Review Article
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Antibacterial mechanism of Ag^+ ions for bacteriolyses of bacterial cell walls via peptidoglycan autolysins, and DNA damages

Abstract

Antibacterial mechanism of bacteriolyses and destructions of bacterial cell walls by silver(I) ions has been considered against *Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli)*. Bacteriolysis against *S.aureus* peptidoglycan (PGN) cell wall by Ag^+ ions is due to inhibition of PGN elongation caused by regulation of PGN synthetic transglycosylase (TG) and transpeptidase (TP), and enhancement of the activation of PGN autolysins of Amidases. On the other hand, bacteriolysis and destruction against *E. coli* cell wall by Ag^+ ions are caused by the destruction of outer membrane structure due to degradative enzymes of lipoproteins at N- and C-terminals, and by the inhibition of PGN elongation owing to inactivation of PGN TP synthetic enzyme endopeptidase and enhancement of the activations of PGN hydrolases and autolysins of Amidase, Peptidase, and Carboxypeptidase. Silver ions induced ROS generations such as O_2^-, H_2O_2,$^+$OH, OH^- producing in bacterial cell wall occur and lead to oxidative stress. DNA damages may be due to Ag^+-coordinated complex formations by Ag^+ substitution within double and triple hydrogen bonds in DNA base pairs.

Keywords: Silver (I) ions, PGN cell wall, outer membrane lipoproteins, bacteriolysis, hydrolase and degradation, PGN synthesis and autolysin, reactive oxygen species (ROS), DNA base-pairs

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Abbreviations: ABNC, active but nonculturable; A, adenine; AgNPs, silver nanoparticles; C, cytosine; *E.coli*, *Escherichia coli;* G, guanine; LP, lipoprotein; LPS, lipopolysaccharide; MTs, metallothioneins; NAG, N-acetyl glucosamine; NAM, N-acetyl-muramic acid; OM, outer membrane; Omp, outer membrane protein; PGRPs, Peptidoglycan Recognition Proteins; Pal, Protein-associated lipoprotein; PGN, peptidoglycan; ROS, reactive oxygen species; *S.aureus, Staphylococcus aureus,* T, thymine; TG, transglycosylase; Tol, tol protein; TP, transpeptidase

Introduction

Silver of transition metal has highly antibacterial activities and is widely utilized as chemotherapy agents. Increasing use of silver as an efficacious chemotherapeutic antibacterial and antifungal agent in wound care products, medical devices textiles, cosmetics, and even domestic appliances in recent years has led to concern as to the safety aspects of the metal and potential risks associated with the absorption of the biologically acting Ag^+ into the human body.[1] Released biologically active Ag^+ shows a strong affinity for sulphydryl groups and other anionic ligands of proteins, cell membranes, and debris that Ag^+ binds protein residues on cell membranes of sensitive bacteria and is absorbed intracellularly by pinocytosis which concentration of 60 ppm Ag^+ should be sufficient to control the majority of bacterial pathogen.[1] Silver exists as silver metal and silver ions of different oxidation states of +1, +2, +3, and +4 that the most common states of silver are silver(0) metal and silver(I) ion and both of them interact with thiols in no redox reaction involved that Ag^{2+}, Ag^{3+},and Ag^{4+} form state are not of relevance for aqueous solutions and under environmental and biological conditions.[2] Recently, with proceeding development in nanotechnology, silver nanoparticles (AgNPs) call attention to potential treatments such as food storage by broad antibacterial effects, prevention of serious diseases, and medical applications.[3] The toxicity of AgNPs is mainly due to release to free silver ions. On the other hand, the antibacterial activity and mechanism of action have been gradually clarified that silver ions may cause *Staphylococcus aureus* (*S. aureus)* and *Escherichia coli* (*E.coli)* bacteria to reach an active but nonculturable (ABNC) state and eventually die, and also have been indicated to the mechanism of inactivation of pathogens by damages and destruction of the bacterial cell membrane.[4,5] The high antibacterial activity factor of Cu^{2+}, Zn^{2+} ions may be thought to be caused by binding bacterial surface proteins, cell membrane, and metal-binding complex formations.[6] However, bactericidal elucidation by metal-binding enzyme degradation due to inhibition of peptidoglycan (PGN) elongation and relationships between PGN synthesis and PGN hydrolase and autolysin has been still remained.

In this review, from the standpoint of bacteriolysis and destruction of bacterial cell wall by imbalance between PGN synthesis and PGN hydorolase/autolysin, antibacterial mechanisms of silver(I) ions could been elucidated against Gram-positive *S. aureus* thick PGN layer cell wall and Gram-negative *E. coli* outer membrane-connecting thin PGN layer cell wall, with additional DNA damage by Ag^+-DNA base pairs interactions.

Molecular structure of *S. aureus* and *E. coli* cell walls, PGN syntheses of both transglycosylase (TG) and transpeptidase (TP), and PGN autolysins

The surface envelop cell structures of *S.aureus* as representative of Gram-positive bacterium and *E.coli* as representative of Gram-negative bacterium, molecular structures of these cell walls, molecular structure of PGN, and PGN biosyntheses and autolysins were searched in detail. Further, the reaction and the behavior of

metallic ions and bacterial cell, and molecular bonding manner also were searched. *S. aureus* surface layer consists of teichoic acids, lipoteichoic acids, and thick PGN envelope cell wall.[7] In the molecular structure of *S. aureus* PGN cell wall, there are the action sites of TG synthetic enzymes of *N-acetylgluco-samidase* cleavage between NAG (N-acetylglucosamine) and NAM (N-acetylmuramic acid), and N-acetylmurami-dase cleavage between NAM and NAG on glycan chain, and TP synthetic enzyme cleavage between Glycine and D-alanine on PGN crosslinking. And there are PGN autolysins of N-acetylmuramyl-L-alanine amidase cleavage, *DD-endopeptidases* cleavages between Glycine and Glycine on pentaglycine (Gly), and in addition, *lysostaphin* cleavage between Glycine and Glycine on PGN cross-linking.[8]

On the other hand, *E. coli* cell wall consists of lipid A, lipopolysaccharide, porin proteins, outer membrane of lipo-protein, and thinner 2-7nm PGN layer in 30-70nm periplasmic space.[7] Degradative enzymes of lipoproteins at N- and C-terminals are *Endopeptidase* between phospholipid Lipoprotein bond and *Amidase* between L-Ala-NAM bond via *E.coli* outer membrane, lipoprotein to PGN. In the molecular bonding manner of *E.coli* cell wall and peri-plasmic PGN, there are *E.coli* PGN synthetic enzymes TG of *Glucosaminidase* cleavage, *Muramidase* cleavage on glycan chain, and TP of *Endopeptidase* cleavage on cross-linking, and the PGN autolysins of the hydrolases and degradative enzymes of *Amidase* cleavage, *Peptidase* cleavage, and *Carboxypeptidase* cleavage. Interactions of PGN molecular structure with PGN syntheses and PGN autolysins influence in any event the bacteriolytic cell walls.[8]

Discussions

S.aureus PGN synthetic enzymes of TG and TP

The released Ag^+ ions from AgNP penetrate into bacterial cells, can inhibit the growth of Gram-positive *B. subtilis* bacterium which exerts toxicity by damaging cellular membrane, degrading chromosomal DNA, lowering reductase activity, and reducing protein expression.[9] Wall teichoic acids are spatial regulators of PGN cross-linking biosynthesis TP,[10] and silver ions could inhibit both TG and TP enzymes of the PGN that Ag^+-induced bacteria may inactivate PGN synthesis transglycosylase TG[11] and transpeptidase TP.[12,13] Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM may function as TP enzyme.[14]

Ag^+ induced amidase of *S.aureus* PGN autolysins

Lytic activity was inhibited by glucosamine, NAG, Hg^{2+}, Fe^{3+}, and Ag^+,[15] and Ag^+ binding Rv3717 showed no activity on polymerized PGN and but, it is induced to a potential role of N-Acetylmuramyl-L-alanine Amidase,[16] PGN murein hydrolase activity and generalized autolysis; Amidase MurA,[17] Lytic Amidase LytA,[18] enzymatically active domain of autolysin LytM,[19] metal-dependent metalloenzyme AmiE,[20] as prevention of the pathogen growth. The activations of these PGN autolysins could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis of *S. aureus* PGN cell wall. $O_2^-$and H_2O_2 permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress.[21]

Bacteriolysis of *S.aureus* PGN cell wall by silver ions

For the sake of growth of *S. aureus* PGN cell wall, there is necessarily required for the adequate balance between PGN synthesis and PGN autolysin. When the balance was broken to be imbalanced, bacteriolysis and destruction of the cell wall should occur. Hence, it became apparent that bacteriolysis of *S. aureus* PGN cell wall by Ag^+ ions is caused by inhibition of PGN elongation due to inactivation of PGN TG or TP and enhancement of activation of PGN autolysins of amidases.

Production of reactive oxygen species (ROS) against *S.aureus*

For the penetration of Ag^+ ions to PGN cell wall, the ROS production such as superoxide anion radical O_2^-, hydroxyl radical ·OH, hydrogen peroxide H_2O_2 occurred from superoxide radical O_2^-molecular.[22]

$$O_2 + e \rightarrow O_2^-$$

$$O_2 + e\text{-} + H^+ \rightarrow \cdot HO_2$$

$$H_2O \rightarrow \cdot OH + \cdot H + e\text{-} \rightarrow H_2O_2$$

$$HO_2 \rightarrow H^+ + O_2$$

$$\cdot O_2^- + 2H^+ + e\text{-} \rightarrow H_2O_2$$

$$H_2O_2 + e\text{-} \rightarrow HO^- + \cdot OH$$

$$\cdot OH + e\text{-} + H^+ \rightarrow H_2O$$

$$2H^+ + 2 \cdot O_2^- \rightarrow H_2O_2 + O_2$$

From above-mentioned results, antibacterial activities for bacteriolytic process of *S.aureus* PGN cell wall by Ag^+ ions are shown in Table 1.

Permeability of silver ions into *E.coli* cell wall

E.coli cell wall is comprised of Lipopolysaccharide (LPS), lipoproteins (LP), and peptidoglycan (PGN) as thinner layer within periplasmic space. When permeability of silver ions in the *E.coli* cell wall, highly anionic LPS with hydrophobic lipid A, core polysaccharide, O-polysaccharide, is liable to be explosive, inhibition of LPS biosynthesis may be possibility to occur by active hydrolases.[23] The OmpA, OmpC, OmpF porins of lipoproteins have metallic cation selective and hydrophilic membrane crossing pore, to be effective for silver transfer.[24] Ag-resistant mutants of *E.coli* display active efflux of Ag^+ and are deficient in porins that active efflux may play a major role in silver resistance, which is likely to be enhanced synergistically by decreases in OM permeability.[25] Physicochemical interaction of *E.coli* cell envelopes suggested that the adsorption of the cell wall or envelope to clay has masked or neutralized chemically reactive adsorption sites normally available to metal ions that metal binding capacity of metal cation bridging in isolated envelopes was determined by atomic adsorption spectroscopy.[26] Silver adsorption by *E.coli* cells displays metallothioneins(MTs) anchored to the outer membrane protein Lam B that the complete MT sequences are anchored by their N-termini and C-termini to the permissive site 153 of the protein.[27] Recently, Ag^+ ions into *E. coli* cell wall are elucidated to be occurred *E. coli* under ionic silver stress which Ag^+-dependent regulation of gene expression is transpeptidase acting on the structural integrity of the cell wall.[28] The addition of glucose as an energy source to starved cell activated the Ag efflux on the increased Ag accumulation in Ag-susceptible and -resist-ant strain. Silver(I) ions reactive with thiol, and then generates silver(I) thiolate compounds. Silver ion complexes with both inorganic and organic thiols with redox reaction involved that with inorganic thiols like $HS^-$and S^{2+}, it is possible to form many

species such as AgSH, $[Ag(SH)_2]^-$and $[Ag_2(SH)_2S]^{2-}$depending on the concentration of the anions present.[29]

$$Ag^+ + (\text{-}SH)^- \rightarrow AgSH \rightarrow [Ag_2(SH)_2S]^{2-}$$

Destruction of outer membrane structure of *E.coli* by hydrolases of lipoproteins at C- and N-terminals

Tol protein $(Tol)^-$protein-associated lipoprotein (Pal) system is composed of five proteins that TolA, TolQ, and TolR are inner membrane proteins, TolB is a periplasmic protein, and Pal, the peptidoglycan-associated lipoprotein, is anchored to the outer membrane.[30] Ag^+ ions induced Tol-Pal complex is antimicrobial agents widely used, it has recently been demonstrated to be essential for bacterial survival and pathogenesis that outer membrane structure may be destroyed.[31,32] It is unclear whether both Amidase and Endopeptidase of lipoprotein at C-, and N-terminals are simultaneously activated by Ag^+ ions. However, outer membrane may be considered to be destroyed probably by predominant activation of lipoprotein-amidase.

Damage of *E.coli* PGN synthetic enzyme of silver-protein amidase in periplasmic space, and amidase, peptidase, and carboxypeptidase of PGN autolysins

Silver ions may be accumulated in *E.coli* periplasmic space, in which the silver ions are spent to the activation of bacteriolysis of the cell wall and efflux activity to extracellular cell. Then, lipoprotein-endopeptidase may be degradative by Ag^+ binding proteins.[33] The other, it is unclear that the silver-induced PGN biosyntheses TG/TP should be inhibited by the silver ions.[34-36] However, silver ions inactivate TP of endopeptidase by because of destructive observation of bacterial cell walls.[28] Silver ions could activate *E.coli* PGN autolysins of amidase, peptidase, Carboxypeptidase,[37,38] such as silver depending PGN autolysin, AmiC,[39] Ami D,[40] Muramidase,[41] Amino acid amidase,[42] Carboxy-peptidase A,[43] zinc metalloenzymes Ami D,[44] Amidase zinc-containing amidase; Amp D,[45] zinc-present PGLYRPs,[46] Carboxypeptidase-degraded aldolase,[47] CarboxypeptidaseY,[48] serve to be effective for the PGN autolysins. It is particularly worth noting that enhancement of the activities of autolysins is characterized on PGN carboxypeptidase and TP-endopeptidase,[37] requiring divalent cations. Accordingly, the inhibition of PGN elongation had occurred by silver ion induced activities of PGN hydrolases and autolysins. Thus, antibacterial mechanism is found that bacteriolysis and destruction of *E.coli* cell wall by silver Ions are caused by the destruction of outer membrane structure owing to the activation of Amidase of lipoprotein at C-, and N-terminals, and inhibition of PGN elongation due to the damage of PGN synthetic enzyme of silver-protein Amidase in periplasmic space, and PGN autolysins of Amidase, Peptidase, and Carboxypeptidase.

ROS production and oxidative stress against *E.coli*

Silver ions reacted with -SH, and H^+ generates. In *E.coli*, free radicals O_2^-,OH^-, $^{\cdot}OH$ and H_2O_2 are formed as follows.[49]

$$O_2 + e \rightarrow O_2^-$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

$$O_2^- + H_2O_2 \rightarrow OH^- + {}^{\cdot}OH + O_2^-$$

In cell wall, reacting with polyunsaturated fatty acids:

$$LH + OH^{\cdot} \rightarrow L^{\cdot} + HOH$$

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

$$LH + LOO^{\cdot} \rightarrow L^{\cdot} + LOOH$$

Ag^+-containing Peptidoglycan Recognition Proteins (PGRPs) induce ROS production of H_2O_2, O^-, $HO^{\cdot}$, and then the ROS occur the oxidative stress, and killing by stress damage.[50] As above-mentioned, antibacterial activities of Ag^+ ions for bacteriolysis and destruction of *E.coli* cell wall are summarized in Table 2.

Damage within DNA base-pairs

Ag^+ ion induced occurrence of generations of ROS and hydrogen peroxide H_2O_2 in bacterial cells and DNA, in which formation of DNA damage resulting from a release of catalytic binding of zinc ion to DNA with generation of $^{\cdot}OH$ radicals, and by reaction of H_2O_2 with the metal produces the strand breaks in DNA as well as DNA base-pairs modifications and deoxyribose fragmentation. Transfer of Ag^+ ions into triple and double hydrogen bonds within DNA base-pairing G(guanine)≡C(cytosine) and A(adenine)=T (thymine) pairs occurs by Ag^+ ion substitution shown in Figure 1**.** Thus, it may be considered that DNA damages due to Ag^+ two-coordinated complex formations within DNA base-pairs of triple hydrogen bond G≡C and double hydrogen bond A=T are subjected in ground state. A=T base pairs are less stable than G≡C base pairs in Ag^+-DNA reaction.[51]

Table 1 Antibacterial activities of Ag^+ ions on *S.aureus* cell wall

Ag^+ ions		*S.aureus* Cell Wall
	Teichoic acid, Lipoteichoic acid	Peptidoglycan layer, Proteins
	Ag^+, O_2^-, H^+, H_2O_2	Ag^+, O_2^-, H^+, $^{\cdot}OH$, H_2O_2, $^{\cdot}HO_2$, $^{\cdot}NO$, $ONOO^-$
Ag^+		$^{\cdot}Ag^+$-induced bacteria may inactivate PGN synthesis transglycosylase TG and transpeptidase TP.
	·Wall teichoic acids are spatial regulators of PGN cross-linking biosynthesis TP	·Activations of PGN autolysins of N-Acetylmura-myl-L-alanine Amidase, Amidase MurA, Lytic Amidase LytA, enzymatically active domain of autolysin LytM, Metalloenzyme AmiE, and Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM.
		·Bacteriolysis of *S.aureus* cell wall caused by inhibition of PGN elongation due to activations of amidases and dd-endopeptidase LytM.
		(DNA molecular is damaged by $O_2^-$and H_2O_2 and leads to oxidative stress.)

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Table 2 Antibacterial activities of Ag^+ ions on the *E coli* cell wall

Ag^+ ion	***E.coli* cell wall**		
	Lipopolysaccharide(LPS) Lipid A, Core polysaccharide	Outer Membrane Lipoprotein, Porins Omp F,A, C	Periplasmic Space Thin PGN layer
	Ag^+, O_2^-, H^+, H_2O_2	Ag^+, O_2^-, H_2O_2, ·OH	$Ag^+, O_2^-, H_2O_2, OH^-$, ·OH
Ag^+	·Negative charge ·Hydrophobic Lipid A ·Inhibition of LPS biosynthesis ·**$Ag^+ + -SH^- \rightarrow AgSH$**	·Porin proteins of hydrophilic channels ·Destruction of outer membrane structure due to degradative hydrolases of lipoprotein at C- and N-terminals · LOO·, L· (Fatty acid)	·Ag accumulation and Efflux activity ·Periplasmic enzymes ·Damage of PGN bio-synthesis TP of Endopeptidase enzymes and activation of PGN autolysins ·Bacteriolysis by inhibition of PGN elongation due to activation of *E. coli* PGN autolysins of amidase, peptidase, and carboxypeptidase.

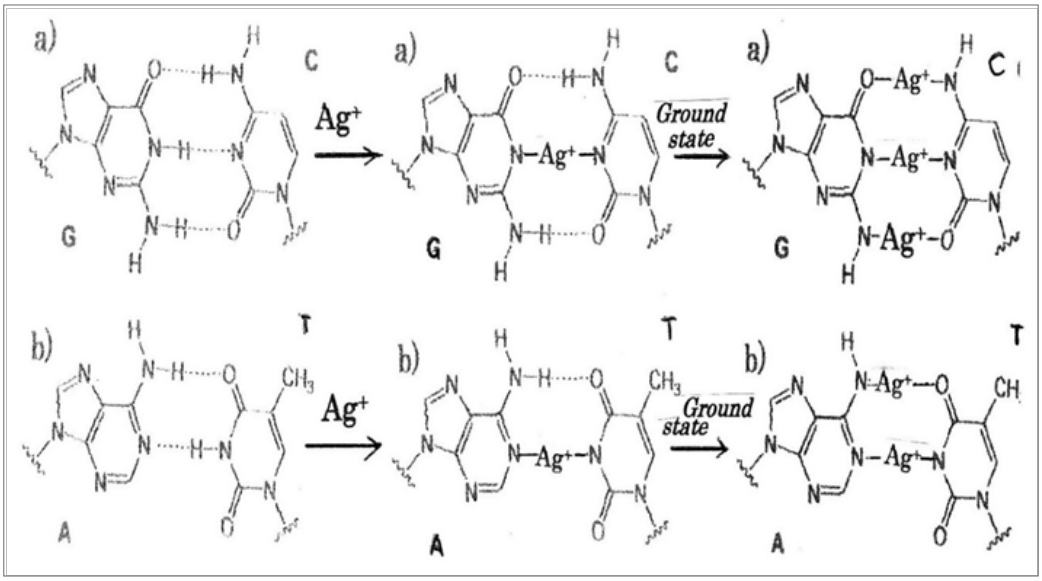

Figure 1 Ag^+ substituting into the triple and double hydrogen bonds within DNA base-pairs G≡C, A=T

Linear coordinated Ag^+ complex formation in G≡C pair ground state; O-Ag^+-N, N-Ag^+-N, N-Ag^+-O (stable)

Planar linear coordinated Ag^+ complex formation in A=T pair ground state; N-Ag^+-O, N-Ag^+-N (stable).

Conclusions

Ag^+-induced *S. aureus* may inactivate PGN synthesis transglycosylase TG and transpeptidase TP. Bacteriolysis of *S. aureus* PGN cell wall, in which wall teichoic acids control PGN synthesis cross-linking TP, is due to the inhibition of PGN elongation by enhancing the activities of PGN autolysins; amidase Ami A and A mi E, and PGN hydrolase Lysostaphin-like endopeptidase (Glycine-Glycine bond cleavage). Bacteriolysis and destruction of *E.coli* cell wall are due to the damage of LPS synthesis, destructing of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to inhibition of PGN formations by inactivation of carboxypeptidase and TP-endopeptidase, and activities of PGN autolysins of amidase, peptidase and carboxypeptidase. By the penetration of silver ion into *S.aureus* cell wall, production of O_2, H^+, H_2O_2, $ONOO^-$ occurs against *S.aureus*. The other, in *E.coli* cell wall, the productions of O_2^-, H^+ in outer membrane, and H_2O_2, OH^-,·OH in periplasmic space occur. These ROS and H_2O_2 give the damages cell membrane proteins and DNA molecular in cytoplasm. DNA damages due to Ag^+ ion-coordinated complex formation within DNA base-pairs of triple hydrogen bond **G≡C,** double hydrogen bond **A=T** may be occurred in cytoplasm of bacterial cells.

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Conflict of interest

The author declares no conflict of interest.

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