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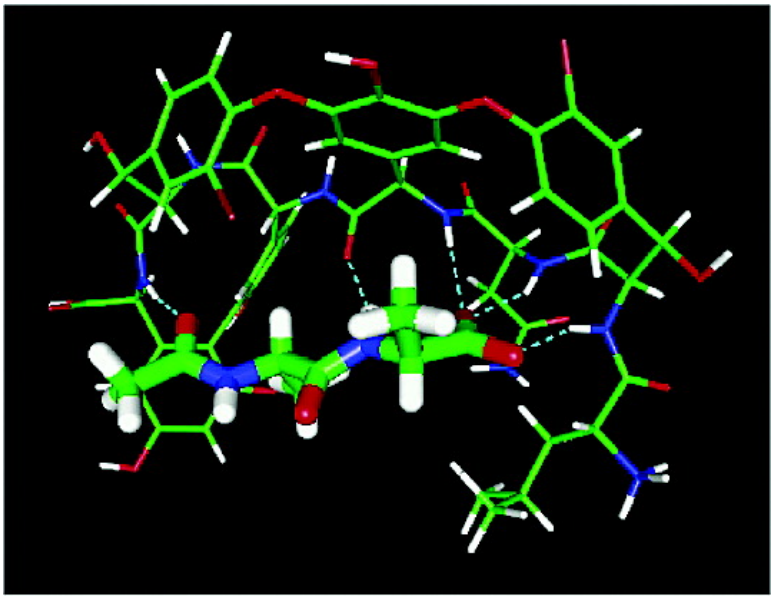

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First Principles Investigation of Vancomycin and Teicoplanin Binding to Bacterial Cell Wall Termini

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The past decade has seen the rise of staphylococcal bacterial strains resistant to virtually all antibiotics, except for vancomycin.[1] Vancomycin is the archetypical member of a family of glycopeptide antibiotics, which includes such important members as teicoplanin, ristocetin, and ramoplanin.[2] Clinically, vancomycin is used worldwide for patients allergic to β-lactam antibiotics, to fight infections during cancer chemotherapy, and as a last resort for the treatment of infections caused by Gram-positive bacteria.[3] Unfortunately, enterococci are becoming increasingly resistant to vancomycin.[4] Hence, there is considerable urgency in understanding the detailed structure and function of vancomycin and related antibiotics, with the aim of designing next-generation drugs to combat these bacterial strains.

It is now understood that vancomycin and other glycopeptide antibiotics act by inhibiting bacterial cell wall biosynthesis.[5] The antibiotics selectively bind to the precursor peptidoglycan peptide terminus N-acyl-D-Ala-D-Ala, thereby preventing the growth and thickening of the bacterial cell wall.[6] In vancomycin-resistant enterococci (VRE), characterized by the vanA, vanB, or vanD gene cluster, some of the D-Ala-D-Ala termini are substituted with D-Ala-D-Lac, so that the antibiotic's affinity for the peptidoglycan layer is reduced by a factor of over 1000.[7,8] VRE levels of resistance have been shown to be directly proportional to the numbers of precursors with D-Ala-D-Lac.[9] Hence, it is clear that the binding between D-Ala-D-Ala versus D-Ala-D-Lac is the key issue that needs understanding in order to overcome the VRE.[10]

To theoretically investigate antibiotic binding, we conducted first principles calculations based on accurate Hartree−Fock (HF) and density functional theory (DFT) simulations. The binding of the aglycon forms of the antibiotics, vancomycin aglycon ($C_{52}H_{51}N_8O_{17}Cl_2$) and teicoplanin aglycon ($C_{58}H_{46}N_7O_{18}Cl_2$), with both Ac-D-Ala-D-Ala ($C_8H_{13}N_2O_4$) and Ac-D-Ala-D-Lac ($C_8H_{12}NO_5$) was studied using Gaussian03[11] calculations. Specifically, HF and DFT calculations with the 6-31G* basis set using 1531 (vancomycin aglycon) to 1611 (teicoplanin aglycon) basis functions were carried out.[12] The DFT calculations also made use of the popular B3LYP[13] gradient-corrected functional for the exchange and correlation energies.

The salient results are shown in Figure 1. Both vancomycin and teicoplanin buckle in such a way as to form a hydrophobic pocket, which makes the important contact with the bacterial cell wall termini. The antibiotic/Ac-D-Ala-D-Ala complex is stabilized through both its dispersive van der Waals interaction, the nonbonding electrostatic interactions, and, most importantly, through a series of five hydrogen bonds. The D-Ala-D-Lac terminus involves the substitution of linking ester for an amide, through the exchange of a single ligand (i.e., X = NH → X = O). This change acts to decrease the binding of the antibiotic/Ac-D-Ala-D-Lac complex through the removal of a single hydrogen bond and through the repulsive interaction between the two oxygen lone-pairs that have now been created (see second position from the left, marked by arrows on Figure 1b,d).

Table 1. Binding Energies (kcal/mol) for Both Vancomycin and Teicoplanin Aglycon to Cell Wall Termini[a]

	vancomycin		teicoplanin	
ligand	HF	DFT	HF	DFT
X = NH	109.8 (4.9)	122.7 (3.6)	119.0 (5.1)	123.4 (3.6)
X = 0	104.9	119.1	113.9	119.8

[a] Numbers in parentheses for the X = NH ligand represent the *difference* in the binding energies between Ac-D-Ala-D-Ala and Ac-D-Ala-D-Lac.

The calculated binding energies for the geometry-optimized structures are given in Table 1. In agreement with the experimental results,[10] both antibiotics bind preferentially to Ac-D-Ala-D-Ala. The difference in the binding between Ac-D-Ala-D-Ala and Ac-D-Ala-D-Lac is about 5.0 (3.6) kcal/mol,[14] using the HF (DFT) estimates, which bracket the recent experimental estimate of 4.4 kcal/mol.[10] At room temperature, one can therefore expect that the binding to Ac-D-Ala-D-Ala is stronger by a factor of about 400−5000 over Ac-D-Ala-D-Lac.[15]

These calculations provide strong support for the previously proposed microscopic picture of the bonding between the antibotics and the cell wall terminus.[10,16] As shown in Figure 1, both antibiotics bond to Ac-D-Ala-D-Ala by means of five hydrogen bonds (whose location and distances are marked), through the dispersive and electrostatic interactions.[17] In both cases, the loss of binding with Ac-D-Ala-D-Lac is due to the O−O lone-pair repulsion. For Ac-D-Ala-D-Ala, the O···HN hydrogen bond (second from the left) is 2.196 Å long; for Ac-D-Ala-D-Lac, the corresponding O−O distance is a much further, 3.362 Å. The lone-pair repulsion has the further effect of altering the other hydrogen bond distances. For the most part (except for the fourth hydrogen bond from the left), these are pushed apart by a small amount, which further serves to decrease the binding energy. While both the HF and DFT bond distances show exactly the same trends, the DFT bond distances are somewhat shorter, reflecting the slight overbinding known to be associated with DFT calculations.[18]

Another interesting feature of the bonding is that both the HF and DFT results predict that the binding of the teicoplanin-based complexes are somewhat stronger than those based on vancomycin. This feature is primarily due to a weak $\pi-\pi$ bonding associated with the stabilization of the "O−C═O" resonance at the end of the D-Ala/D-Lac terminus and the phenyl sidegroups of teicoplanin. The small arrows on Figure 1c,d mark the specific overlap between the $\pi-\pi$ bonds.

In summary, we have investigated the binding of two antibiotics, vancomycin and teicoplanin, to bacterial cell wall termini D-Ala-D-Ala and D-Ala-D-Lac, with HF and DFT methods. In agreement with recent experimental results, the binding of both antibiotics to

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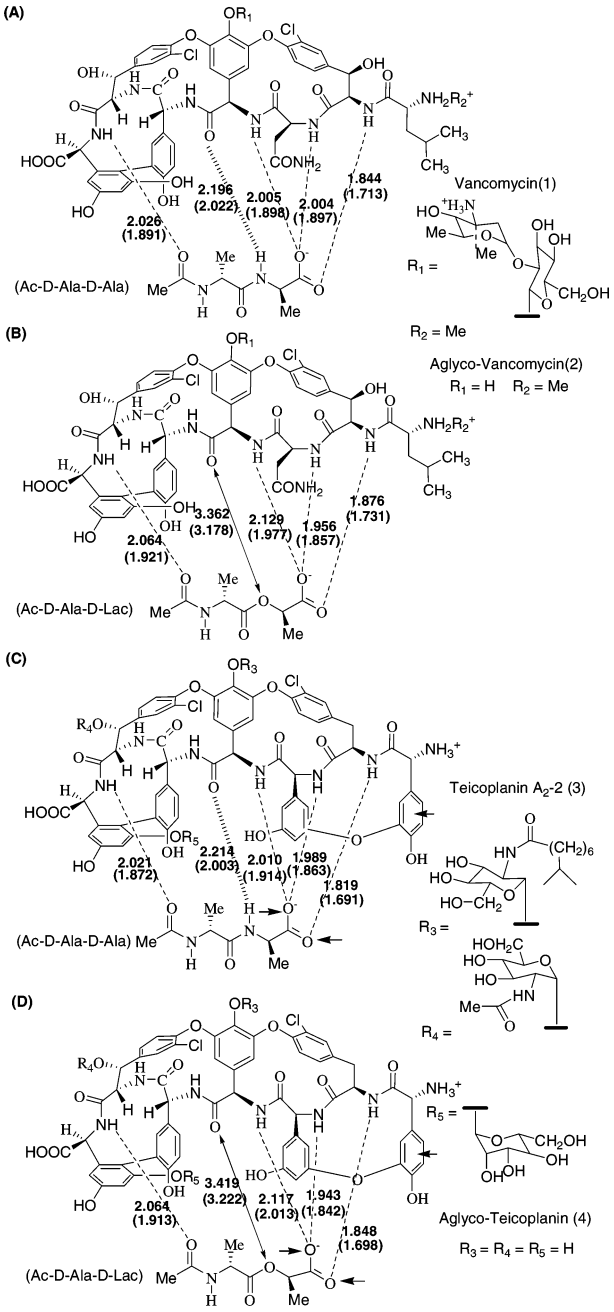

Figure 1. Schematic of (a) vancomycin/Ac-D-Ala-D-Ala, (b) vancomycin/Ac-D-Ala-D-Lac, (c) teicoplanin/Ac-D-Ala-D-Ala, and (d) teicoplanin/Ac-D-Ala-D-Lac complexes. The dotted lines mark the hydrogen bonds stabilizing the complexes, while the arrow in b and d marks the O−O lone-pair repulsion chiefly responsible for the loss of binding with Ac-D-Ala-D-Lac. HF (DFT) bond distances in Å are also marked. In c and d, the small arrows mark the weak interaction between the resonating teicoplanin bonds and the D-Ala/D-Lac terminations (see discussion in text).

Ac-D-Ala-D-Lac is estimated to be weaker by about 3−5 kcal/mol, which accounts for the diminished potency of the drugs toward combating VRE strains. Having understood the origins of this loss of binding, current efforts are underway to theoretically redesign the drugs so as to recover their antibacterial fighting power. These studies are ongoing and will be reported in the future, along with more results on different configurations of the antibiotic/cell wall termini complexes.[19]

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Supporting Information Available: Coordinates and structural information as to the binding of aglycon vancomycin to Ac-D-Ala-D-Ala and Ac-D-Ala-D-Lac (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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