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Conformational changes of peptidoglycan fragments during their interactions with vancomycin

Research Article

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Abstract: Six complexes of vancomycin and peptidoglycan precursors were studied via molecular dynamics simulations. The interactions between the antibiotic and peptidoglycan fragments were identified and described in detail. All six studied modifications of the peptidoglycan precursor resulted in a weakening of the interaction with vancomycin when comparing to the native D-Ala-D-Ala-terminated fragment. It was confirmed that the N-terminus of the vancomycin is directly responsible for peptidoglycan recognition and antimicrobial activity. In simulated systems, the saccharide part of the antibiotic interacts with peptide precursors, thus it could also be important for antimicrobial activity. The complex terminated with D-Lac is the only one in which there is a weak interaction with the sugar moiety in the simulated systems. Analysis of conformational changes is a major scope of this work. The lack of interactions resulting from modification of the peptidoglycan precursors (D-Lac, D-Ser or other substitution) would be counterbalanced by proper modifications of the vancomycin moiety, especially the saccharide part of vancomycin.

Keywords: *Modified peptidoglycan • Molecular dynamics of sugar • RMSd • Trajectory analysis • Vancomycin interaction*

1. Introduction

Vancomycin is a glycopeptide antibiotic used to treat bacterial infections caused by many drug-resistant enterococci and staphylococci, such as methicilin-resistant Staphylococcus aureus [1,2].

Vancomycin inhibits the last steps of the cell wall biosynthesis in Gram-positive bacteria. It binds to the C-terminal D-Ala-D-Ala fragment of peptidoglycan precursors, preventing their incorporation into the cell wall [3]. This significantly impairs the structural integrality of the cell wall and results in osmotic lysis. In the last 20 years, extensive use of vancomycin and other glycopeptide antibiotics resulted in the development of high-level vancomycin resistance in several strains of Enterococcus (named VRE, vancomycin-resistant Enterococcus). Bacterial resistance to vancomycin results from the synthesis of peptidoglycan precursors, ending in D-Ala-D-Lac (for VanA, VanB, and VanD resistance types) or in D-Ala-D-Ser (for VanC, VanE, and VanG resistance types), which exhibit reduced affinity for vancomycin [4-9]. The substitution with D-Lac is achieved due to the activity of two enzymes: D-lactate dehydrogenase and ligase, which synthesize D-Ala-D-Lac rather than D-Ala-D-Ala [10]. The strategy to achieve D-Ser substitution is more complicated and involves, among others, DD-carboxypeptidase and serine racemase [11].

It is known, that the substitution of C-terminal D-Ala with D-Lac/D-Ser results in the loss/impairment of the critical interactions within the binding pocket of the vancomycin, and, as a consequence, in a 1000-fold decrease in the affinity for the D-Lac terminated precursor [12-14]. It is also known, that the mutation of the C-terminal D-Ala residues to D-Ser results in a six-fold drop in affinity for vancomycin [15,16]. Several NMR studies showed that the complex of the peptide cell wall precursor and antibiotic is stabilized by five hydrogen bonds [17-19]. Moreover, it has been demonstrated that vancomycin and its analogs have an ability to form homodimers in aqueous solution and a cooperative effect between dimerization and cell wall precursor binding has been observed [20-23].

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In this study the complexes of vancomycin and six various pentapeptide peptidoglycan precursors have been studied *via* molecular dynamics simulations to identify the conformational changes during their binding and to identify interactions between the antibiotic and peptidoglycan precursors. Points of interactions have been identified and the stability of properly formed interactions has been investigated. Based on the type and magnitude of the identified changes, some vancomycin modification will be proposed, especially in the saccharide part of vancomycin, to counterbalance the bacterial defense systems.

2. Experimental Procedure

2.1. Construction of the models

The three-dimensional structure of vancomycin (Fig. 1) has been constructed on the basis of X-ray structure (resolution 0.98 Å), PDB code 1AA5 [24].

The fragments of peptidoglycan investigated in this work were constructed to obtain the following sequences: (1) Ala-D-Glu-Lys-D-Ala-D-Ala – abbreviated here as DA-DA; (2) Ala-D-Glu-Lys-D-Ala-D-Lac – abbreviated here as DA-DLac; (3) Ala-D-Glu-Lys-D-Ala-D-Ser – abbreviated here as DA-DSer; (4) Ala-D-Glu-Lys-D-Ala-L-Ala – abbreviated here as DA-LA; (5) Ala-D-Glu-Lys-L-Ala-D-Ala – abbreviated here as LA-DA; (6) Ala-D-Glu-Lys-L-Ala-L-Ala – abbreviated here as LA-LA.

All parameters have been evaluated using the corresponding data from existing force field parameters in *Amber 9* [25], *glycam04* [26,27], and *ff03* [28]. Atomic partial charges of all non-standard vancomycin fragments have been calculated by fitting them to the *ab initio* molecular electrostatic potential (6-31G* basis set, *Gamess* molecular orbital program package [29]) for two different conformations of each calculated fragment, followed by consecutive averaging of the charges over all conformations, as recommended by the *RESP* protocol [30]. Full-atom models, supplemented by the newly parameterized fragments, were relaxed and energy-minimized using the *Amber glycam04* and/or *ff03* force fields.

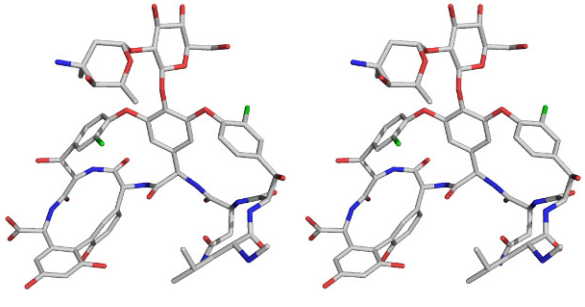

Figure 1. Structure of vancomycin (stereo view).

2.2. Docking and molecular dynamics

The pentapeptide peptidoglycan representative of bacterial cell-wall (DA-DA) has been docked in the binding cavity of vancomycin. The docking procedure involved enclosing the peptidoglycan fragment using distance constraints from force fields, defined in accordance to the crystal structure of vancomycin-DA-DA complex (PDB code 1FVM) [31]. The resulting structure has been relaxed and optimized.

This structure, which was properly docked to correspond to the crystallographic data, was subsequently modified by substituting one or both C-terminal amino acid residues with respective residues to obtain the desired, modified representatives of bacterial cell-wall (DA-DLac, DA-DSer, DA-LA, LA-DA and LA-LA). All of the ensuing complexes of pentapeptides embedded into the vancomycin binding cavity were subsequently relaxed and their energies minimized. This common starting position allowed us to compare future changes in position and conformation of pentapeptides bound to vancomycin.

In the next step, the unconstrained molecular dynamics (MD) of all previously obtained complexes was driven in generalized Born continuous solvent model (as implemented in *Amber*; [32,33]) using 12 Å as the cutoff for non-bonding, in standard conditions, without periodicity. The MD simulated 1 ns of the complex behavior. Snapshots were taken every 1 ps. The MD simulation was driven in order to explore the principally available conformational space of pentapeptide fragments, relax them and obtain a stable, representative conformation of investigated pentapeptide docked into the binding cavity of vancomycin. Formation of polar interactions has also been observed and described.

2.3. Energy calculations

After the MD run, the postprocessing software has been applied to calculate free energies of binding. This has been done with the molecular mechanics Poisson-Boltzmann surface area (*MM/PBSA*) method [34] utilized by the *Amber* package. The *mm_pbsa* module does not contain any parameters that vary for different complex systems and it involves a set of physically well-defined terms. This method restricts the simulations to the states before and after binding, so the required modification was to apply calculations from the initial to the final state of each modeled complex through all collected snapshots in every MD run. The binding affinity has been estimated as a difference between the

free energy of the vancomycin-peptidoglycan fragment complex and the sum of free energies of vancomycin and peptidoglycan.

3. Results and Discussion

3.1. Changes in peptidoglycan fragment conformation during MD

During the unconstrained MD simulations of vancomycin-peptidoglycan fragment complex, the changes of the Cα positions of all amino acid residues of peptidoglycan fragments have been measured. The root mean square deviations (RMSd) have been measured for all Cα atoms in each snapshot, using the starting structures as a reference. Trajectories built from these snapshots are presented in Figs. 2-7. These trajectories lead to some important findings.

The modeled interaction of vancomycin with DA-DA (the native peptidoglycan fragment, which is known to be prone to vancomycin) is stable during the simulation. The positions of Cα atoms remain almost unchanged. The main changes are present in the beginning of the MD. Positions of the residues ("res 1" through "res 5" - see the sequences in the "2.1. Construction of the models" paragraph) do not change much (about 0.2 – 0.3 Å, see Fig. 2) and the observed changes are mainly the result of the adaptation of interacting moieties to each other (for about 150 ps of the initial MD) and are almost absent in the RMSd (root mean square deviation) plot. The most distorted trajectory concerns the last residue in the sequence (D-Ala). It stabilizes after the initial period of MD. This native peptidoglycan fragment is known to form stable interactions with vancomycin. These trajectories are presented here only for comparison with other plots.

In the trajectory of the MD of the vancomycin-DA-DLac complex (Fig. 3) only the position of residue 1 remains stable and almost unchanged during whole the MD. This suggests proper binding of this residue to the vancomycin. The next residue (D-Glu) changes its position after about 300 ps of MD and remains in the new position. The third residue in the sequence (Lys) rapidly changes its position during the whole simulation. The changes of Lys and following residues of peptidoglycan oscillate at the level of 0.3-0.6 Å adapting to the changes in the geometry of vancomycin (compared to the energetic effects discussed below). Therefore, the rapid changes of the position of all residues during the simulation (except residue 1, see above) suggest weak and unstable interaction between the peptide and antibiotic.

From the trajectories of DA-DSer in complex with vancomycin (Fig. 4) one can see that the trajectory of the residue in the middle of the peptidoglycan representative (Lys) is the most distorted.

The most important finding is that residues 5 and 4 change their positions the most from the starting position – known to be favorable for the native peptidoglycan (see the discussion above). This proves that this specific modification of the peptidoglycan structure easily destabilized its overall binding to the vancomycin. The rapid (lasting about 50 ps from the beginning of the MD) and significant (0.9 Å) change of the position of the last residue in the sequence (D-Ser) does not stimulate the changes of vancomycin structure which means that this residue loses its significant role in vancomycin binding. During the MD of DA-LA in complex with vancomycin,

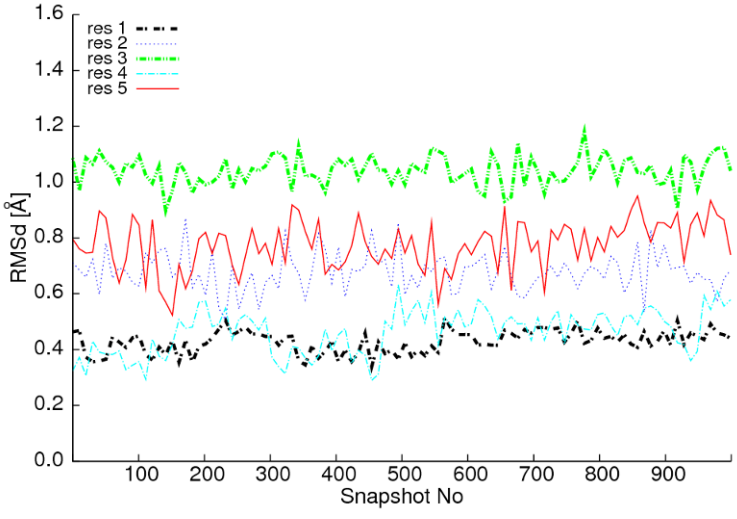

Figure 2. Plot of RMSd changes of DA-DA during unconstrained MD.

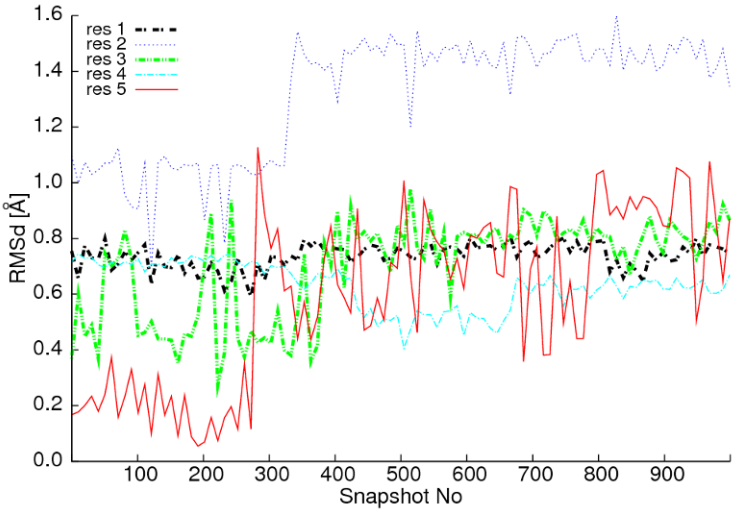

Figure 3. Plot of RMSd changes of DA-DLac during unconstrained MD.

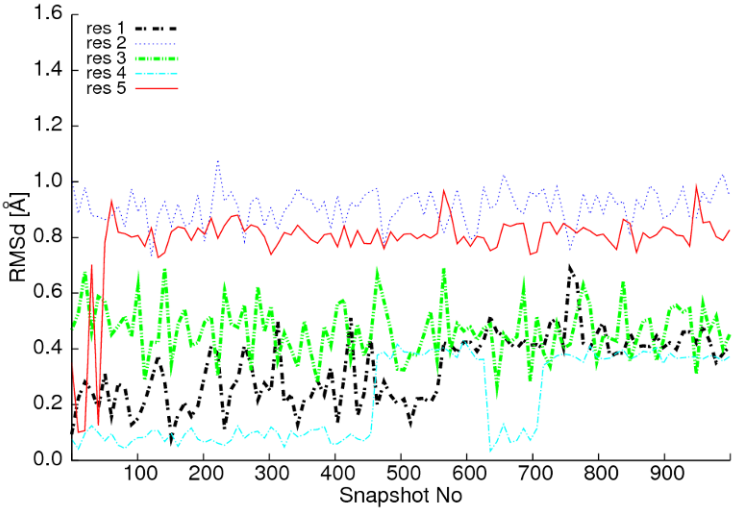

Figure 4. Plot of RMSd changes of DA-DSer during unconstrained MD.

the positions of all residues change rapidly (Fig. 5). The changes are almost 1 Å for all residues, including residue 3 (Lys), which is situated in the middle of the sequence and should not change its position much. Residue 1, the most stable in all other simulations, changes its position frequently in this MD run. These changes oscillate between 0.2 - 0.8 Å. The last residue in the sequence (L-Ala) changes its position for 1.2 Å, which suggests lack of important interactions with vancomycin and impossibility of achieving any other point of stabilizing interaction. Such an instability leads to a drop in vancomycin efficacy and remains in agreement with empirical observations [5].

Considering the trajectory of LA-DA from the MD with vancomycin (Fig. 6), the biggest changes are observed again within residue 3 (Lys). The last residue in the sequence (D-Ala) also changes its position (for about 0.6 Å) suggesting a lack of important interactions formed by the preceding L-Ala which can also destabilize the interactions with vancomycin (see below). The position of residue 3 – not bonded with vancomycin in the native peptidoglycan fragment (DA-DA) in this complex is quite stable, but the residue moves almost 1 Å before reaching the final position (see Fig. 6). Such a displacement of the middle part of LA-DA from its geometry-optimized position signifies large distortion within this part of the molecule. In a biological environment, such a dislocation of residue 3 together with residues 1 and 2 in peptidoglycan (also present in discussed trajectory) while the position of the remaining part of peptidoglycan remains almost unchanged will not occur. The simulated fragment is only a C-terminal part

Table 1. Number of identified polar interactions per vancomycin part.

pentapeptide	vancomycin C-terminal part	vancomycin saccharide group	vancomycin N-terminal part	total number
DA-DA	2	3	6	11
DA-DLac	3	2	2	7
DA-DSer	3	3	5	11
DA-LA	3	3	2	8
LA-DA	4	3	2	9
LA-LA	3	3	3	9

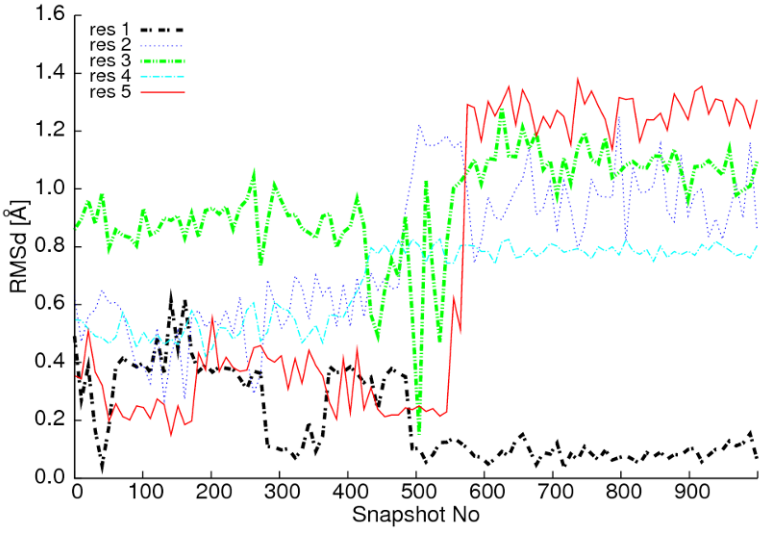

Figure 5. Plot of RMSd changes of DA-LA during unconstrained MD.

of the whole peptidoglycan and the observed change in the pentapeptide fragment would require almost impossible adaptation of its preceding fragment.

In the trajectory of the last investigated vancomycin partner – LA-LA (Fig. 7), residues 2 and 5 change their positions the most, which demonstrates an overall incompatibility with vancomycin binding site. Mutation from D-Ala-D-Ala to L-Ala-L-Ala in the C-terminal part of peptidoglycan destabilizes binding of this fragment to the peptide region of vancomycin, and properly formed interactions of the Lys residue disallow sufficient bonding to the saccharide region of vancomycin – as found in other complexes. After the adjustment of both interacting partners (about 300 ps of MD simulation) the structure of LA-LA stabilizes and almost does not change. This adjustment is correlated with the formation of 4 hydrogen bonds within the C-terminus of the peptidoglycan – as discussed below.

All identified interactions within the sugar moiety are present in our simulation systems since there is a possibility that these may occur. In a natural environment, where both interacting objects are explicitly solvated, and the vancomycin could form dimers or oligomers [20-23] such interactions could be absent. This could explain some additional interactions *e.g.* those with the carboxylate C-terminal part of vancomycin, as discussed, which were absent in previous findings.

3.2. Vancomycin-peptidoglycan interactions.

After 1 ns of continuous MD simulations, the relaxed structures of vancomycin-peptidoglycan fragments have been obtained. Final, optimized structures are shown in Fig. 8.

The energy of binding in all resultant complexes has been analyzed. Calculations of free energies were based on vancomycin monomer in the continuous solvent model. In this environment both interacting moieties could easily adapt to each other. Under these conditions, the averaged free energies of binding have been found to be similar in all complexes with some exceptions – as discussed below. This similarity induced the necessity of searching for other differences between the modeled complexes. Numerous polar interactions (including hydrogen bonds) between the vancomycin and peptidoglycan fragments have been identified: 11 for DA-DA and DA-DSer, 9 for LA-DA and LA-LA, 8 for DA-LA and 7 for DA-DLac (Table 1).

These identified interactions were chosen on the basis of the overall MD analysis, where all contacts were monitored. Tables 1 and 2 include only those interactions

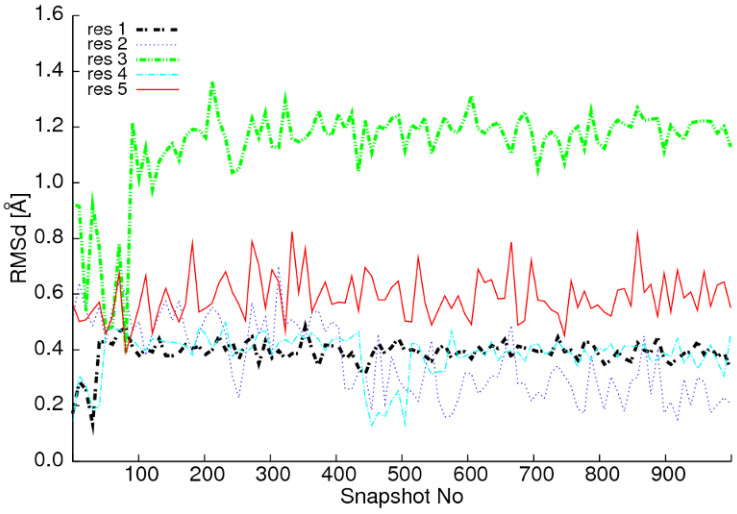

Figure 6. Plot of RMSd changes of LA-DA during unconstrained MD.

which were present for more than 95% of the MD time and they have been found in the final complex. The exceptions were: LA-LA, DA-LA and DA-DLac, where 80%, 50% and 70% of MD time respectively has been taken as the limit, as no other interactions have been present longer.

The total number of the interactions between vancomycin and the representative fragments investigated were classified into three subgroups: interactions formed within the C-terminal part of vancomycin, interactions formed with the saccharide part of the vancomycin and finally - those involving other parts of vancomycin (described here as "N-terminal part of vancomycin"; see Table 1). In this classification the most important region is the N-terminal part of vancomycin because it recognizes the C-terminal part of peptidoglycan. Simultaneously, the identified interactions within the peptidoglycan fragment were classified to distinguish between the interactions formed by the crucial C-terminal dipeptide, where mutations occur, versus those formed within the immediately preceding unchanged tripeptide part (see Table 2).

In analyzing Table 1, it has been noticed that in all simulated systems there are the same number of interactions formed by the saccharide part of vancomycin with the small exception of DA-DLac peptide, where one less interaction is formed (see Table 1). This could suggest that the interactions within this region are weakly dependent on the mutations of the peptidoglycan C-terminus, which is not true. The answer comes from binding energy calculations: the interaction energy in this complex is almost 30% higher than in all other complexes, which proves that the interaction between DA-DLac and vancomycin is weaker.

The number of interactions within the C-terminal part of vancomycin remains similar in all simulated systems (see Table 1). This emphasizes the importance of these two parts of vancomycin for interaction with peptidoglycan; however, it is not sufficient for antimicrobial activity. It is also a good confirmation that the simulations have been properly performed [35-37]. The variable number of interactions formed within the N-terminus of vancomycin is directly connected with the vancomycin antimicrobial activity. These interactions are mainly formed within the C-terminal dipeptide part of the peptidoglycan (Table 2) which is responsible for interaction with vancomycin and is where amino acid mutations occur in bacterial cell walls. As expected, the highest number of hydrogen bonds is observed in the system containing the natural, unmodified peptidoglycan fragment. There are six polar interactions formed within DA-DA dipeptide, including the same hydrogen bonds as observed in experimental studies [17-19], (see Tables 1, 2 and Fig. 8, panel A). Three hydrogen bonds have been formed between the C-terminal carboxyl group of D-Ala and the three neighboring amide groups of the C-terminal part of the antibiotic. One more hydrogen bond has been found between the amide group of the C-terminal D-Ala and the carbonyl oxygen of the vancomycin (see Fig. 8, panel A). Moreover, some polar interactions between the C-terminal carboxylate of D-Ala and amino group of the antibiotic have been observed, which have not been described before [14].

The number of hydrogen bonds formed between the N-terminal region of vancomycin and C-terminal dipeptide of peptidoglycan is variable and smaller in other complexes than in the vancomycin-DA-DA complex (see Fig. 8). In the complex with DA-DSer, there are 5

Table 2. The number of polar interactions per pentapeptide fragment.

pentapeptide	tripeptide [Ala-D-Glu-Lys]	pentapeptide C-terminus	total interactions
DA-DA	5	6	11
DA-DLac	6	1	7
DA-DSer	6	5	11
DA-LA	5	3	8
LA-DA	7	2	9
LA-LA	5	4	9

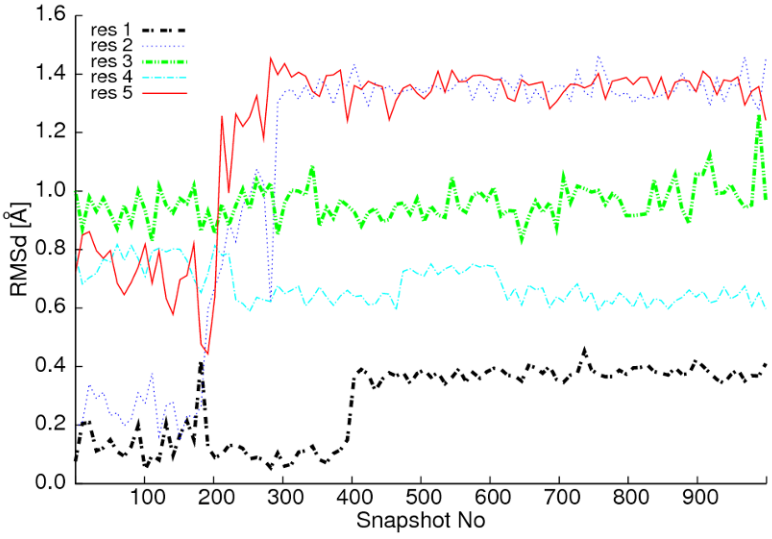

Figure 7. Plot of RMSd changes of LA-LA during unconstrained MD.

points of interaction (Fig. 8, panel C). However, there is no possibility to form an efficient hydrogen bond with the carbonyl oxygen of the vancomycin as a result of the steric hindrance of the hydroxymethyl group in D-Ser residue. There is only weak interaction observed. This is consistent with the data that the mutation of the C-terminal D-Ala residues to D-Ser results in a six-fold drop in affinity toward vancomycin [38]. The presence of the additional hydroxyl group of D-Ser, not only disturbs the formation of important hydrogen bonding with the antibiotic as described above, but also has a negative effect on conformation of the sugar part of the vancomycin – as observed in the final complex. In the vancomycin LA-LA complex, there are 4 interactions identified within the crucial C-terminal regions of the interacting molecules (Fig. 8, panel F). Three hydrogen bonds are formed between the C-terminal carboxylate group of the C-terminal L-Ala and simultaneously with the amino group of the vancomycin and the neighboring amide group and the sugar part of the vancomycin. A fourth hydrogen bond is formed between the carbonyl oxygen of the C-terminal L-Ala and the amino group of the vancomycin. There is no available data about the L-Ala-L-Ala terminated peptidoglycan, but from the results of this study, the conformation of the LA-LA and position of the vancomycin toward this pentapeptide as well as their weak interaction allows for the prediction that such a mutation would also result in the resistance to vancomycin. The complex of vancomycin with DA-LA peptidoglycan fragment is very similar to the complex of vancomycin with LA-LA fragment. There is similar reciprocal position of the two interacting parts and only 3 interactions have been observed within the C-terminal dipeptide part (Table 2), which are identical to three of the four interactions in the vancomycin-LA-LA complex (Fig. 8, panels F and D). In the vancomycin-DA-LA complex, the lowest acceptance limit was applied for selecting formed interactions (see above in the current paragraph). Therefore, it seems that the single mutation of C-terminal D-Ala to L-Ala also would result in resistance to vancomycin, probably stronger than mutation of both D-alanyl residues. Similar conclusion may be drawn from analysis of the interactions between the vancomycin and LA-DA peptidoglycan fragment. Only 2 interactions have been formed within the C-terminal dipeptide and only 9 interactions within whole pentapeptide. Finally, as expected, in the vancomycin-DA-DLac complex, a smaller number of interactions have been observed: 7 within the whole pentapeptide, only 1 interaction within the C-terminal crucial part and

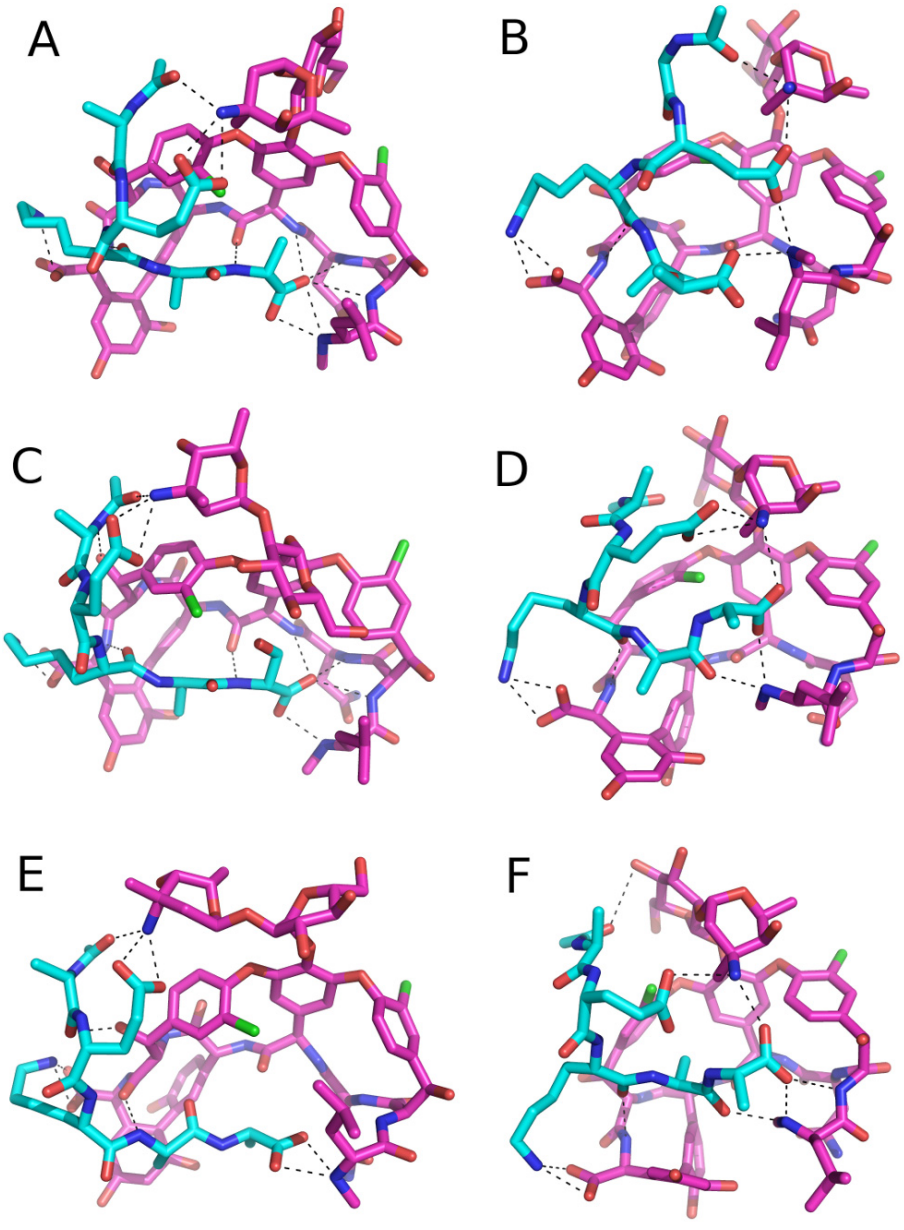

Figure 8. The polar interactions (including hydrogen bonds) identified in particular (natural and mutated) peptidoglycan fragments of peptidoglycan and vancomycin. Vancomycin – magenta; peptidoglycan – cyan; the polar interactions have been marked with dashed lines. Panel A – DA-DA; Panel B – DA-DLac; Panel C - DA-DSer; Panel D - DA-LA; Panel E - LA-DA; Panel F - LA-LA.

only one interaction with the saccharide part of the vancomycin (Fig. 8, panel B). This is in agreement with the experimental data showing that the replacement of the D-Ala-D-Ala with D-Ala-D-Lac results in a 1000-fold lowering of the affinity of vancomycin for its target [10,14,17]. It has been believed that this drop in activity is connected only with the loss of a one crucial hydrogen bond in the binding site [12]. Interestingly, the results of this study show that the substitution of D-Ala with D-Lac induces such an extensive conformational change of the peptidoglycan fragment that its efficient interaction with the antibiotic is impossible (only 7 interactions observed versus 11 in "native" complex, see Fig. 8).

4. Conclusions

Six complexes of vancomycin and peptidoglycan precursors modified at the C-terminal D-Ala-D-Ala have been studied *via* molecular dynamics simulations. The interactions between the antibiotic and peptidoglycan fragments have been identified and described in detail. The strength and classification of the identified

interactions remains in agreement with recent findings, both theoretical and experimental, and for the interactions within C-terminal part of peptidoglycan even with those about dimerization of antibiotics [39-43]. Analysis of the interactions, MD trajectories as well as energy calculations show that all studied modifications of the peptidoglycan precursor result in the impairment of the interaction with vancomycin. We have concluded that the bacterial resistance to vancomycin *via* D-Lac substitution may not only be a result of loss of a crucial hydrogen bond, but also due to an extensive conformational change that occurs in the peptidoglycan, making its strong interaction with antibiotic impossible. To counterbalance this effect, the structure of vancomycin should be more flexible. The central aromatic ring, bonding the sugar moiety, could be substituted with an aliphatic ring. The rotation of aromatic rings substituted with chlorine atoms also should be taken into consideration. This change should allow for a reduction of the steric hindrance of the N-terminal part of peptidoglycan representative. The N-terminus of vancomycin appears to be directly responsible for peptidoglycan recognition and antimicrobial activity. It has been confirmed that in the simulated systems the saccharide part of the antibiotic interacts with peptide precursors, thus it could also be important for antimicrobial activity. Weak interaction with the sugar moiety was found only in the complex terminated with D-Lac. Thus, there is a possibility to modify the saccharide part of the vancomycin to design new analogues with improved strength of binding within this part of the molecule to counterbalance the loss of the interactions resulting from D-Lac modifications of the peptidoglycan precursors. This will be studied in our future work.

Acknowledgments

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