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Antibacterial Mechanism of Bacteriolyses of Bacterial Cell Walls by Zinc(Ⅱ) Ion Induced Activations of PGN Autolysins, and DNA damages

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Abstract

Bacteriolyses and destructions of bacterial cell walls by zinc (Ⅱ) ion solutions were investigated against *Staphylococcus aureus* and *Escherichia coli*. Bacteriolysis of *S. aureus* peptidoglycan (PGN) cell wall by zinc ion solution is due to the inhibition of PGN elongation by the activations of PGN autolysins of amidases. The other, bacteriolysis and destruction of *E. coli* cell wall are caused by the destruction of outer membrane structure due to degradative enzymes of lipoproteins at N- and C-terminals, and also inhibition of PGN elongation is dependent on the activities of PGN hydrolases and autolysins of amidase and carboxypeptidase-transpeptidase. Zinc ions induced ROS such as O_2^-, H_2O_2, ·OH, OH^- producing in bacterial cell wall occur oxidative stress. DNA damages may be due to Zn ion complex formation by Zn^{2+} substitution into hydrogen bonds in DNA base pairs.

Keywords

Zinc ions; PGN cell wall; Outer membrane lipoproteins; Bacteriolysis; Degradation; Biosynthesis and autolysin; Reactive oxygen species (ROS); DNA base-pairs

Introduction

Silver, copper, and zinc of transition metals have highly antibacterial activities and are utilized as chemotherapy agents. Recently, antibacterial activities of zinc and its complexes call attention to potential treatments such as prevention of serious diseases [1], exploitation during bacterial pathogenesis [2,3], virus counter- measure [4], anti-cancer and anti-tumor cells [5]. Halo inhibitory tests against *Staphylococcus epidermidis* were carried out various sulfate solutions, in which the result was also obtained that the antibacterial effect of zinc ions was the highest in the sulfate system with halo inhibitory large zone for zinc ion solutions [6]. In this study, considering such as the highest antibacterial activity for Zn^{2+} ions obtained from halo inhibitory tests of metallic sulfate solutions, the processes of bacteriolyses and destructions by antibacterial activities of Zn^{2+} ions are analyzed and considered against thick peptidoglycan(PGN) layer cell wall, and outer membrane-connecting thin PGN layer cell wall. Further, the bacteriolytic mechanisms by zinc (Ⅱ) ions solutions have been revealed against Gram-positive *S. aureus* and Gram-negative *E. coli* cell walls, with additional DNA damage by Zn^{2+}-DNA base pairs interactions. These Zn^{2+}-proteins and Zn^{2+}-DNA interactions are considered to enhance the persistence in several forms of Zn-ligand bonds (O, S, NH_3, H_2O, OH, SCH_3, and H ligands) with Zn coordination chemistry and molecular orbital theory.

Analyses of Bacterial Cell Walls, PGN Biosyntheses and Autolysins, and the action sites of PGN syntheses and 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 bacteria cell, molecular bonding manner, and zinc ion characteristics also were searched.

Molecular structure of *S. aureus* and *E. coli* cell walls, and action sites of PGN biosyntheses of both transglycosylase TG and transpeptidase TP; TG/TP and PGN autolysins

S. aureus surface layer consists of teichoic acids, lipoteichoic acids, and thick PGN cell wall [6]. The schematic structure of *S. aureus* cell wall is shown in Figure 1, in which the molecular structure of *S. aureus* PGN cell wall and the action sites of TG/TP synthesis enzymes, PGN forth autolysins, and lysostaphin are represented in Figure 2.

The other, *E.coli* cell wall consists of lipid A, lipopolysaccharide, porin proteins, outer membrane of lipoprotein, and thinner 2-7nm PGN layer in 30-70nm periplasmic space [7]. Figure 3 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 are represented in Figure 4. Furthermore, Figure 5 shows *E. coli* PGN synthetic enzymes TG/TP and the action sites of the autolysins such as amidase, peptidase, carboxy- peptidase, etc. Interactions of PGN molecular structure, synthesis, autolysin molecular structure, synthesis, and autolysin influence in any event the bacteriolysis of bacterial cell walls (Figures 1-3).

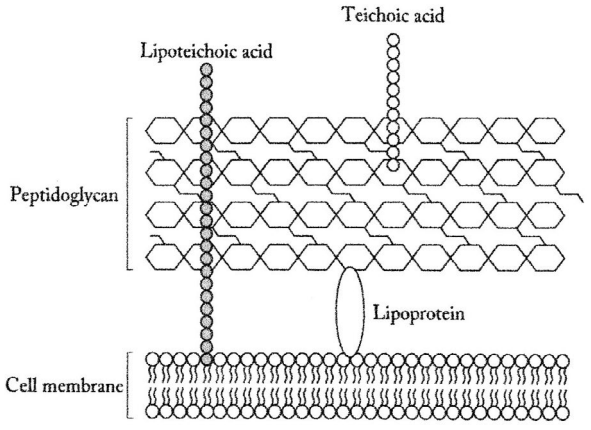

Figure 1: Schematic structure of S. aureus cell wall.

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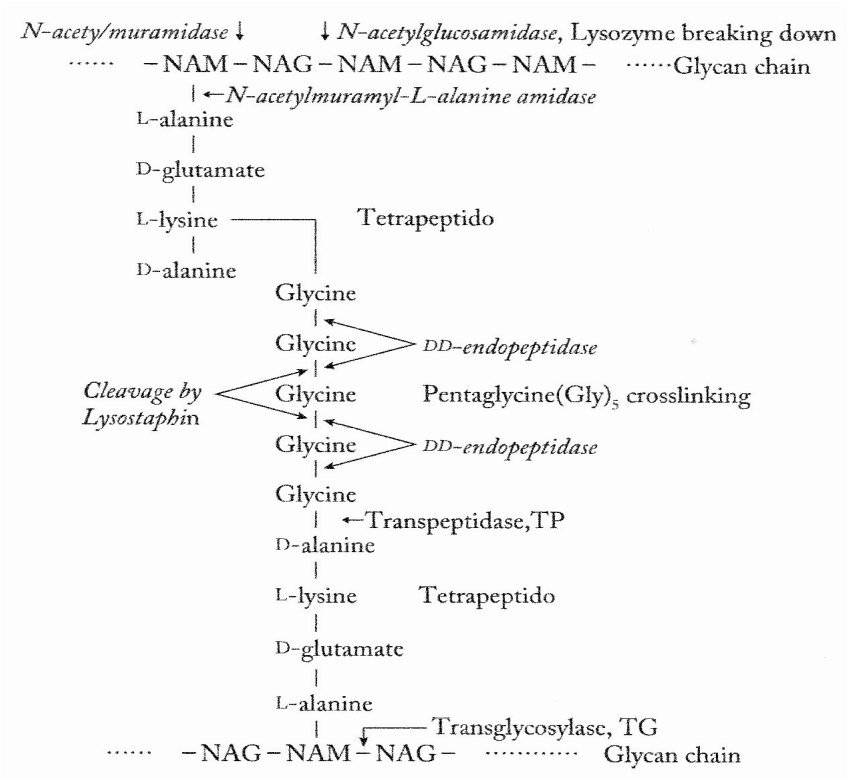

Figure 2: Molecular structure of S. aureus PGN cell wall and the action sites of PGN syntheses TG/TP enzymes, forth autolysins, and lysostaphin against S. aureus.

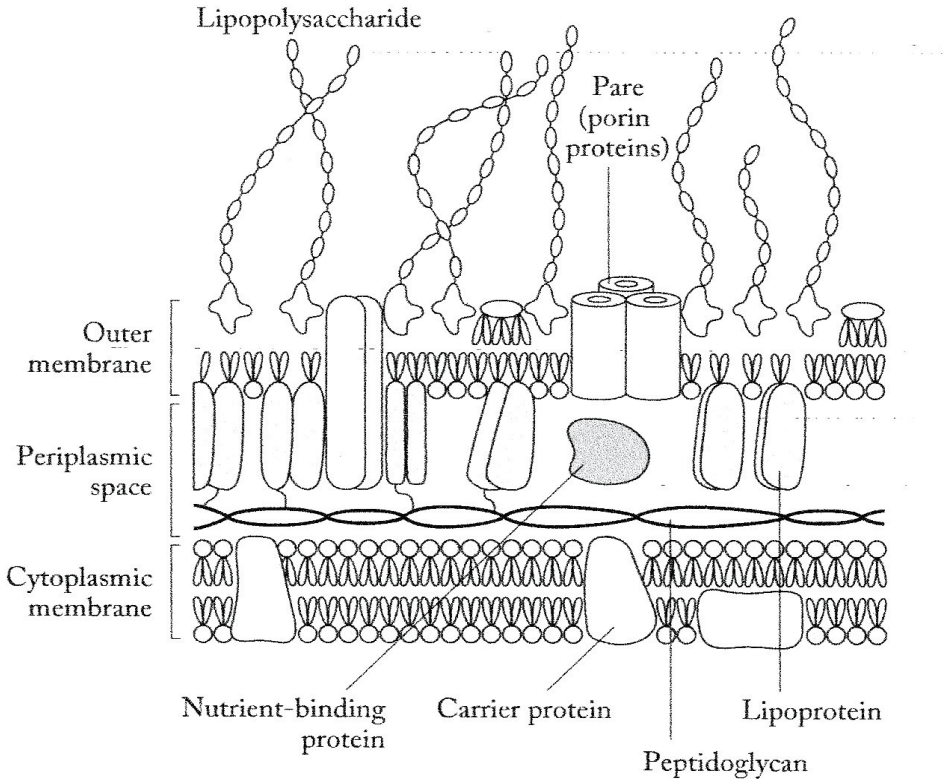

Figure 3: Schematic structure of outer membrane (OM) and periplasmic space of *E. coli* cell wall.

Discussions on bacteriolyses by zinc ions

Bacteriolysis and destruction of *S. aureus* PGN cell wall by zinc ions: For the sake of growth of *S. aureus* PGN cell wall, there is necessarily required for the adequate balance between PGN biosynthesis and PGN autolysin. When the balance was broken to be imbalanced, bacteriolysis and destruction of the cell wall should become to occur.

PGN biosynthesis enzymes of transglycosy-lase TG and transpeptidase TP: Wall teichoic acids are spatial regulators of PGN cross-linking biosynthesis TP [8]; however, it is not explicit whether zinc ions could inhibit both TG and TP enzymes of the PGN.

Inhibition of PGN elongation due to the activations of autolysins: 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 [9], PGN murein hydrolase activity and generalized autolysis; Amidase MurA [10] (Figure 4).

Lytic Amidase LytA [11], enzymatically active domain of autolysin LytM [12], Zinc-dependent metalloenzyme AmiE [13] as prevention of the pathogen growth, and Lysostaphin-like PGN hydrolase and glycylglycine endopeptidase LytM [14]; 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. O_2^- and H_2O_2 permeates into membrane and cytoplasm, and then, DNA molecular is damaged by oxidative stress [15] (Figure 5).

Production of reactive oxygen species (ROS) against *S. aureus*: 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 [16].

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

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

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

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

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

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

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

From above-mentioned results, the bacteriolytic process of *S. aureus* PGN cell wall is shown in Table 1.

Bacteriolysis and destruction of *E. coli* cell wall by zinc ions

Permeability of zinc ions into *E. coli* cell wall: *E. coli* cell wall is comprised of lipopolysaccharide (LPS), lipoproteins (LP), and peptidoglycan (PGN) thinner layer within periplasmic space. When permeability of zinc ions in the *E. coli* cell wall, highly anionic LPS with hydrophobic lipid A, core polysaccharide, O-polysaccharide, inhibition of LPS biosynthesis may be possibility to occur [17].

The OmpA, OmpC, OmpF porins of lipoproteins have metallic cation selective and hydrophilic membrane crossing pore, to be effective for zinc transfer [18]. Zinc (Ⅱ) ions reactive with -SH base, and then generate H_2. Zinc bivalent is unchangeable as 4-coodinated -SZn—S—bond.

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

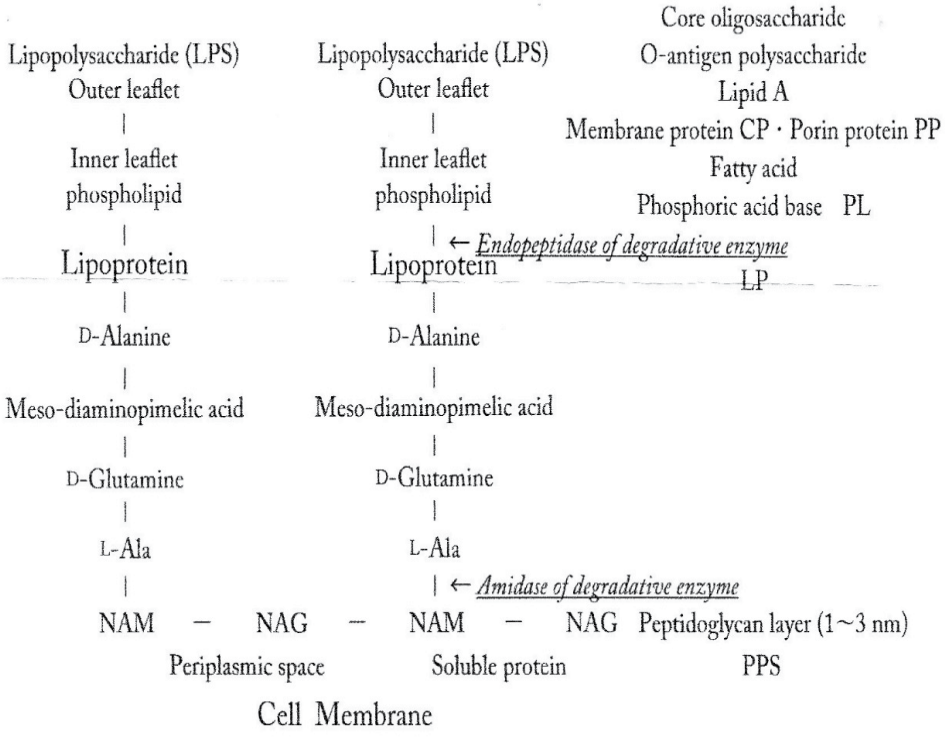

Figure 4: Molecular bonding manner of *E. coli* cell wall, outer membrane, lipoprotein, peptidoglycan, periplasm and the sites of the action of degradation enzymes of lipoproteins at N- and C-terminals.

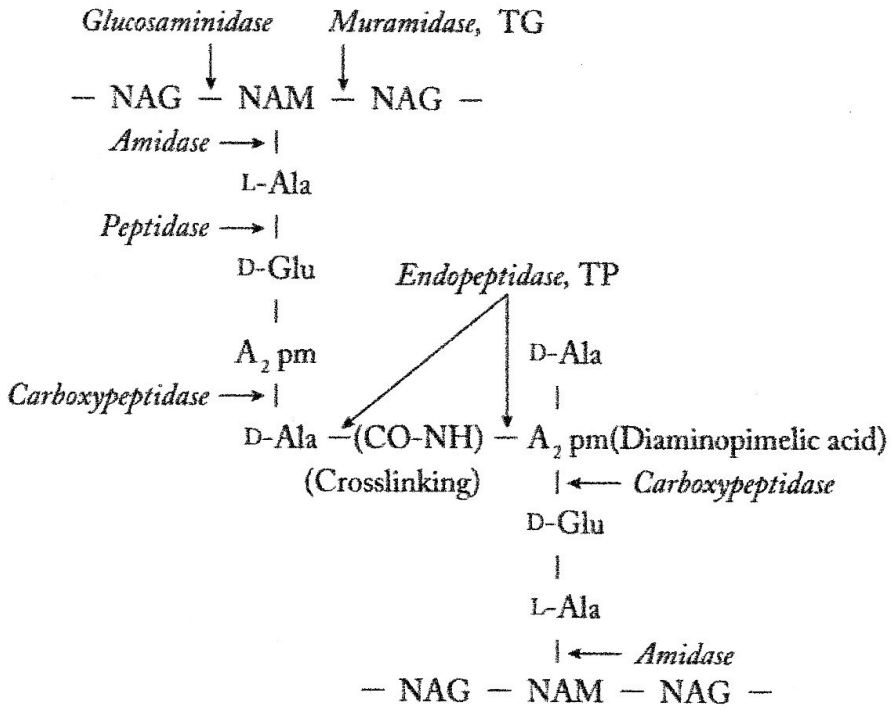

Figure 5: Molecular structure of *E. coli* peptidoglycan and the action sites of TG, TP synthesis enzymes and autolysins

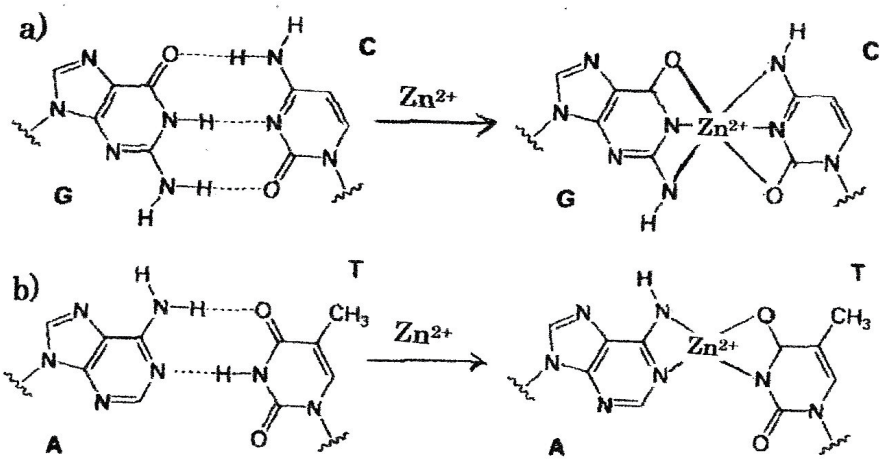

Figure 6: Zn^{2+} substitution into the triple and double hydrogen bonds in DNA base-pairing G:C, A:T pairs.

a) **G≡C** base pair, 6-coodinated Zn complex formation (stable)
b) **A=T** base pair, 4-coodinated Zn complex formation (unstable)

Destruction of *E .coli* cell wall outer membrane structure by hydrolases of lipoproteins at C- and N-terminals: 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 [19,20].

Inhibition of PGN elongation due to the damage of PGN synthesis enzyme of zinc-protein amidase in periplasmic space, and the activities of PGN autolysins: The zinc-induced decrease of protein biosynthesis led to a partial disappearance of connexin-43 of protein synthesis in neurons [21], but it is unknown whether PGN biosynthesis is inhibited. Further, it is also unclear whether the both TG/TP should be inhibited by the zinc ions [22-24].

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 [25], zinc metalloenzymes AmiD [26], amidase zinc-containing amidase;

Table 1: Bacteriolysis of *S. aureus* PGN cell wall by the permeability and antibacterial activities of Zn^{2+} ions.

Zn^{2+} ions solution	***S. aureus* Cell Wall**
Zn^{2+}	**Teichoic acid, Lipoteichoic acid, Peptidoglycan layer, Proteins**
	Zn^{2+} O_2^-, H_2O_2, ·OH, · NO, $ONOO^-$
	·Teichoic acids are spatial regulators of biosynthesis of PGN cross -linking TP enzyme
	·Zn^{2+} binding proteins
	·It is unknown whether zinc ions inhibit the PGN biosynthesis TG/TP enzyme
	·Activation of PGN autolysins
	·Bacteriolysis of PGN cell wall due to inhibition of PGN elongation
	·ROS productions and the oxidative stress

Table 2: Bacteriolysis and destruction of the *E.coli* cell wall by the permeability and antibacterial activities of Zn^{2+} ions.

Zn^{2+} ion solution	***E. coli* cell wall**		
Zn^{2+}	**Lipopolysaccharide(LPS) Lipid A, Core polysaccharide**	**Outer Membrane Lipoprotein, Porins Omp F, A, C**	**Periplasmic Space PGN layer**
	Zn^{2+}, H^+	**Zn^{2+}, O_2^-, H_2O_2,**	**Zn^{2+}, O_2^-, H_2O_2, OH^-, ·OH**
	·Negative charge	·Porin proteins of hydrophilic channels	·Zn accumulation
	·Hydrophobic Lipid A	·Zn binding proteins	·Periplasmic enzymes
	·Inhibition of LPS biosynthesis	·Destruction of outer membrane structure due to degradative hydrolases of lipoprotein at C- and N-terminals	·Damage of PGN bio-synthesis TG/TP enzymes and activation of PGN autolysins
	·Zn^{2+} + 2(-SH) → -SZn－S－ + H^+	· LOO·, L·(Fatty acid)	·Inhibition of PGN elongation

AmpD [27], zinc-present PGLYRPs [28] 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 [29] requiring divalent cations. Accordingly, the inhibition of PGN elongation had occurred by zinc ion induced activities of PGN hydrolases and autolysins.

ROS production and oxidative stress against *E. coli*: 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 [30]:

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

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

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

In cell wall, reacting with polyunsaturated fatty acids:

$$LH + OH\cdot \rightarrow L\cdot + HOH$$

$L\cdot + O_2 \rightarrow LOO\cdot$

$LH + LOO\cdot \rightarrow L\cdot + LOOH$

Zinc-containing Peptidoglycan Recognition Proteins (PGRPs) induce ROS production of H_2O_2, O_2-, $HO\cdot$, and then the ROS occur the oxidative stress, and killing by stress damage [31].

As above-mentioned, the bacteriolysis and the destruction of *E. coli* cell wall in process of the permeability and antibacterial activities of zinc ions are summarized in Table 2.

Damage of DNA base-pairs

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 $\cdot OH$ 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 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 6. 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. **A=T** base pairs are less stable than **G**≡**C** base pairs in Zn-DNA [32]. These considerations in the Zn-DNA base-pairs interactions must account for Zn^{2+}-ligands (6-coordination and 4-coodination complex formations) in DNA base pairs of **G**≡**C** and **A=T**, according to coordinated chemistry and molecular orbital theory [33,34].

Conclusion

(1) Bacteriolysis and destruction of *S. aureus* PGN cell wall, in which wall teichoic acids control PGN synthesis cross-linking TP, are due to the inhibition of PGN elongation by the activities of PGN autolysins; amidase AmiA and AmiE, PGN hydrolase Lysostaphin-like endopeptidase (Glycine cutting).

(2) Bacteriolysis and destruction of *E. coli* cell wall are due to the damage of LPS biosynthesis, destructing of outer membrane structure by degrading of lipoprotein at C-, N-terminals, owing to inhibitions of PGN formations by activities of PGN autolysins of amidase and carboxypeptidase.

(3) By the penetration of zinc 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^-, $\cdot OH$ in periplasmic space occur. These ROS and H_2O_2 give the damages cell membrane proteins and DNA molecular in cytoplasm.

(4) DNA damages due to zinc ion complex formation within DNA base-pairs **G**≡**C**, **A=T** may be occurred in cytoplasm of bacterial cells.

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