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COMMUNICATIONS

Published on Web 01/10/2006

Glutathione-Mediated Delivery and Release Using Monolayer Protected Nanoparticle Carriers

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Drug delivery systems (DDSs) provide important tools for enhancing the efficacy of chemotherapeutics. Nanoscopic materials, such as polymeric micelles,[1] mesoporous silica nanorods[2] and nanotubes,[3] have seen growing interest as drug carriers. Although numerous examples have shown successful drug encapsulation and cellular internalization, the release of the drugs in a controlled fashion presents a challenge. Stimuli such as hydrolysis under low pH and enzymatic degradation have been the major mechanisms for drug release.[4] Recently, specific chemical reactions, such as the disulfide reduction, have emerged as alternative mechanisms for drug release.[2,5]

In previous research, we have established glutathione (GSH)-mediated release of biomolecules from monolayer protected gold nanoparticle surfaces and demonstrated manipulation of their bioactivities in vitro.[6] GSH is the most abundant thiol species in the cytoplasm and the major reducing agent in biochemical processes, providing a potential in situ releasing source in living cells.[7] The intracellular GSH concentration (1−10 mM) is substantially higher than extracellular levels (2 μM in plasma),[8] providing a mechanism for selective intracellular release. In this communication, we report a monolayer protected gold nanoparticle-based delivery system utilizing GSH-mediated release (Figure 1). We demonstrate that GSH serves as an effective trigger to release a payload (dye molecules) from nanoparticle surfaces both in vitro and in cell cultures. We also demonstrate that the payload release can be manipulated by external stimulus, further strengthening the applicability of the GSH-mediated release as a DDS.

Gold nanoparticles possess distinctive attributes that make them promising as drug carriers. First, the ability to formulate mixed monolayers provides direct access to systems.[9] For instance, the surface of nanoparticles can be tailored to realize tumor specificity and cell membrane penetration.[10] Second, the surface monolayer is stable under most physiological conditions, thus providing a reservoir of hydrophobic drugs, yet allowing controlled release by GSH though place exchange reactions of thiols on gold nanoparticle surfaces.[11] Third, gold has low toxicity, a feature exploited by Paciotti for protein delivery to tumors.[12] Finally, the small size of nanoparticles and the capability to construct biocompatible surface monolayers provide long circulation time, low cytotoxicity, and high payload-to-carrier ratios.[13]

The gold nanoparticles (**AuNP**) used in this work feature a 2-nm core and a mixed monolayer composed of a tetra(ethylene glycol)-lyated cationic ligand **TTMA** and a thiolated Bodipy dye, **HSBDP** (Figure 1). The **TTMA** ligand is used to generate a cationic surface to enhance cellular uptake. The dye molecule doped into the particle monolayer provides an analogue for hydrophobic drugs and allows facile detection of payload release.

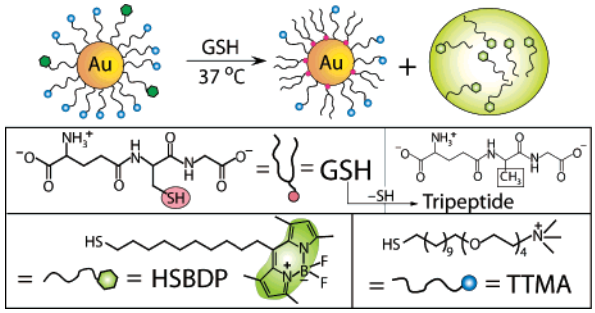

Figure 1. Structure of the **AuNP** carrier and schematic depiction of the GSH-mediated surface monolayer exchange reaction which releases the payload, in this case a hydrophobic dye.

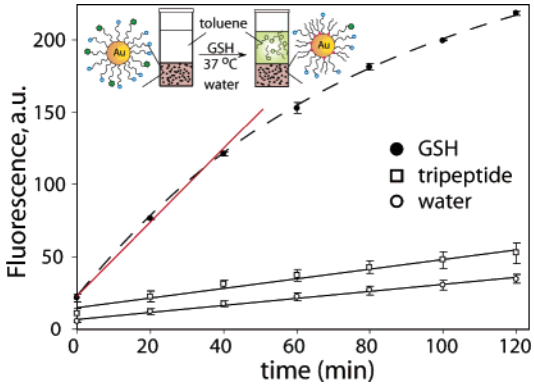

Figure 2. In vitro release of **HSBDP** mediated by GSH. Samples of nanoparticles in pure water, in tripeptide (10 mM), and in GSH (10 mM) were incubated at 37 °C, and the fluorescence spectra of the toluene phase were recorded. The fluorescence intensities at 507 nm of the toluene phase were plotted against the incubation time. The slopes were 2.5 (for the initial period), 0.33, and 0.24 for the GSH, tripeptide, and water sample, respectively.

Gold nanoparticles provide excellent fluorescent quenchers, allowing the in vitro release of **HSBDP** from nanoparticles to be followed by fluorescence spectroscopy. The strong tendency of the hydrophobic **HSBDP** ligands to aggregate necessitated a toluene−water two-phase system in which released **HSBDP** ligands were transferred into the toluene phase (Figure 2).[14]

It is clear that the release of **HSBDP** was much more pronounced in the presence of GSH (Figure 2). A nearly 8-fold increase of releasing rate was recorded in GSH solution than in the tripeptide, suggesting that the thiol group is responsible for releasing **HSBDP** ligands from the **AuNP** surface through the place-exchange reaction. The marginal increase of fluorescence in the absence of GSH is attributed to the interfacial stress applied on the **AuNP** monolayer with the concomitant release of weakly associated ligands.[15] The two-phase setup used here is a reasonable analogue to in vivo

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10.1021/ja056726i CCC: $33.50

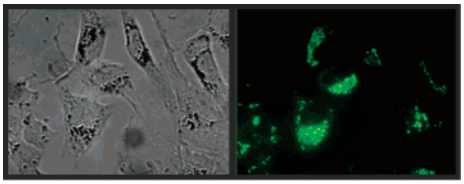

Figure 3. Representative bright field and fluorescence images of human Hep G2 cells incubated with **AuNP** and imaged after 96 h.

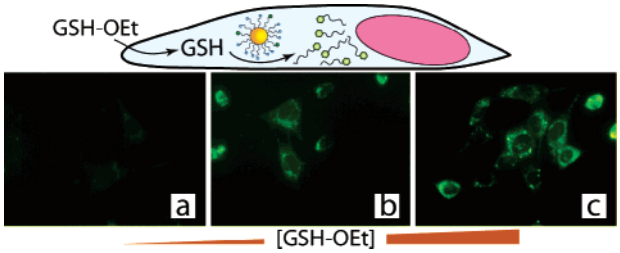

Figure 4. Schematic representation and fluorescence images when using GSH−OEt as an external stimulus to release **HSBDP** from **AuNP**. The GSH−OEt concentrations were varied from 0, 5, and 20 mM in panels a, b, and c, respectively.

environments, where released dyes (prodrugs) can partition into the hydrophobic domains in living cells (such as lipid membranes and vesicles).

GSH-mediated release was likewise observed in living cells. Human liver cells (Hep G2) were incubated with **AuNP** (1.67 μM) for 4 h and washed twice with PBS. The cells were allowed to grow and imaged at certain time points. Strong fluorescence from Bodipy was observed after 96-hour incubation (Figure 3). No fluorescence was observed in the control experiment without any **AuNP**. The results clearly show that the multifunctional cationic nanoparticles efficiently penetrated cell membranes and that the payload dye molecules were successfully released in living cells.

The GSH-mediated payload release from **AuNP** surfaces was confirmed by using glutathione monoester (GSH−OEt) as an external stimulus to trigger **HSBDP** release. GSH is not able to internalize cell membranes due to its anionic nature. As a neutral molecule, GSH−OEt is efficiently internalized into cells and rapidly hydrolyzed to generate GSH, thus offering a method to transiently manipulate intracellular GSH concentrations.[16] In this experiment, mouse embryonic fibroblast cells containing ∼50% lower GSH levels than Hep G2[17] were first treated with varied concentrations of GSH−OEt (0, 5, and 20 mM) for 1 h and washed. Cells were then incubated with **AuNP** (1.67 μM) for 8 h and imaged after nanoparticle removal as above. Increased fluorescence intensity was clearly observed with increasing GSH−OEt concentration (Figure 4). Without any GSH−OEt, the cells were only weakly fluorescent. Increasing GSH−OEt to 5 mM promoted the release of **HSBDP**, with further increase of fluorescence observed with 20 mM of GSH−OEt. This dose-dependent increase in fluorescence effectively demonstrates that GSH is responsible for releasing dye molecules from the **AuNP** carrier. In all experiments, **AuNP** did not seem to have deleterious effect on cells as determined by their retention of normal morphology.

The above results have significant implications in both understanding the release mechanism and achieving controlled release in nanoparticle-based delivery systems. The results demonstrate that GSH can be used as a reliable in vivo releasing agent in nanoparticle-based delivery systems. As the most abundant thiol species in living cells, GSH is the most likely candidate for the disulfide reduction in previously reported DDSs.[2,5] The manipulation of GSH concentration in living cells as demonstrated here conclusively proves that GSH-mediated release is a viable mechanism for releasing payloads from nanocarriers.

In summary, we have developed a nanoparticle-based delivery and release system using GSH as the releasing agent. We have shown that GSH is responsible for releasing payloads from nanoparticles carrier both in vitro and in cell cultures. The capability to tune the nanoparticle surface monolayer and incorporate targeting functionality will further enhance the efficiency of nanoparticle-based DDSs in vivo, and further efforts on optimization of these systems are underway. The GSH-mediated release, combined with the controlled interactions of biomolecules with surface functionalized nanoparticle scaffolds, can be further utilized to realize delivery of proteins and enhance transfection of genetic materials.

Acknowledgment. This research is supported by the National Institutes of Health (Grant EB004503).

Supporting Information Available: Synthesis of gold nanoparticles; experimental protocols. This information is available free of charge via the Internet at http://pubs.acs.org.

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JA056726I