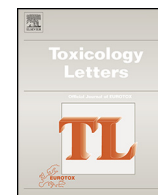




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Amino acid-dependent transformations of citrate-coated silver nanoparticles: Impact on morphology, stability and toxicity

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HIGHLIGHTS

- Different AAs have different effects on the physicochemical properties of the AgNPs.
- The change of physicochemical properties will affect the toxicity of AgNPs.
- There may be different transformations and toxicity behaviors for AgNPs in different cells and tissues.

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ABSTRACT

Humans face the risk of exposure to silver nanoparticles (AgNPs) due to their extensive application in consumer products. AgNPs can interact with many substances in the human body due to their chemically unstable nature and high activity properties, which might result in unknown hazards and even some serious diseases for humans. As the basic constituent element of human bodies, amino acids (AAs) differ in concentration and variety in different cells and tissues. Thus, understanding the transformation of citrate-coated AgNPs in the presence of AAs is crucial for determining their fate and toxicity in the human body. Our study focused on the transformation of the morphology, dissolution behavior and reaction product of AgNPs in different AA-containing systems and then evaluated the effect of these transformations on the cytotoxicity of AgNPs. The obtained results indicated that the addition of glycine with the lowest Ag^+ binding energy had little effect on the transformations and toxicity of AgNPs. While in the presence of histidine with higher Ag^+ binding energy, the Ag^+ release and particle size of AgNPs obviously increased. These transformations resulted in a decrease in the cytotoxicity of AgNPs due to the formation of Ag–His complex and the growth of AgNPs. Furthermore, L-cysteine with the highest Ag^+ binding energy could easily interact with AgNPs, transforming them completely to form $[\text{Ag}(\text{Cys})_n]^+$ and Ag_2S precipitates, which induced the largest decrease in AgNP toxicity. In summary, our results may provide useful information to understand the fate, transformation, and toxicity of citrate-coated AgNPs in the human body.

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1. Introduction

Nanomaterials provide novel prospects for commercial and scientific applications due to their specific physicochemical properties, which may differ from those of bulk substances (Burda et al., 2005). Currently, nanomaterials are widely used in our daily lives, such as in food, medicine, textiles and imaging (Barreto et al., 2011; Hong et al., 2012; Sanguansri and Augustin, 2006; Windler et al., 2013). Although these applications have provided many

benefits to humans, biologically adverse effects of nanomaterials must be evaluated carefully (Sharifi et al., 2012). However, to date, information about nanotoxicology remains incomplete (Lewinski et al., 2008; Winnik and Maysinger, 2012).

Currently, silver nanoparticles (AgNPs) are widely used in consumer products because of their excellent antibacterial, antiviral, and antifungal properties (Windler et al., 2013). However, AgNPs have been proven to be a potential threat to human health (Wijnhoven et al., 2009). In addition, many researchers have reported the toxicity of AgNPs toward both cells and mammals (Eom and Choi, 2010; Mukherjee et al., 2013; Nymark et al., 2012; Rahman et al., 2009). For example, Hackenberg et al. presented evidence that AgNPs can induce gene toxicity and cytotoxicity in

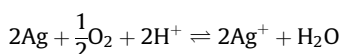
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human mesenchymal stem cells (Hackenberg et al., 2011). In addition, the research of Sung et al. also showed that exposure to AgNPs for 90 days can induce both an obvious decrease in lung function and the occurrence of inflammatory lesions in the lung (Sung et al., 2008).

Regrettably, many of the toxicity studies on AgNPs did not consider the transformations of AgNPs existing in cells and tissues, where AgNPs could be affected by many substances in the human body. Therefore, once AgNPs have entered into the human bodies, their composition, structure, and surface properties will be affected greatly, resulting in many changes in the physicochemical properties. This will inevitably affect their transport, reactivity, and toxicity. It is well known that amino acids (AAs) are the basic constituent element of human bodies, and they play a key role in growth and development (Wu, 2009). Many studies have indicated that several AAs can interact with Ag⁺ and that this interaction depends on the identity of the AA (Lee et al., 1998; Shoeib et al., 2002). The release of Ag⁺ has often been regarded as the most probable toxic mechanism of AgNPs (Liu and Hurt, 2010; Shi et al., 2013). Thus, interactions between AgNPs and various AAs are inevitable when AgNPs enter the human body, which affects the physicochemical properties and toxicity of AgNPs. However, to date, little attention has been focused on the interaction between AgNPs and AAs or on the possible toxic changes induced by this interaction. There are approximately 20 AAs serving as the building blocks of proteins in human bodies, and the concentration and variety of AAs are different in various tissues and cells (Rose et al., 1942; Wu, 2009). There may be different transformations and biological behaviors for AgNPs in different cells and tissues. Thus, it is very significant to investigate the interaction between AgNPs and AAs and to evaluate the transformation and cytotoxicity of AgNPs in the human body.

Citrate is one of the most common stabilizer in the synthesis of AgNPs (Zhang et al., 2011). The main focus of this study is first to determine the transformation of AgNPs when influenced by different AAs and understand the mechanism of this transformation and then to evaluate the toxicity of AgNPs when influenced by different AAs. As reported in previous studies (Lee et al., 1998; Shoeib et al., 2002), various AAs possess different relative Ag⁺ binding energies ($\Delta\Delta G_{Ag}$), indicating different reaction abilities between Ag⁺ and each AA. At the same time, many research studies have demonstrated that there is an equilibrium reaction between Ag⁺ and Ag⁰ in a AgNP solution, as follows (Liu and Hurt, 2010; Zhang et al., 2011):



This means that the existence of various AAs can break the equilibrium and induce different transformations of citrate-coated AgNPs. To further investigate the transformation of AgNPs with different AAs, three typical AAs, glycine (Gly), histidine (His) and L-cysteine (Cys), were chosen as the experimental model due to their common existence in the human body and their different relative Ag⁺ binding energies ($\Delta\Delta G_{Ag}^{Gly} < \Delta\Delta G_{Ag}^{His} < \Delta\Delta G_{Ag}^{Cys}$) (Lee et al., 1998; Shoeib et al., 2002). The transformation and the toxicity of AgNPs with these three AAs were investigated in detail to understand the potential risks of AgNPs in different cells and tissues.

2. Materials and methods

2.1. Materials

Silver nitrate, sodium citrate, sodium borohydride, glycine, histidine, and L-cysteine (with >99.0% purity) were purchased from

Sinopharm Chemical Reagent (Shanghai, China). Human umbilical vein endothelial cells (HUVECs) were obtained from the cell resource center, Shanghai Institutes for Biological Sciences (SIBS, China). Fetal bovine serum (FBS) and RPMI-1640 were purchased from Life Technologies (Gibco, USA).

2.2. Synthesis of AgNPs

AgNPs were prepared as reported previously (Chen and Carroll, 2004). Briefly, 3 mL of 10 mM NaBH₄ was injected into a stirred mixture containing 2.5 mL of 10 mM AgNO₃ and 100 mL of 1.25 mM sodium citrate. Then, the solution was stirred for 3 h at room temperature and aged for 7 days at 4 °C. Silver ions were removed by centrifugation using Amicon centrifugal ultrafilters with pore diameters of 1–2 nm (Millipore, USA). The concentration of AgNPs was determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Optima 7000DV, USA).

2.3. Cytotoxicity assay of AgNPs

HUVECs were cultured in RPMI-1640 containing 10% FBS and incubated at 37 °C in a CO₂ incubator (Heraeus, Germany). To determine the cytotoxicity of AgNPs, cell viability was assessed using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) (Dojindo Laboratory, Japan) according to the manufacturer's protocol. Briefly, cells were cultured in a 96-well plate with approximately 5000 cells per well. The cultured cells were treated with different samples (culture medium containing AgNPs or AgNPs and different AAs) after 12 h. Then, the cells were incubated for 24 h at 37 °C. Subsequently, a volume of 10 μL of WST was added to each well, and the plate was incubated for 4 h in a CO₂ incubator. Finally, the absorbance was measured at 490 nm using a microplate reader (Molecular Devices, SpectraMAX M5, USA), and the reference wavelength was 630 nm.

2.4. AA-dependent dissolution of AgNPs

AgNPs containing different AAs were prepared for Ag⁺ release experiments in ultrapure water. In this experiment, 1 mM was chosen as the experiment concentration for the AAs (Bergström et al., 1974). Then, the pH of the samples was adjusted to neutral (6.5–7.5) to be consistent with the pH of the human body (Zhang et al., 2006). Briefly, 10-mL samples were taken at different times and centrifuged (Heraeus, Germany) for 10 min at 4 °C (4500 × g) using fresh centrifugal ultrafiltration (Millipore, USA). Finally, the 3-mL filtrate was collected and mixed with 0.15 mL of 67% HNO₃ for inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx, USA) analysis to determine the Ag⁺ concentration.

2.5. TEM and EDX measurements

The change in the AgNP morphologies was determined using a transmission electron microscope (TEM, Hitachi, H-7650, Japan). AgNP samples in different AA solutions were deposited on a copper grid. Then, TEM images of the AgNPs were obtained. At the same time, energy dispersive spectroscopy (EDS) analysis was performed on the sample sections.

2.6. UV–vis, FTIR and XRD measurements

The ultraviolet–visible (UV–vis) spectra of the AgNPs were obtained using an ultraviolet and visible spectrophotometer (Thermo Scientific, USA) at different times and in the presence

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