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A comparative study of the antibacterial mechanisms of silver ion and silver nanoparticles by Fourier transform infrared spectroscopy

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ABSTRACT

In this study, we performed the first comparative study of the antibacterial mechanisms of silver ion (Ag^+) and silver nanoparticles (AgNPs) on *Escherichia coli* (*E. coli*) using Fourier transform infrared (FTIR) spectroscopy. Through a thorough analysis of the FTIR spectra of *E. coli* after silver treatment in the spectral regions corresponding to thiol group, protein, lipopolysaccharide (LPS), and DNA, we were able to reveal a multifaceted antibacterial mechanism of silver at the molecular level for both Ag^+ and AgNPs. Features of such mechanism include: (1) silver complexes with thiol group; (2) silver induces protein misfolding; (3) silver causes loss of LPS from bacterial membrane; (4) silver changes the overall conformation of DNA. Despite the similarities between Ag^+ and AgNPs with respect to their antibacterial mechanisms, we further revealed that Ag^+ and AgNPs display quite different kinetics for silver-thiol complexation and loss of LPS, with Ag^+ displaying fast kinetics and AgNPs displaying slow kinetics. At last, we proposed a hypothesis to interpret the observed different behaviors between Ag^+ and AgNPs when interacting with *E. coli*.

1. Introduction

Silver is perhaps the oldest antibacterial agent known by human being. Historical evidence can date back to around 400 BCE when Hippocrates, the Father of Medicine, described the use of silver for infection prevention and food preservation [1,2]. Today, in this antibiotic era, we still see silver-based antibacterial agents employed in a wide range of applications, such as wound dressing, medical implant, textile, cosmetics, food packaging, and domestic appliance [3–5]. Furthermore, silver's broad-spectrum antibacterial capability makes it a promising weapon against antibiotic-resistant bacterial strains. This point is well illustrated in a recent study where scientists show that the combined use of the antibiotic drug and silver ion (Ag^+) can significantly enhance the antibiotic susceptibility of a drug-resistant *Escherichia coli* (*E. coli*) strain [2].

In recent years, silver nanoparticles (AgNPs) have been increasingly used in medical and consumer products as an effective silver-based antibacterial agent [3,4,6]. The wide use of AgNPs has sparked great interests among scientists in the antibacterial mechanism of AgNPs. One interesting issue regarding the antibacterial mechanism of AgNPs is whether AgNPs exert identical antibacterial actions on bacterial cells like Ag^+ ions do. In fact, this issue has been under debate for more than a decade and it is still not fully resolved. As shown in two recent seminal works, in the work by Xiu et al., people investigated the antibacterial activity of AgNPs under aerobic environment and anaerobic environment [7]. They found that under anaerobic environment where Ag° oxidation and Ag^+ release are prohibited, AgNPs displayed no antibacterial activity. This observation supports the argument that Ag^+ ions dissociated from AgNPs contribute to the toxicity of AgNPs and implies that Ag^+ and AgNPs should have identical antibacterial actions; yet, in the work by Ivask et al., people used a genome-wide library of *E. coli* consisting of ~4000 single gene deletion mutants to elucidate the pathways involved in the antibacterial actions of Ag^+ (i.e., $AgNO_3$) and AgNPs. They found that Ag^+ and AgNPs affect cellular response pathways in *E. coli* differently [8].

In this study, we will perform a comparative study of the antibacterial mechanisms of Ag^+ and AgNPs on *E. coli* by Fourier transform infrared (FTIR) spectroscopy with the aim to gain more insights into the similarities and differences between Ag^+ and AgNPs regarding to their actual antibacterial behaviors. FTIR spectroscopy along with other vibrational techniques such as Raman spectroscopy has now developed into a valuable tool in

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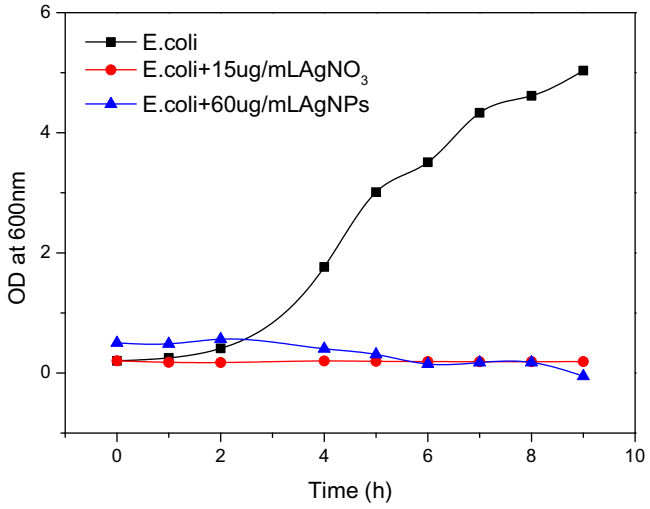

Fig. 1. Growth curves of *E. coli* in LB medium with and without Ag^+ and AgNPs treatments.

antibacterial mechanistic studies. Some pioneering works are briefly surveyed here. Nadtochenko et al. used attenuated total reflection FTIR (ATR-FTIR) spectroscopy to follow the structural changes of the *E. coli* cell membranes during TiO_2 photocatalysis [9]. They found that the C—H symmetric stretching band can be used as a valuable probe for photo-induced cell membrane damage and proposed the progressive membrane disordering in *E. coli* as the precursor event of the bacterial lysis. Saulou et al. used synchrotron FTIR microspectroscopy to study the antibacterial effect of $AgNO_3$ on *E coli* at single-cell scale. By analyzing the C—H stretching and protein spectral regions, they proposed that the inhibitory action of Ag^+ on *E. coli* was due to silver's effects on fatty acids and proteins [10]. Cui et al. performed an *in situ* study of the antibacterial mechanism of AgNPs on *E. coli* using surface-enhanced Raman (SERS) spectroscopy. Through the observation of the dramatic change in protein, hypoxanthine, adenosine, and guanosine bands, they suggested that AgNPs had a significant impact on protein and metabolic processes of purine [11]. Kardas et al. examined the antibacterial effect of cobalt using ATR-FTIR spectroscopy and revealed consistent, wide-spread changes in bacterial cell membrane, including a decrease in peptidoglycan content and increased lipid ordering of the membrane. They also observed a decrease in RNA and protein concentrations and slight alterations in DNA conformations due to cobalt effect on bacterial cells [12]. Neugebauer et al. characterized the influence of antibiotics on bacterial growth by means of UV resonance Raman spectroscopy. By monitoring the Raman scattering of the aromatic amino acids and the nucleic acid bases in bacterial cells, they were able to track the metabolic changes that occur during bacterial growth. With the help of chemometrical methods, they observed the small changes in the UV resonance Raman spectra due to the interaction between the antibiotic with bacterial cell [13]. Despite these previous antibacterial mechanistic studies using vibrational spectroscopic techniques, to our knowledge, there is no reported comparative study of the antibacterial mechanisms of Ag^+ and

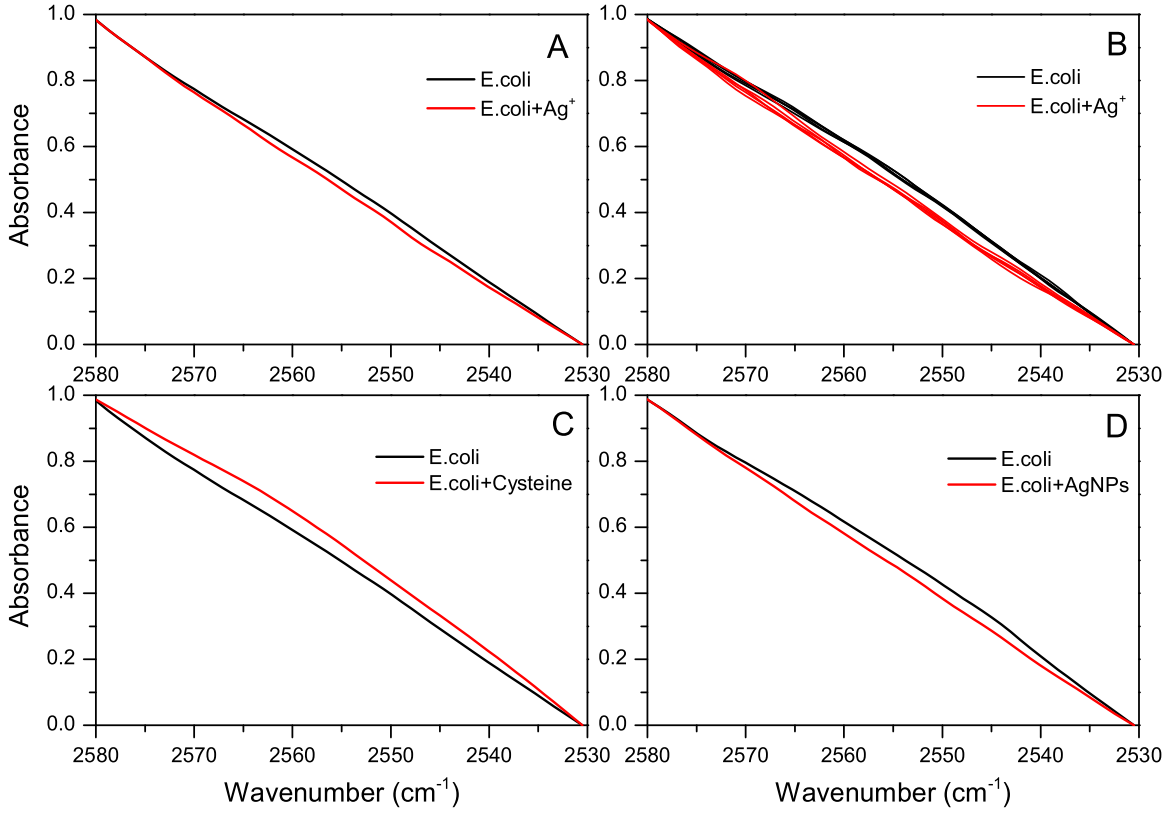

Fig. 2. FTIR spectra of *E. coli* in the spectral region of 2580–2530 cm^{-1} corresponding to the S—H stretching mode of thiol groups. (A) Spectra of the *E. coli* sample treated with Ag^+ and the *E. coli* control sample; (B) Spectra of the *E. coli* samples treated with Ag^+ and the *E. coli* control samples from five different experimental runs; (C) Spectra of the *E. coli* control sample with and without added cysteine; (D) Spectra of the *E. coli* sample treated with AgNPs and the *E. coli* control sample. The *E. coli* spectra were taken at the end of the four-hour incubation. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)