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Linear self-assembly formation between gold nanoparticles and aminoglycoside antibiotics

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ABSTRACT

Ribostamycin is a broad-spectrum aminoglycoside antibiotic with a molecular weight of 454.5 g/mol. Under neutral pH conditions, ribostamycin is highly positive charged because it carries multiple amino groups in its structure. Negatively charged citrate ligand capped-gold nanoparticles (AuNPs) have been studied extensively for their interactions with a wide range of biomolecules including proteins, carbohydrates, and small drug compounds. These studies are aimed at developing new therapeutics and diagnostics by exploiting the unique properties of gold nanoparticles. Under this general aim, we studied the interaction between ribostamycin and AuNPs. Using a suite of analytical techniques including dynamic light scattering (DLS), UV–vis absorption spectroscopy, and dark field optical microscope imaging (DFM), we analyzed the mixture products of AuNPs with various sizes and ribostamycin under different concentrations. Our study revealed for the first time that ribostamycin has a tendency to self-assemble into linear oligomers at increased concentrations (above 250–500 μM). Such self-assembled oligomers then interact with negatively charged AuNPs to produce rod-like AuNP assemblies. Similar findings were observed from another structurally related aminoglycoside antibiotic, amikacin. It is technically challenging to detect and characterize oligomer formation of small molecules. It is especially challenging when the interactions that are holding the oligomers are not very strong. Through their interaction with gold nanoparticles that have exceptionally strong light scattering properties, we were able to observe the self-assembling of ribostamycin and amikacin in solution using various spectroscopic and microscopic techniques. This concentration-dependent self-assembling behavior of ribostamycin and amikacin may have direct relevance to the antibiotic effect of ribostamycin, amikacin and other structurally similar antibiotics.

1. Introduction

Ribostamycin is an aminoglycoside antibiotic agent derived from *Streptomyces ribosifidicus* [1,2]. It can be used to treat both Gram-negative and Gram-positive bacterial infection [3,4]. In comparison with other major aminoglycoside antibiotics, ribostamycin possessed comparable efficacy and weakened ototoxicity. It is also used to treat certain types of diseases, including pelvic inflammatory disease, cystitis, and upper respiratory tract infections [5]. The chemical structure of ribostamycin is shown in Fig. 1A. Under neutral pH condition, ribostamycin is heavily positive charged because of the presence of multiple amino groups in its structure. Ribostamycin is believed to bind with bacterial ribosomes, leading to mistranslation of mRNA, and ultimate killing of the bacteria [6,7]. Amikacin is another structurally related aminoglycoside antibiotic with similar biological activities (Fig. 1B). Because of their extensive clinical usage, there has been a growing concern over the increasing resistance of bacteria to aminoglycoside antibiotics. The need to further understand the molecular mechanisms of aminoglycoside antibiotics including drug resistance has propelled continuous study of these antibiotic agents both *in vivo* and *in vitro*.

Gold nanoparticles (AuNPs), especially the citrate ligand-protected, water-soluble AuNPs, are among the most extensively studied nanoparticle materials for biomedical applications [8–11]. For example, AuNPs that have been conjugated with antibodies have a long history of being used as gold immunoprobes in lateral flow immunoassay-based diagnostic devices such as home pregnancy testing dipsticks. AuNPs may also be used as carriers for

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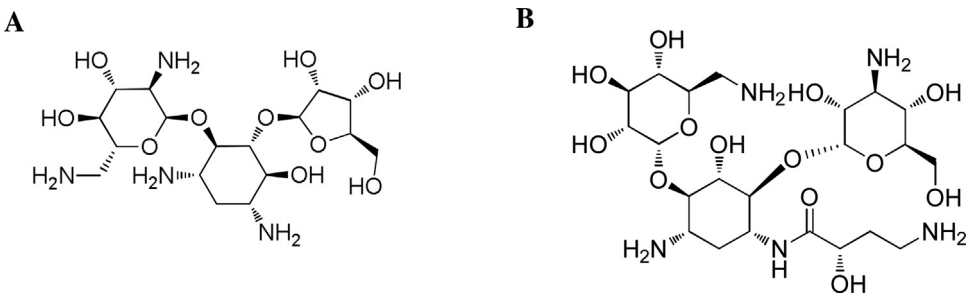

Fig. 1. Chemical structure of (A) ribostamycin molecule and (B) amikacin molecule.

drug delivery, or for targeted phototherapy [12–14]. Because of the strong light scattering property of AuNPs, they have been applied more recently in cell imaging using dark field optical microscope [15–18]. The combined use of AuNP probes with the dynamic light scattering technique has led to the development of a new analytical platform for chemical and biological target detection and analysis by monitoring AuNP aggregate formation upon binding with target analytes [19–28].

The use of AuNPs in all of these applications requires a more thorough understanding on the molecular interactions between various biomolecules and AuNPs. These studies are important for improving the activity and stability of the AuNP-biomolecule conjugate, controlling bio-retention and decreasing their toxicity *in vivo*. Indeed, the interactions between many biomolecules, such as proteins, DNAs, RNAs, and even small molecules with AuNPs have been extensively studied [29–33]. Most biomolecules bind with AuNPs through non-covalent interactions including electrostatic interaction, van der Waals interactions, as well as Au-S, Au-N bonding [34,35]. In one of our own recent studies, we revealed different binding modes occurring between bovine serum albumin, immunoglobulin G (IgG), immunoglobulin M (IgM), and the anionic biopolymer hyaluronan with citrate-capped AuNPs [36].

While continuing to study the interactions between different bio-relevant molecules with AuNPs, aminoglycoside antibiotics such as ribostamycin have attracted our attention. Because ribostamycin is an aminoglycoside, a polycationic amino sugar, it is natural to surmise that ribostamycin may adsorb to the negatively charged citrate-AuNPs through electrostatic interactions. A literature survey has returned almost no information on such studies. Only a few studies were reported on using AuNPs as a potential carrier of aminoglycosidic antibiotics, with an aim to improve the antibacterial efficacy of the antibiotics [37–39].

In our first attempt to study the ribostamycin-AuNP interactions by simply mixing ribostamycin with a 40 nm (diameter) citrate-capped AuNPs in aqueous solution, we discovered a very interesting self-assembled linear structure from such interactions. The anisotropic structure was confirmed by hydrodynamic diameter analysis of the mixture using dynamic light scattering, and the surface plasmon resonance of the AuNPs using UV–vis absorption spectroscopy. Encouraged by our initial finding, we extended the study to AuNPs with different sizes and ribostamycin under different concentrations. Additional analysis of the interaction product using dark field optical microscope (DFM) imaging further confirmed the presence of an anisotropic linear self-assembling structure. On the basis of these observations, we conclude that ribostamycin tends to form a concentration-dependent linear self-assembling, and such self-assembled oligomer structure further interacts with AuNPs through electrostatic interactions to produce a rod-like AuNP assembly. Similar findings were observed from the interaction product between AuNPs and amikacin.

To our best knowledge, this is the first time that oligomeric self-assemblies of aminoglycoside antibiotics were observed directly in aqueous solution. This finding could bear potential relevance to the biological function-structure relation of aminoglycoside antibiotics such as ribostamycin, amikacin and other structurally similar antibiotics.

2. Experimental methods

2.1. Chemical and materials

Citrate ligand-capped AuNPs with varied diameters (40, 60, 80, and 100 nm) were purchased from Ted Pella Inc. (Redding, CA). All the AuNPs were manufactured by British Biocell International (BBI). Corresponding concentrations of each AuNP solution are 9.00×10^{10}, 2.60×10^{10}, 1.10×10^{10}, and 5.60×10^{9} particle/mL for the 40, 60, 80, and 100 nm AuNPs, respectively. CTAB (cetyl trimethylammonium bromide)-stabilized gold nanorods was purchased from Nanopartz Inc. (Cat. No. A12-10-600, Loveland, Co). This GNR has an aspect ratio of 1.9. Both ribostamycin sulfate salt (156543, lot number QR11874) and amikacin (150342, lot number QR12294) were in the form of powder and purchased from MP Biomedicals (Solon, OH). Ribostamycin solutions as well as amikacin solutions at concentrations of 1 mM, 500 μM and 100 μM were prepared in nanopure water (Barnstead Nanopure Diamond Purifier: model # D11931) at room temperature.

2.2. Dynamic light scattering measurements

DLS analysis of all ribostamycin-AuNP solutions were performed using a Zetasizer Nano ZS90 DLS system equipped with a green laser (532 nm, 4 mW) and an avalanche photodiode detector (APD) (quantum efficiency >50% at 532 nm) (Malvern Instruments Ltd., England). The measured scattering light intensity is displayed as photon count rate with a unit of kilo count per second (kcps). To obtain an optimum photon count rate, the power of the incident laser beam needs to be adjusted due to the different scattered light intensities of AuNPs with different sizes. The incident laser power was adjusted to specific levels as needed by using a built-in attenuator. The attenuation level is indexed by an attenuation number corresponding to a particular attenuation level. The attenuation numbers 11, 10, 9, and 8 correspond to laser powers 4, 1.2, 0.4, and 0.12 mW, respectively. Attenuation numbers 10 and 9 were utilized for AuNP–40 nm and AuNP–60 nm studies, respectively. Both AuNP–80 nm and AuNP–100 nm were analyzed under an attenuation number of 8. The Malvern DTS 5.10 software was applied to process and analyze the data. All average particle sizes reported here are based on scattered light intensity weighted averages. For each sample solution, two DLS measurements were made with a fixed run time of 20 s. The scattering angle was set at 90°.

To initiate the study, 6 μL of ribostamycin or amikacin solution was mixed with 100 μL of AuNP suspension in an Eppendorf microtube. After 10 min of incubation at room temperature, the mixture solution was then transferred to a cuvette (catalog num-