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Anti-Bacterial Mechanism for Metallic Ag^{+}, Cu^{2+}, Zn^{2+} Ions-Induced Bactertiolysis on Disruptive OM Lpp and PGN Inhibitive Elongations Against *S. aureus* and *E. coli*

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ABSTRACT

Anti-bacterial mechanism for complete-ionized Ag^{+}, Cu^{2+}, Zn^{2+} ion solutions has been established against *S. aureus* and *E. coli*. Anti-bacterial mechanism against *S. aureus* is involved that bacterolysis and destruction of *S. aureus* cell wall occur by inhibition of PGN elongation through metallic Ag^{+}, Cu^{2+}, Zn^{2+} ions-induced PGN inhibitive transglycosylase (TG) and transpeptidase (TP) syntheses (TG for Zn^{2+}) and PGN activated major autolysin of amidase. The other, anti-bacterial mechanism against *E. coli* has been clarified that bacteriolysis and destruction of *E. coli* cell wall occur by disruption of *E. coli* outer membrane (OM) structure with OM lipoprotein-endopeptidase activation, and by inhibition of PGN elongation through inhibitive TG and TP syntheses (TG for Zn^{2+}) and PGN activated major autolysins. Ag^{+}, Cu^{2+}, Zn^{2+} ions-induced ROS generation of O_2^{-} and H_2O_2 and ROS-mediated oxidative stress in bacterial cell lead to killing by stress damage for silver ions, cell membrane damages due to high reactive •OH and OH^{-} are formed by Haber-Weiss and Fenton reactions for Cu^{2+} ions, and DNA molecular damage for Zn^{2+} ions.

Keywords: Ag^{+}; Cu^{2+}; Zn^{2+} ions; Bacteriolysis; PGN synthesis and autolysin; PGN elongation; Autolysin amidase; ROS-mediated oxidative stress

ABBREVIATIONS

BLP: Braun's lipoprotein; CTD: C-terminal domain; *E. coli: Escherichin coli*; IMP: integral membrane protein; LdtF: l,d- transpeptidase factor; Lpp: lipoprotein; LPS: lipopolysaccharide; MBP: maltose-binding protein; NAG: N-acetylglucosamine; NAM: N-acetylmuramic acid; NTD: N-terminal domain; OM: outer membrane; OMP: outer membrane protein; Omp: outer membrane porin; Pal: Proteinasso-ciated lipoprotein; PGN: peptidoglycan; PGRPs: peptidoglycan recognition proteins, ROS: reactive oxygen species; *S. aureus*: *Staphylococcus aureus*; SNF: silver nanoformulation form; TG: transglycosylase; Tol: Tol proteins; TP: transpeptidase; ZnPT: zinc pyrithione.

INTRODUCTION

Silver, copper, and zinc of transition metals have highly antibacterial

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activities and are utilized as chemotherapy agents. The high antibacterial activities for these Ag^{+}, Cu^{2+}, Zn^{2+} ion solutions have the processes of bacteriolyses and destructions of bacterial cell walls against *StaphylococcuS. aureus* (*S. aureus*) peptidoglycan (PGN) and *Escherichia coli* (*E. coli*) outer membrane cell walls.

Anti-bacterial activity of silver (I) ions depend on bacteriolysis and destruction of bacterial cell walls that silver ions inhibit PGN elongation and PGN biosynthesis, and enhance PGN autolysin activation [1]. Especially, the interaction of silver ions with *Escherichia coli* (*E. coli*) used as a model microorganism is characterized by energy-filtering transmission electron microscopy (EFTEM) that the outer membrane and the interior cell membrane with cytoplasmic protein were destructed by silver ions [2], in which bacterial killing of silver ions is shown to have a strong highest function for the destructions of *E. coli* outer membrane lipoprotein and inner membrane protein.

Copper ions destroy the bacterial cell wall, which becomes thick and coarse, the cytoplasm is then degraded and disappears, leading finally to cell death. The antibacterial mechanism is attributed mainly to the strong adsorption of copper ions to bacterial cells, which imparts antibacterial efficacy in a concentration-dependent manner [3]. The bacteriolytic mechanisms by copper (II) ions had been revealed that bacteriolysis of *S. aureus* PGN cell wall by Cu^{2+} ions is ascribed to the inhibition of PGN elongation due to the damages of PGN biosynthesis of transglycosylase (TG) and transpeptidase (TP), and the Cu^{2+} ions-induced activated PGN autolysins, whereas bacteriolysis of *E. coli* outer membrane cell wall by Cu^{2+} ions is attributed to the destruction of outer membrane structure and the inhibition of PGN elongation due to the damage of PGN biosynthesis TP and the activations of PGN autolysins [4].

Zn^{2+} ions can be internalised into the bacterial cell and disrupt the enzymatic system. ROS production (causing the destruction of cellular components such as DNA, proteins and lipids): O_2^- and HO_2^- do not penetrate the membrane, but direct contact causes damage and H_2O_2 is internalised. Internalisation within the bacteria cell and direct contact cause damage such as the loss of cellular integrity [5]. Zinc ions-induced anti-bacterial mechanism also may be clarified. It had been appeared that the anti-bacterial effects had the order of $Zn^{2+} > Cu^{2+} > Ag^{+} > Al^{3+}$ in metallic ion concentration 100 mL of the sulfate solution under the halo inhibitory tests, in which Zn^{2+} ion indicated to be the highest effect in the sulfates [6].

As described above, Ag^{+}, Cu^{2+}. Zn^{2+} ion solutions having very high antibacterial abilities call attention to potential treatments such as preventions of serious diseases, restriction of viral infection, and regulation of cancer tumor cells. Furthermore, antibacterial Ag, Cu, Zn metallic ion solution materials are raised such as silver compound (silver chloride), silver nanoparticles for Ag^{+} ion solutions, copper sulfate, copper chelators, CuO nanoparticles for Cu^{2+} ion solutions, and zinc chloride, zinc sulfate, zinc pyrithione, zinc oxide for Zn^{2+} ion solutions.

In this semi-review article, silver (I)-, copper (II)-, zinc (II)-induced, respectively, bacteriolytic functions of inhibition or activation of *E. coli* outer-membrane lipoprotein, bacterial PGN synthesis and PGN major autolysins are investigated against *S. aureus* and *E. coli*, subsequently anti-bacterial mechanisms for silver, copper, and zinc ion solutions is elucidated from the bactericidal viewpoint that relates metallic ions-induced bacteriolytic denaturation of outer-membrane lipoprotein (Braun's lipoprotein), bacterial PGN elongation, syntheses, and autolysins.

Molecular structures of bacterial cell walls, PGN synthesis and PGN autolysins in *S. aureus* and *E. coli*

The bacterial cell walls are a strong flexible meshwork of PGN that gives a bacterium structural integrity, in which to accommodate a growing cell, the walls are remodeled by PGN synthesis and PGN autolysin. PGN is the main constituent of bacterial cell walls and must be continuously synthesized and degraded to maintain the integrity and viability of the cells that bacterial cell wall hydrodases of amidase, gycosidase, and peptidase display a modular architecture combining multiple and different catalytic domains, including some lytic transglycosylases as well as cell wall binding domains [7]. Bacterial PGN structure of both Gram-positive and Gram-negative bacteria comprises repeating disaccharide backbones of N-acetylglucosamine (NAG) and β-(1-4)-N-acetylmuramic acid (NAM) that are cross linked by peptide stem chains attached to the NAM residues [8].

S. aureus surface layer consists of teichoic acids, lipoteichoic acids, and thick PGN cell wall, in which the molecular structure of *S. aureus* PGN cell wall and the action sites of synthesis TG/TP enzymes and PGN forth autolysins, as shown in Figure 1. For StaphylococcuS. aureus (*S. aureus*) PGN layer, there are biosynthesis TG/TP and forth autolysins of *N-acetylmuramidase* and *N-acetylglucosamidase*, *N-acetylmuramidase-L-alanine* amidase and PGN chain cross-linkage *DD-endopeptidase*.

The other, *E. coli* cell wall consists of lipid A, lipopolysaccharide, porin proteins, outer membrane of lipoprotein, and thinner 2-7 nm PGN layer in 30-70 nm periplasmic space [9]. *E. coli* cell wall is constituted of lipopolysaccharide (LPS), lipoproteins (LPT), and PGN, thinner layer within periplasmic space. The first permeability barrier of zinc ions in the *E. coli* cell wall is highly anionic LPS with hydrophobic lipid A, core polysaccha-ride, O-polysaccharide, in which zinc ions may be possible for the inhibition of LPS biosynthesis, owing to that promotes formation of metal-rich precipitates in a cell surface [10]. *E. coli* Braun's lipoprotein (BLP) of outer-membrane (OM) lipoprotein that BLP is anchored in the OM via a lipidated N-terminus, whereas the C-terminus is covalently attached to the peptide chain of PGN and that BLP exists in PGN-bound and PGN-unbound states, the length of BLP has a direct influence on the distance between the peptidoglycan layer and the outer membrane of *E. coli* [11]. BLP facilitates interactions of OmpA monomer with PGN, in which The OmpA dimer readily binds to PGN, in which The *E. coli* outer membrane porin OmpA is a multidomain protein whose N-terminal domain (NTD) is made of a b-barrel and C-terminal domain (CTD) is a globular periplasmic unit that binds to PGN, connected by an unstructured 20-residue linker region and that BLP and OmpA CTD are able to form nonspecific electrostatic interactions in the periplasm [11]. Penicillin binding protein4 (PBP4) localizes specifically at midcell as part of the division machinery that PBP4 is a periplasmic endopeptidase with a C-terminal amphipathic alpha-helix that associates with membranes and has three domains [12]. Despite its conservation throughout evolution among pathogenic and non-pathogenic bacteria, OmpA interacts with specific receptors for initiating pathogenesis in some Gram-negative bacterial infections [13].

The gram-negative bacterial cell envelope is made up of an outer membrane (OM), an inner membrane (IM) that surrounds the cytoplasm, and a periplasmic space between the two membranes containing peptidoglycan (PGN or murein). PGN is an elastic polymer that forms a mesh-like sacculus around the IM, protecting cells from turgor and environmental stress conditions. In several bacteria, including *Escherichia coli*, the OM is tethered to PGN by an abundant OM lipoprotein, Lpp (or Braun's lipoprotein), that functions to maintain the structural and functional integrity of the cell envelope. Since its discovery, Lpp has been studied extensively, and although L,D-transpeptidases, the enzymes that catalyze the formation of Lpp-PGN linkages, have been earlier identified, it is not known how these linkages are modulated. Recently, LdtF is identified as an endopeptidase that cleaves the Lpp-PGN cross-links and as a glycine-specific carboxypeptidase [14]. For *Escherichia coli* (*E. coli*) cell wall, there are endopeptidase and aminopeptidase of degrading enzyme at lipoprotein of N- and C-terminals, and amidase, peptidase, and caboxypeptidase at thin PGN layer in periplasmic space [15].

Figure 2 shows the schematic structure of *E. coli*, in which the molecular bonding manner of *E. coli* cell wall and periplasmic PGN, and the action sites of the hydrolases and degradative enzymes of lipoproteins *E. coli* PGN synthetic enzymes TG/TP and the PGN autolysins such as *Muramidase, Glucosamidase, Amidase, Peptidase,* and *Carboxypeptidase*. Interactions of PGN molecular structure, PGN biosynthesis TG/TP and PGN autolysins influence in any event for the bacteriolysis of bacterial cell walls.

Bacterial PGN biosynthesis autolysins against *S. aureus* and *E. coli* are summarily represented in Table 1 that these PGN biosynthesis and autolysin sites are shown in Figure 1 and Figure 2. The *S. aureus* killing mechanism was more likely due to activation autolysins along with minimum membrane disruption [16]. In these autolysins, zinc-dependent PGN major autolysin of amidases chiefly may be enhanced induced anti-bacterial activities.

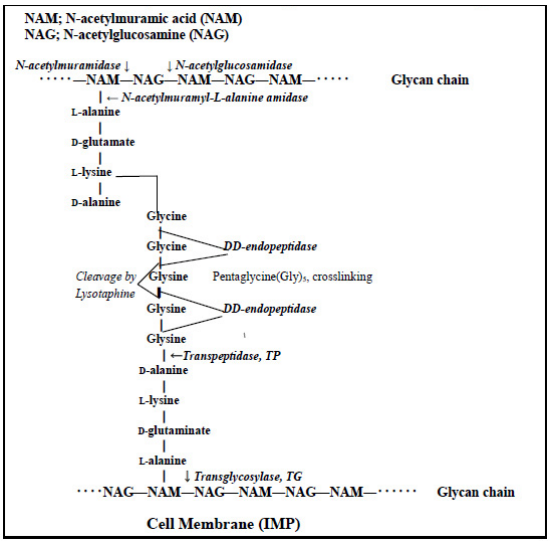

Figure 1. PGN molecular structure and the action sites of PGN synthesis TG/TP and PGN autolysins of N-acetylmuramidase, *N-acetylglucosamidase, N-acetylmuramyl-L-alanine amidase*, DD-endopeptidase against *S. aureus* thick PGN layer.

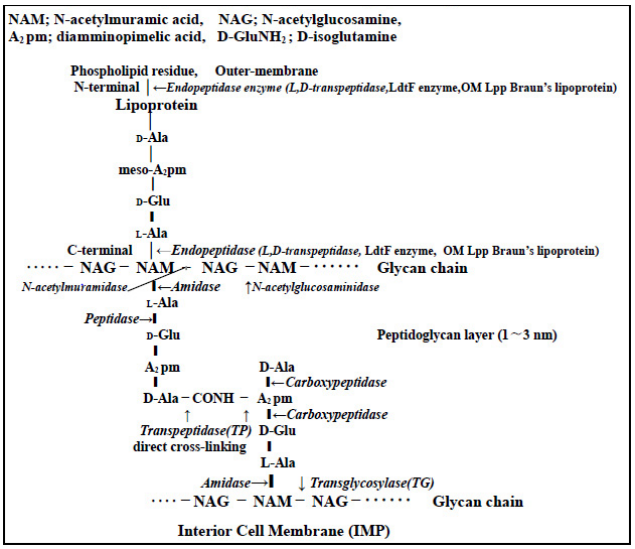

Figure 2. *E. coli* outer-membrane and periplasmic space PGN molecular structures, and the action sites of degrading endopetidase enzyme of outer-membrane lipoprotein at C-and N-terminals, PGN synthesis TG/TP enzymes, and PGN autolysins of *N-acetylglucosaminidase, N-acetylmuramidase*, *amidase, peptidase*, and *carboxypeptidase* against *E. coli* cell wall.

Table 1. Bacterial PGN synthesis and autolysins against *S. aureus*, and outer membrane lipoprotein degrading enzyme, PGN synthesis and autolysins against *E. coli*

PGN synthesis *TG/TP*and PGN autolysins against *S. aureus*	**Outer membrane lipoprotein degrading enzymes, and PGN synthesis *TG/TP* and PGN autolysins against *E. coli***
·**PGN synthesis** *N-acetylmuramidase, TG Transpeptidase, TP*	·*Endopeptidase* of degrading enzyme at lipoprotein of N-terminal and *Endopeptidase or* OM lipoprotein, Lpp(Braun's lipoprotein), *L,D-transpeptidase,LdtF* of degrading enzyme at lipoprotein of C-terminal.
·**PGN autolysins** *N-acetylmuramidase and N-acetylglucosamidase* *N-acetylmuramidase-L- alanine amidase,* *PGN chain cross-linkage* *DD-endopeptidase.*	·**PGN synthesis** *N-acetylmuramidase, TGTranspeptidase, TP* ·**PGN autolysins** *Muramidase, Glucosamidase, Amidase, Peptidase,* and *Carboxypeptidase.*

Anti-bacterial activity for silver ions against *S. aureus* and *E. coli*

1. Silver ions induced PGN inhibitive synthesis TG/TP against *S. aureus* cell wall

Silver (I) ions-induced PGN inhibitive synthesis TG/TP and PGN activated major autolysin against *S. aureus* and destructive outer membrain lipoprotein, PGN inhibitive synthesis TG/TP and PGN activated major autolysin against *E. coli* are described. In proteins, the coordination is limited by His, Cys, Glu, and sulfur donors from the side chains of a few amino acids. In silver nitrate solution, $AgNO_3$ is dissociated into aqua silver ion $[Ag(H_2O)_2]+$ and nitrate ion $(NO_3)^-$, aqua silver ions are liable to be bound to ligand L having negative charge. The nitrate ion has bactericidal inactivity.

For silver nitrate in solution is

$$AgNO_3 + 2H_2O = [Ag(H_2O)_2]^+ + (NO_3)^-$$

$$[Ag(H_2O)_2]^+ + L^- = Ag(H_2O)_2L$$

$$Ag(H_2O)_2L = AgL + 2H_2O$$

or

$$AgNO_3 \xrightarrow{+2H_2O} \left[Ag\left(H_2O\right)_2\right]^+ \xrightarrow{+L^-} Ag\left(H_2O\right)_2 L \xrightarrow{-2H_2O} AgL$$

The released Ag^+ ions from $AgNO_3$ solution 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 of transpeptidase (TP), and silver ions could inhibit both transglycosylase (TG) and TP enzymes of the PGN that Ag^+-induced bacteria may inactivate PGN biosynthesis TG and TP [17]. Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM may function as TP enzyme. Silver ions could inhibit both TG and TP enzymes of the PGN that Ag^+-induced bacteria may inactivate PGN synthesis transglycosylase TG [17] and transpeptidase TP [18,19]. Thus, Ag^+-induced *S. aureus* can inactivate PGN synthesis of TG and TP.

2. Silver ions induced PGN major autolysins against *S. aureus* cell wall

Silver ions enhance activation of PGN autolysins of amidases [20]. For the sake of growth of *S. aureus* thick PGN layer 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 [17] and enhancement of activation of PGN autolysins of amidases [15].

Thus, Ag^+ ions activate PGN major autolysins of 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 AmiA and AmiE, and PGN hydrolase Lysostaphin-like endopeptidase (Glycine-Glycine bond cleavage).

3. Silver ions induced disruption of *E. coli* outer membrane structure by hydrolases of lipoproteins at C- and N-terminals

E. coli outer-membrane lipoprotein structure had been observed to be destructed by silver ions [2], in which silver ion is shown to have interaction with protein Braun lipoprotein. Silver nitrate has interaction with protein Braun lipoprotein and is capable of making interaction with many proteins by that bioinformatic interaction of silver nitrate with Braun lipoprotein [21].

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. 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 disrupted [22].

It is unclear whether both Aminopeptidase and Endopeptidase (or ***L,D-transpeptidase, LdtF***) of lipoprotein at C- and N-terminals are simultaneously activated by Ag^+ ions. However, outer membrane may be considered to be disrupted probably by predominant activation of lipoprotein-endopeptidase. There is no data about Ag-lipoprotein aminopeptidase, LdtF enzyme interactions, hence, whether Ag^+ ion react with *endopeptidase* enzyme or not [14].

Both silver nanoparticles and ionic silver may interact with proteins associated to the bacterial cell wall and membrane disrupion and thereby form detrimental complexes that alter its physicochemical properties. Silver quickly reacts with the sulfhydryl groups on the bacterial cell membrane by exchanging the terminal hydrogen atom, generating a stable S–Ag bond and thereby fully blocking the respiratory chain, electron transfer, protein secretion and lipid biosynthesis [23].

Silver inhibits outer membrane proteome (OMP) that The molecular mechanism of the antibacterial activity of silver and molecular changes in bacterial cells strongly depend on the physical and chemical properties of the tested silver nanoformulation form (SNF) [24]. A silver-binding peptide, AgBP2, was identified from a combinatorial display library and fused to the C terminus of the *E. coli* maltose-binding protein (MBP) to yield a silver-binding protein exhibiting nanomolar affinity for the metal [25]. Silver ions may be accumulated and damaged in *E. coli* PGN synthetic enzyme of silver protein endopeptidase in 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, ***Endopeptidase (L,D-transpeptidase, LdtF)*** of lipoprotein *endopeptidase* is degradative by Ag^+ binding proteins.

4. Silver ions-induced PGN activated major autolysins of amidase, peptidase, and carboxypeptidase against *E. coli*

It is unclear that the silver-induced PGN syntheses TG/TP should be inhibited by the silver ions. However, silver ions inactivate TP of endopeptidase by because of destructive observation of bacterial cell walls. Silver ions could activate *E. coli* PGN autolysins of amidase, peptidase, Carboxypeptidase, such as silver depending PGN autolysin, AmiC, AmiD, Muramidase, Amino acid amidase, Carboxypeptidase A, Bacteriolysis and destruction for *E. coli* cell wall also are considered to be due to the damage of LPS synthesis, destructing of outer membrane structure by degrading of lipoprotein at C-, N-terminals, and to be owing to inhibition of PGN formations by inactivation of *carboxypeptidase* and *TP-endopeptidase*, and activities of PGN autolysins of amidase, peptidase and carboxypeptidase.

Thus, the anti-bacterial mechanism of Ag^+ ion solution has been 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 endopeptidase of lipoprotein at C-, and N-terminals, and inhibition of PGN elongation due to the damage of PGN synthetic TG/TP enzyme and PGN major activated autolysins of Amidase, Peptidase, and Carboxypeptidase in silver-protein amidases in periplasmic space. Specially, the inhibition of PGN elongation had occurred by silver ion induced activities of PGN hydrolases and autolysins.

5. Siver ions induced ROS generation in *S. aureus* and *E. coli*

For the penetration of Ag^+ ions to *S. aureus* 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. O_2^- and H_2O_2 permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress [26]. Silver ions react with -SH, and H^+ in *E. coli* that free radicals O_2^-, OH^-, ·OH and H_2O_2 are formed as follows:

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

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

In cell wall, reacting with polyunsaturated fatty acids:

$$LH + OH\cdot \rightarrow L\cdot + HOH \quad L\cdot + O_2 \rightarrow LOO\cdot \quad LH + LOO\cdot \rightarrow L\cdot + LOOH$$

Thus, Ag^+-containing peptidoglycan recognition proteins (PGRPs) induce ROS production of H_2O_2, O^-, HO·, and then the ROS occur the oxidative stress, and killing by stress damage [27].

Accordingly, anti-bacterial mechanism for silver ion solution is found that bacteriolysis and destruction of *S. aureus* cell wall occur by inhibition of PGN elongation through Ag^+ ions-induced damages PGN both synthetic TG/TP and PGN major activated autolysins of amidase, the other, bacteriolysis destruction of *E. coli* cell wall occur by the disruption of outer membrane structure owing to the activation of *endopeptidase* of lipoprotein at N-terminal, inhibition of PGN elongation due to Ag+ ions induced damage of PGN synthetic TG/TP enzyme and PGN major activated autolysins of *Amidase, Peptidase*, and *Carboxypeptidase* in silver-protein amidases in periplasmic space.

Anti-bacterial activity for copper (II) ions against *S. aureus* and *E. coli*

1. Copper (II) ions-induced *S. aureus* with coordinated limited ligand

Copper is redox-inert and has only one valence state of Cu (II). In proteins, the coordination is limited by His, Cys, Glu, and sulfur donors from the side chains of a few amino acids. In copper sulfate solution, $CuSO_4$ is dissociated into aqua Cu ion $[Cu(H_2O)_6]^{2+}$ and sulfuric ion $(SO_4)_2^-$ aqua Cu ions are liable to be bound to ligand L having negative charge. The sulfuric ion has bactericidal inactivity

$$Cu(NO_3)_2 + 6H_2O = [Cu(H_2O)_6]^{2+} + 2(NO^3)^-$$

$$[Cu(H_2O)_6]^{2+} + 2L^- = CuL_2 + 6H_2O$$

2. Inhibition of polymerization of glycan chains bonding and cross-linking of side peptide

Cu^{2+} ions may inhibit polymerization of glycan chains, forming copper complex in which is partial action sites of glycan saccharide chains [4]. L is coordinated molecular.

$Cu^{2+} + LH \rightarrow CuL^{+} + H^{+}$ $CuL^{+} + LH \rightarrow CuL_2 + H^{+}$

Copper-complexes on saccharide chains may be,

Glycan chaine: —NAG-(NAM-Cu-2O-2N-NAG)-NAM—.

The other, Cu^{2+} ions may inhibit cross-linked reaction by peptide copper complex formation bonding to sidepeptide chains.

$Cu^{2+} + 2LH \rightarrow CuL_2 + H^{+}$

Peptide copper complex may be 3N-Cu-O, Cu (Gly-L-Ala) H_2O. Specially, Cu^{2+} ions react with cross-molecular penta glycine $(Gly)_5$, copper-glycine complex may be formed.

Amino acid: $Cu^{2+} + Gly^{-} \rightarrow Cu(Gly)^{+}$, $Cu(Gly)^{+} + Gly^{-} \rightarrow Cu(Gly)^{2}$,

Peptido: $Cu^{2+} + GlyGly \rightarrow Cu(GlyGly)$, $Cu(GlyGly) + Gly^{-} \rightarrow Cu(GlyGlyGly)^{-}$.

3. Cu^{2+} Ions induced Bacteriolysis of *S. aureus* PGN Cell Wall by inhibition of PGN elongation through inhibitive TG/TP enzymes and PGN activated major autolysins

Bacteriolysis by balance deletion between synthesis enzyme and decomposition enzyme (autolysin) in PGN cell wall: 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 is broken by Cu^{2+} penetration, Cu^{2+} ions are self-catalytically treated as coenzyme, that this is indicated that activation of autolysin is preceded, in which bacteriolysis and killing may result.

Copper ions inhibit PGN synthesis TG/TP against *S. aureus* that damages PGN synthetic TG/TP [28]. Cu2+ ions could activate PGN autolysin, AmiA [29,30]. Hence, bacteriolysis of *S. aureus* PGN cell wall by Cu^{2+} ions is due to inhibition of PGN elongation owing to the damages of PGN synthetic TG/TP and the activation of PGN major autolysins of AmiA.

4. Bacteriolysis and Destruction of *E. coli* Outer Membrane Cell Wall by Cu^{2+} Ions

Inhibition of outer membrane cell wall: Cu^{2+} ions inactivate catalyst enzyme with forming Cu^{+} ions.

$Cu^{2+} + {}^{-}SH \rightarrow {}^{-}SCu(I) + H^{+}$

By the penetration of Cu^{2+} ions, the activations of amidase enzyme of N-terminal and endopeptidase enzyme of C-terminal are enhanced. Interaction of copper ion with *E. coli* Braun lipoprotein is considered that copper dramatically decreases the minimal inhibitory concentration of ampicillin in *E. coli* strain with a resistance mechanism relying on LD-transpeptidases (LDTs) and inhibits purified LDTs at submillimolar concentrations [31].

Accordingly, the activations of decomposition at N-, C-terminals of lipoproteins may occur with the disruption of outer membrane structure. Hence, bacteriolysis of *E. coli* cell wall by Cu^{2+} ions occurs by disruption of outer membrane structure due to degradation of lipoprotein at N-, C-terminals, damage of TP enzyme and activations of PGN autolysins. Furthermore, deletion of PGN autolysin also becomes bacteriolytic factor.

5. Cu^{2+} ions-induced ROS production in *S. aureus* and *E. coli*

Cu^{2+} ions-induced reactive oxygen species (ROS) O_2^{-} and H_2O_2 generated in the cell wall, and permeate into cell membrane and cytoplasm, in which in cell membrane high reactive •OH and OH^{-} are formed by Haber-Weiss and Fenton reactions.

Haber-Weiss reaction: $H_2O_2 + O_2^{-} \rightarrow \bullet OH + OH^{-} + O_2$

Fenton reaction: $Cu^{+} + H_2O_2 \rightarrow \bullet OH + OH^{-} + Cu^{2+}$

Furthermore, new ROS productions occur by Fenton-like type. L=Ligand

$LCu(II) + H_2O_2 \rightarrow LCu(I) + {}^{\cdot}OOH + H^{+}$ $LCu(I) + H_2O_2 \rightarrow LCu(II) + \bullet OH + OH^{-}$

Production of reactive oxygen species (ROS) against *S. aureus*. O_2^{-} and H_2O_2 permeate into membrane and cytoplasm, that DNA molecular is damaged by oxidative stress [32]. By the penetration of copper ions into bacterial cell wall, productions of O_2^{-}, H^{+}, H_2O_2, $ONOO^{-}$ occurs. The other, in *E. coli* cell wall, the productions of O_2^{-}, H^{+} in outer membrane, and H_2O_2, OH^{-}, ${}^{\cdot}OH$ in periplasmic space occur. These ROS and H_2O_2 damage the cell membrane and the DNA molecules by oxidase stress [33].

Anti-bacterial activity for Zinc (II) ions against *S. aureus* and *E. coli*

1. Zinc induced zinc-proteins complex formation against *S. aureus*

In Bacteriolysis of *S. aureus* PGN Cell Wall by Zn^{2+} Ions against *S. aureus*, zinc is redox-inert and has only one valence state

of Zn(II). In proteins, the coordination is limited by His, Cys, Glu, and sulfur donors from the side chains of a few amino acids. In zinc sulfate solution, $ZnSO_4$ is dissociated into aqua zinc ion $[Zn(H_2O)_6]^{2+}$ and sulfuric ion $(SO_4)_2^-$ aqua zinc ions are liable to be bound to ligand L having negative charge. The sulfuric ion has bactericidal inactivity [34].

$ZnSO_4 + 6H_2O \rightarrow [Zn(H2O)_6]^{2+} + (SO_4)^-$

$[Zn(H_2O)_6]^{2+} + 2L- \rightarrow [Zn(H_2O)L_2] + 5H_2O$

$Zn(H_2O)L_2 \rightarrow ZnL_2 + H_2O$

Structural Zn^{2+} ions are most commonly coordinated by cysteine, followed by histidine, aspartate, and glutamate that Zn-cysteine complex in bacteria and Zn^{2+} chelation represents a potential therapeutic approach for combating biofilm growth in a wide range of bacterial biofilm-related infections [35].

2. Zinc induced PGN inhibitive synthesis enzymes of transglycosylase TG and transpeptidase TP against *S. aureus*

Zinc disrupts PGN synthesis in bacterial cell wall [36] and wall teichoic acids are spatial regulators of PGN cross-linking biosynthesis TP, however, it is not explicit whether zinc ions could inhibit both TG and TP enzymes of the PGN, wherein is due to uncertain relation between wall teichoic acids biosynthesis and PGN biosynthesis [37].

Metallation of Per R with Zn(II) disrupts this coordination, resulting in depression of heme synthesis but continued repression of catalase that Zn(II) intoxication leads to intracellular heme accumulation from measurement of heme content of crude extract of cells treated with zinc concentration 50 μM Zn(II) [38]. Zinc intoxication also is observed to disrupt or inhibit PGN biosynthesis [39].

The bactericidal activity of Zn^{2+}-dependent peptidoglycan recognition proteins (PGLYRPs) is salt insensitive and requires N-glycosylation of PGLYRPs that the LD99 of PGLYRPs for Gram-positive and Gram-negative bacteria is 0.3–1.7 M, and killing of bacteria by PGLYRPs does not involve permeabilization of cytoplasmic membrane, namely, zinc may be shown to inhibit PGN biosynthesis TG [40]. But, these limited PGLYRPs don't be applicable for Gram-negative bacteria. Thus, zinc ions could inhibit PGN synthesis TG against *S. aureus.*

3. Zinc induced PGN inhibitive elongation due to the activations of autolysins against *S. aureus*

Zn^{2+} binding Rv3717 showed no activity on polymerized PGN and however, it is induced to a potential role of N-Acetylmuramyl L-alanine Amidase [41], PGN murein hydrolase activity and generalized autolysis; Amidase MurA [42], Lytic Amidase LytA [43], enzymatically active domain of autolysin LytM [44], Zinc-dependent metalloenzyme AmiE [45] as prevention of the pathogen growth, and Lysostaphin-like PGN hydrolase and glycylglycine *endopeptidase* LytM [46].

Zn^{2+} ions-induced bacteriolysis and destruction of *S. aureus* PGN cell wall could be enhanced by the inhibitions of PGN elongation simultaneously with the activations of these PGN autolysins. Thus, zinc(II) ions can impair the activity of PGN biosynthesis TG and PGN elongation by bacteriolytic destruction of bacterial cell walls, causing bacterial lysis [47].

Accordingly, zinc induced PGN inhibitory biosynthesis corresponds to disruption of bacterial cell wall, but zinc ions may be possible to inhibit PGN synthesis TG and PGN elongation by PGN activated major autolysin of *amidase* against *S. aureus*.

4. Zinc induced disruption of outer membrane structure by hydrolases of lipoproteins at C-, N-terminals against *E. coli*

In zinc ion uptake across the outer membrane, the lipoproteins of Omp A, Omp C, Omp F porins have a role for at least some of these proteins in Zn^{2+} uptake, in which the lipoproteins have metallic cation selective and hydrophilic membrane crossing pore, to be effective for zinc transfer [48]. Zinc (II) ions react with -SH base, and then H_2 generates. Zinc bivalent is unchangeable as

-SZn—S— bond 4-coordinated.

$Zn^{2+} + 2(-SH) \rightarrow -SZn(II) -S- + 2H^+$

ZnPT (zinc pyrithione) and Tol (Tol proteins)-Pal (Proteinassociated lipoprotein) complex are antimicrobial agents widely used, however, it has recently been demonstrated to be essential for bacterial survival and pathogenesis that outer membrane structure may be disrupted [49,50]. Interaction zinc ions with *E. coli* Braun lipoprotein may be considered that Lpp as a new target of antimicrobial peptides is Gram-negative bacterial cell surface receptor for cationic antimicrobial peptides [51].

5. Zinc induced PGN inhibitive elongation through the damage of PGN synthesis enzyme of zinc-protein in periplasmic space and the activation of PGN autolysins against *E. coli*

The zinc-induced decrease of protein biosynthesis led to a

partial disappearance of connexin-43 of protein synthesis in neurons [52], but it is unknown whether PGN synthesis is inhibited. Further, it is also unclear whether the both TG/TP should be inhibited by the zinc ions [53-55]. The other, zinc ions were accumulated in *E. coli* periplasmic space, in which the zinc ions are spent to the activation of bacteriolysis of the cell wall. Zinc depending PGN autolysin, amidase PGRPs [56], zinc metallo enzymes AmiD [57], zinc-containing amidase; AmpD [58], zinc-present PGLYRPs [59] 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-transpeptidase IIW [60] requiring divalent cations. Thus, the inhibition of PGN elongation had been occurred by zinc ion-induced activa tions of PGN hydrolases and autolysins.

Accordingly, bacteriolysis of *E. coli* cell wall by Zn^{2+} ions is due to disruption of outer membrane structure by degrading of lipoprotein at C-, N-terminals through PGN formation inhibition by PGN inhibitive synthesis TG and PGN activated autolysins of *amidase* and *carboxypeptidase-transpeptidase*

6. Zinc induced ROS generation against *S. aureus* and *E. coli*

Zinc induced production of reactive oxygen species (ROS) against *S. aureus*: O_2^- and H_2O_2 permeate into membrane and cytoplasm, that DNA molecular is damaged by oxidative stress [61]. For the penetration of zinc 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 [62]. O_2^- and H_2O_2 permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress [61].

$O_2 + e^- + H^+ \rightarrow \bullet HO_2$

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

$H_2O_2 + e^- \rightarrow HO^- + \bullet OH$

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

$H_2O \rightarrow \bullet OH + \bullet H + e^- \rightarrow H_2O_2$

Zinc induced ROS production and oxidative stress against *E. coli*: Zinc ions react with -SH, and H^+, ROS generate. In *E. coli*, free radicals O_2^-, OH^-, •OH) and H_2O_2 are formed as follows [63]:

$O_2 + e \rightarrow O2^-$ $2\ O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ $O_2^- + H_2O_2 \rightarrow OH^- + \bullet OH + O_2^-$

In the cell wall, reacting with polyunsaturated fatty acids:

$LH + OH\bullet \rightarrow L\bullet + HOH$ $L\bullet + O_2 \rightarrow LOO\bullet$ $LH + LOO\bullet \rightarrow L\bullet + LOOH$

Zinc-containing Peptidoglycan Recognition Proteins (PGRPs) induce ROS production of H_2O_2, O_2^-, HO•, the ROS occur the oxidative stress, and killing by stress damage [64].

Accordingly, as mentioned above, metallic Ag^+, Cu^{2+}, Zn^{2+} ions-induced PGN inhibitive synthesis TG/TP, distruptive OM Lpp, and PGN activated autolysin against *S. aureus* and *E. coli* cell walls are summarized in Table 2, including bactericidal mechanism for metallic Ag^+, Cu^{2+}, Zn^{2+} complete-ionized ion solutions.

Table 2. Metallic Ag^+, Cu^{2+}, Zn^{2+} ions-induced PGN inhibitive synthesis TG/TP, disruptive OM Lpp, and PGN activated autolysin against *S. aureus* and *E. coli* cell walls

Ag^+,Cu^{2+}, Zn^{2+} Ions	*S. aureus* Cell Wall		*E. coli* Cell Wall		
	PGN SynthesisTG/TP	**PGN Autolysins**	**OM lipoprotein-endopeptidase**	**PGN Synthesis TG/TP**	**PGN Autolysins**
	➡ Ag^+, Cu^{2+}, Zn^{2+}, ROS, O_2^-, OH^- H_2O_2, O^-	**➡ Ag^+, Cu^{2+}, Zn^{2+},ROS, O^-, OH^- H_2O_2, O^-**	**➡Ag^+, Cu^{2+}, Zn^{2+},ROS**	**➡Ag^+, Cu^{2+}, Zn^{2+}, ROS, O_2^-, OH^-, ·OH and H_2O_2**	**➡ Ag^+, Cu^{2+}, Zn^{2+}, ROS O_2^-, OH^-, ·OH and H_2O_2**
Ag^+ ➡	Both inhibitive TG and TP	PGN activated majorautolysins of AmiA, AmiE	Disruptive OM Lppby *Endopeptidase (L,D-transpeptidase,LdtF)*	Inhibitive both TG and TP	PGN activated major autolysins
Cu^{2+} ➡	Both inhibitive TG and TP	PGN activated majorautolysin of AmiA	Disruption of outer membranestructure	Inhibitive both TG and TP	PGN activated major autolysins

Zn^{2+} ➡	Inhibitive TG	PGN activated majorautolysin of AmiD	Interaction zinc ions with *E. coli* Braun lipoproteinoccurs	Inhibitive synthesis TG	PGN activated autolysins of amidase, carboxypeptidase-transpeptidase

Bactericidal mechanism: Bacteriolysis and destruction of *S. aureus* cell wall by metallic Ag^{+},Cu^{2+}, Zn^{2+} ions occur by inhibition of PGN elon-gation through PGN inhibitive TG/TP (TG for Zn^{2+} ion) and PGN activated major autolysins.

Batericidal mechanism: Metallic Ag^{+}, Cu^{2+}, Zn^{2+} ions can disrupt OM Lpp and inhibit PGN elongation through PGN inhibitive TG/TP (TG forZn^{2+} ion) and PGN activated major autolysins against *E. coli.*

CONCLUSIONS

Anti-bacterial activity for Ag^{+} ion solution against *S. aureus* has been found that bacteriolysis and destruction of *S. aureus* PGN cell wall occurs by the inhibition of PGN elongation by Ag^{+}-induced *S. aureus* inactivating PGN synthesis transglycosylase TG and transpeptidase TP and enhancing the activation of PGN autolysins of amidase AmiA and AmiE. The other, against *E. coli*, the anti- bacterial activity for Ag^{+} ion solution has been found that bacteriolysis and destruction of *E. coli* cell wall by silver ions are caused by the disruption of outer membrane structure owing to the activation of *Endopeptidase, L,D-transpeptidase, LdtF* of lipoprotein at C- and N-terminals, and inhibition of PGN elongation through the damage of PGN TG/TP synthetic enzyme of silver-protein Amidase in periplasmic space, and PGN activated autolysins of amidase, peptidase, and carboxypeptidase.

Thus, antibacterial mechanism for Ag^{+} ions solution is clarified that bacteriolysis and destruction of bacterial cell wall occur by the disruption of *E. coli* outer membrane structure owing to the activation of *Endopeptidase (L,D-transpeptidase, LdtF)* of lipoprotein at C- and N-terminals, and by inhibition of PGN elongation through the damage of PGN synthetic TG/TP enzymes and PGN activated autolysins of amidase against *S. aureus* and *E. coli*.

Bacteriolysis of *S. aureus* PGN cell wall by Cu^{2+} ions are thought to be due to inhibition of PGN elongation owing to the damages of PGN both synthetic TG/TP and the activations of PGN major autolysin of AmiA. The other, bacteriolysis of *E. coli* cell wall by Cu^{2+} ions occur by disruption of outer membrane structure due to degradation of lipoprotein at N-, C-terminals, damage of PGN syntheses TG and TP enzyme, and activations of PGN major autolysins. Furthermore, deletion of PGN autolysin also becomes bacteriolytic factor.

Anti-bacterial activity of Zn^{2+} ions against *S. aureus* has been found that Zn^{2+} ions-induced PGN autolysin activation could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis and destruction of *S. aureus* PGN cell wall. The activations of these PGN autolysins by Zn^{2+} ions could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis of *S. aureus* PGN cell wall. The other, antibacterial mechanism of Zn^{2+} ions against *E. coli* was found that Bacteriolysis and destruction of *E. coli* cell wall by Zn^{2+} ions are due to disruption of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to PGN formation inhibition by damage of PGN synthesis TG and PGN autolysins of amidase and carboxypeptidase-transpeptidase.

Ag^{+}, Cu^{2+}, Zn^{2+} ions-induced ROS generation of O_2^- and H_2O_2 and ROS-mediated oxidative stress in bacterial cell lead to killing by stress damage for silver ions, cell membrane damages due to high reactive •OH and OH^- are formed by Haber-Weiss and Fenton reactions for Cu^{2+} ions, and DNA molecular damage for Zn^{2+} ions.

Accordingly, bactericidal mechanism for complete-ionized metallic Ag^{+}, Cu^{2+}, Zn^{2+} ions solutions has been established that Ag^{+}, Cu^{2+}, Zn^{2+} ions, respectively, induced the bacteriolyses and destructions of bacterial cell walls occur by disruption of *E. coli* outer- membrane lipoprotein and by inhibition of PGN elongation through PGN both inhibitory syntheses TG/TP (TG for Zn 2+ ion) and PGN activated major autolysin of amidase. Ag^{+}, Cu^{2+}, Zn^{2+} ions-induced ROS generation of O_2^- and H_2O_2 and ROS-mediated oxidative stress in bacterial cell lead to killing by stress damage, cell membrane damages due to high reactive •OH and OH^-, and DNA molecular damage.

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