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Interaction Between Cell Wall Model of Vancomycin Resistant Strain and Aglucovancomycin Synthetic Analogs

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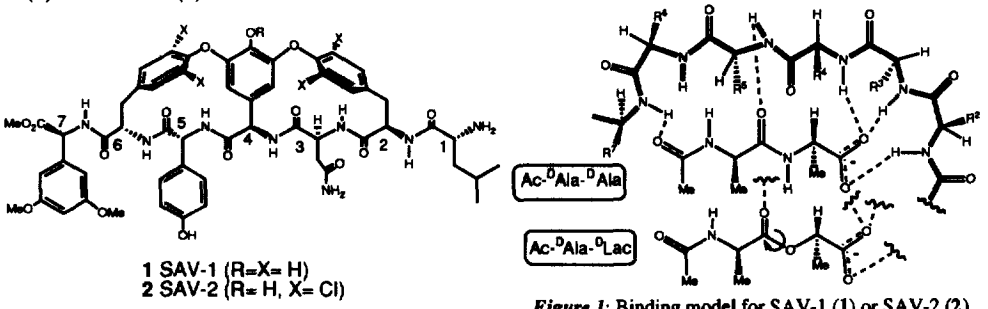
Abstract: The binding nature of the bacterial cell wall model N-acetyl-D-alanyl-D-lactate with synthetic analogs of vancomycin [SAV-1 (**1**) and SAV-2 (**2**)] is discussed.

The glycopeptide antibiotic vancomycin is one of the most potent drugs for clinical use as a bactericidal agent, especially against methicillin resistant *Staphylococcus aureus* (MRSA). The mode of action of this antibiotic is established by a specific binding to the bacterial cell wall (peptide glycan) precursor possessing a terminal D-alanyl-D-alanine residue by a five-point interaction.[1] In the preceding papers,[2] we described synthesis of vancomycin analogs, SAV-1 (**1**) and SAV-2 (**2**), possessing the bicyclic aryl ether moiety, by the phenolic oxidation methodology using thallium (III) salts.[3] Their conformational analysis and binding properties to a D-alanyl-D-alanine residue were discussed by means of a spectroscopic method, coupled with computational calculation.

In further investigation, our attention was focused on D-alanyl-D-lactate as a bacterial cell wall model bearing no proton-donative amide bond; more importantly, a vancomycin-resistant strain can synthesize this residue instead of D-alanyl-D-alanine as a peptide glycan terminal.[4] We describe herein its binding properties to SAV1 (**1**) and SAV-2 (**2**).



1 SAV-1 (R=X= H)
2 SAV-2 (R= H, X= Cl)

Figure 1: Binding model for SAV-1 (**1**) or SAV-2 (**2**) and two bacterial cell wall models

The schematic binding models of **1** and **2** to the two cell wall models are depicted in Fig. 1. The replacement of the C-terminal alanine of the ligand to a lactic acid causes no change in the donor-acceptor relationships of hydrogen bondings towards **1** and **2**. The interaction between the cell wall model and **1** or **2** was spectroscopically monitored by employing ^{1}H NMR as previously described.[2] Intermolecular hydrogen bondings were estimated by broadenings and downfield shifts of the NMR signals at 5 ~ 35 °C.

When aglucovancomycin was mixed with N-acetyl-D-alanyl-D-lactate,[5] its ^{1}H NMR exhibited an additive spectrum of each component, implying no interaction. Contrary to this observation, in the case of **1** and **2**, the ^{1}H NMR spectra of the complex indicated that the hydrogen bonds were mainly constructed in N(5)-H, N(4)-H and N(2)-H of the substrates. In particular, a hydrogen bonding at N(2)-H is observed even at a higher temperature (35 °C). These observations indicate that the interaction is initiated between carboxylate and N(2)-H. Finally, a hydrogen bonding network is constructed, as depicted in Fig. 1.[6]

To evaluate the structural features of the complexes, molecular dynamics calculation using CHARMm (ver. 23.0) / QUANTA (ver. 4.0) program package (Molecular Simulation Inc.) was applied to these systems.[7] For the formation of a complex between **1** or **2** and N-acetyl-D-alanyl-D-lactate, four-point hydrogen bonding constraints were given as mentioned above. Energy minimization considering the hydrogen bonding constraints was carried out in the whole system to make an applicable complex.[8] Molecular dynamics simulation was carried out under the following conditions: the initial molecule was heated to 300 K over 3 ps with 3 ps equilibration followed by 25 ps productive simulation at the same temperature. During this simulation, the intermolecular hydrogen bond constraints were maintained for the first 20 ps, then the constraints were released for the last 5 ps. A longer simulation period provides an accurate intermolecular interaction of complexes. The averaged structure was calculated from the last 5 ps of the productive simulation, then energy minimization was applied to the whole system.

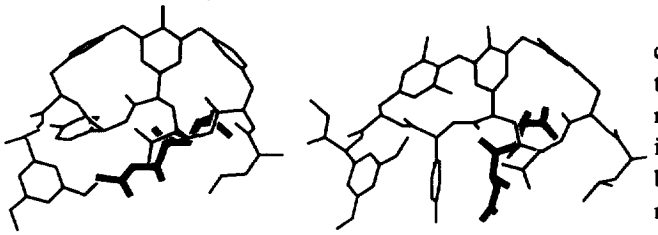


Figure 2: The computer-generated view of the complexes between SAV-1 (left) or SAV-2 (right) and D-alanyl-D-lactate

As can be seen in Fig. 2, the calculation provided the conformations of the complexes of **1** and **2** to the cell wall model D-alanyl-D-lactate. In each case, the interaction to the ligand is achieved at the backside of the molecule. The acetyl-methyl residue of the ligand is exposed to the solvent accessible surface at the lower site of the complex, thus no interaction to the substrates takes place. This seems to be due to flexibility of the ester linkage.

In conclusion, the properties of SAV-1 or SAV-2 and the bacterial cell wall model were established by means of a spectroscopic method and molecular dynamics calculation. The possibility of an effect on the vancomycin-resistant strain was also suggested.

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5. The cell wall model N-acetyl-D-alanyl-D-lactate was synthesized from Z-D-alanine and D-lactic acid benzyl ester by the DCC-DMAP method for esterification. Gilon, C.; Klausner, Y; Hassner, A. *Tetrahedron Lett.* **1979**, *20*, 3811. ^{1}H NMR data (ref. 4a) should be corrected as following: δ (D_2O, TSP= 0 ppm) 1.42 (3H, d, J= 7.3 Hz), 1.51 (3H, d, J= 7.0 Hz), 2.01 (3H, s), 4.42 (1H, q, J= 7.3 Hz), 5.03 (1H, q, J= 7.0 Hz).
6. Although the dissociation constant K_d can be estimated by a differential UV spectrum (ref. 4), the differential spectra in this case is obscure because an interaction of the chromophores might be weak. The measurement of K_d is now under way.
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8. When the ligand was brought from the lower site of the molecule, consistent hydrogen bonds were achieved (see ref. 2).

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