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Acylsulfonamide-Functionalized Zwitterionic Gold Nanoparticles for Enhanced Cellular Uptake at Tumor pH

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Abstract

We report here a nanoparticle design featuring pH-responsive alkoxyphenyl acylsulfonamide ligands. Due to ligand structure, this nanoparticle is neutral at pH 7.4, becoming positively charged at tumor pH (< 6.5). The particle uptake and cytotoxicity increases over this pH range. This pH-controlled uptake and toxicity makes this particle a promising tool for cancer therapeutics.

Keywords

pH responsive; zwitterionic; gold nanoparticles; acylsulfonamide; cellular uptake

The pH difference between normal tissue (pH 7.2–7.4) and tumor tissue (pH 6.0–6.8)¹ provides an attractive strategy for selective accumulation of nanoparticles (NPs) into tumor tissues for cancer treatment and/or imaging. To supply high tumor selectivity, a pH-responsive bio-interactive surface must be generated with appropriate responses at normal and tumor pH.² The most common strategy is the use of a non-interactive functional group bearing an acid-cleavable unit; however, difficulty in controlling the acid sensitivity can result in the cleavage reaction taking place even at neutral pH,³ or requiring quite low pH ($\approx$ 5.0) for activation.⁴ The use of reversible protonation/deprotonation systems provides an alternate strategy that features high tunability.⁵ Recent reports employing polymers such as poly-histidine and polyamines as a pH-responsive moieties provided high tumor pH sensitivity.⁶ Solubility can be a challenge, however, with these neutral-to-cationic systems, requiring the use of additional functional group such as poly(ethylene)glycol chains at neutral pH.

Zwitterionic surfaces have recently received considerable attention as non-interacting chemical surfaces.⁷ NPs with zwitterionic surfaces exhibit a long circulatory half-life,⁸ low

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cytotoxicity,⁹ and high biocompatibility. Integration of these properties with a pH dependent zwitterionic-to-cationic charge conversion system would make them attractive scaffolds for therapeutics. The low cellular uptake of zwitterionic particles¹⁰ makes them excellent candidates for pH-controlled tumor uptake upon protonation to the resulting cationic particle. Moreover, concomitant cytotoxicity resulting from these cationic NPs¹¹ would occur only in the tumor environment, leading to the possibility of tumor-selective therapy.

A few examples of pH-responsive zwitterionic chemical surfaces such as carboxybetaine¹² and phosphorylcholine have been reported.¹³ However, the charge switching abilities of these structures are not sensitive enough to respond to stimuli such as weakly acidic tumor pH, with carboxybetaines protonated at pH <2 and phosphorylcholine at pH <5. The structure of the negatively charged group is the key for precise pH-responsiveness, and been reported using mixed-monolayer particles featuring carboxylic acid and ammonium ligands.¹⁴ Here, we report a new pH-responsive zwitterionic surface structure engineered by derivatization of acylsulfonamide group. This designed zwitterionic group becomes cationic at tumor pH (< 6.5), resulting in dramatically enhanced cellular uptake and cytotoxicity. Significantly, these particles show no hemolytic activity at blood pH (7.4).

The acylsulfonamide group features pH behavior similar to that of carboxylic acid groups.¹⁵ We chose this group as the negatively charged part of the zwitterionic ligand, with pH-responsiveness controlled by the functional group attached to the sulfonyl group. AuNP **1** features an aryl acylsulfonamide while AuNP **2** provides an alkyl analog (Figure 1). The ligands have trimethylammonium termini, and a tetra(ethylene glycol) spacer between the negative group and the hydrophobic chain was used as a passivating group to avoid irreversible adsorption and denaturation of serum proteins.¹⁶ These particles were synthesized from pentanethiol-capped AuNPs (ca. 2 nm core) by means of place-exchange reactions.¹⁷

We initially measured zeta potential to determine the pH dependence of the AuNP surface charges (Figure 2). The surface charge of both AuNP **1** and **2** were close to neutral at physiological pH (7.4), consistent with the zwitterionic structure of these NPs. AuNP **1** features a sharp transition from neutral to cationic centered at pH 6.5, with consistent changes observed by ¹H-NMR (Supplementary Figure 1).¹⁸ In contrast, AuNP **2** displayed a zwitterionic surface even at acidic pH, consistent with the reported pKa value of an alkyl acylsulfonamide group (pKa ≈ 4.5)¹⁹ and providing a permanently zwitterionic control particle for our studies. Zeta potentials for particles with higher coverage obtained by two exchange reactions were similar to those formed with only one exchange. (Supplementary Figure 2 and 3). Recent reports on a pH-responsive zwitterionic AuNP with a mixed monolayer of carboxylic acid and quaternary ammonium ligands showed that the neutralization of surface charge during the charge alteration process results in the formation of precipitates.^{14,20} Therefore, we investigated if similar aggregates formed in our case. Dynamic light scattering data revealed that no aggregation of these NPs was observed in the pH range from 7.4 to 6.0 (see Supporting Information), preventing potential complications. The stability the particles to degradation in serum was confirmed by MALDI-MS (Supplementary Figure 4) after the incubation in 10% serum containing media (37 °C, 24 h).

The change of AuNP **1** from zwitterionic to positively charged surface at weakly acidic pH should enhance cellular uptake of AuNP. Both AuNPs **1** (switchable) and **2** (non-switchable) were incubated with HeLa cells for 3 h at three different pH values,²¹ with the resulting intracellular amount of gold measured using inductively coupled plasma mass spectrometry (ICP-MS) (Figure 3). At pH 7.4, very low uptake (~10 ng/well) was observed for both AuNPs **1** and **2**, similar to the cellular uptake of recently reported highly nonfouling sulfobetaine functionalized AuNPs.^{7e} Uptake of AuNP **1** increased with decreasing pH value, reaching a four-fold increase at pH 6.0. In contrast, no significant change in uptake was observed with control AuNP **2** over this pH range. Transmission electron microscopy (TEM) analysis was used to evaluate the intracellular localization of AuNPs at different pH. AuNP **1** was located only on cell membrane at pH 7.4, while endosomal uptake was observed at pH 6.0 (Supplementary Figure 5), confirming that acidic pH leads to protonation and triggers concomitant endosomal uptake of AuNP **1** in HeLa cells. Taken together, these studies establish the role of acylsulfonamide protonation in determining cellular uptake.

We next investigated the pH dependent cellular uptake in human microvascular endothelial cells (HMEC-1). At pH 7.4, the amount of intracellular gold was similar with that with HeLa cells. In contrast to HeLa cells, no significant difference in uptake was observed even at pH 6.0 for HMEC-1 cells (Figure 3). This is presumably due to differential uptake mechanisms in HeLa and HMEC-1 cells.²² Significantly, these results indicate that the cationic charge on the nanoparticle surface can provide different affinity and selective uptake in HeLa cells relative to HMEC-1 cells, which could be a critical parameter for selective delivery of nanomaterials in tumor microenvironment.

Positively charged AuNPs show higher cytotoxicity than their zwitterionic counterpart,¹⁰ which indicates that cytotoxicity of AuNP **1** could be triggered by weakly acidic pH. Therefore, we next evaluated the cytotoxicity of AuNPs **1** and **2** at varying pH values and concentrations using the Alamar blue viability assay (Figure 4). At pH 7.4, no cytotoxicity of AuNPs **1** and **2** was observed in HeLa cells due to their non-interactive zwitterionic properties. Enhanced cytotoxicity of AuNP **1** on HeLa cells was observed at pH 6.0, as expected due to the enhanced cellular uptake resulting from the conversion of the particle from zwitterionic to cationic. G6PD assay indicated no cell membrane damage (Supplementary Figures 6), indicating that cytotoxicity arose from intracellular mechanisms, e.g. oxidative stress or DNA damage.¹¹ In contrast, AuNP **2** did not show any significant toxicity at low pH values. In addition, no cytotoxicity was also observed in HMEC-1 cells at both pH 7.4 and pH 6.0 (Supplementary Figures 7).

Hemolysis is an important issue for therapeutic nanomaterials.²³ Therefore, we performed a hemolytic assay to investigate the blood compatibility of our NPs. AuNP mediated cell lysis events were not observed on either AuNP **1** or **2** in both the presence and absence of plasma proteins (Supplementary Figures 8 and 9).²⁴

In conclusion, we have developed a new pH-responsive zwitterionic ligand based on the alkoxyphenyl acylsulfonamide group. Due to its precisely designed surface structure, zwitterionic AuNPs functionalized with this ligand reversibly becomes cationic at tumor pH, with concomitant enhancement of cellular uptake and cytotoxicity on HeLa cells. This

combination of pH-regulated tumor selective cellular uptake and cytotoxicity could make this ligand design promising for imaging, delivery, and self-therapeutic applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Chemical structure of the monolayer-protected gold nanoparticles (AuNPs) and our strategy for a pH-responsive delivery system into tumors.

Figure 2.

Zeta potential vs. pH curves obtained for AuNPs 1 and 2 (both 1 μ M). Zeta-potentials were measured in 5 mM phosphate buffer at different pH values. Error bars represent standard deviations based on three independent measurements per pH value.

Figure 3.

Cellular uptake of AuNPs 1 and 2 (both 1 μ M) after 3 h incubation with HeLa (30,000 cells/well) or HMEC-1 cells (100,000 cells/well) in the presence of 10% serum. All experiments were performed in triplicate, and error bars represent standard error of the mean.

Figure 4.

Cell viability of HeLa cells after 72 h incubation with AuNPs 1 and 2 (0.5 μM–4.0 μM) in the presence of 10% serum. All experiments were performed in triplicate. Error bars are standard error of the mean.