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A Rational Strategy for Enhancing the Affinity of Vancomycin towards Depsipeptide Ligands

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Abstract—Glycopeptide antibiotics with enhanced affinity for model depsipeptide ligands may also exhibit enhanced efficacy against bacteria exhibiting the vanA resistance phenotype. To design modified agents with enhanced affinity for these ligands, and better understand why traditional agents have low affinity for depsipeptide ligands, free energy perturbation studies were performed on vancomycin derivatives by means of molecular dynamics simulation. The results suggest that modifications of the asparagine side chain on residue 3 of the antibiotic which enhance its hydrophobicity will enhance the affinity of glycopeptide antibiotics for depsipeptide ligands, and act synergistically with other modifications that enhance the efficacy of these agents against vanA-positive bacteria.

Introduction

Vancomycin is a clinically important glycopeptide antibiotic which inhibits normal cross-linking activity in the peptidoglycan of bacterial cell walls by binding to C-terminal D-Ala-D-Ala intermediates.[1] One form of high-level acquired resistance to the action of vancomycin (known as vanA) is mediated by a plasmid which provides for the synthesis of bacterial cell walls through C-terminal D-Ala-D-lactate (depsipeptide) intermediates.[2] Because this form of resistance is increasingly common, derivatives of vancomycin and related glycopeptide antibiotics with activity against vanA resistance are of considerable interest.

Some progress in developing such derivatives has been made. Activity against bacteria with vanA resistance has been reported for vancomycin dimers which have been covalently linked at their C-termini.[3] C-terminal amide derivatives of a compound in the teicoplanin group of glycopeptide antibiotics,[4] and derivatives of A82846B in which the disaccharide amino function has been N-alkylated,[5] are both active against vanA-positive enterococci. Nevertheless, the activity of the best of these agents is variable, and marginal against many pathological strains. The antimicrobial activity of glycopeptide antibiotics against susceptible strains generally parallels the affinity of the drug for di- and tri-peptide ligands terminating in D-Ala-D-Ala. Although this correlation is not perfect,[6] it suggests that model studies of the complexes formed between glycopeptide antibiotics and small peptide ligands can yield valuable insight into the manner by which these antibiotics recognize their targets.

Therefore, it is significant that molecular mechanics models of these complexes have been developed which not only propagate in a manner consistent with structural data from 2D NMR, but which accurately predict the relative affinity of different ligands.[7] With such models, and new techniques which enhance the precision of thermodynamics calculations,[8] we have powerful tools available with which to undertake rational modification efforts.

This report focusses on the interaction between vancomycin and a depsipeptide ligand representing the C-terminal peptidoglycan intermediate involved in vanA resistance. Free energy calculations were performed, comparing the affinity of a dipeptide (Ac-D-Ala-D-Ala, AcDADA) and a depsipeptide (Ac-D-Ala-D-lactate, AcDADL) ligand for the heptapeptide core of

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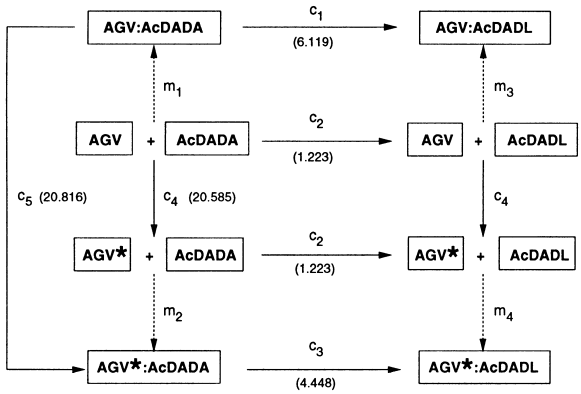
vancomycin. In agreement with crystallographic evidence suggesting that the asparagine (Asn) side chain of residue 3 plays an important, albeit indirect, role in ligand recognition,[9] our results indicate that modifications of the residue 3 side chain may significantly enhance the affinity of vancomycin and related compounds for depsipeptide ligands.

Methods

In these studies, the disaccharide group of vancomycin was removed to yield aglycovancomcyin (AGV, Figure 1). This modification reduces the antimicrobial activity of vancomycin somewhat,[10] but it yields a more computationally tractable system. All-hydrogen models of AGV and peptide ligands were prepared, validated, and propagated as described previously.[7] Molecular dynamics simulations were performed at 300 K, with 8 Å non-bond cutoffs and a step size of 0.001 ps, using a locally modified version of CHARMM22g2.[11] A 19 Å stochastic boundary was centered on the methyl carbon of the C-terminal alanine/lactate residue of the ligand, and filled with TIP3 water as detailed elsewhere.[7] Since the complexes fit within a 12.5 Å sphere, this provided for a $\geq$6.5 Å shell of water around the complex at all points.

Relative affinities between various antibiotic:ligand complexes were calculated using thermodynamic cycles (Figure 2), and a dual-topology free energy perturbation approach in which a scalar coupling parameter, λ, represents the non-physical conversion of one molecular



$$m_4 - m_2 = c_3 - c_2$$

$$m_3 - m_1 = c_1 - c_2$$

$$m_2 - m_1 = c_5 - c_4$$

$$m_4 - m_3 = c_5 - c_4 + c_3 - c_1$$

Figure 2. The thermodynamic cycles pertaining to Tables 1–3. Solid line arrows connecting various states of the system indicate calculated free energy changes (ΔA), while dotted line arrows are measurable changes. Since different paths to the same state must have identical free energy changes, differences between measurable changes may be related to differences between calculated changes as indicated. The calculation results for each path are indicated in parentheses (kcal/mol). Note that the calculation results given in the figure are for single paths (ΔA), whereas Tables 1–3 report the corresponding path differences (ΔΔA).

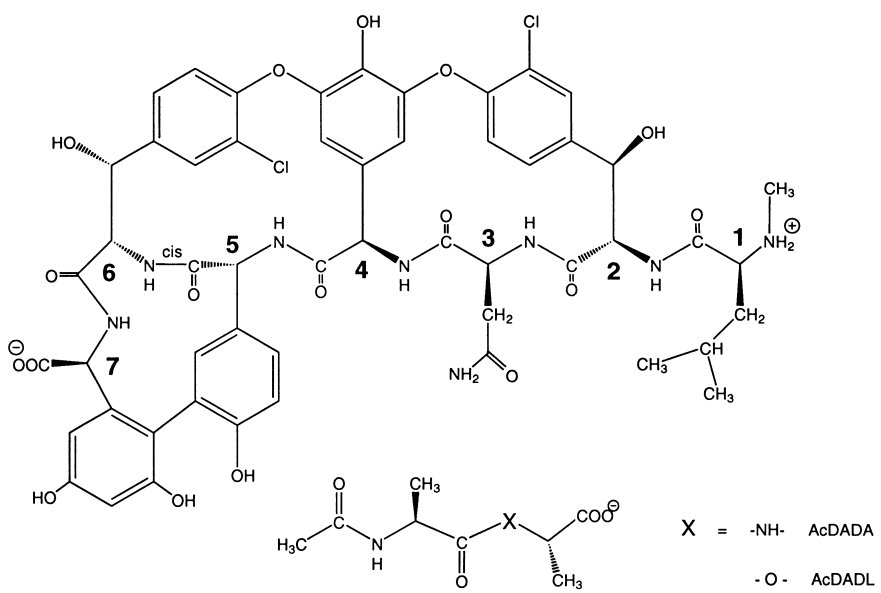


Figure 1. Chemical structure of AGV, AcDADA, and AcDADL. The α-carbon atom of each residue is numbered.

species ($\lambda = 0$) to another ($\lambda = 1$).[12] The calculation was performed in a series of windows, $\lambda^- < \lambda < \lambda^+$, and the free energy change of each window is summed to yield a total free energy for the conversion from $\lambda = 0$ to $\lambda = 1$. Each window was pre-equilibrated for 200 ps to eliminate hysteresis with respect to λ in the free energy results, and propagated for 10-30 intervals of 10 ps. For each interval,

$$\Delta A_{\lambda^- \rightarrow \lambda^+} = -k_B T ln\left[\langle e^{-\beta\lambda^+(V_p - V_R)}\rangle_\lambda / \langle e^{\beta\lambda^-(V_p - V_R)}\rangle_\lambda\right]$$

was calculated (i.e. double-wide sampling[13]) at each step. The values reported in the tables for each window are the mean and standard error of the mean (s.e. $= \sqrt{\sigma^2/n}$) for these n = 10–30 intervals, in units of kcal/mol, expressed as $\Delta\Delta$A, the free energy difference between the two corresponding paths of the thermodynamic cycle. The calculations reported in Tables 1–3 required approximately four processor-months of CPU time on SGI R8000 processors.

The mutation of Ac-D-Ala-D-Ala (AcDADA) into Ac-D-Ala-D-lactate (AcDADL) was accomplished by substituting an ester oxygen ($\lambda = 1$) for the peptide N–H ($\lambda = 0$) of AcDADA. A partial charge of -0.15 was assigned to the oxygen, in lieu of -0.40 and $+0.25$ for the N and H atoms, respectively. Other parameters were unchanged from those described previously.[7] The ensemble was biased by means of a harmonic constraint with a force constant of 20 kcal/mol Å^2 constraining the oxygen and nitrogen atoms involved in this substitution to each other's position. As we have shown,[8] this bias is automatically removed from the final result, yet while eliminating divergent entropy contributions and dramatically enhancing its precision.

Table 1. The free energy difference $c_1 - c_2$

Window			$c_1 - c_2$	
λ	λ	λ^+	$\Delta\Delta$A	s.e.
0.00	0.01	0.02	0.076	0.014
0.02	0.03	0.04	0.061	0.005
0.04	0.05	0.07	0.193	0.010
0.07	0.10	0.15	0.459	0.020
0.15	0.20	0.25	0.671	0.030
0.25	0.30	0.35	0.688	0.018
0.35	0.40	0.45	0.645	0.021
0.45	0.50	0.55	0.585	0.020
0.55	0.60	0.65	0.413	0.029
0.65	0.70	0.75	0.648	0.029
0.75	0.80	0.85	0.010	0.019
0.85	0.90	0.93	0.183	0.033
0.93	0.95	0.96	0.073	0.020
0.96	0.97	0.98	0.128	0.021
0.98	0.99	1.00	0.063	0.023
Total $\Delta\Delta_{0.00\rightarrow1.00}$			4.90	0.09

Table 2. The free energy difference $c_3 - c_2$

Window			$c_3 - c_2$	
λ	λ	λ^+	$\Delta\Delta$A	s.e.
0.00	0.01	0.02	0.031	0.014
0.02	0.03	0.04	0.037	0.005
0.04	0.05	0.07	0.037	0.010
0.07	0.10	0.15	0.156	0.020
0.15	0.20	0.25	0.239	0.030
0.25	0.30	0.35	0.522	0.018
0.35	0.40	0.45	0.448	0.021
0.45	0.50	0.55	0.499	0.020
0.55	0.60	0.65	0.314	0.029
0.65	0.70	0.75	0.284	0.029
0.75	0.80	0.85	0.329	0.019
0.85	0.90	0.93	0.121	0.033
0.93	0.95	0.96	−0.031	0.020
0.96	0.97	0.98	0.146	0.021
0.98	0.99	1.00	0.093	0.023
Total $\Delta\Delta_{0.00\rightarrow1.00}$			3.225	0.085

When indicated, the Asn side chain amide group was depolarized in two stages. Completely unpolarized alkyl groups are not typical of the Charmm22 parameter set, so we did not depolarize atoms to zero. In stage 1, partial charge assignments of $+0.10/-0.10$ were substituted for $+0.55/-0.55$ on the C=O moiety. In stage 2, partial charge assignments of $+0.10$ for H and -0.20 for N were substituted for charges on the NH_2 moiety of $+0.30$ and -0.60, respectively. The coupling parameter for these calculations represents the change in partial charge assignment (i.e. $\lambda = 0.00 \rightarrow 0.45$ for the O atom,

Table 3. The free energy difference $c_5 - c_4$

Window			$c_5 - c_4$	
λ	λ	λ^+	$\Delta\Delta$A	s.e.
(Stage 1)				
0.00	0.025	0.05	–0.038	0.026
0.05	0.075	0.10	0.116	0.021
0.10	0.125	0.15	0.117	0.019
0.15	0.175	0.20	0.013	0.022
0.20	0.225	0.25	0.077	0.026
0.25	0.275	0.30	−0.007	0.026
0.30	0.325	0.35	−0.037	0.017
0.35	0.6375	0.40	0.027	0.070
0.40	0.425	0.45	0.027	0.021
Total $\Delta\Delta_{0.00\rightarrow0.45}$			−0.295	0.095
(Stage 2)				
0.00	0.025	0.05	−0.009	0.010
0.05	0.075	0.10	−0.012	0.013
0.10	0.125	0.15	−0.006	0.009
0.15	0.175	0.20	−0.038	0.008
Total $\Delta\Delta_{0.00\rightarrow0.20}$			−0.065	0.020

and $\lambda = 0.00 \rightarrow 0.20$ for the H atoms) with corresponding changes in the C and N atoms. Because they involved only changes in partial charge assignments, these calculations required fewer windows to achieve a suitable level of precision. The resulting, depolarized, side chain model is approximately isosteric and isoelectric with that of leucine.

Results

As expected, the affinity of AGV for AcDADA is considerably better than for AcDADL. The relative free energy difference (c_1-c_2) favors AcDADA by approximately 4.9 kcal/mol (Table 1 and Figure 2). With an experimentally measured value for the affinity of AGV for AcDADA of 4.4 kcal mol,[7] this suggests that the unbound form of AcDADL is energetically favored. There are at least three factors contributing to this difference.

First, there is loss of the hydrogen bond between the NH of AcDADA and the backbone CO of AGV residue 4 (Figure 3a). Second, there is electrostatic repulsion between the electronegative ester O of AcDADL and the backbone CO of AGV residue 4 (Figure 3b). Third, there was a tendency for water to interpose between the Asn side chain (residue 3) of AGV and the ligand (Figure 3b) during a relatively long dynamics trajectory generated at $\lambda = 1$. This did not occur, however, in the 500 ps trajectories on which the free energy calculation was based (for which $\lambda \leq 0.99$).

This last observation suggested that reducing the tendency of the Asn side chain to associate with solvent may increase the overall affinity of AGV for depsipeptide ligands. Accordingly, we calculated the relative free energy difference between the dipeptide ligands and a model of AGV in which atoms of the Asn side chain have been substantially depolarized (AGV*). Our results show that this decreases the relative affinity of AGV for AcDADA versus AcDADL (c_3-c_2) from 4.9 to 3.2 kcal/mol (Table 2 and Figure 2). Furthermore, when the Asn side chain is hydrophobic, it associates with the alanyl methyl group of AcDADL and excludes water from between the antibiotic and the ligand (Figure 3c). Without additional information, however, one cannot know whether the decrease in relative affinity is due to a lesser affinity for AcDADA, or a greater affinity for AcDADL. The direct calculation of m_4-m_3 (by mutating AGV to AGV* with and without AcDADL present) was not feasible because complexes of AGV: AcDADL were not sufficiently stable, as noted above.

For this reason, we calculated the relative affinity of AGV versus AGV* for AcDADA. This calculation was performed in two stages, one in which the carbonyl group is substantially depolarized (Table 3a), and a subsequent calculation in which the amine is similarly depolarized (Table 3b). The overall calculation yields a net difference (c_5-c_4) of 0.2 kcal/mol, indicating that depolarizing the Asn side chain only slightly decreases the affinity of AGV for AcDADA. Therefore, the affinity of AcDADL for AGV* is greater than for AGV by 1.4 kcal/mol ($=c_5-c_4+c_3-c_1$).

Discussion

These results suggest that substituting a hydrophobic side chain (e.g. Leu) for Asn at position 3 will significantly enhance the affinity of glycopeptide antibiotics for the depsipeptide ligands of vanA-resistant bacteria. While this substitution is synthetically challenging,

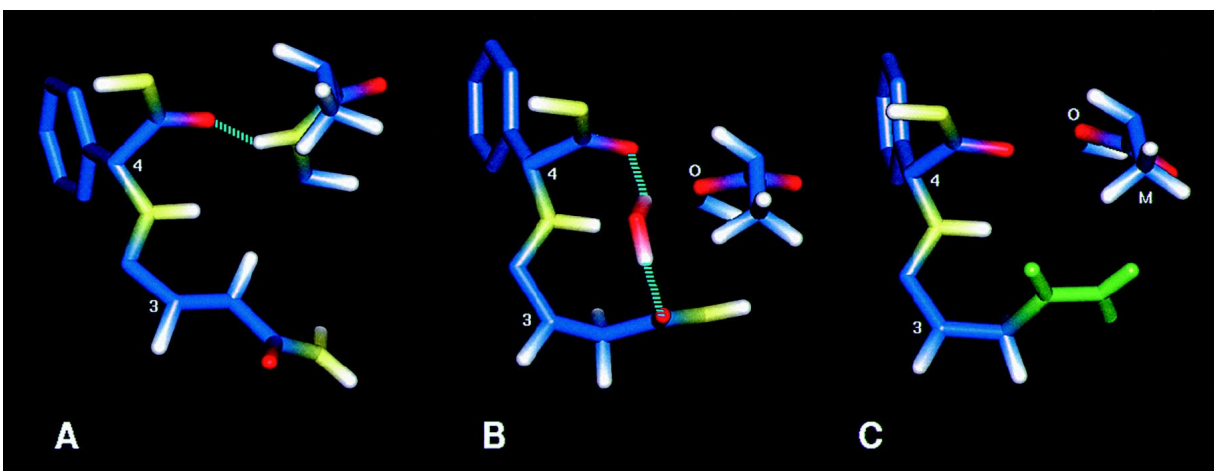


Figure 3.

complete removal and substitution of residues 1 and 3 has already been reported in a closely related antibiotic.[14] Other strategies involving deamidation and functionalization of the Asn side chain are conceivable after suitable protection of other reactive moieties, or with suitable direction of the derivatizing reagents. In this light, there are four significant aspects to our result. First, it is chemically reasonable. The ester group in AcDADL precludes any hydrogen bond formation on that aspect of the ligand which interfaces with the antibiotic and, for this reason, it may be considered to render that aspect more hydrophobic than AcDADA. Thus, greater hydrophobicity in this portion of the ligand binding pocket matches greater hydrophobicity in adjacent portions of the ligand, and should enhance affinity.

Second, the result is consistent with known structure–activity relationships. Some derivatives of antibiotics in the ristocetin/teicoplanin family exhibit activity against vanA-positive bacteria, and have an aryl side chain on residue 3.[4] We suspect that these aryl groups contribute to the affinity of these agents for depsipeptide ligands for the same reason as the modification proposed herein, namely increased hydrophobic interaction with the lactate methyl group. However, we have shown in other systems that aromatic π-electron systems have significant polar character and hydrogen bonding potential.[15] Therefore, replacing an aryl group at position 3 with an even more hydrophobic group should further improve target ligand affinity.

Third, the substitution of a more hydrophobic side chain at position 3 should be additive or synergistic with other modifications known to enhance the anti-vanA activity of vancomycin. This is especially advantageous considering that these other modifications involve derivatizing the sugar and the N-terminal amino functions,[4] and it may be necessary to complete these modifications before deamidation or functionalization of the Asn side chain can be accomplished.

Fourth, the enhancement factor, 1.4 kcal/mol, translates roughly into a one order-of-magnitude improvement in affinity constant. This gain would be sufficient to boost the effectiveness of existing agents well into the range of clinically useful activity.

Modification of the Asn side chain at position 3 is not an obvious or straightforward strategy for altering the ligand affinity of glycopeptide antibiotics. Yet, it may represent a critically significant conceptual step as efforts to overcome emerging resistance among pathogenic bacteria become urgent. Rigorously conducted free energy calculations have an important role in guiding these efforts onto paths which would otherwise not be taken due to synthetic difficulties, or conceptual subtlety.

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