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Comparative Bacteriolytic Mechanism for Ag^+ and Zn^{2+} Ions against *Staphylococcus Aureus and Escherichia Coli*: A review

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ABSTRACT

Bactericidal influences of highly antibacterial silver(I) ions and zinc(II) ions on bacteriolyses of bacterial cell walls by activated imbalance between peptidoglycan (PGN) syntheses and PGN autolysins have been compared against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). Bacteriolytic activity of S. aureus PGN cell wall by Ag^+ ions is considered to be caused by inhibition of PGN elongation due to regulation of PGN synthetic transglycosylase (TG) or transpeptidase (TP) and enhancement of the activation of amidase of PGN autolysins, but, the bacteriolysis for Zn^{2+} ions may be caused that Zn^{2+} ions inhibit the PGN elongation due to the activations of PGN autolysins. On the other hand, bacteriolytic destruction of E. coli cell wall is regarded to be caused by disrupting of outer membrane structure due to degradative amidase for Ag^+ ion, and degradative endopeptidase for Zn^{2+} ion at the lipoproteins at N- and C-terminals. Further, the bacteriolyses by Ag^+ and Zn^{2+} ions occur by the inhibition of PGN elongation owing to the inactivation of PGN TP synthetic enzyme endopeptidase and enhancement of the activations of PGN autolysins of amidases and carboxypeptidase. Ag^+ and Zn^{2+} ions induced reactive oxygen species (ROS) generations such as O_2^-, H_2O_2, ·OH, OH^- producing in bacterial cell wall occur and lead to oxidative stress and DNA damage. The DNA damages are ascribed to two-coordinated linear Ag^+ complex formations by Ag^+ substitution, and four-coordinated planar Zn^{2+} complex and six-coordinated Zn^{2+} complex formations by Zn^{2+} substitution, within double and triple hydrogen bonds in DNA base pairs

Keywords: *Silver (I) and zinc (II) ions, Bacteriolysis, PGN synthesis and autolysin, ROS, DNA damage.*

Abbreviations: *ABNC: Active but nonculturable, A-Adenine, AgNPs-Silver nanoparticles, C-Cytosine, E. coli-Escherichia coli, G-Guanine, LP-Lipoprotein, LPS-Lip polysaccharide, MTs-Metallothioneins, MBC-Minimum bactericidal concentration, MIC-Minimum inhibitory concentration, NAG-N-acetyl glucosamine, NAM-N-acetylmuramic acid, OM-Outer membrane, Omp-Outer membrane protein, PGRPs-Peptidoglycan recognition proteins, Pal-Protein-associated lipoprotein, PBP2-Penicillin-binding protein 2, PEL-Permissible exposure limit, PGN-Peptidoglycan, ROS-Reactive oxygen species, S. aureus-Staphylococcus aureus, T-Thymine, TG-transglycosylase, Tol-Tol protein, TP-Transpeptidase.*

INTRODUCTION

Silver, copper, and zinc of transition metals have highly antibacterial activities and are utilized as chemotherapy agents.

Recently, antibacterial activities of silver, copper, zinc and these complexes call attention to potential treatments such as prevention of serious diseases, exploitation during bacterial pathogenesis, and cancer and tumor cell. Silver absorbed into the body as Ag^+ readily binds to intracellular proteins, serum albumins and macroglobulins for metabolism that Ag^+ actively absorbed from silver nitrate or silver sulphadiazine induces and binds the cysteine-rich proteins —Metallothioneins (MTs) Ⅰ and Ⅱ in metabolically active cells of the wound margin [1]. Bactericidal activity of Ag^+ ions is apparent to be strong from the experimental data that minimum inhibitory concentration (MIC) = 8 ppm, minimum bactericidal concentration (MBC) = 32 ppm for Ag^+ in silver sulfate solutions against *S. aureus* are obtained [2].

The other, MIC= 625 ppm, MBC= 1250 ppm for copper nitrate solutions against *S. aureus* are gained [3]. Owing to their antibacterial properties, recently advanced silver nanoparticles (AgNPs) are the most commonly used that an oxidation reaction at nanoparticle surface, from the elemental Ag (0) to

Ag^+, and a subsequent binding of Ag^+ to a ligand L^-, AgL in which important ligands to be considered are chloride, sulfide, and organic ligands with thiol groups [4]. These results of reaction of Ag^+ ion with Ligand are caused by antibacterial factors.

In the metallic sulfate solutions, Al^{3+}, Zn^{2+}, Cu^{2+}, Ag^+ have higher antibacterial activities. From these observations, the antibacterial order is $Zn^{2+} > Cu^{2+} > Ag^+ > Al^{3+}$, in which Zn^{2+} ions have indicated the highest antibacterial effect [5].

Zn^{2+} ions exhibit antimicrobial activity against various bacteria that released Zn^{2+} contributes inhibition of bacterial cell growth and DNA damage which the effectiveness of Zn inhibition of bacterial growth results from changing the active transport system and impeding the initial phase of bacterial mating [6].

Synergistic antibacterial effect of Ag^+ and Zn^{2+} is dependent upon the amount of Zn^{2+} present in the medium that atomic $Ag^+ - Zn^{2+}$ ratios higher than 1:6 appear to be optimum ratios against Gram-positive *E. faecalis* that the mechanism by which Ag^+ and Zn^{2+} interacts synergistically with each other is not clearly understood [7].

And then, it is unclear whether the antibacterial factor for these highly antibacterial silver and zinc ions is caused by inhibition of peptidoglycan (PGN) elongation for bacterial cell wall growth or not. However, the bacteriolysis of bacterial cell wall should be considered to relate directly with PGN synthesis and autolysin.

For the sake of growth of bacterial PGN cell wall, there is necessarily required for the adequate balance between PGN biosynthesis and PGN autolysin.

When the balance is broken, bacteriolysis and destruction of the bacterial cell wall may become to occur. Realization of PGN growth inhibition is found to be bacteriolytic phenomenon of bacterial cell wall that specially, PGN autolysin has important role for bacteriolyses. The PGN autolysin is peptidoglycan hydrolase that can collectively cleave almost any glycoside and amide bond which hydrolase sculpt the shape, size, and thickness of peptidoglycan and amidase have a prominent role in septum cleavage [8, 9].

In this review, bacteriolysis and destruction of bacterial cell walls by silver (I) and zinc (II) ions are compared with molecular structures of bacterial cell walls, bacteriolysis of *S. aureus* PGN cell wall, destruction of *E. coli* outer membrane lipoprotein, and activation or inactivation at action sites of PGN syntheses and PGN autolysins.

Molecular Structures of Bacterial Cell Walls and Peptidoglycan Syntheses/Autolysins against *S. Aureus*

Bacterial PGN syntheses are present in chiefly Transglycosylase (TG) and Transpeptidase (TP), and the other, PGN autolysin is hydrolyzing enzymes. Molecular structure of *S. aureus* PGN cell wall and action sites of synthetic TG and TP, and autolysins are shown in **Figure 1.**

S. aureus surface layer consists of teichoic acids, lipoteichoic acids, and thick PGN envelope cell wall [10]. There are action sites of transglycosylase (TG) and transpeptidase (TP) mainly on thick PGN layer.

The TG is the synthetic enzymes of *N-acetylglucosamidase* cleavage between NAG (N-acetylglucosamine) and NAM (N-acetyl-muramic acid), and N-acetylmuramidase cleavage between NAM and NAG on glycan chain. The TP is the synthetic enzyme cleavage between Glycine and D-alanine on PGN crosslinking.

The other, there are PGN autolysins of N-acetylmuramyl-L-alanine amidase cleavage,*DD-endopeptidases* cleavages between Glycine and Glycineon penta-glycine $(Gly)_5$, and in addition, *lysostaphin* cleavage between glycine and glycineon PGN cross-linking [11].

PGN synthesis requires glycosytransferases to polymerize the glycan chain and DD-transpeptidase to crosslink the peptides, and PGN cleavage of PGN hydrolases and autolysins is required for reductive cell division and cell separation that amidase has a prominent role in septum cleavage, but lytic transglycosylases and endopeptidases contribute to cell separation, and their role is probably understated owing to their greater redundancy, in which they play a vital role in regulating cell wall growth as well as other lysis phenomena [9].

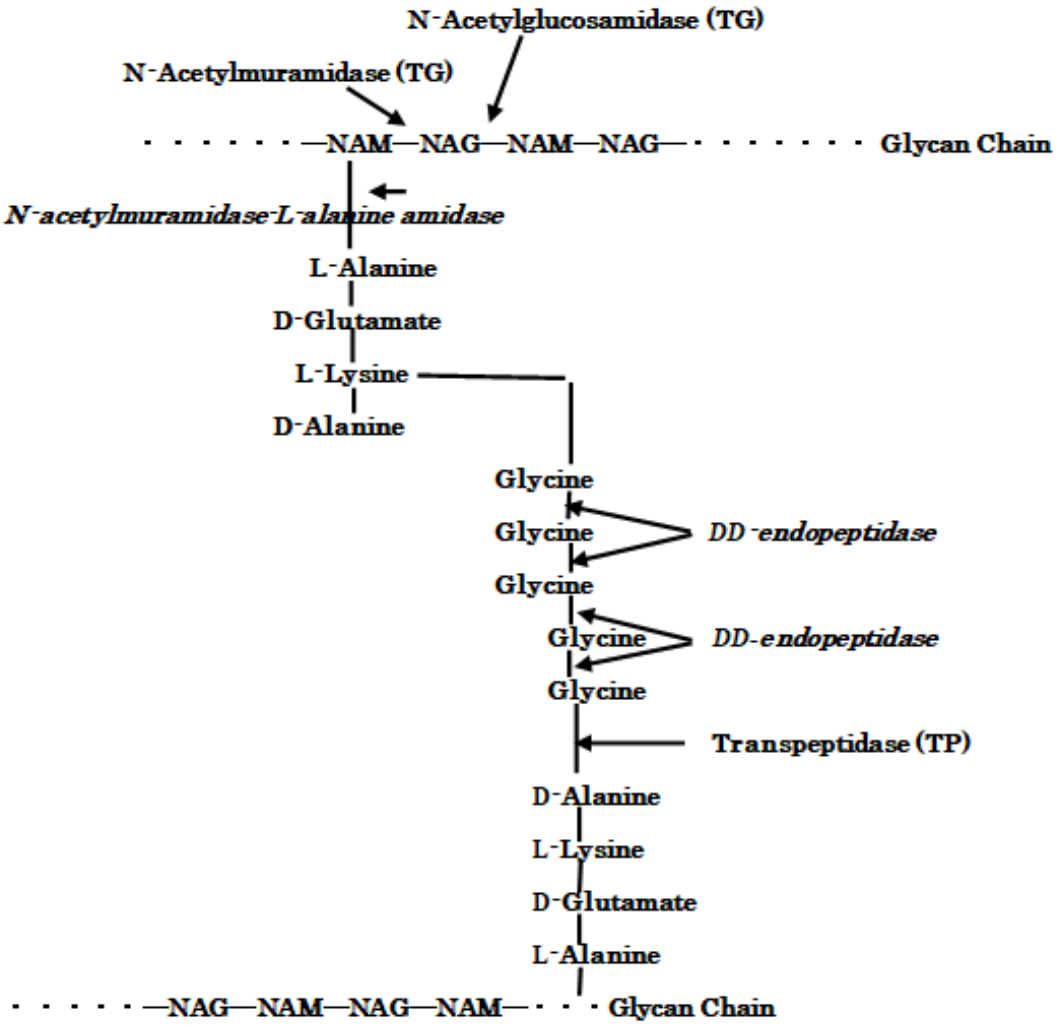

Fig1. ***Molecular structure of PGN cell wall and action sites of PGN synthetic TG/TP and autolysins against S. aureus***

Degradative Enzymes of Lipoprotein at N- and C-Terminals, PGN TG·TP Syntheses and PGN Autolysins against *E. coli*

Molecular structure of *E. coli* outer membrane cell wall and action sites for degradative enzymes in outer membrane lipoprotein at N- and C-terminals and for PGN synthetic TG/TP and autolysins are represented in **Figure 2.**

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 [10]. 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 periplasmic 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. The other, PGN hydrolases and autolysins are degradative enzymes of *Amidase* cleavage, *Peptidase* cleavage, and *Carboxypeptidase* cleavage [12]. Penicillin-binding protein 2 and Rod A (encoded downstream of the PBP2 gene) are required for the PGN synthesis of glycan strands during elongation and the periplasmic amidase cleave only one-sixth of the PGN that is turned over by the lytic trans-glycosylases [13]. *E. coli* has at least 13 periplasmic PGN hydrolases (autolysins), which can collectively cleave almost any glycoside and amide bond [9]. Interactions of PGN molecular structure with PGN syntheses and autolysins influence in any event the bacteriolytic cell walls.

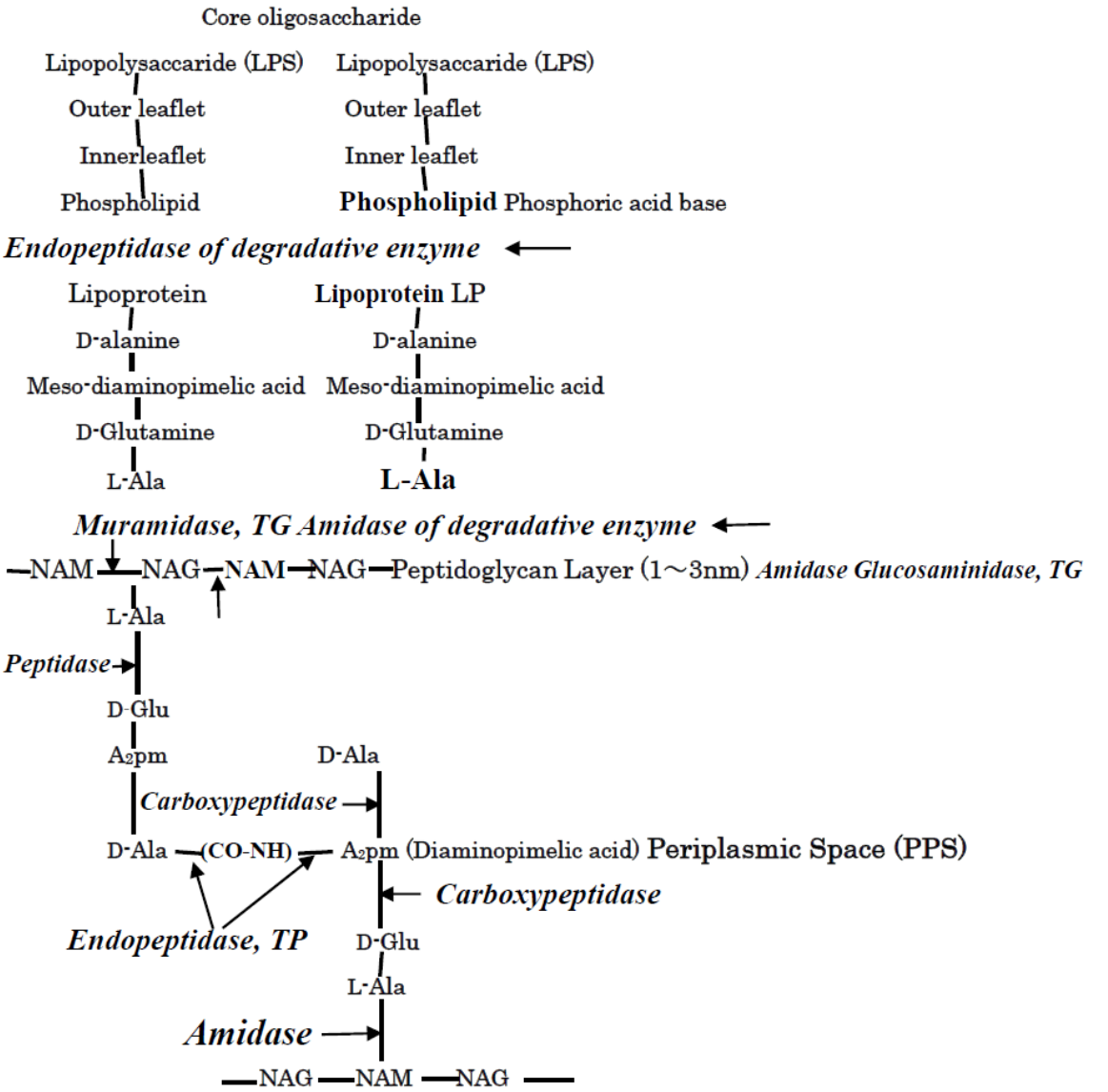

Fig2. ***Molecular structure of outer membrane cell wall and PGN thin layer in periplasmic space against E. coli, and action sites of degradative enzymes of lipoprotein at N- and C- terminals and PGN synthetic TG/TP and autolysins***

Discussion

Ag^{+} and Zn^{2+} Ions Induced S. Aureus PGN Cell Wall and *E. Coli* Outer Membrane with PGN Thin Layer within Periplasmic Space

Ag^{+} and Zn^{2+} Ions Induced PGN Synthetic Enzymes of TG and TP against S. Aureus

The released Ag^{+} ions that penetrated from AgNP 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 [14]. Wall teichoic acids are spatial regulators of PGN cross-linking biosynthesis TP [15].

Silver ions could inhibit both TG and TP enzymes of the PGN that Ag^{+}-induced bacteria may inactivate PGN synthesis transglycosylase TG[16] and transpeptidase TP[17,18].The other, 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.

Ag^{+} and Zn^{2+} Ions Promote Amidase Activations of S. Aureus PGN Autolysin

Lytic activity was inhibited by glucosamine, NAG, Hg^{2+}, Fe^{3+}, and Ag^{+} [19], and Lytic Amidase LytA [20], enzymatically active domain of Autolysin LytM [21], 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.

The other, Zn^{2+} binding Rv3717 showed no activity on polymerized PGN and but, it is induced to a potential role of N-Acetylmuramyl-L-alanine Amidase [22], PGN murein hydrolase activity and generalized autolysis; Amidase MurA [23], Lytic Amidase LytA [20], enzymatically active domain of autolysin LytM [21], Zinc-dependent metallo-enzyme AmiE [24] as prevention of the pathogen growth, and Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM [25]. These autolysins are an important role in the killing of bacteria [26].

Ag^+ and Zn^{2+} Ions Enhance Inactivation of *S. Aureus* PGN Syntheses and Activation of PGN Autolysins

Ag^+ ions induced inactivating PGN synthesis and activated amidase of PGN autolysin occur simultaneously, subsequently, bacteriolysis of *S. aureus* PGN cell wall results with suppression of PGN growth. Accordingly, the cause of bacteriolysis of *S. aureus* PGN cell wall by Ag^+ ions is due to the inhibition of PGN elongation by inactivation of PGN TG or TP and enhancement of activation of amidases of PGN autolysins. The other, Zn^{2+} induced the activations of PGN autolysins are considered to enhance inhibitions of PGN elongation with bacteriolysis and destruction of *S. aureus* PGN cell wall.

Production of Reactive Oxygen Species (ROS) against S. Aureus

For the penetration of Ag^+ or Zn^{2+} 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 [27]. $O_2^-$and H_2O_2 permeate into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress [28].

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

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

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

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

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

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

$$\cdot OH + e^- + H^+ \rightarrow H_2O$$

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

Ag^+ and Zn^{2+} Ions Induced Outer Membrane, Lipoprotein, and PGN within Periplasmic Space against *E. coli*

Permeability of Ag^+ and Zn^{2+} 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 [29]. The OmpA, OmpC, OmpF porins of lipoproteins have metallic cation selective and hydrophilic membrane crossing pore.

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 [30].

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 [31].

Silver adsorption by *E. coli* cells displays metallothioneins (MTs) anchored to the outer membrane protein LamB that the complete MT sequences are anchored by their N-termini and C-termini to the permissive site 153 of the protein [32]. 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 [33]. The addition of glucose as an energy source to starved cell activated the Ag efflux on the increased Ag accumulation in Ag-susceptible and-resistant 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 [34];

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

The other, in permeability of zinc ions into *E.coli*

cell wall, the first permeability barrier of zinc ions in the *E.coli* cell wall is highly anionic LPS with hydrophobic lipid A, core polysaccharide, 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 [29]. 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 [35]. Zinc (II) ions react with -SH base, and then H_2 generates. Zinc bivalent is unchangeable as -SZn－S－bond 4-coodinated;

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

Ag^+ and Zn^{2+} Ion Induced Destruction of Outer Membrane Structure by Degradation of Lipoproteins at C- and N-Terminals against *E. Coli.*

Tol protein (Tol)－protein – associated lipoprotein (Pal) system is composed of five proteins that Tol A, Tol Q, and Tol R are inner membrane proteins, Tol B is a periplasmic protein, and Pal, the peptidoglycan-associated lipoprotein, is anchored to the outer membrane [36].

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 [37,38]. It is unclear whether both Endopeptidase and Amidase of lipoprotein at C- and N-terminals are simultaneously activated by Ag^+ ions. However, the outer membrane structure is considered probably to be destroyed by predominant activation of lipoprotein-amidase.

Zinc ions induced destruction of *E. coli* cell wall outer membrane structure occurs by hydrolases of lipoproteins at C-, N-terminals that ZnPT (zinc pyrithione) and Tol (Tol proteins)-Pal (Protein-associated 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 destroyed [39].

Ag^+ and Zn^{2+} Ion PGN Synthesis in Periplasmic Space and PGN Autolysins against E. coli

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 function to be degradative by Ag^+ binding proteins [40]. The other, it is unclear that the silver-induced PGN syntheses TG/TP should be inhibited by the silver ions [41, 42], but silver ions inactivate probably TP of endopeptidase from destructive observation of bacterial cell walls [33]. Silver ions enhance activation of *E. coli* PGN autolysins of amidase, peptidase, carboxypeptidase [43,44], such as silver depending PGN autolysin, Ami C [45], Ami D [46], Muramidase [47], Amino acid amidase [48], Carboxypeptidase-degraded aldolase [49], Carboxypeptidase Y [50] that serve to be effective for the PGN autolysins. It is particularly worth noting that enhancement of the activities of autolysins is characterized on PGN carboxy peptidase and TP-endopeptidase [40] requiring divalent cations. Degradation of *E. coli* PGN during elongation may be turned over by lytic TG and periplasmic amidase [13]. Thus, the inhibition of PGN elongation had occurred by silver ion induced activities of PGN hydrolases and autolysins. Ag^+ ions promote damage of *E. coli* PGN synthetic enzyme of silver-protein amidase in periplasmic space and the activations by amidase, peptidase, and carboxypeptidase of PGN autolysins. Accordingly, antibacterial mechanism for *E. coli* by Ag^+ ions is found that bacteriolysis and destruction of *E.coli* cell wall are caused by the destruction of outer membrane structure owing to the activation of amidase of lipoprotein at C- and N-terminals, and by inhibition of PGN elongation due to the damage of PGN synthetic enzyme of silver-protein amidase in periplasmic space and the activations of PGN autolysins of amidase, peptidase, and carboxypeptidase. The other, the zinc-induced decrease of protein biosynthesis led to a partial disappearance of connexin-43 of protein synthesis in neurons [51], 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 [41,52]. 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 [44], zinc metalloenzymes AmiD [46], amidase zinc-containing amidase; AmpD [53], zinc-present PGLYRPs [54] 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[55] requiring divalent cations.

Accordingly, the inhibition of PGN elongation had occurred by zinc ion induced activities of PGN hydrolyses and autolysins.

ROS Production and Oxidative Stress against E. Coli

Silver or zinc ions reacted with -SH, and H^+ generates. In *E.coli*, free radicals O_2^-, OH^-, ·OH and $H_2O_2$are formed as follows [56]:

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

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

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

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·, and then the ROS occur the oxidative stress, and killing by stress damage [57].

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.

As mentioned above, bacteriolyses and destructions of bacterial cell walls by antibacterial Ag^+ and Zn^{2+} ions are caused of inhibition of PGN elongation by which the activations of degradative hydrolases and PGN autolysins against *S. aureus* and *E. coli* are summarized in **Table 1.**

Table1. ***Antibacterial Ag^+ and Zn^{2+} ions induced activations of degradative hydrolase and PGN autolysins, and ROS generations against S. aureus and E. coli***

Ag^+ and Zn^{2+} ions	*S. Aureus* Cell Wall	*E. Coli* Cell Wall	
	PGN Layer Cell Wall	**Lipoprotein at C- and N-Terminal**	**Periplasmic Space Thin PGN Layer**
Ag^+ ion	**Ag^+, O_2^-, H^+, ·OH, H_2O_2, ·HO_2, ·NO, ONOO$^-$**	**Ag^+, O_2^-, H_2O_2,·OH**	**Ag^+, O_2^-, H_2O_2, OH^-, ·OH**
	·PGN TG or TP inactivation ·Autolysins; Amidases Mur A, Lyt A. $Ag^+ + (-SH)^- \rightarrow AgSH \rightarrow [Ag_2(SH)_2S]^{2-}$	·Activation of degradative amidase	·PGN autolysins; Amidase, peptidase and carboxy-peptidase
Zn^{2+} ion	**Zn^{2+},O_2^-,H_2O_2,·OH,·NO,ONOO$^-$**	**Zn^{2+}, O_2^-, H_2O_2**	**Zn^{2+}, O_2^-, H_2O_2, OH^-, ·OH**
	·PGN TG/TP inactivation is unclear. ·Amidase and endopeptidase	·Endopeptidase activation $Zn^{2+} + 2(-SH) \rightarrow -S\ Zn(II) - S + 2H^+$	·PGN autolysins; Amidases and carboxypeptidase-transpeptidase.

Damage within DNA Base-Pairs

DNA Damage by Ag^+ Ions

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 · 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 3.** Ground state-structure in Figure 1 is considered that the gain in electronic charge is mainly localized on the noble silver metal atom for anions and the charge is donated partially by the metal and partially by the nucleobase for cations. In neutral case, binding occur through positive and negative centers, where the s electronic orbital in the metal atom hybridizes with the d orbital by redistributing the charge to favor the dipolar interaction [58]. Silver ion can bond to consecutive guanines that when the metal bonds to a non-hydrogen-bonding site it has only a very small effects on the structure of the base pair and on the strengths of the hydrogen bonds of the base pair, in which the bonding of the metal atoms to two consecutive base pairs leads mechanism of popular cisplatin-based anti-

cancer drugs [59]. Thus, it may be considered that DNA damages due to linear two-coordinated Ag^+ 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 [60].

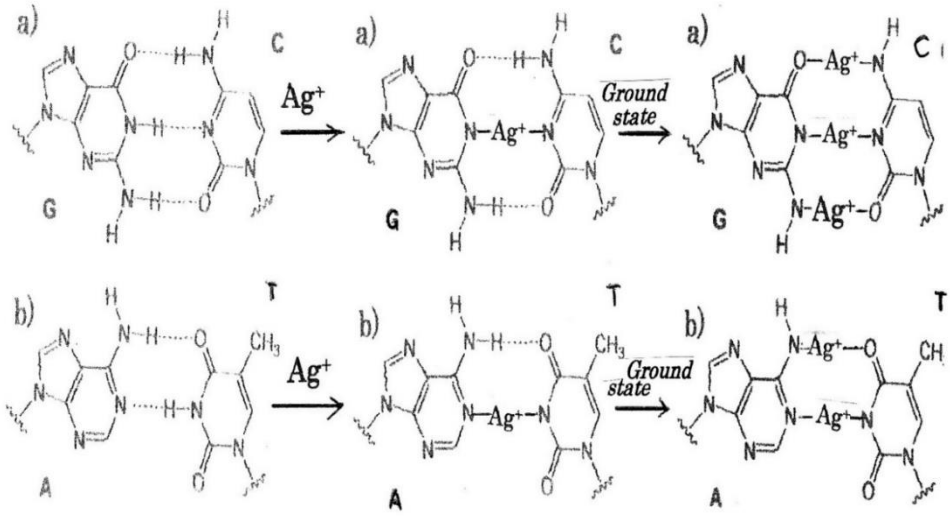

Fig3. ***Ag^+ substitution into the triple and double hydrogen bonds within DNA base-pairs G≡C and A=T pairs***

a) 2-coordinated linear Ag^+ complex formation in G≡C pair Ground state; O-Ag^+-N, N-Ag^+-N, N-Ag^+-O (stable)

b) 2-coordinated planar linear Ag^+ complex formation in A=T pair Ground state; N-Ag^+-O, N-Ag^+-N (stable).

Damage of DNA Base-Pairs by Zn^{2+}Ions

Zn^{2+} ion induced occurrence of generations of ROS and hydrogen peroxide H_2O_2 in bacterial cells damages DNA, in which formation of DNA damage resulting from a release of catalytic binding of zinc ion to DNA with generation of 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 Zn^{2+} ions into hydrogen bonds in DNA base-pairing **G** (guanine) ≡ **C** (cytosine) and **A** (adenine) = **T** (thymine) pairs occurs by Zn^{2+} ion substitution shown in **Figure 4**. Thus, it may be considered that DNA damages due to Zn-complex within DNA base-pairs **G≡C, A=T** is formed in the hydrogen bonds.

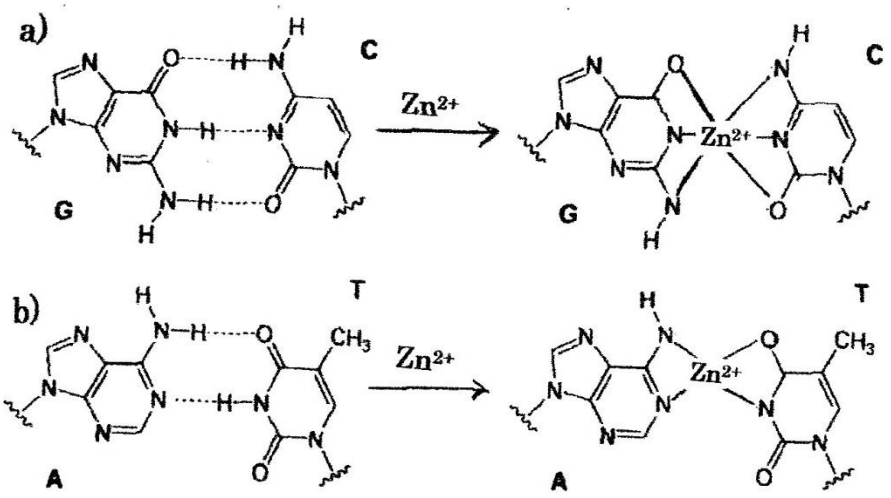

Fig4. ***Zn^{2+} substitution into the triple and double hydrogen bonds in DNA base-pairing G: C and A: T pairs***

a) G≡C base pair, 6-coodinated Zn^{2+} complex formation (stable)

b) A=T base pair, 4-coodinated planar Zn^{2+} complex formation (unstable)

Conclusion

Ag^+-induced PGN synthesis TG or TP on S. aureus PGN cell wall is inactivated, and simultaneously, PGN autolysin is activated. Bacteriolysis of *S. aureus* PGN cell wall that the wall teichoic acids control PGN synthesis cross-linking TP, may be caused by the inhibition of PGN elongation due to enhancement of the activities of PGN autolysins; amidase Ami A and Ami E, and PGN hydrolase Lysostaphin-like endopeptidase. The other, Zn^{2+} ions are thought that the activations of these PGN autolysins could be enhanced the inhibitions of PGN elongation simultaneously, with bacteriolysis and destruction of *S. aureus* PGN cell wall. Accordingly, Zn^{2+} ions inhibit the PGN elongation due to the activations of PGN autolysins. Bacteriolysis and destruction of *E.coli* outer membrane lipoprotein cell wall by Ag^+ ions are considered that the damage of LPS synthesis and destructing of outer membrane structure by degrading of lipoprotein at C-, N-terminals occur, and bacteriolytic cause due to the inhibition of PGN growth formation by enhancement of activation of amidase, peptidase, and carboxypeptidase of PGN autolysins results with inactivation of TP-endopeptidase. The other, Zn^{2+} ions are particularly worth noting that enhancement of the activities of autolysins is characterized on PGN carboxypeptidase-transpeptidase IIW requiring divalent cations. Accordingly, the inhibition of PGN elongation had occurred by zinc ion induced activities of PGN hydrolases and autolysins. By the penetration of silver or zinc ion into *S. aureus* cell wall, production of O_2, H^+, H_2O_2, $ONOO^-$occurs against *S. aureus*. The other, in silver and zinc ions into *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 of cell membrane proteins and DNA molecular in cytoplasm. The DNA damages may be considered that are ascribed to two-coordinated linear Ag^+ complex formations by Ag^+ substitution, and four-coordinated planar Zn^{2+} complex and six-coordinated Zn^{2+} complex formations by Zn^{2+} substitution, within DNA base pairs of A=T double and G≡C triple hydrogen bonds in cytoplasm of bacterial cells.

Conflict of Interest

The author declares no conflict of interest.

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