Structural insight into the binding mode between the targeting domain of ALE-1 (92AA) and pentaglycine of peptidoglycan

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ALE-1 is a glycolylglycine endopeptidase that selectively targets and lyases Staphylococcus aureus, and is expected to be a next generation antibiotic agent because of its substrate specificity to pathogenic bacteria. It has a central catalytic domain and a targeting domain called 92AA. 92AA has been shown to recognize pentaglycine, but the molecular mechanism by which it recognizes and interacts with pentaglycine has not been elucidated. To predict the binding modes of pentaglycine is important for estimating the catalytic reaction mechanism of ALE-1. In the present study, we characterized the binding cleft of 92AA by a computational method and modeled the complexes formed between 92AA and the pentaglycine of peptidoglycan by a binding simulation. In addition, we performed precise simulations of the molecular dynamics by which the complexes identify the amino acid residues interacting with the pentaglycine. We also experimentally constructed mutants in which the amino acid residues present in the binding cleft were changed by site-directed mutagenesis and assessed their ability to bind to peptidoglycan by ELISA. Based on the results of these analyses, we proposed a mode of binding between 92AA and the pentaglycine of peptidoglycan, and modeled the energetically stable complexes between 92AA and the pentaglycine.

Keywords: binding simulation/interpeptide bridge of peptidoglycan/molecular dynamics (MD) simulation/systematic conformational search/targeting domain of ALE-1

Introduction

ALE-1 is a unique bacteriolytic enzyme that selectively targets and lyases Staphylococcus aureus by digesting pentaglycine bridges of its peptidoglycan. Staphylococcus aureus is a human pathogen that is leading cause of nosocomial infections. Like methicillin-resistant S. aureus (MRSA), S. aureus shows resistance to a variety of antibiotics, and is becoming a worldwide threat to public health. ALE-1 is a lysostaphin homolog produced by S. capitis EPK1 (Sugai et al., 1997; Fujiwara et al., 2005). Because of its high selectivity, ALE-1 is expected to be a next generation antibacterial agent, as well as an enzybiotic against S. aureus infection. The total length of ALE-1 is 362 aa, including an N-terminal 13-amino-acid repeat domain followed by a central catalytic domain and a C-terminal targeting domain called 92AA. The central catalytic domain of ALE-1 exhibits glycolylglycine endopeptidase activity, which selectively cleaves the interpeptide of the peptidoglycan cell wall in S. aureus. The X-ray crystallographic structure of the targeting domain 92AA has been determined (Lu et al., 2006). The 92AA has been experimentally shown to selectively recognize S. aureus by discriminating pentaglycine; however, the molecular mechanism of this recognition and interaction has not yet been elucidated. Moreover, the amino acid residues related to the binding of the pentaglycine have not been identified. It is important to predict the binding modes of 92AA to pentaglycine to understand the catalytic reaction mechanism of ALE-1.

Peptidoglycan is an essential structural element in the cell wall of most bacteria. The main structural features of peptidoglycan are linear glycan strands cross-linked by short peptides (interpeptide bridges). The glycan strands are made up of alternating N-acetylmuramic (GlcNAc) and N-acetylmuramic acid (MurNAc) residues linked by β-1,4 bonds. In S. aureus, the interpeptide bridges are made up of pentaglycine (Vollmer et al., 2008). Dmitriev et al. suggested a model of the overall architecture of the peptidoglycan of S. aureus (Dmitriev et al., 2004). Meroueh et al. reported the three-dimensional structure of the 2-kDa NAG-NAM(pentapeptide)-NAG-NAM(pentapeptide) synthetic fragment of the cell wall as determined by NMR, and proposed a structural model for the bacterial cell wall (Meroueh et al., 2006). The three-dimensional structures of the moieties of the pentaglycine have been analyzed in complex states with various enzymes. For example, Cho et al. determined the three-dimensional structure of the complex between the NAG-NAM-L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala synthetic fragment and the human peptidoglycan recognition proteins, which have bactericidal activities against Gram-positive bacteria (Cho et al., 2007). However, the overall three-dimensional structure of peptidoglycan, including the interpeptide bridges, has not yet been determined. We previously attempted to analyze the three-dimensional structure of the 92AA-peptidoglycan complex, but we found that this complex was difficult to crystallize. Therefore, we must computationally generate the three-dimensional structure of the pentaglycine and further predict the three-dimensional structure of the complex between the pentaglycine and 92AA by molecular modeling.

In the present study, we first identified the binding cleft of 92AA by a computational method and modeled the structures of the pentaglycine by systematic conformational search. Next, we excluded the pentaglycines whose internal energies were high and whose conformations were invalid (i.e. the N-terminal was close to C-terminal) and modeled the complexes with 92AA. According to the interaction energy
between 92AA and the pentaglycine, we estimated the direction of the N-terminal of the pentaglycine in the binding cleft. We performed molecular dynamics (MD) simulations for the complexes to precisely identify the amino acid residues interacting with the pentaglycine. To test the computational model, we experimentally constructed mutants with amino acid residues implicated in the binding cleft changed by site-directed mutagenesis and analyzed their ability to bind with peptidoglycan by ELISA. Finally, we compared the binding activities by ELISA and the results of the binding simulations and MD simulations to build a structural model of the complex between 92AA and pentaglycine.

Materials and methods

Three-dimensional structure of the targeting domain of ALE-1

Coordinates for the X-ray crystallographic structure of the targeting domain of ALE-1 (92AA) were obtained from the Protein Data Bank (PDB code: 1R77) (Lu et al., 2006). There are two chains, the A- and B-chains (1R77A and 1R77B) in 1R77, and they were determined at high resolution (1.75 Å). The positions of the amino acid residues with coordinates in 1R77B were at 260–362 in the primary structure of ALE-1, while those in 1R77A were at 264–362. We selected 1R77B for the following simulations because the flag-TAG was completely attached.

Binding simulation between pentaglycine and the targeting domain of ALE-1

Simulation of the binding between the peptidoglycan pentaglycine and 92AA was performed by using the ASEDock 2005 module of the MOE program (Chemical Computing Group, Montreal, Canada) through the following five steps (Goto et al., 2008).

Generation of conformations for peptidoglycan pentaglycine The three-dimensional structure of the pentaglycine was modeled, since no X-ray crystallographic data have been reported for this pentaglycine. The initial three-dimensional structure of the pentaglycine was modeled by the Edit/Build/Protein command in the MOE program. The structure was modeled as a standard extended form. The eight dihedral angles (φ and ψ) in the backbone were rotated 120 degrees. Consequently, we modeled 3^8 (=6561) structures for the pentaglycine. To reduce the number of energetically unstable structures, the pentaglycines above 100 kcal/mol (vdW contact) were excluded. Further, the pentaglycines that showed a distance of <11 Å between the N atom at the N-terminal and O atom at the C-terminal were excluded, since it was considered invalid for these two terminals to be in close proximity.

Concavity search on the surface of 92AA The alpha spheres used in the binding simulations were first detected by using the Alpha Site Finder function of the MOE program (Tønдел et al., 2006). An alpha sphere is a sphere that contacts four protein atoms on the surface of a protein and has no atoms contained internally. The alpha spheres are determined geometrically using only the positions and radii of the heavy atoms and are classified as hydrophobic or hydrophilic. Then the alpha spheres are clustered using a single linkage clustering algorithm to form the alpha sites. ASEDock is a docking program based on a shape similarity assessment between a concave portion (i.e. concavity) of a protein and a ligand. Surrounding a concavity where a ligand is bound, there is a region which the ligand cannot access. The volume of such a region is called the ‘extended volume’. The extended volume is evaluated using the non-hydrogen atoms of the protein within 5.5 Å from the alpha spheres. The excluded volume of each non-hydrogen atom is calculated using a sphere with a radius of 1.4 Å for the lone-pair atoms (such as nitrogen and oxygen atoms) or 1.8 Å for the non-lone-pair atoms (such as carbon atoms). The sphere is called an ‘exclusion sphere’. An ASE model is defined by the combination of alpha spheres generated at a concavity in a protein and the excluded volumes around the concavity.

ASE score To evaluate the bound state by superimposing it onto the ASE model, the following simple Gaussian overlap fraction was applied as an ASE score.

\[ \text{ASE score} = - \sum_{i} \sum_{j} w_{ij} \exp(-\alpha d_{ij}^{2}), \]

Here, the parameter \( d \) is the distance between a ligand atom and an alpha sphere or an exclusion sphere in the ASE model, and \( \alpha \) is an adjustable parameter to attenuate the influence of a ligand atom when it is aligned with the alpha sphere or the exclusion sphere. The sums are taken over the alpha spheres (a), the exclusion spheres (e) and the ligand atoms (l). The parameter \( w \) is a weighting factor, and is defined as \( +r_{a}r_{l} \) and \( -r_{e}r_{l} \) for the alpha and exclusion spheres, respectively; here, \( r_{a} \), \( r_{e} \) and \( r_{l} \) are the radii of the alpha spheres, the radii of the exclusion spheres and the van der Waals radii of the ligand atoms, respectively. The smaller the ASE score, the larger the overlap among the alpha spheres, the ligand atoms and the exclusion spheres.

Superimposing on and rigid-body alignment of the ligand conformations to the ASE model The selected independent conformers are superimposed onto the ASE model. These alpha spheres are randomly used for candidates in the binding clefts, thereby creating a guide on which various ligand conformations treated as rigid bodies can be superimposed.

Energy minimization of the superimposed conformations of the pentaglycine in the concavity The configuration of the pentaglycine bound to the alpha spheres was optimized by the energy minimization, and the \( U_{\text{dock}} \) value was calculated as the sum of the \( U_{\text{ele}} \) (electric energy), \( U_{\text{vdw}} \) (vdw energy), \( U_{\text{solv}} \) (generalized Born/solvent accessible solvation interaction) and \( U_{\text{strain}} \) (difference of the minimal energies between the docked ligand and the ligand which is located nearest the docked ligand).

MD simulation of the complex

To investigate the interaction and identify the amino acid residues in 92AA interacting with the pentaglycine, an MD simulation was performed on the complex. Amber ff99 force
field parameters were used (Wang et al., 2000). The system was solvated in a periodic cubic box filled with TIP3P water molecules (Jorgensen et al., 1983).

To begin the MD simulation, the modeled structure was optimized by the following five steps using the AMBER7 package (Pearlman et al., 1995). First, the coordinates of the water molecules were optimized by the steepest-descents and conjugate gradients methods until the maximum derivative was less than 0.01 kcal/(mol Å). Second, the MD simulations at 300 K for 20 ps were performed for only water molecules to optimize the positions of the water molecules. Third, the coordinates of the heavy atoms of 92AA were tethered loosely and the positions of hydrogen atoms were optimized by the steepest-descents method and the conjugate gradients method until the maximum derivative was less than 0.01 kcal/(mol Å). Fourth, the coordinates of the backbone atoms of proteins were tethered loosely, and the positions of the amino acid residues were optimized. Fifth, the positions of all atoms were optimized without being tethered until the maximum derivative was less than 0.01 kcal/(mol Å).

In the MD simulation, the following two steps were applied by using the AMBER7 package. First, in the equilibration stage, the system was heated to 300 K for 20 ps in the NVT ensemble. Second, the simulation was continued at 300 K for 2.0 ns with a 1.0 fs time step in the NVT ensemble. After the systems had reached the sufficiently equilibrated state shown in the Supplementary figure, the last 1000 conformations sampled at 1 ps intervals were used for structural analysis.

Investigation of the conformational changes of the binding clefts in the mutants by MD simulations

To investigate the conformational change of the binding cleft in each mutant relative to the wild type, the three-dimensional structures of N274A, Y276A, T278A, Y280A, T298A, G299A, P300A, F301A, M304A, E320A, M322A, Q324A and Y341A were modeled by using the replace command in Insight II (Accelrys Co., San Diego, CA, USA) based on that of the wild-type 92AA (PDB id: 1R77B). The direction of the side-chain of the mutated amino acid residue was optimized by the autorotamer command in Insight II. Next, the modeled structure was optimized by using the same procedure that was previously applied before MD simulation. MD simulations were performed for each mutant and for the wild type. The parameters of the MD simulation were the same as those used for the simulation of the complexes. During the MD simulation, the last 200 conformations were sampled at 1 ps intervals, and the averaged conformation was calculated. To quantify the conformational change of the binding cleft, the backbone atoms between the average MD structure of the wild type and the average MD structure for each of the mutants were superimposed, and the RMSDs for each of the 13 amino acid residues in the binding site were further averaged.

Site-directed mutagenesis

The targeting domain 92AA (residues 271–362) was expressed as Hisx6 tagged fusion proteins. The corresponding sequences were amplified with PCR using S. capitis EPK1 chromosomal DNA as a template with the primers ALE-U5 (ATGGATCCTATAAAAACACTAATAAA) and ALEL4 (TTTGTGCAATATGGGTAGTGATA), and cloned in-frame downstream of the pQE30 expression vector (Qiagen). The recombinant Escherichia coli was grown to mid-log phase in 500 ml of LB medium at 37°C, induced with 1 mM IPTG and incubated for 4 h. Cells were harvested by centrifugation, suspended in 10 ml of buffer B (8 M urea, 0.1 M NaH2PO4, 0.01 M Tris–Cl, pH 9.0) and incubated for 30 min at room temperature. The cells were disrupted by ultrasonication (Ultrasonic Disruptor; TOMY SEIKO, Tokyo). After the undisrupted cells were removed by centrifugation at 25 000g and 4°C for 20 min, the supernatant was subjected to affinity chromatography on Ni-NTA agarose (Qiagen) pre-equilibrated with buffer B. The column was washed with a 5 x bed volume of buffer B and eluted with buffer E (8 M urea, 0.1 M NaH2PO4, 0.01 M Tris–Cl, pH 4.5). The eluted fraction was dialyzed against 4 M urea, 0.1 M phosphate buffer (pH 6.8) and, finally, 0.1 M phosphate buffer (pH 6.8).

ELISA

Protein bound to bacterial peptidoglycan was detected by an ELISA procedure. Polystyrene enzyme immunoassay plates (96 wells; Nalge Nunc) were coated with bacterial peptidoglycan digested with mutanolysin in 10 mM sodium citrate (pH 7.0) and 4 mM MgCl2 to a concentration of 18.8 μg/ml, and left overnight with 100 μl per well. After coating, the wells were washed three times with distilled water, and then 1% BSA diluted in PBS was added to the wells and left overnight. After blocking, the wells were washed three times with distilled water, and then 7.5 μg/ml protein (100 μl) diluted in PBS was added to the wells and incubated at 4°C for 1 h. After incubation with protein, the wells were washed three times with PBS containing 0.05% Tween 20. Anti-ALE-1 Serum (100 μl) diluted in PBS containing 4 μg/ml human IgG and 0.1% BSA was added to the wells and incubated at 37°C for 2 h. After three washes with PBS-Tween 20, 100 μl of diluted horseradish peroxidase-conjugated goat IgG fraction to Rabbit IgG F(AB')2 was added and the mixture was incubated for 2 h at 37°C. Unbound conjugate was removed by washing three times with PBS-Tween 20 and two times with PBS. The substrate (100 μl) was then added (25 mg of o-phenylenediamine dihydrochloride and 5 μl of H2O2 in 10 ml of sodium phosphate-citrate buffer). The enzymatic reaction lasted for 15 min at room temperature and was stopped by adding 100 μl of 2 N H2SO4. The optical density (OD) was read at 490 nm on a TiterTek Multiscan Spectrophotometer.

Results and discussion

Identification of the binding cleft

Previously, the binding cleft of 92AA was only inferred by the conservation of amino acid residues among lytic enzymes targeting S. aureus: ALE-1 (from S. capitis EPK1), lysostaphin (from S. simulans ATCC 1362), autolysin (from S. aureus) and amidase (from Staphylococcus phages Twort and PVL) (Lu et al., 2006). The binding cleft of 92AA was estimated by using the Alpha Site Finder function of the MOE program.

The five alpha sites were detected in 1R77B. The numbers of the hydrophobic and hydrophilic sites were 6 and 12 (site 1), 3 and 22 (site 2), 0 and 15 (site 3), 9 and 14 (site 4) and

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2 and 13 (site 5), respectively. The alpha spheres in site 1 were used for the binding simulation, because site 1 corresponded to the binding cleft inferred in the previous study (Lu et al., 2006). As shown in Fig. 1, site 1 included the 13 amino acid residues. These amino acid residues are classified into three groups: the polar (Asn274, Tyr276, Thr278, Tyr280, Thr298 and Gln324), non-polar (Gly299, Pro300, Phe301, Met304, Met322, and Tyr341) and negatively charged (Glu320).

Binding simulation of the pentaglycine to 92AA

As described in Materials and Methods, each of the 3070 pentaglycines modeled by the systematic conformational search randomly bound to the alpha spheres in site 1. After the energy minimization, 82 complexes between 92AA and the pentaglycine were modeled. The complexes were sorted in ascending order of their $U_{\text{dock}}$ values, and, respectively, designated C1 to C82.

It is important to infer the direction of the N-terminal of pentaglycine in the bound state in order to understand the binding mode of the peptidoglycan to ALE-1. The distributions of the $U_{\text{dock}}$ values and the directions of the N-terminal of the pentaglycine for the 82 complexes are shown in Fig. 2.

As shown in Fig. 1, we defined the direction of the pentaglycine based on the position of its N-terminus. Twenty-one pentaglycines were bound to the right side, while 61 pentaglycines were bound to the left side. The average $U_{\text{dock}}$ of the complexes bound to the right and left sides were $-68.8$ (SD = 9.178) and $-43.6$ (SD = 10.599) kcal/mol, respectively. Thus, the $U_{\text{dock}}$ of the complexes in which the pentaglycine was bound to the right side tends to be lower than that of those in which the pentaglycine was bound to the left side. This indicates that the N-terminal of the pentaglycine is energetically stable when it binds to the right side. The binding modes of the 21 pentaglycines bound to the right side and the amino acid residues interacting with the 21 pentaglycines are shown in Fig. 3.

Among the 21 complexes, Asn274 (12), Tyr276 (10), Thr278 (2), Thr298 (6), Ser303 (1), Glu320 (34) and Tyr341 (1) interacted with the pentaglycines. The number of complexes which bound with hydrogen bonding is shown in parentheses. Glu320 was considered to be important, since it interacted with the pentaglycines more frequently than any other amino acid. The N-terminal of all of the pentaglycines bound to the right side. The values of $U_{\text{dock}}$ of the complexes C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C13, C14, C15, C16, C18, C20, C22, C24, C33 and C36 were $-83.4$, $-81.5$, $-80.2$, $-79.9$, $-75.8$, $-73.7$, $-73.3$, $-72.9$, $-71.6$, $-71.2$, $-70.9$, $-68.7$, $-67.4$, $-64.8$, $-63.9$, $-62.6$, $-61.2$, $-59.6$, $-58.2$, $-52.4$ and $-50.8$ kcal/mol, respectively. The complex C1 was the most energetically stable, because the $U_{\text{dock}}$ of C1 was the lowest among the 21 complexes.
Identification of amino acid residues related to the binding of pentaglycine by site-directed mutagenesis and ELISA

To evaluate whether the 13 amino acid residues in site 1 are related to the binding of the pentaglycine, the mutants for each of the 13 amino acid residues (N274W, Y276A, T278A, Y280A, T298A, G299A, P300A, F301A, M304A, E320A, M322A, Q324A and Y341A) were constructed by site-directed mutagenesis. In addition, the mutants for each of the four amino acid residues around the 13 amino acid residues (N272A, T273A, K275A and S303A) were constructed as references. Thus, the influence of the mutation on the binding activities of the amino acid residues in the binding cleft of 92AA was investigated by analyzing the binding of the 92AA mutant to peptidoglycan using ELISA.

Binding simulation of the targeting domain of ALE-I

Fig. 3. Twenty-one binding modes and the amino acid residues interacting with the pentaglycines estimated by the binding simulations and the MD simulations. The pentaglycines in the complexes are indicated by lines. The amino acid residues interacting with these 21 pentaglycines and the direction of the N-terminal are indicated. The polar, non-polar and negatively charged amino acid residues are colored yellow, green and magenta, respectively.

MD simulation of the complex C1, the most energetically stable structure

We performed an MD simulation for the complex C1 to investigate the interaction between the amino acid residues. Among the 1000 complexes sampled from the MD simulation, Asn274 (197), Tyr276 (2), Thr278 (120), Thr298 (38) and Glu320 (140) interacted with the pentaglycine. The number of complexes in which the amino acid residue interacted with the pentaglycine by hydrogen bonding is shown in parentheses.

As a result of the docking simulation and the MD simulation, Asn274, Tyr276, Thr278, Thr298, Ser303, Glu320 and Tyr341 were considered to interact with pentaglycine. Asn274, Tyr276, Thr278, Thr298 and Glu320 were especially important for the substrate binding, because these amino acid residues interacted with the pentaglycine in the docking simulation and frequently interacted in the MD simulation.

Investigating the conformational changes of the binding clefts in the mutants using MD simulations

The three-dimensional structures of N274A, Y276A, T278A, Y280A, T298A, G299A, P300A, F301A, M304A, E320A, M322A, Q324A and Y341A were modeled based on that of the wild-type 92AA (PDB id: 1R77B). As described earlier, to quantify the conformational change of the binding cleft, the backbone atoms between the average MD structure of the wild type and the average MD structure for each of the mutants were superimposed, and the RMSDs for each of the 13 amino acid residues in the binding site were further averaged. The conformational changes of the binding clefts in the mutants are shown in Fig. 5.

The RMSD values of T298A, G299A, P300A and Y341A were higher than those of the other mutants, which indicates that the conformations of the binding clefts were significantly changed. The conformational change of the binding cleft in T298A was particularly large. According to the B-factor of the X-ray crystallographic structure (PDB id: 1R77B), the atomic fluctuation of Thr298 was the highest. The mutation of an amino acid residue with high fluctuation might cause a large conformational change. The mutations of Gly299, Pro300 and Tyr341 might be directly related to the changes in solvent accessibility, because they were located in the hydrophobic regions which were calculated using DSSP (Kabsch and Sander, 1983). Thr298, Gly299, Pro300 and Tyr341 are located on the upper side of the binding cleft. The changes of the binding activities of these mutants determined by ELISA might have been caused by the conformational changes of the binding clefts. On the other hand, the conformational changes of the binding clefts for the remaining mutants were small. These amino acid residues were located at the bottom of the binding cleft. In these mutants, the binding activities determined by ELISA might have been affected by the changes in the interaction caused by mutation and the conformational changes of the binding clefts.
have been affected by the interaction between the pentaglycine and 92AA.

The binding activities of the amino acid residues on the structure are shown in Fig. 6. The directions of the side-chains of Asn274, Tyr276, Thr278, Tyr280, Pro300, Met322, Gln324 and Tyr341, mutations which significantly influenced the binding activity, were oriented toward the binding cleft. However, the directions of the side-chains of Lys272, Thr273, Lys275, and Ser303, mutations which did not affect the binding activity, were not oriented toward the binding cleft and were far from the binding cleft. The 92AA has one \(\alpha\)-helix, \(\alpha\)1 (residues 264–268) and eight \(\beta\)-sheets, \(\beta\)1 (residues 272–273), \(\beta\)2 (residues 279–289), \(\beta\)3 (residues 293–296), \(\beta\)4 (residues 306–310), \(\beta\)5 (residues 315–324), \(\beta\)6 (residues 327–333), \(\beta\)7 (residues 339–346) and \(\beta\)8 (residues 359–361). As shown in the figure, the amino acid residues that moderately influenced the binding activity were also directed toward the binding cleft and are located on the turn between \(\beta\)-sheets \(\beta\)3 and \(\beta\)4 or on \(\beta\)5, all of which were adjacent to the binding cleft.

In the present study, in which the binding simulations for 3070 pentaglycines were performed, 82 complexes were modeled. Estimating the direction of the N-terminal of the pentaglycine should help to elucidate the binding modes of the complex between the peptidoglycan and ALE-1. We found that the complexes in which the N-terminal of the pentaglycine bound to the right side were energetically stable. Lu et al. have shown that the binding of 92AA to Gly2-Ser-Gly2 was significantly reduced to 29.4%. This biological observation would be useful for building a complex between 92AA and pentaglycine (Lu et al., 2006). Finally, we succeeded in modeling the complexes in which the
predicted by using the X-ray structure and the docking and MD simulations. However, the catalytic reaction mechanism has not been estimated, because the X-ray structure of 36AA and the pentaglycine were not analyzed. To clarify the catalytic reaction mechanism of the ALE-1, the structural information of the complex formed between the peptidoglycan, 92AA and 36AA is necessary. In future studies, we should make a model for the complex between L-Lys-(Gly5)-D-Ala and 92AA by overcoming the high degree of freedom in L-Lys and D-Ala. By clarifying the catalytic mechanism of ALE-1, we will extend the possibility of using ALE-1 as a next generation antibacterial agent and an enzybiotic against S. aureus infection.

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