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Effect of gold nanoparticle morphology on adsorbed protein structure and function

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ABSTRACT

Many biomedical applications of gold nanoparticles (NPs) rely on proteins that are covalently attached or adsorbed on the NP surface. The biological functionality of the protein-NP conjugate depends on the protein's ability to interact with target molecules, which is affected by NP characteristics such as size, curvature, aspect ratio, morphology, crystal structure, and surface chemistry. In the present study, the effect of gold nanoparticle morphology on the structure and function of adsorbed enzymes, lysozyme (Lyz) and α-chymotrypsin (ChT), has been investigated. Gold nanospheres (AuNS) were synthesized with diameters 10.6 ± 1 nm, and gold nanorods (AuNR) were synthesized with dimensions of (10.3 ± 2) × (36.4 ± 9) nm. Under saturating conditions, proteins adsorb with a higher surface density on AuNR when compared to AuNS. In the case of Lyz, adsorption on AuNS and AuNR resulted in a 10% and 15% loss of secondary structure, respectively, leading to conjugate aggregation and greatly reduced enzymatic activity. ChT retained most of its secondary structure and activity on AuNS and AuNR at low surface coverages; however, as protein loading approached monolayer conditions on AuNR, a 40% loss in secondary structure and 86% loss of activity was observed. Subsequent adsorption of ChT in multilayers on the AuNR surface allowed the conjugates to recover activity and remain stable. It is clear that AuNP morphology does affect adsorbed protein structure; a better understanding of these differences will be essential to engineer fully functional nanobioconjugates.

1. Introduction

Gold nanoparticles (AuNP) have been used for centuries [1] and possess many properties that are now useful in biomedical applications. Developments in the past two decades have led to reproducible synthetic methods for producing AuNP with a variety of morphologies and narrow size distributions [2–5]. Precise control of AuNP properties such as size and morphology has enabled a better understanding of how these characteristics determine material properties, such as nanoparticle crystal and defect structure [6–8], surface plasmon resonance [9,10], and optical absorption cross-section [11]. Many of these properties are important for applications in biomedicine, such as targeted drug and gene delivery [12–14], imaging diagnostics [15,16], and photothermal therapy [17]. In particular, recent applications of gold nanorods in cancer imaging [15] and tumor ablation [18] have resulted in a strong motivation for further evaluation of these particles in biological systems.

Nanoparticle interactions with biological systems are often mediated through proteins covalently attached or physically adsorbed onto the nanoparticle surface. Targeted therapeutic nanomaterials depend on attached biomolecules in order to carry out their function, and most nanoparticles will typically adsorb blood serum proteins upon introduction to an *in vivo* environment [19,20], creating a protein corona around the nanoparticle [21,22]. It is, therefore, essential to achieve a comprehensive understanding of how protein binding, structure, and function are influenced by nanoparticle characteristics such as size, curvature, aspect ratio, morphology, crystal structure, and surface chemistry. Nanoparticle size and curvature can have substantial effects on protein structure and stability compared to relatively 'flat' supports. It has been shown, for example, that lysozyme retains more of its native

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secondary structure and enzymatic activity on smaller, highly curved hydrophilic silica nanospheres than on larger particles [23]. This behavior was investigated further, and similar results were found on hydrophobic single-wall carbon nanotubes, C_{60} fullerenes, and gold nanospheres [24], with adsorbed soybean peroxidase and the protease subtilisin, particularly under denaturing conditions [25]. It has been proposed that the observed greater stability under denaturing conditions is due to a decrease in lateral protein–protein and protein-surface interactions on highly curved surfaces; however, this effect may not be universal for all proteins, and greatly depends on the protein's affinity for the NP surface [26]. Further studies have revealed that the charge of the stabilizing ligand on the particle surface [27], as well as the chemical nature of the nanoparticle [28], have a strong effect on immobilized protein structure and function. Computational molecular dynamics simulations of cytochrome c adsorbed onto monolayer-protected metal nanoparticles indicate the importance of both ligand chemistry and packing structure on the nanoparticle surface in determining the orientation and conformation of adsorbed proteins [29].

Simplified descriptions of nanoparticles as hard spheres completely passivated by surface ligands do not account for nanoparticle crystalline structure, atomic-scale topography, and surface ligand density. These characteristics can account for increased nonspecific interactions between the protein and the nanoparticle surface [30], altering the structure and potential activity of the attached protein. Nanoparticles exhibit specific crystal facets determined by their method of synthesis [8], which also has an effect on ligand surface density [31,32]. Indeed, varying material and facet structure has been shown to produce a different 2D packing structure of adsorbed self-assembled monolayers [33]. This packing structure can be controlled further through ligand chemistry, creating a unique interfacial environment at the nanoscale [34] that may affect subsequent protein attachment. Changes in protein conformation upon attachment can have unanticipated effects on the function of the protein-nanoparticle conjugate, with potential for toxicological repercussions or decreased viability.

While many current biomedical studies focus primarily on exploiting nanoparticle material properties, poor identification and control of nanoparticle characteristics and the variables involved at the protein-NP interface hinder the development of optimized nanobiomaterials. To fully realize the potential of these structures, it is essential to understand and quantify the effects of individual NP characteristics on conjugated protein structure and function. In the present work, we have investigated the effect of gold nanosphere (AuNS) and gold nanorod (AuNR) morphology on the adsorbed proteins lysozyme (Lyz) and α-chymotrypsin (ChT). A variety of physical and spectroscopic techniques were used to characterize changes in protein structure and activity upon adsorption, as well as the stability and morphology of the resulting protein-nanoparticle conjugates. Understanding how nanoparticle morphology and protein characteristics affect adsorbed protein structure will assist in the engineering of functional nanobioconjugates for biomedical applications.

2. Materials and methods

2.1. Materials

Gold (III) chloride trihydrate 99.9%, silver nitrate ACS reagent 99+%, sodium citrate, L-ascorbic acid 99+%, sodium borohydride, hexadecyltrimethylammonium bromide (CTAB), and 16-mercaptohexadecanoic acid 90% (MHDA) were purchased from Sigma (St. Louis, MO). Lysozyme from chicken egg white and α-chymotrypsin from bovine pancreas, type II, were purchased from Sigma and used without further purification. Mass spectrometry 10 ppm NIST-traceable gold standards were purchased from Absolute Standards (Hamden, CT). Oregon Green 488 was purchased from Invitrogen (Carlsbad, CA). All synthesis glassware was cleaned with *aqua regia* before use, and DI water (from a Milli-Q filtration unit) was used in all experiments.

2.2. Instrumentation

All transmission electron micrographs were obtained using a Philips CM-12 operating at 120 kV. Samples were drop-cast on carbon-coated copper grids (Electron Microscopy Services), and observed particle dimensions were evaluated using NIH ImageJ software (http://rsb.info.nih.gov/ij). Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was performed on a Varian 820-MS spectrometer. X-ray photoelectron spectroscopy (XPS) was performed on a PHI 5000 Versaprobe, and absorption measurements were made on a Hitachi U-2910 spectrophotometer. Protein pseudo-isotherms were determined using a Molecular Devices SpectraMax M5 Platereader, and protein-nanoparticle aggregates were imaged using a Zeiss LSM 510Meta Confocal Microscope. The far UV-CD spectra were recorded using a Jasco J-815 automatic recording spectrapolarimeter.

2.3. Nanoparticle synthesis

Spherical gold nanoparticles (AuNS) with a narrow size distribution were prepared using a seed-mediated growth method [35]. Using two-20 ml glass vials, 40 ml of 2.5×10^{-4} M $HAuCl_4$ and 2.5×10^{-4} M trisodium citrate aqueous solution was prepared in an ice bath. Under constant stirring, 0.6 ml of ice cold 0.1 M $NaBH_4$ was added to each vial. Stirring was stopped after 10 min and the gold seeds sat undisturbed for 1 h, yielding a reddish–orange solution. To obtain the average sphere size of interest, the particle growth solution was prepared containing 36 ml solution of 0.08 M CTAB and 2.5×10^{-3} M $HAuCl_4$, and 2 ml of 0.1 M ascorbic acid was swirled in until the solution became clear, followed by addition of 40 ml of the seed solution. The particle solution was kept overnight before further use in a 35 °C water bath to ensure that the CTAB remained soluble. The resulting solution was dark red and produced nanopsheres with diameters of ~10 nm.

Gold nanorods (AuNR) were also prepared via a seed-mediated growth process [36]. A typical AuNR solution containing nanorods with average dimensions 10 × 36 nm was prepared as follows: 0.25 ml of 0.01 M $HAuCl_4$ was added to 7.5 ml of 0.1 M CTAB solution. Then, 0.6 ml of ice cold 0.01 M $NaBH_4$ was added and the solution was inverted rapidly for 2 min, resulting in a pale brown color. The resulting seed solution then sat in a 35 °C water bath for 2 h before further use. Following seed preparation, 120 ml of growth solution was prepared in a conical flask, consisting of 0.1 M CTAB, 4.2×10^{-4} M $HAuCl_4$, and 1.25×10^{-4} M $AgNO_3$. To the growth solution, 1.6 ml of 0.1 M ascorbic acid was added, and the flask was swirled gently until the solution turned clear. Then 2 ml of the as-prepared seed solution was added. The growth solution was allowed to sit in a 35 °C water bath overnight before further use. The final solution of AuNR was reddish–brown in color and used without further purification.

2.4. Surface functionalization

The aqueous-organic ligand exchange procedure was adapted from methods previously described in the literature [37]. First, 20 ml of the as-prepared NP solution was incubated with 0.5 ml of dodecanethiol (DDT) in a 50 ml centrifuge tube, while shaking on a orbital shaker at 150 rpm for 20 min. Particles were transferred to the organic phase by adding alternating 5 ml aliquots of acetone and toluene to the DDT-NP mixture. After the second addition of acetone, the particles visibly rose from the aqueous phase into the toluene layer, resulting in a darker organic phase that was removed via a micropipette and transferred to a separate centrifuge tube. The stepwise transfer of the AuNP continued until the aqueous phase was clear. Care was taken not to remove any NP aggregates that may form at the aqueous-organic interface. The particles were then washed 4x in a 1:1.5 solution of toluene:MeOH via centrifugation and decantation (4000 rpm, 15 min). After each centrifugation cycle, the supernatant was discarded and the particles redispersed in 10 ml of toluene via brief sonication. The NP remained stable for at least one week, when stored at 4 °C in toluene, by visible inspection. In order to transfer the particles back to an aqueous phase, the DDT ligand was exchanged with 16-mercaptohexadecanoic acid (MHDA). In this process, 2 ml of 0.1 M MHDA solution was added to 18 ml of the DDT-AuNP in toluene and allowed to stir for 4 h (125 rpm, 25 °C). At the end of this period, all particles had precipitated at the bottom of the vial. The aggregate-toluene solution was then recovered from the vial and washed 3x in toluene via centrifugation (4000 rpm, 5 min). The AuNPs were then washed with isopropanol to deprotonate the MHDA (4000 rpm, 20 min), and then redispersed in H_2O or 10 mM phosphate buffer (pH 7.4). The MHDA-AuNP were stable for at least two weeks after dispersion, monitored through particle surface plasmon resonance (SPR). Surface chemistry was confirmed using XPS; 50 μl of AuNP dispersed in isopropanol was drop-cast onto a silica wafer. The isopropanol was allowed to evaporate and the sample was immediately analyzed.

2.5. Nanoparticle characterization

The final Au concentration in NP samples was determined using ICP-MS. Samples were prepared by allowing a small aliquot (50 μl) of particles to dissolve