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NANODRUGS (ATY LAU, SECTION EDITOR)

Antibacterial Mechanism of Nanosilvers

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Abstract
Due to antibiotic abuse, multiple drug-resistant bacteria have become the most threatening killers in the clinic. The alternative metal drugs, silver nanoparticles (AgNPs), thus exhibit huge antibacterial potential. Many studies have proved that the antibacterial efficacy of AgNPs depends on their concentration, size, surface charge, and coated material. In recent years, with the development of omics technology including genomics, proteomics, and transcriptomics, several antibacterial molecular mechanisms of AgNPs are proposed. Collapsing cell membrane/wall; inducing ROS, photocatalytic protein damage, and genotoxicity; inhibiting the respiration chain; and interfering with protein biosynthesis and folding are considered as the main molecular mechanisms till date. However, there are contradictions among these views and a lack of the complete theory. A global understanding of the antibacterial mechanisms of AgNPs is necessary for wider application of AgNPs to the clinic.

Keywords Nanosilver · Antibacterial · Omics technology

Introduction

In recent years, drug-resistant bacteria have progressively become a major threat with the clinical abuse of antibiotics [1], and an increasing number of drug-resistant bacteria have been found, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococci* [2, 3]. The increasing spread of drug-resistant bacteria has posed several challenges to clinical treatment. Efficient drugs against infection with drug-resistant bacteria are thus an urgent need.

Silver has been known as an antibacterial material for more than a century. Ancient Egyptians and Romans used silver plates to boost the wound healing and treatment of ulcers [4]. Silver nitrate was applied for treatment of wounds by Paracelsus, who even used silver internally [5]. However, they did not know how silver nitrate could accelerate the healing of wounds until John Higginbottom suggested that silver nitrate can retard inflammation to alleviate complications and cure erysipelas in 1847 [6]. This phenomenon demonstrated that silver nitrate has antibacterial potential because erysipelas is caused by β-hemolytic *Streptococci* of group A [7]. Afterward, in 1849, silver nitrate was found useful to treat laryngeal ulcers [8]. The antibacterial properties of silver were reported in a study involving treatment of *Staphylococcus aureus* (*S. aureus*) with 0.5 to 2% silver nitrate solution [9]. Meanwhile, various forms of silver compounds also show antibacterial effects, including protargol, largin, ichthargan, albargin, argonin, and argentamine, which could inhibit *Neisseria gonorrhoeae* that causes conjunctival infection [10, 11]. Direct evidence of the effectiveness and safety of silver nitrate solution in the clinic was obtained by the observation that 0.5% silver nitrate solution could treat burns as it would not inhibit epidermal proliferation but could kill *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, and *S. aureus* [12].

However, silver has been progressively replaced by antibiotics since their emergence. Silver was then regarded as a positive control to measure the antibacterial activity of antibiotics such as penicillin [13]. Reliance on antibiotics increased because of their strong antibacterial activity. In recent years, because of this dependence, antibiotic abuse has emerged in the clinic, which has directly resulted in the appearance of antibiotic-resistant bacteria such as methicillin-resistant

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S. aureus (MRSA), penicillin-resistant *S. pneumoniae* (PRP), and vancomycin-resistant *enterococcus* (VRE) [3, 14]. Therefore, owing to the harsh situation of bacterial drug resistance, silver has again attracted attention as an antibacterial.

With the development of nanotechnology, nanomaterials have demonstrated powerful potential in a wide range of fields, including renewable energy, medicine, cosmetics, environmental remediation, and biomedical devices [15–17]. Thus, nanodrugs and metal drugs like nanosilvers (AgNPs) are strongly considered for use against drug-resistant bacteria. It has been identified that a composite of silver and nanomaterials shows vigorous antimicrobial activity [18, 19]. Furno et al. investigated that AgNP impregnation into medical polymers resulted in stable antibacterial activity, especially against drug-resistant bacteria [20]. Aymonier et al. in 2002 found that AgNP complexes exert significant antibacterial activity, which was modified by amphoteric hyperbranched macromolecules [21]. Nowadays, AgNPs are widely applied to the biomedical field because of their broad-spectrum antibacterial effects, for example, AgNPs are doped in the baseplates of orthodontic appliances to inhibit the multiplication of caries causing of bacteria [22].

Until now, AgNPs have been found to kill many species of bacteria including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhus*, *Enterococcus faecalis*, *Klebsiella* sp., *Listeria* sp., and *Acinetobacter* sp. [23–25]. Tables 1 and 2 list the inhibition and minimal inhibitory concentrations (MIC) of AgNPs for sensitive and drug-resistant bacteria, respectively. In addition, because of their characteristics, antibacterial drugs such as ampicillin (Amp), vancomycin, linezolid, nisin, sulfonamides, and quorum-sensing inhibitors are attached as ligands on the surface of AgNPs, which could enhance their effects against antibiotic-resistant bacteria [27, 28, 39–42]. These results indicate that AgNPs could improve the antibacterial efficiency of antibiotics. Furthermore, our group has an interesting phenomenon that superbugs are more sensitive to AgNPs [9].

With the development of omics techniques, many molecular mechanisms of AgNPs have been progressively reported. However, there are many controversial theories about the action mechanism of AgNPs. It is necessary to deeply investigate the mechanism of AgNP action to employ them properly in medicine. In this review, we focus on the recent studies related to the action mechanism of AgNPs, aiming to summarize and expound a complete mechanism to guide the improvement and utilization of AgNPs.

Bactericidal Mechanisms of AgNPs

The actual activity form of AgNPs is controversial as it is unclear whether AgNPs act by themselves or by releasing silver ions. Meanwhile, reactive oxygen species (ROS) are also claimed to be the key pathogen-killing mechanism induced by AgNPs whereas some propose contrasting opinions.

First, to confirm whether Ag^+ is the key antibacterial agent that can produce free oxygen radicals, *E. coli* was treated with AgNPs or silver nitrate, both in combination with *N*-acetyl-L-cysteine (NAC), an antioxidant. The result showed that the antibacterial effectiveness of both nanosilver and the Ag^+ processing group was decreased [43]. Another group used different size AgNPs to treat *E. coli* and *S. aureus* and measured the Ag^+ content of each sample at every 8 h using the anodic stripping voltammetry (ASV) method [44]. As the incubation time increased, the Ag^+ content also increased and the release concentration of Ag^+ exhibited a linear function with action time. In other words, as the action time is prolonged, Ag^+ is released from AgNPs during their activity against the test strain. Based on these results, the antibacterial activity was concluded to arise from the Ag^+ released from AgNPs. It thus seems that Ag^+ is the actual active form in the antibacterial activity of AgNPs.

ROS was also considered as a key factor for the antibacterial activity of nanosilver based on proteomics and bioinformatic approaches in *Pseudomonas aeruginosa* treated with

Table 1 Activity of AgNPs against sensitive bacteria

			Bacteria species	Zone of inhibition/MIC	Size of AgNPs	Reference
Kirby–Bauer disc diffusion method	G^+	1	*S. aureus* ATCC 6538	9.1 mm	7 nm	[26]
	G^-	2	*P. aeruginosa* ATCC9027	9.1 mm	7 nm	[26]
		3	*E. coli* MTCC 443	6.00 ± 0.24 mm	66.7–73.9 nm	[27]
Microdilution method	G^+	4	*S. aureus* ATCC 6538	3.0 ± 0.10 μg/mL	8 nm	[28]
		5	*Staphylococcus aureus* CCM 3953	3.38	26 nm	[29]
		6	*Enterococcus faecalis* CCM 4224	6.75 μg/mL	26 nm	[29]
		7	*Pseudomonas aeruginosa* CCM 3955	3.38 μg/mL	26 nm	[29]
		8	*B. subtilis* MTCC 441	30–50 μg/mL	5–20 nm	[30]
	G^-	9	*P. aeruginosa* ATCC 9027	2.5 ± 0.21 μg/mL	8 nm	[28]
		10	*Escherichia coli* CCM 3954	1.69 μg/mL	26 nm	[29]

Table 2 Activity of AgNPs against drug-resistant bacteria

			Bacteria species	Zone of inhibition/MIC	Size of AgNPs	Reference
Kirby–Bauer disc diffusion method	G^+	1	Multidrug-resistant *Micrococcus luteus*	11 mm	55–83 nm	[31]
		2	Multidrug-resistant *Klebsiella pneumoniae*	18 mm	55–83 nm	[31]
		3	Multidrug-resistant *S. aureus*	10 mm	55–83 nm	[31]
		4	Methicillin-resistant *S. aureus*	9–11 mm	5–20 nm	[32]
		5	Multidrug-resistant *S. pneumoniae*	21 mm	80–98.56 nm	[33]
	G^-	6	Multidrug-resistant *Salmonella typhi*	18 mm	80–98.56 nm	[33]
Microdilution method	G^+	7	Methicillin-resistant *S. aureus*	5.6 μg/mL, 15.6–62.5 μg/mL	10–30 nm, 15.11–16.54 nm	[34, 35]
		8	Erythromycin-resistant *S. pyogenes*	50–83.4 mM	100 nm	[36]
		9	*Enterococcus faecium* VRE	6.75 μg/mL	26 nm	[29]
	G^-	10	Multidrug-resistant *P. aeruginosa*	6.25–12.5 μg/mL	16–32 nm	[37]
		11	Multidrug-resistant *P. aeruginosa*	66.6–100 mM	100 nm	[36]
		12	Ampicillin-resistant *E. coli* O157:H7	66.6–100 mM	100 nm	[36]
		13	Multiple antibiotic-resistant *E. coli*	40 μg/mL	1.31–4.25 nm	[38]
		14	*Klebsiella pneumoniae* ESBL	6.75 μg/mL	26 nm	[29]

AgNPs [45]. In contrast, our group did not find increasing ROS and Ag^+ levels after co-incubation of AgNPs and *E. coli* for 30 min and 1 h [9].

Many factors influence the antibacterial efficacy of AgNPs including size, shape, charge, coating material, chemical stability, concentration, and other physicochemical properties of nanosilver [30, 46, 47]. The antibacterial efficacy of AgNPs is highly dependent on the particles' physicochemical properties, for example, adhesion reaction is the premise of their function, as AgNPs with positive surface charge can easily to adhere to the cell membrane of microorganisms with a negative charge [30]. Several AgNPs with different coatings were tested, among which positively charged coating could significantly increase the antibacterial efficiency of AgNPs [46]. Simultaneously, in the study of antibacterial activity, AgNPs are always exposed to a variety of liquid environments, both in vivo and in vitro, such as blood and culture media. Therefore, the chemical stability of AgNPs could be affected, which in turn could significantly affect the size, surface-to-volume ratio, and silver ion release kinetics of AgNPs, thereby affecting antimicrobial activity [46]. It is also claimed that plate and rod AgNPs show higher antibacterial activity compared to spherical particles [48, 49].

In summary, AgNPs with positive charge, chemical stability, high surface-to-volume ratio, and small size show high antibacterial activity. Furthermore, the comprehensive key action mechanisms of AgNPs are significant for their global application in a safe manner. Thus, we next focus on the molecular mechanisms obtained with omics technologies including cell wall/membrane damage, inhibition of the respiration chain and polypeptide synthesis, induction of genotoxicity and protein misfolding, ROS induction, and photocatalytic toxicity.

Collapsing Cell Membrane/Wall

The cell membrane/wall is the first line of defense to protect bacteria from external influences. The cell wall has different structures due to the differences in bacterial species, which are roughly divided into two types: gram-positive (G^+) and gram-negative (G^-) [50]. AgNPs can break the bacterial defense in two ways: disorganizing the cell wall and leakage of cytoplasmic content in G^+ bacteria, or changing the membrane permeabilsity of G^- bacteria [50]. Some studies on both *E. coli* (G^-) and *S. aureus* (G^+) showed that G^- bacteria were more sensitive to AgNPs than G^+ bacteria and some small holes and "pit" were observed on *E. coli* cells by electron microscopic after treatment with negatively charged AgNPs [51–53]. Several phenomenons including broking cell membrane, leaking cytoplasm, and increasing cell membrane permeability indicated that AgNPs infiltrate cells, release LPS and cell membrane proteins, and thus lead to cell death. According to these results, almost all *E. coli* cells exhibited varying degrees of cytoplasmic damage, regardless of whether the outer membrane was disintegrated. On the contrary, before cell wall disintegration, the cytoplasm of *S. aureus* did not show significant changes. The two kinds of bacteria have different cell wall structures; G^+ bacteria have a thicker cell wall that acts as a barrier to protect bacteria from the attacking AgNPs and maintains the cell membrane permeability [54, 55]. However, upon losing the protection of the cell wall, G^+ bacteria only have the cell membrane which easily undergoes breakdown. In contrast, G^- bacteria have three lines of defense which could greatly protect the cytoplasm from leaking. At the same time, Hwang et al. used a fluorescent *E. coli* sensitive to protein/membrane damage as a model and showed that nanosilver can damage proteins and membranes [56] through

free radicals that formed by AgNPs attacking the membrane proteins and causing lipid peroxidation, oxidative damage, and interference with the liquidity and stability of the cell membrane, finally killing the cells [57, 58].

One transcriptome analysis on *Bacillus cereus* ATCC14579 showed that many genes that encode osmoprotectant transporters, proton-dependent di-, tri-, oligopeptide transporters, and membrane proteins showed increased expression after AgNP treatment [59]. In other words, osmotic pressure changes with destruction of cell membrane and cell wall in the bacteria. Another proteomic study found that several envelope proteins were overexpressed, including OmpA, OmpC, OmpF, OppA, and MetQ in *E. coli* after AgNPs treatment, suggesting that OmpF and OmpC were the pathways used by AgNPs to enter G^- bacteria [18, 60]. According this result, the existing form of these proteins was investigated, which revealed that they existed in their precursor form, indicating that the proteins were released before losing their signal peptide in the inner membrane. A study confirmed this conclusion by knocking out these genes [60]. On the other hand, peptidoglycan synthase was found to be upregulated in *Bacillus thuringiensis* after incubation with AgNPs indicating that the synthesis of peptidoglycan was blocked [61].

AgNPs were thus confirmed to destroy the bacterial first line of defense by collapsing the cell membrane/wall, in two different ways: directly attacking the cell wall of G^+ bacteria or changing the membrane permeability of G^- bacteria which is already proven by observing the diversification of morphology of AgNP-treated bacteria and transcriptomics analysis. Two proteomic analyses confirmed that OmpF and OmpC were the main pathways through which AgNPs enter to G^- bacteria [18, 60].

Inhibiting Respiration Chain

The respiration chain provides ATP for bacteria to maintain normal biochemical reactions and growth. Determining the proteins interacting with AgNPs is important to learn how the AgNPs influence growth and their action mechanism and targets. However, interactions between silver ions and proteins are difficult to detect; in order to find the proteins directly binding with silver, Wang et al. established a unique system, namely liquid chromatography gel electrophoresis inductively coupled plasma mass spectrometry (LC-GE-ICP-MS), which identified 34 silver binding proteins [62]. Used in conjunction with the omics tool among them, the above partial proteins were found to be enriched in the TCA cycle (or Krebs cycle) and glyoxylate cycle. They found that the activity of isocitrate dehydrogenase (Icd) was decreased over 80% from 5 min to 1 h, and indicated that the enzymes involved in the TCA cycle were major targets of Ag^+. Whereas the activity of Icd was decreased, genes encoding isocitrate lyase (AceA) and malate synthase (AceB) were upregulated, converting the TCA cycle to the glyoxylate cycle. However, the activity of AceA and AceB was not increased because the adaptive glyoxylate cycle was also destroyed by Ag^+; finally, ATP would be exhausted in useless metabolic branches. Our group also found that differentially expressed proteins (DEPs) in AgNP-treated *E.coli* were significantly enriched in the TCA cycle [9]. One early study found another target of Ag^+ in the respiration chain [63]: the terminal oxidase cytochrome BD oxidase subunit II (CydB), whose inactivation could trap electrons and induce ROS.

Meanwhile, aerobic respiration was regarded as the target of AgNPs. Transcriptome analysis of *E. coli* and a proteomic research of *S. aureus* ATCC 6538P indicated that many anaerobic respiration-related reductases (such as Formate acetyltransferase) were upregulated upon AgNP treatment, whereas aerobic respiration-related oxidases (such as glycerol-3-phosphate dehydrogenase) were suppressed [64, 65].

Thus, AgNPs lead to bacterial cell death by damaging the respiration chain, leading to energy exhaustion in the bacteria cell, which was proven by both proteomic and transcriptome analysis. Compared with collapsing cell membrane/wall, inhibition of the respiration chain seems more hazardous to bacterial cells and shows the huge antibacterial potential of AgNPs.

Inducing Genotoxicity

Genotoxicity is the most deathful action to bacteria, usually through inhibiting the synthesis and replication of DNA and damaging DNA via damaging the related protecting proteins. For direct evidence to prove that AgNPs can cause genotoxicity, Wang et al. first found that SodA and Dps as DNA protecting proteins were classified as AgNP-binding proteins [62, 66]. They then discovered that the levels of adenosine monophosphate (AMP), inosine-5′ monophosphate (IMP), hypoxanthine, guanosine, and uridine were decreased by Ag^+ exposure.

Besides, DNA fragmentation was observed when AgNPs existed in *E. coli* and *S. aureus* [53, 64, 67]. Other than this method to generate genotoxicity, AgNPs also block the synthesis of DNA [68, 69]. It has been shown that AgNPs stimulate *E. coli* cells and induce filamentation, a marker of cell division arrest in *E. coli* [70, 71]. Therefore, Bao et al. used the BrdU dye to detect the newly formed DNA levels in cells upon exposure to AgNPs and observed that higher AgNP concentration resulted in lower rates of new DNA synthesis [68]. Related to the above study, *Corynebacterium glutamicum* showed the upregulation of DNA repair proteins by proteomics analysis after AgNP treatment [72] and the expression of many cell division proteins was decreased in *Bacillus thuringiensis* [73].

The above results indicate that AgNPs could induce genotoxicity in bacteria by blocking mitosis through

inhibiting the synthesis of newborn DNA and by binding with DNA-protecting proteins resulting in loss of their activity.

Interfering with Protein Biosynthesis and Folding

Some drugs can block bacterial growth by binding with the active sites or directly inhibiting the biosynthesis and folding of proteins. Misfolding can lead to improper functioning of the newly formed polypeptide chains. Proteomics analysis of *Pseudomonas* after exposure to Ag^+ or AgNPs showed that 12 proteins involved in translation were overexpressed, in which 8 proteins were overexpressed upon treatment with AgNPs but reversed upon treatment with Ag^+ [74]. These proteins are mainly involved in the assembly of small or large ribosomal subunits. At the same time, the content of ribosome was increased (did not show the detecting result) [9, 74]. A transcriptomics study showed that genes of several protein chaperones including GroEL, GroES, DnaJ, DnaK, and DnaE and proteolytic enzymes like htpX, hflK, and hflX, which promote protein folding, were upregulated after treatment with AgNPs [59, 74, 75].

These results conclude that AgNPs interfere with the synthesis of polypeptide chain and promote polypeptide misfolding. Bacteria fight against AgNPs stress by overexpressing the related proteins participating in protein biosynthesis and folding.

ROS Induction

ROS is the general name for oxygenates such as superoxides, hydrogen peroxide, and hydroxyl radical that could trigger a variety of cellular biological reactions [76]. Excessive ROS can cause lipid peroxidation, enhancement of membrane permeability, DNA and RNA damage, impairment of oxidative phosphorylation processes, and ATP decrease, leading to changes in the direction of electron transport in the respiratory chain [53, 62, 68]. Excess ROS has been demonstrated in a variety of cell models after exposure to AgNPs [77].

GSH participates in the redox process in organisms and can bind peroxides or free radicals to protect the sulfhydryl groups of sulfhydryl proteins or enzymes on the membrane from oxidative damage and avoid free radical attack on important organs [78, 79]. When GSH is oxidized, GSSG is produced. Thus, GSSG could reflect the consumption of GSH. The content of GSSG increased with increasing ROS in AgNP-treated *Phanerochaete chrysosporium* [78] and enhancing toxicity induced by ROS.

Liao et al. showed that ROS induced by AgNPs could be eliminated after adding antioxidants like GSH and that bacteria could then resist the toxicity of AgNPs [79, 80]. The most attractive result showed that the activity of catalase (CAT) and peroxidase (POD) was notability decreased and that the activity of superoxide dismutase (SOD) was distinctly high in multidrug-resistant *Pseudomonas aeruginosa* after AgNPs treatment. SOD, CAT, and POD are antioxidases that can clear ROS and are the first line of defense against oxidative stress. Among them, SOD promotes the conversion of highly toxic O_2^- to H_2O_2, which is degraded into H_2O and O_2 by CAT and POD. So, AgNPs induce ROS in bacteria by suppressing the activity of CAT and POD and increasing SOD.

Theoretically, to counter the toxicity of accumulated peroxides, bacteria should remove peroxides by antioxidant reactions. Some proteomics research has found these countermeasures of bacteria. Many proteins were involved in antioxidants such as methionine suffix reductase A (MsrA), which belongs to the Msr enzyme family, and were overexpressed in AgNP-treated *S. aureus* [61, 75, 81]. The transcriptional regulatory gene, soxS, was found to be significantly (600-fold) upregulated in *E. coli* on a gene expression microarray [75]. Once this is induced, the antioxidant reaction is initiated.

Another countermeasure to eliminate the peroxides in *S. aureus*, the Thioredoxin Reductase−Thioredoxin System, composed of NADPH, thioredoxin reductase (TrxR), and thioredoxin (Trx), is irreplaceable for *S. aureus*, which lacks a natural glutathione/glutaredoxin (Grx) system [82]. Previous studies have shown that AgNPs mainly act on TrxR and Trx by binding with their redox active site—the thiols of proteins [83, 84].

However, Wang et al. held the opposite opinion that metal–protein interactions were too complicated to obtain the direct evidence like characterization of the binding protein to prove the above theory [62]. Further investigation is needed to learn about the binding sites of AgNPs and proteins. They thus proposed that silver ions induced ROS at a later stage of its toxicity. Other studies also confirmed this conclusion about the period of ROS action by detecting the level of ROS when bacteria were co-incubated with AgNPs, which was significantly increased after 5 h and 12 h treatment [53, 85].

AgNPs induce ROS by blocking the key antioxidase, resulting in peroxide accumulation. However, how the AgNPs inhibit the activity of these enzymes was an unsolved puzzle, until our group found that AgNPs induced photocatalytic protein damage [9].

Inducing Photocatalytic Protein Damage

In recent years, our group first suggested that a novel key mechanism of the antibacterial effects of AgNPs was photocatalytic protein damage induced under visible light rather than ROS damage [9]. Moreover, AgNPs carry out their antibacterial function via directly binding to proteins, helping the light catalyzed oxidation of cellular proteins, which circumvent the bacterial protection mechanisms. AgNPs absorb the energy from visible light, and their catalytic activity is excited, which catalyzes the redox reaction of proteins and intracellular oxides. According to this result, the antibacterial activity of

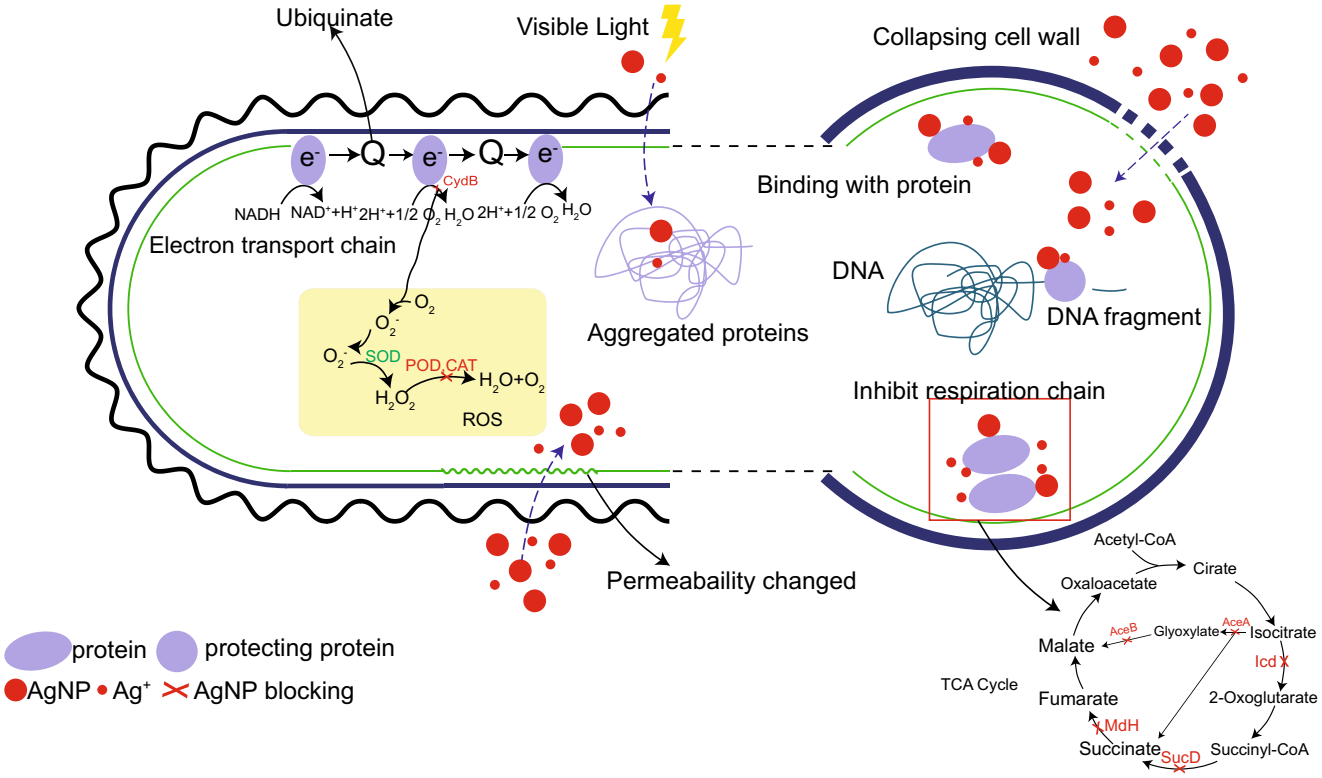

Fig. 1 Antibacterial mechanism of AgNPs

AgNPs is dependent on light intensity, showing a positive correlation. On the other hand, ROS was not increased after treatment of AgNPs with 15 min and 30 min in *E. coli*; thus, we suggested that oxidative stress is not the main bacterial mechanism of AgNPs during the early stage. The binding assay indicated that AgNPs can directly bind with proteins, depending on the shape of the AgNPs, the surface heterogeneity, and the quaternary structure of the protein [86]. After interaction in the cell, the physiological functions of the metal–protein complex could be seriously damaged. This direct physical mechanism is unlikely to be counteracted by any known drug resistance mechanism of bacteria and therefore may serve as a last resort against drug resistance and viable option for the treatment of multidrug-resistant bacteria.

Thus, UV light as well as visible light could motivate the antibacterial activity of AgNPs, which expands their scope of application. AgNPs can absorb energy from visible light and transfer it to their binding proteins, making them lose their physiological activity, finally enhancing the efficiency of other antibacterial mechanisms.

Conclusion

In this review, we summarized the six proposed antibacterial mechanisms of AgNPs (Fig. 1). However, the main antibacterial mechanism of AgNPs is still controversial, as it is uncertain whether ROS or photocatalysis is primarily responsible for bacterial death. According to the above summary, we speculate that photocatalysis is an early mechanism and ROS acts at the later stage of AgNP toxicity. We also know how ROS is produced and accumulated in bacterial cells exposed to AgNPs for 90 min and 12 h [74, 79, 85]. That is why our group did not detect the increasing ROS in *E. coli* after AgNP treatment to *E. coli* for 15 min and 30 min [9]. It thus seems that the content of ROS is related to the time of treatment.

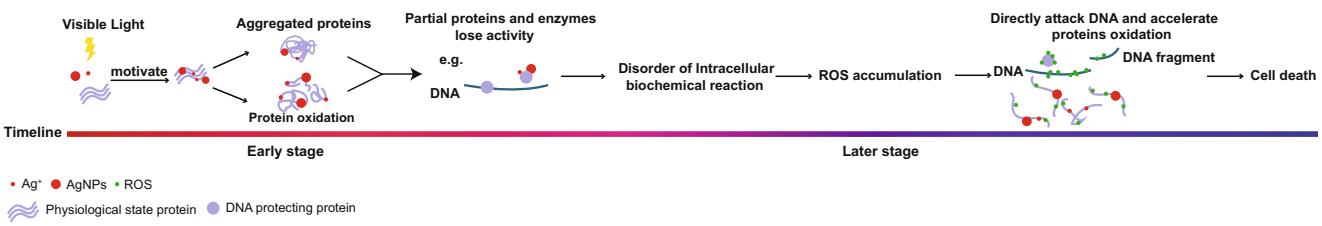

Fig. 2 Antibacterial sequence-wise mechanism of AgNPs

If our hypothesis is established, we could draw a slightly complete antibacterial mechanism of AgNPs: first, AgNPs absorb energy from visible light and cause protein aggregation or bind with proteins to disrupt their activity and thus damage the bacterial cell wall or cell membrane or change their permeability. Then, antioxidases lose their activity and cause H_2O_2 accumulation in the cell. At the same time, AgNPs destroy the respiration chain by blocking the enzymes of the TCA cycle and inducing the adaptive glyoxylate cycle, which is also destroyed by AgNPs, resulting in ATP exhaustion. Meanwhile, AgNPs also inhibit the biosynthesis of DNA, causing the genotoxicity and impaired biosynthesis or folding of polypeptides. In the end, excessive peroxide induces the high formation of ROS that further damages the cell wall/membrane, DNA, enzymes, respiration chain, and so on (Fig. 2). However, the proposed complete antibacterial mechanism of AgNPs still needs more research to provide direct evidences and will promote the application of AgNPs in clinical treatment.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent The article does not contain any studies with human or animal subjects performed by any of the authors.

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