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\(_2\cdot\), H\(_2\)O\(_2\), OH\(^-\), OH\(^{1/-}\) 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, hydrolysis and degradation, PGN synthesis and autolysin, reactive oxygen species (ROS), DNA base-pairs

**Abbreviations:** ABNC, active but nonculturable; A, adenine; AgNPs, silver nanoparticles; C, cytosine; *E. coli*, *Escherichia coli*; G, guanine; LP, lipoprotein; LPS, lipopolysaccharide; MTS, metallothionines; 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. Released biologically active Ag\(^+\) shows a strong affinity for sulphydryl groups, 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\(^+\) is 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. 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. 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 hydrolase/autolysin, antibacterial mechanisms of silver(I) ions could be 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 pair 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 effects, prevention of serious diseases, and medical applications. The toxicity of AgNPs is mainly due to release of 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.
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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. In the molecular structure of S. aureus PGN cell wall, there are the action sites of TG synthetic enzymes of N-acetylgluco-sami diase cleavage between NAG (N-acetylgucosamine) and NAM (N-acetylmuramic acid), and N-acetyluramidase-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 amide cleavage, DD-endopeptidases cleavages between Glycine and Glycine on pentaglycine (Gly), and in addition, lysostaphin cleavage between Glycine and Glycine on PGN cross-linking.

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. 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.

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. Wall teichoic acids are spatial regulators of PGN crosslinking biosynthesis TP, and silver ions could inhibit both TG and TP enzymes of the PGN that Ag⁺-induced bacteria may inactivate PGN synthesis transglycosylase TG and transpeptidase TP; Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LymtM function as TP enzyme.

Ag⁺ induced amidase of S. aureus PGN autolysins

Lytic activity was inhibited by glucosamine, NAG, Hg²⁺, Fe⁺³, and Ag⁺; and Ag⁺ binding Rs3717 showed no activity on polymerized PGN and but, it is induced to a potential role of N-Acetylmuramyl-L-alanine Amidase, PGN murein hydrolase activity and generalized autolysis; Amidase MurA, Lytic Amidase LytA, enzymatically active domain of autolysin LymtM, metal-dependent metalloenzyme AmiE, 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. O₂⁻ and H₂O₂ permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress.

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 autolysis. 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 O₂⁻, hydroxyl radical ·OH, hydrogen peroxide H₂O₂ occurred from superoxide radical O₂⁻ molecular.

O₂⁻ + e⁻ → O₂⁻
O₂⁻ + e⁻ + H⁺ → HO₂⁻
H₂O → ·OH + H + e⁻ → H₂O₂
HO₂⁻ → H⁺ + O₂
·O₂⁻ + 2H⁺ + e⁻ → H₂O₂
H₂O₂ + e⁻ → HO⁻ + OH
·OH + e⁻ + H⁺ → H₂O
2H⁺ + 2·O₂⁻ → H₂O₂ + O₃

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 hydrolyses. The OmpA, OmpC, OmpF porins of lipoproteins have metallic cation selective and hydrophilic membrane crossing pore, to be effective for silver transfer. 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. 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. 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. 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. 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 thiol with redox reaction involved that with inorganic thiol like HS⁻ and S²⁻, it is possible to form many.
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species such as AgSH, [Ag(SH)]⁻ and [Ag₂(SH)₂]⁻ depending on the concentration of the anions present.²⁹

\[ \text{Ag}⁺ + (-SH)⁻ → \text{AgSH} → [\text{Ag}_2(\text{SH})_2]⁻ \]

** 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, TolO is a periplasmic protein, and TolP, 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 degradated by Ag⁺ ions.³³ The other, it is unclear that the silver-induced PGN biosyntheses TG/TP should be inhibited by the silver ions.³⁴ However, silver ions inactivate TP of endopeptidase by because of destructive observation of bacterial cell walls.³⁵ Silver ions could activate E.coli PGN autolysins of amidase, peptidase, Carboxypeptidase,³⁶ such as silver depending PGN autolysin, AmiC,³⁷ AmiD,³⁸ Muramidase,³⁹ Amino acid amidase,³¹ Carboxy-peptidase A,³² zinc metalloenzymes Ami D,³² Amiase zinc-containing amidase; Amp D,³⁸ zinc-present PGLYRPs,⁴⁰ Carboxypeptidase-degraded aldolase,⁴¹ CarboxypeptidaseV,⁴² 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,³³ 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 O₂⁻, OH⁻, 'OH and H₂O₂ are formed as follows.⁴³

\[ \begin{align*}
O₂ + e^- & \rightarrow O₂^- \\
2O₂^- + 2H^+ & \rightarrow H₂O₂ + O₂ \\
O₂^- + H₂O₂ & \rightarrow OH^- + 'OH + O₂-
\end{align*} \]

In cell wall, reacting with polyunsaturated fatty acids:

\[ \text{LH} + \text{OH}^- \rightarrow \text{L}^- + \text{HOH} \]

\[ \text{L}^- + O₂ \rightarrow \text{LOO}^- \]

\[ \text{LH} + \text{LOO}^- \rightarrow \text{L}^+ + \text{LOOH} \]

Ag⁺-containing Peptidoglycan Recognition Proteins (PGRPs) induce ROS production of H₂O₂, O₂⁻, HO⁻, and then the ROS occur the oxidative stress, and killing by stress damage.⁴⁴ 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 H₂O₂ 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 O₂⁻, HO⁻ radicals, and by reaction of H₂O₂ 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.⁴⁵

<table>
<thead>
<tr>
<th>Ag⁺ ions</th>
<th>S.aureus Cell Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teichoic acid, Lipoteichoic acid</td>
<td>Peptidoglycan layer, Proteins</td>
</tr>
<tr>
<td>Ag⁺, O₂⁻, H⁺, H₂O₂</td>
<td>Ag⁺, O₂⁻, H⁺, OH, H₂O₂, HO₂, NO, ONOO⁻</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>Ag⁺-induced bacteria may inactivate PGN synthesis transglycosylase TG and transpeptidase TP, Activations of PGN autolysins of N-Acetylgluaramy-myl-L-alanine Amidase, Amidase MurA, Lytic Amidase LytA, enzymatically active domain of autolysin LytM, Metalloenzyme AmiE, and Lysostaphin-like PGN hydrolase and glycyglycine endopeptidase LytM, Bacteriolysis of S.aureus cell wall caused by inhibition of PGN elongation due to activations of amidases and dd-endopeptidase LytM.</td>
</tr>
</tbody>
</table>

(DNA molecular is damaged by O₂⁻ and H₂O₂, and leads to oxidative stress.)

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

** Citation: ** Ishida T. Antibacterial mechanism of Ag⁺ ions for bacteriolyses of bacterial cell walls via peptidoglycan autolysins, and DNA damages. MOJ Toxicol. 2018;4(5):345–350. DOI: 10.15406/mojotox.2018.04.00125
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Table 2 Antibacterial activities of Ag⁺ ions on the E.coli cell wall

<table>
<thead>
<tr>
<th>Ag⁺ ion</th>
<th>E.coli cell wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopolysaccharide(LPS)</td>
<td>Outer Membrane</td>
</tr>
<tr>
<td>Lipid A, Core polysaccharide</td>
<td>Lipoprotein, Porins Omp F,A, C</td>
</tr>
<tr>
<td>Ag⁺, O₂⁻, H⁺, H₂O₂</td>
<td>Ag⁺, O₂⁻, H₂O₂, OH⁻, OH</td>
</tr>
<tr>
<td>Negative charge</td>
<td>Oxidation of LPS</td>
</tr>
<tr>
<td>Hydrophilic Lipid A</td>
<td>Porin proteins of hydrophilic channels</td>
</tr>
<tr>
<td>Inhibition of LPS biosynthesis</td>
<td>Destruction of outer membrane structure due to degradative hydrolases of lipoprotein at C- and N-terminals</td>
</tr>
<tr>
<td>Ag⁺ + -SH → AgSH</td>
<td>'LOO', L (Fatty acid)</td>
</tr>
</tbody>
</table>

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 the 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 O₂, H⁺, H₂O₂, ONOO⁻ occurs against S.aureus. The other, in E.coli cell wall, the productions of O₂⁻, H⁺ in outer membrane, and H₂O₂, OH⁻, OH in periplasmic space occur. These ROS and H₂O₂ 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.

References


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