Agency (JST), 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan, and Department of Materials Science, Faculty of Science, Kanagawa University, 2946 Tsuchiya, Hiratsuka, Kanagawa 259-1293, Japan

Received November 26, 2008; E-mail: nakanishi.jun@nims.go.jp

Methods for the administration of drugs and physiological compounds at high spatiotemporal resolutions in living systems are useful for studying intertwined inter- and intracellular signal transduction networks. Some of the most powerful tools are caged compounds, whose activities are suppressed by the covalent linkage of photocleavable protecting groups but are restored upon photoirradiation.[1−4] However, there are only a limited number of commercially available products, and their de novo construction often requires precise design, complex synthesis, and purification. In addition, a change in solubility due to caging has been troublesome in some cases. As an alternative approach, nanocarriers with photoresponsive cargo-releasing features have been developed to expand the repertoire of targets; these are especially focused on the delivery of plasmid DNA and anticancer drugs on time scales of minutes and hours.[5−12] We demonstrate herein colloidal gold nanoparticles (GNPs) presenting a photocleavable succinimidyl ester that allows for the delivery of amines to study cellular signaling on the millisecond to second time scale. Under this molecular design, we expected that the GNP would function as a bulky caging group that suppressed the activity of the cargo as well as a solid-phase support that facilitated the synthesis and purification of the caged compounds. In addition, the conjugation of cargoes to self-assembled monolayers (SAMs) on the GNP surface should be advantageous in minimizing release-rate variations among the molecules. As a proof of concept, caged histamine was developed and its ability to evoke intracellular signaling examined by fluorescence imaging.

The GNPs (caging-GNPs) bear mixed SAMs of disulfide **1** [bis(12-(4-(1-(succinimidyloxycarbonyloxy)ethyl)-2-methoxy-5-nitrophenoxy)dodecyl) disulfide] and disulfide **2** [a condensation reaction product of ω-methoxy-poly(ethylene glycol) (PEG) amine (MW = 5000) and disuccinimidyl 11,11′-dithiobisundecanoate] on its surface (Figure 1). Disulfide **1** reacts with an amine via the succinimidyl ester and releases it as the corresponding carbamate via the photocleavage reaction of the 2-nitrobenzyl group upon near-UV irradiation.[13,14] The released carbamate decarboxylates quickly and becomes the original amine. Meanwhile, disulfide **2** introduces PEG to the GNP surface and prevents self-aggregation of the GNPs and nonspecific adsorption of proteins and other biological species on their surface.

Caging-GNPs were prepared by the place-exchange reaction of citrate-capped GNPs with disulfides.[15] Commercial citrate-capped GNPs (ϕ = 5.8 nm) were reacted with disulfides **1** and **2** (both at 0.96 mM) in 9:1 DMSO/water at room temperature. The GNPs were washed with DMSO and water by centrifugation (see the Supporting Information). We also prepared control GNPs whose surfaces were modified only by disulfide **2** (PEG-GNPs).

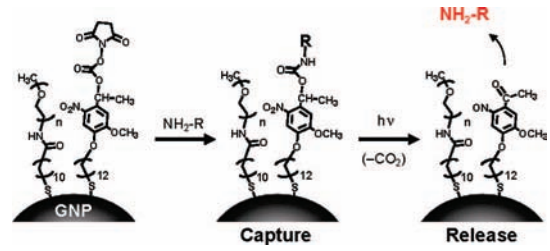

Figure 1. Capture and release of amines on GNPs having a photocleavable succinimidyl ester. Disulfides **1** (right) and **2** (left) are shown in their thiol forms for clarity. The average value of *n* is 113.

Caging-GNPs and PEG-GNPs showed similar absorption peaks at ∼520 nm, corresponding to the surface plasmon resonance of gold nanoparticles, but showed a difference in the near-UV region (Figure 2a). This difference was attributed to the presence of disulfide **1** on the caging-GNP surface, as their difference spectrum resembled the absorption spectrum of disulfide **1** (Figure S7 in the Supporting Information). On the basis of the molar extinction of disulfide **1** (12 000 at 345 nm) and the GNPs (1.7×10^7 for 5.8 nm GNPs),[16] the number of caging ligands (thiol form of disulfide **1**) per caging-GNP was 200. Thermogravimetric analysis (TGA) of caging-GNPs yielded 34% weight loss (Figure S8). From these results, the number of PEG per caging-GNP was 95, and the loading ratio of the caging ligand and PEG was 200:95.

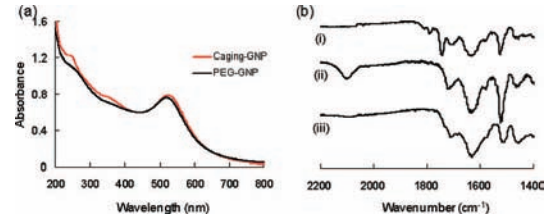

Figure 2. Characterization of the GNP having a photocleavable succinimidyl ester. (a) UV−vis spectra of (black) PEG-GNPs and (red) caging-GNPs. (b) IR spectra of the caging-GNPs (i) before and (ii) after immobilization of the azidoamine and (iii) after photoirradiation.

Next, we examined the reactivity of the caging-GNPs with an amine and their response to near-UV irradiation via IR spectrometry by using a KBr pellet (Figure 2b and Figure S9). As an example, we used 11-azido-3,6,9-trioxaundecan-1-amine because the azide group has a characteristic IR absorption at ∼2100 cm^{-1}. The caging-GNPs showed absorption peaks typical of succinimidyl ester at

† NIMS.
‡ JST.
§ Kanagawa University.

10.1021/ja809236a CCC: $40.75

1743, 1790, and 1811 cm^{-1} [Figure 2b(i)].[17] These peaks were replaced with those of carbamate (1722 cm^{-1})[18] and azide (2100 cm^{-1}) after mixing with the azidoamine [Figure 2b(ii)]. The observed IR absorption changes indicated that the azidoamine reacted with the succinimidyl ester. Irradiating the GNPs at 365 nm and removing the released amine by spin filtration caused the azide peak to disappear and the nitro group (1525 cm^{-1}) to be replaced with the nitroso group (1512 cm^{-1}) [Figure 2b(iii)]. These results indicate that upon near-UV irradiation, immobilized azidoamine was released from the GNP surface by the photochemical reaction typical of the 2-nitrobenzyl group.

We prepared caged histamine simply by mixing the amine with the caging-GNPs and spin filtering. The rate of uncaging was determined from the fluorescence of the derivatized product of released histamine with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (2ME).[19] As Figure 3a shows, the near-UV irradiation caused a dose-dependent increase in the histamine concentration. On the basis of this time profile, the quantum yield (ϕ) of the uncaging reaction was calculated to be 0.028,[20] and the product of the quantum yield and molar extinction ($\phi\varepsilon$) was 170. These values were comparable to those of caged compounds with the DMNPE caging group.[2] Therefore, conjugation of the photolabile group to the GNP surface did not significantly alter its photochemical properties.

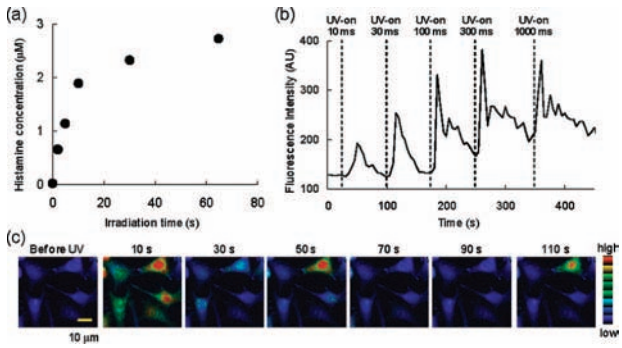

Figure 3. Photorelease of histamine from the GNPs. (a) Dose-dependent histamine release determined by OPA−2ME derivatization (λ_{ex} = 350 nm, λ_{em} = 450 nm). (b) Fluorescence change of Fluo 3-loaded HeLa cells in response to various exposure times. (c) Fluorescence (pseudocolor) images of the cells in response to the 1000 ms UV irradiation. The upper-right cell shows an oscillatory action.

To evaluate the biological activity of the uncaged histamine, we investigated the intracellular Ca^{2+} concentration in HeLa cells. This cell type exhibits histamine-induced Ca^{2+} oscillations and Ca^{2+} waves due to stimulation of the endogenous histamine H_1 receptor.[21] HeLa cells were loaded with the Ca^{2+} indicator Fluo 3 AM, and their fluorescence images were obtained under an inverted fluorescence microscope. The histamine-immobilized GNPs were added to the extracellular medium of the cells, and imaging was conducted immediately after the addition (at time −25 s in Figure 3b). The cells exhibited larger responses to near-UV irradiation as the exposure time was increased from 10 to 1000 ms (λ = 365 nm, 100 mW/cm^2; Figure 3b,c). Similar results were obtained for cells where histamine solution was added to the extracellular culture at various concentrations, but their dose dependency was not a simple progression because of the low spatiotemporal resolution in the histamine application (Figure S10). On the other hand, Ca^{2+} did not increase by the addition of the caging-GNPs or by irradiation of the cells in the presence of PEG-GNPs (Figure S11). These results demonstrate that histamine has no biological activity during its linkage to the GNP surface but becomes active only when it is released from the GNP; that is, the GNP actually serves as a caging group for histamine's function. To our knowledge, this is the first report of caged histamine. It will be useful to study the spatiotemporal dynamics of histamine in the nervous system.[22]

In this study, we have demonstrated GNPs with a photocleavable succinimidyl ester as a photoresponsive nanocarrier for amines. Caged compounds for other amines, such as neurotransmitters, peptides, and inhibitors, will become available. The present approach will be useful for constructing caged compounds via a simple procedure (i.e., mixing and centrifuging), thus enabling researchers to prepare them immediately prior to use. Furthermore, since it is easy to add chemical functionalities to GNPs through thiol linkages, we will be able to deliver the GNPs inside living cells by introducing membrane-translocating peptides such as oligoarginines.[23,24] Such modifications could lead to new possibilities for the direct regulation of intracellular signaling.

Acknowledgment. This work was supported in part by KAKENHI (207004010001) and the High-Tech Research Center Project from MEXT, Japan. We thank Dr. H. Kobayashi (NIMS) for the spectrofluorometer.

Supporting Information Available: Syntheses, UV−vis, NMR, and MS spectra, TGA results, and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org/.

References

(1) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900–4921.
(2) Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619–628.
(3) Pelliccioli, A. P.; Wirz, J. *Photochem. Photobiol. Sci.* **2002**, *1*, 441–458.
(4) Kobayashi, T.; Urano, Y.; Kamiya, M.; Ueno, T.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 6696–6697.
(5) Mal, N. K.; Fujiwara, M.; Tanaka, Y. *Nature* **2003**, *421*, 350–353.
(6) Radt, B.; Smith, T. A.; Caruso, F. *Adv. Mater.* **2004**, *16*, 2184–2189.
(7) Nishiyama, N.; Iriyama, A.; Jang, W. D.; Miyata, K.; Itaka, K.; Inoue, Y.; Takahashi, H.; Yanagi, Y.; Tamaki, Y.; Koyama, H.; Kataoka, K. *Nat. Mater.* **2005**, *4*, 934–941.
(8) Han, G.; You, C. C.; Kim, B. J.; Turingan, R. S.; Forbes, N. S.; Martin, C. T.; Rotello, V. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 3165–3169.
(9) Skirtach, A. G.; Javier, A. M.; Kreft, O.; Kohler, K.; Alberola, A. P.; Mohwald, H.; Parak, W. J.; Sukhorukov, G. B. *Angew. Chem., Int. Ed.* **2006**, *45*, 4612–4617.
(10) Park, H.; Yang, J.; Seo, S.; Kim, K.; Suh, J.; Kim, D.; Haam, S.; Yoo, K. H. *Small* **2008**, *4*, 192–196.
(11) Wu, C. L.; Chen, C.; Lai, J. P.; Chen, J. B.; Mu, X.; Zheng, J. S.; Zhao, Y. B. *Chem. Commun.* **2008**, 2662–2664.
(12) Ghosh, P.; Han, G.; De, M.; Kim, C. K.; Rotello, V. M. *Adv. Drug Delivery Rev.* **2008**, *60*, 1307–1315.
(13) Nakanishi, J.; Kikuchi, Y.; Takarada, T.; Nakayama, H.; Yamaguchi, K.; Maeda, M. *J. Am. Chem. Soc.* **2004**, *126*, 16314–16315.
(14) Nakanishi, J.; Kikuchi, Y.; Inoue, S.; Yamaguchi, K.; Takarada, T.; Maeda, M. *J. Am. Chem. Soc.* **2007**, *129*, 6694–6695.
(15) Rouhana, L. L.; Jaber, J. A.; Schlenoff, J. B. *Langmuir* **2007**, *23*, 12799–12801.
(16) Liu, X. O.; Atwater, M.; Wang, J. H.; Huo, Q. *Colloids Surf., B* **2007**, *58*, 3–7.
(17) Grubisha, D. S.; Lipert, R. J.; Park, H. Y.; Driskell, J.; Porter, M. D. *Anal. Chem.* **2003**, *75*, 5936–5943.
(18) Cameron, J. F.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1991**, *113*, 4303–4313.
(19) Krasnova, I. N.; Kartsova, L. A.; Cherkas, Y. V. *J. Anal. Chem.* **2000**, *55*, 58–65.
(20) Adams, S. R.; Kao, J. P. Y.; Grynkiewicz, G.; Minta, A.; Tsien, R. Y. *J. Am. Chem. Soc.* **1988**, *110*, 3212–3220.
(21) Sauve, R.; Diarra, A.; Chahine, M.; Simoneau, C.; Morier, N.; Roy, G. *Cell Calcium* **1991**, *12*, 165–176.
(22) Haas, H. L.; Sergeeva, O. A.; Selbach, O. *Physiol. Rev.* **2008**, *88*, 1183–1241.
(23) Futaki, S.; Suzuki, T.; Ohashi, W.; Yagami, T.; Tanaka, S.; Ueda, K.; Sugiura, Y. *J. Biol. Chem.* **2001**, *276*, 5836–5840.
(24) Koch, A. M.; Reynolds, F.; Merkle, H. R.; Weissleder, R.; Josephson, L. *ChemBioChem* **2005**, *6*, 337–345.

JA809236A