Structural Basis of Synercid® (Quinupristin-Dalfopristin) Resistance in Gram-positive Bacterial Pathogens*

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Synercid®, a new semisynthetic streptogramin-derived antibiotic containing dalfopristin and quinupristin, is used in treatment of life-threatening infections caused by glycopeptide-resistant Enterococcus faecium and other bacterial pathogens. However, dissemination of genes encoding virginiamycin acetyltransferases, enzymes that confer resistance to streptogramins, threatens to limit the medical utility of the quinupristin-dalfopristin combination. Here we present structures of virginiamycin acetyltransferase D (VatD) determined at 1.8 Å resolution in the absence of ligands, at 2.8 Å resolution bound to dalfopristin, and at 3.0 Å resolution in the presence of acetyl-coenzyme A. Dalfopristin is bound by VatD in a similar conformation to that described previously for the streptogramin virginiamycin M1. However, specific interactions with the substrate are altered as a consequence of a conformational change in the pyrrolidine ring that is propagated to adjacent constituents of the dalfopristin macrocycle. Inactivation of dalfopristin involves acetyl transfer from acetylcoenzyme A to the sole (O-18) hydroxy group of the antibiotic that lies close to the side chain of the strictly conserved residue, His-82. Replacement of residue 82 by alanine is accompanied by a fall in specific activity of >100-fold, indicating that the imidazole moiety of His-82 is a major determinant of catalytic rate enhancement by VatD. The structure of the VatD-dalfopristin complex can be used to predict positions where further structural modification of the drug might preclude enzyme binding and thereby circumvent Synercid® resistance.

The challenge presented by life-threatening nosocomial bacterial infections involving multiply antibiotic-resistant, Gram-positive pathogens has prompted renewed interest in the medical use of streptogramins (1) (also called virginiamycins, pristinamycins, and synergistins). These are a group of natural-product antibiotics that contain two distinct active components, streptogramin A and B. The individual compounds act as bacteriostatic agents, but combinations of streptogramins A and B demonstrate bactericidal activity against Gram-positive microorganisms as a result of synergistic inhibition of the ribosomal peptide elongation cycle (2). Synercid®, a new semi-synthetic streptogramin formulation, was approved recently by the United States Food and Drug Administration for the treatment of bacteremia associated with glycopeptid (vancomycin)-resistant Enterococcus faecium and of postoperative infections caused by Staphylococcus aureus and Streptococcus pyogenes (3). Synercid® is a mixture of dalfopristin and quinupristin, derivatives of streptogramin A and streptogramin B, respectively, each of which has been modified to enhance the bioavailability of the antibiotic (4).

A number of streptogramin resistance mechanisms have been identified that are directed to either A or B components (5), but resistance to streptogramin A alone is sufficient to dramatically reduce efficacy of the combined antibiotic (6). Streptogramin A resistance is achieved by a putative active efflux mechanism or, more commonly, by inactivation attributable to O-acetylation of the single hydroxy group of the antibiotic. Efflux may be mediated by the product of the vra (virginiamycin factor A) gene, whereas acetylation is catalyzed by virginiamycin acetyltransferase (Vat)1 proteins using acetyl-CoA (AcCoA) as the acetyl-donor substrate (6–8). Vats are members of a superfamily of trimeric enzymes containing a left-handed β-helical (LβH) protein domain built from a repeating hexad amino acid sequence motif (9–11). Genes (vat) encoding such acetyltransferases are frequently encountered in veterinary strains of staphylococci and enterococci, probably reflecting the widespread use of virginiamycin as a growth promoter in animal husbandry, but at present are relatively rare in clinical isolates (12). However, as dalfopristin is also a substrate of Vat enzymes, dissemination of vat determinants via inter- and intraspecific horizontal gene transfer has the potential to severely limit the utility of the quinupristin-dalfopristin combination in the treatment of glycopeptide (vancomycin)-resistant E. faecium and other serious infections.

The crystal structure of VatD from E. faecium strain BM1445 (6) was determined recently at 2.5 Å resolution in the...
absence of ligands and in complex with substrates AcCoA and virginiamycin M1 (VM) at 2.7 and 2.8 Å resolution, respectively (11). In this report, we extend the structural analysis of the apo-enzyme to 1.8 Å resolution and present 2.8 Å data for a complex of VatD and dalfopristin. The latter define important contacts between the antibiotic and amino acids of the VatD active site, including a strictly conserved histidyl residue, His-82. Mutational analyses indicate that deletion of the imidazole side-chain of His-82 reduces the activity of VatD by a minimum of five orders of magnitude. In addition to identifying important determinants of catalytic rate enhancement, the structure of the dalfopristin-VatD complex also provides a structural basis for the rational design of new streptogramin A-derived antibiotics that are no longer susceptible to inactivation by Vat-mediated acetylation.

EXPERIMENTAL PROCEDURES
Preparation and Assay of Native VatD Protein—Native VatD protein was expressed in Escherichia coli JM101 from plasmid pAT421 (6) and purified to apparent homogeneity (>95%) using a fast protein liquid chromatography system (Amersham-Pharmacia) by anion-exchange (Q-Sepharose FF and Mono-Q 10/10) and hydrophobic interaction (Phenytoin-Sepharose HP) column chromatography. The concentration of homogeneous VatD was determined at 280 nm using the theoretical extinction coefficient of 4.7 × 10^4 M^{-1} cm^{-1} calculated according to Edelhoch (13). VatD activity was measured spectrophotometrically at 420 nm in 50 mM Tris-HCl, pH 7.5, at 25 °C, containing 100 mM NaCl and 0.1 mM EDTA with standard substrate concentrations of 0.1 mM VM and 0.4 mM AcCoA and in the presence of 1 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (dNBA), 5,5'-dithiobis(2-nitrobenzoic acid) react stoichiometrically with the free thiol of the CoA produced by VatD catalysis, yielding the thionitrobenzoate anion that absorbs strongly at 412 nm (E = 1.36 × 10^4 M^{-1} cm^{-1}) (28).

Preparation of Selenomethionyl VatD—To produce selenomethionine-substituted VatD protein, a pT728a selenomethionine-expressed plasmid (28) was introduced into the methionine auxotrophic E. coli strain B834 (DE3). Cell-free extracts were prepared from isopropyl-1-thio-galactopyranoside-induced exponential-phase cultures grown in M9 minimal medium supplemented with selenomethionine (20 μg ml^{-1}) and the other 19 l-amino acids. Selenomethionine-substituted VatD protein was purified as described for the native enzyme.

Site-directed Mutagenesis—The His-82 codon (CAT) of the VatD gene in pAT421 was replaced by GCG (encoding alanine) using paired mutagenic oligonucleotides IM144 (5′-TATGAGACCAACAGCAGAAATG-GATGCGCTC-3′) and IM145 (5′-GAGCCCCATCTTCGCTTTT-GTCTCATTACA-3′) (mismatched nucleotides are underlined) using the QuickChange protocol (Stratagene). The additional nucleotide substitution introduces a silent (T to C) mutation in the third position of codon 81 (Asn), generating a new BsuRI restriction site that was exploited to screen for plasmid derivatives containing the desired mutation. Nucleotide sequence analysis of the entire vatD coding and 5′ noncoding sequences was then used to confirm the presence of the correct nucleotide substitutions (CAT to GCG at codon 82) and the absence of additional mutations in the modified plasmid, pAT421/Ala-82 (data not shown).

Crystallization and Data Collection—Crystallization trials were conducted using the hanging-drop method of vapor diffusion; 5 μl of protein solution (0.15 to 0.25 mM in 1 mM Tris-HCl buffer, pH 7.5) was mixed with an equal volume of precipitant. Crystals of native and selenomethionine-incorporated enzyme were obtained after two to five days of incubation at 17 °C using 2.7 to 3.0 mM sodium formate as a precipitant. The free enzyme crystallized in the space group P2_12_1 with cell dimensions a = 181.4 Å, b = 183.0 Å, c = 181.4 Å, and α = β = γ = 90°. The crystals have six copies of the monomer in the asymmetric unit and a solvent content of 48%. The crystals were stabilized in 20% glycerol and 3 mM sodium formate before flash cooling at 100 K. Multi-wavelength anomalous dispersion data were collected to 2.7 Å (2.8 Å for the injection wavelength) from a single crystal on station BM14 at the European Synchrotron Radiation Facility in Grenoble. Three wavelengths were chosen near the selenium absorption edge (see Table I) based on an x-ray fluorescence scan of the frozen crystal to maximize the f'′ component (λ̄ peak), to minimize the f′ component (λ̄ remote), and to maximize the Δf′ component (λ̄cutoff). The data collected at the European Synchrotron Radiation Facility were processed and merged using DENOZO/SCALEPACK from the HKL suite of programs (14).

Subsequently, 1.8 Å native data were collected at 100 K on station 9.6 at the CLRC Daresbury Synchrotron Radiation Source Laboratory. Data were processed and merged using the DENZO/SCALEPACK package (14).

Cocrystals of selenomethionine VatD (0.15 mM) with AcCoA (1 mM) were grown in 2.9 mM sodium formate and stabilized in Paratone-N before flash cooling at 100 K. The cocrystals were isomorphous with those of the free enzyme, and data were collected to 3.0 Å on a Mar345 image plate using copper Kα x-rays on a Rigaku RU200 rotating anode generator. Data were processed and merged using the DENZO/SCALEPACK package (14).

Structure Determination and Refinement—The diffraction data from the multi-wavelength anomalous dispersion experiment were analyzed, and estimates of heavy atom structure factors, F_A values derived from an analysis of the anomalous differences between reflections at the three wavelengths, were determined using the program XPREP (Bruker, AXS, Madison, WI). The anomalous signal was found to extend to three wavelengths, were determined using the program XPREP (Bruker, AXS, Madison, WI). The anomalous signal was found to extend to three wavelengths, were determined using the program XPREP (Bruker, AXS, Madison, WI). The anomalous signal was found to extend to three wavelengths, were determined using the program XPREP (Bruker, AXS, Madison, WI).
and TLS refinement gave a final pristin molecule was fitted to the density, and further individual XYZ revealed clear and unambiguous density for the dalfopristin. The dalfopristin-applied Difference electron density maps were generated, which re-performed, and 3-fold non-crystallographic symmetry restraints were structure as the starting model. Subsequent rigid body refinement was.

The final model contained 10,313 atoms, including 759 of solvent, and gave R = 0.176 and Rfree = 0.207 (see Table II for refinement statistics). The program PROCHECK (22) was used to monitor the quality of the final model. The overall average B was 16.1 and 22.6 Å² for the protein main chain and side chain atoms, respectively, and 34.4 Å² for the water molecules.

Molecular replacement using AMoRe (23) was successfully carried out on the 2.8 Å VatD plus dalfopristin data using the 1.8 Å free enzyme structure as the starting model. Subsequent rigid body refinement was performed, and 3-fold non-crystallographic symmetry restraints were applied. Difference electron density maps were generated, which revealed clear and unambiguous density for the dalfopristin. The dalfopristin molecule was fitted to the density, and further individual XYZ and TLS refinement gave a final R = 0.272 and Rfree = 0.303.

Rigid body refinement of the free enzyme model against the 3.0 Å VatD data with AcCoA bound was performed with initial B-factor values set to 70 Å² as suggested by a Wilson plot of the data, and 6-fold non-crystallographic symmetry restraints were applied. Difference electron density maps were generated, which revealed clear and unambiguous density for the AcCoA. AcCoA was fitted to the density, and further individual XYZ and TLS refinement gave a final R = 0.245 and Rfree = 0.295.

RESULTS

Quality of the Models—The 1.8 Å structure of VatD contains six copies of the polypeptide chain (two trimers) in the asymmetric unit (labeled A, B, C, X, Y, and Z). The electron density is of high quality throughout the majority of the map (Fig. 1a), and the final model is composed of 1,217 of an expected total of 1,254 residues. A Ramachandran plot shows that all but six of the non-glycine residues lie either in the most favorable or additional allowed regions. Examination of the χ1-ψ2 plots for all residue types showed that 99% of side chains lie in favorable conformations. The improved resolution of our free enzyme structure determination compared with those published previously (11) allows greater confidence in defining side chain conformations and provides a more complete description of the solvent structure around the enzyme that may have relevance for its catalytic activity and/or substrate affinity. There is an average of 126 solvent molecules per subunit in our higher resolution structure compared with 47 and 27 per subunit in the earlier 2.5 and 2.7 Å free enzyme structures, respectively (11).

The 2.8 Å structure of VatD with dalfopristin bound contains a trimer in the asymmetric unit (the individual polypeptide chains being labeled A, B, and C). The electron density is of good quality throughout the majority of the map (Fig. 1b), and the model is composed of 612 of an expected 627 residues. Clear electron density is observed for dalfopristin molecules (labeled H, I, and J) bound at all three active sites of the VatD trimer.

The 3.0 Å structure of VatD with AcCoA bound is isomorphous with the 1.8 Å free enzyme structure. The electron density is of good quality for this resolution (Fig. 1c), and the final model is composed of 1,211 of 1,254 residues. Electron density corresponding to five complete AcCoA molecules and one partial molecule lacking the acetyl group (labeled H to M) was observed at the six active sites of the two trimers.

Structure of the VatD Subunit—The enzyme is a homotrimer with overall dimensions of 75 Å × 75 Å × 35 Å, and the monomers are related by a non-crystallographic 3-fold axis. Each monomer contains four α-helices, seven β₃-helices, and 16 β-strands with a substantial number of turns as defined using the program PROMOTIF (24) (Fig. 1d). The units of regular secondary structure together make up ~46% of the VatD polypeptide chain. The monomer structure comprises four elements: the N-terminal region, a left-handed parallel β-helix (LβH) domain, an extended loop, and a C-terminal helical domain.

The N-terminal region, residues 1–29, includes a single β-strand (βA) and α-helix (αA) and caps one end of the LβH domain (residues 30–75 and 114–167). The LβH domain is the largest structural element of VatD and folds as a left-handed spiral into five triangular coils (25). It contains 14 short β-strands (βB–βO) and two 3₁₀ helices (B and C). The LβH domain is interrupted by the extended loop region (residues 76–113), which includes four short 3₁₀ helices (D–G) and is extruded from the LβH domain at the tight turn position in the second coil. The LβH domain terminates in a β-strand (βP) that is not part of a coil but instead forms an antiparallel β-interaction with the final strand (βO) of the fifth coil. The C-terminal domain (residues 168–202) contains three α-helices (αH, αJ, and αK) and a single 3₁₀ helix (L).

Quaternary Structure and Intersubunit Contacts—The solvent-accessible surface areas of the VatD monomer and trimer (calculated using AREAIMOL (26) with a probe radius of 1.4 Å) are ~11,000 and 25,000 Å², indicating that ~30% of the monomer becomes buried on formation of the trimer. Sixty-one of 209 residues are involved in intersubunit contacts, the majority being either interactions between the extended loop region and the LβH domain or between the LβH domains of adjacent subunits. There are 10 intersubunit hydrogen bonds, 9 of which involve donors or acceptors provided by residues located in the extended loop.

His-82 Is a Catalytic Residue of VatD—The GenBank database currently contains gene sequences encoding 11 distinct classes (discounting minor allelic variants) of Vat proteins that are present in Gram-positive bacteria (data not shown). Of 15 VatD residues that form direct contacts (<4 Å) with dalfopristin and/or VM (those shown in Fig. 2e plus Gly-79 and Asn-92), only His-82 and Tyr-54 are absolutely conserved in these Vat variants. Another candidate catalytic group, the side chain hydroxyl of Tyr-37* that forms a hydrogen bond with the O-18...
FIG. 1. **Stereo images.** 

- **a**, the final $|2Fo-Fc|$ electron density map contoured at 1.0 $\sigma$ of a representative portion of the 1.8 Å *E. faecium* VatD structure. 
- **b**, the final $|2Fo-Fc|$ electron density map contoured at 1.0 $\sigma$ of dalfopristin molecule H at 2.8 Å. 
- **c**, the final $|2Fo-Fc|$ electron density map contoured at 1.0 $\sigma$ of AcCoA molecule H at 3.0 Å. 
- **d**, arrangement of secondary structural elements in the VatD monomer. This figure was produced using TURBO-FRODO (18), MOLSCRIPT (40), and Raster3D (41).
hydroxyl, which is the site of antibiotic acetylation, also appears to be dispensable because the gene encoding a Vat enzyme in Clostridium acetobutylicum contains an isoleucine codon at this position (27). Because His-82 is the only absolutely conserved amino acid residue positioned close to the acetylated hydroxy group of dalfopristin and VM (O-18 to Ne2 distances of 3.9 and 4.4 Å, respectively), we decided to address the consequences of deleting this imidazole group by using site-directed mutagenesis. The results showed that Ala-82 VatD behaved identically to the wild-type enzyme at each chromatographic step, suggesting that the replacement of His-82 by alanine did not result in any major rearrangement of the catalytic competence. In the former case, His-195 acts as a general base function of His-64 in subtilisin (32). In the latter, the general base function of His-64 in subtilisin is reprogrammed to activate the side chain hydroxy group of Ser-221 that is the target of acetylation by VatD. The second H-bond interaction involves Tyr-54 and the O7 carbonyl oxygen of dalfopristin located between the oxazoline and pyrrolidine rings of the substrate. In addition to these hydrogen bonds, a number of hydrophobic and van der Waals interactions are observed in the complex.

The conformation adopted by dalfopristin is generally similar to that reported for VM in its binary complex with VatD (11), with contacts involving the main-chain atoms of residues Gly-79 to Asn-81 and the side chains of Tyr-37* and Asp-39* being observed in both structures. However, there are some differences, including the movement of two regions of polyepptide chain involved in antibiotic binding. The first is a displacement of residues 102–111 toward atoms C-26–C-31 of dalfopristin to relieve close contacts with symmetry-related molecules and facilitate crystal packing in space group P2₁2₁2₁. The second is a movement of residues 11–15 of the N-terminal region, resulting in new contacts between the side chains of Asn-14* and Val-17* and atoms of the oxazoline ring of dalfopristin, which are not observed in the VM complex. In addition, a hydrogen bond involving Nδ2 of Asn-92 and the O-27 carbonyl oxygen of VM (11) is not observed in the dalfopristin complex.

There are no direct contacts between residues of VatD and atoms of the sulfonyl trimethylamine moiety that is present in dalfopristin but not in natural-product streptogramin A antibiotics such as VM. Instead, this substituent projects from the enzyme active site and is solvent-accessible, consistent with its clinical role in enhancing the solubility of the antibiotic. However, the modified structure of dalfopristin does lead to changes in the contacts between enzyme and substrate when compared with those observed in the VatD-VM complex (Fig. 2d). Such differences arise as a consequence of the loss of planarity of the pyrrolidine ring of VM that occurs when the additional substituent is added at C-2 to create dalfopristin. Accommodation of the changed pyrrolidine conformation results in a reorientation of proximal elements of the dalfopristin macrocycle compared with their positions in VM.

AcCoA Binding Site—The model determined for the structure of the complex of VatD and AcCoA contains two trimers in the asymmetric unit and six substrate molecules, five of which are complete. The following discussion is centered on AcCoA molecule H, which shows the clearest and most readily interpretable electron density.

The overall conformation of bound AcCoA is very similar to that presented previously by Roderick and co-workers (11) with the substrate adopting a bent conformation, folding back upon itself at the pyrophosphate group (Fig. 2c). Contacts between the protein and substrate atoms, from the C-2 carbon of the β-mercaptoethylamine moiety to the adenine ring of CoA, are essentially the same in both structures. The conformation of the terminal acetyl group, however, is quite different. In our structure the acetyl group torsion angle is −168°, positioning the acetyl group adjacent to Tyr-52, Gly-79, and Ala-80. In the published structure, this angle is −15°, and the acetyl group contacts residues Tyr-37*, Ser-68* to Gly-70*, and Trp-121* of Dalfopristin is a macro cyclic lactone (see Fig. 2b for structure) that binds to VatD in a cleft formed primarily by residues from the extended loop region and from the short insertion loop at the end of the first β-helical coil of the same monomer. Contacts with the adjacent subunit are limited to the side chains of Asn-14* and Val-17* in the N-terminal region and Tyr-37* and Asp-39* of the first coil of the LβH domain (Fig. 2c). Two hydrogen bonds are observed in the enzyme-substrate complex, each involving the hydroxy groups of conserved tyrosyl residues. The first is between the side chain of Tyr-37* and the O-18 hydroxy group on the substrate that is the target of acetylation by VatD. The second H-bond interaction involves Tyr-54 and the O7 carbonyl oxygen of dalfopristin located between the oxazoline and pyrrolidine rings of the substrate. In addition to these hydrogen bonds, a number of hydrophobic and van der Waals interactions are observed in the complex.

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DISCUSSION

Location of the Dalfopristin and AcCoA Binding Sites—In common with other CoA-dependent acyltransferases containing an LβH domain (33–35) and as demonstrated previously for VatD (11), both substrates are bound at the interfaces between subunits. The structure of the VatD apoenzyme reveals a short tunnel formed, at the N-terminal end, by residues of the extended loop region of one monomer and the LβH domain of the symmetry-related subunit and, at the C-terminal end, by residues provided by two adjacent LβH domains. The substrates dalfopristin and AcCoA bind at the N-terminal (Fig. 2a) and the C-terminal ends of the tunnel, respectively, thereby bringing together the target (O-18) hydroxy group of the antibiotic and the acetyl moiety of AcCoA at the enzyme catalytic center. Dalfopristin-binding Site—The model of VatD with dalfopristin contains three monomers in the asymmetric unit, and the binding of dalfopristin was detected in all three subunits. The following discussion will focus on dalfopristin molecule H, which showed the clearest electron density.

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FIG. 2. The active site of VatD. a, stereo image of the VatD trimer showing AcCoA superimposed on the model of the dalfopristin complex. Carbon atoms are colored yellow (AcCoA) and gray (dalfopristin), with non-carbon atoms colored according to atom type (N blue, O red, P purple, and S black). b, chemical structure and atom numbering scheme for dalfopristin. Natural product streptogramin A antibiotics (e.g. VM) lack the sulfonyl triethylamine substituent (atoms 39 through 48) on the pyrrolidine ring. c, stereo image of dalfopristin bound at the VatD active site. Amino acid residues shown are those making contacts (4 Å) with the substrate. Asterisks denote residues from a symmetry-related subunit. Atoms, other than S (yellow) and the carbon atoms of dalfopristin (green), are colored as in a, and secondary structural elements of VatD are colored in red, green, and yellow for helices, β-strands, and turns, respectively. d, stereo image showing the conformation of dalfopristin (green) bound to VatD superimposed on the complex of the same enzyme with VM (pink) (11). Non-carbon atoms are colored as in c. The altered positions of the pyrrolidine groups (and proximal elements of the macrocycle) arise because the C-1-C-2 double bond of VM becomes saturated, when the additional C-2 substituent is introduced to produce dalfopristin, changing the pyrrolidine ring from a planar to a puckered conformation. e, stereo image of
the adjacent subunit. Although this difference demonstrates that the active site of VatD can accommodate significant rotational flexibility of the acetyl group, it is not possible to predict which (if any) of the two acetyl-group conformers is functionally relevant. However, because both AcCoA structures were determined as binary complexes, it is likely that formation of the productive (ternary) complex imposes additional constraints on the orientation of the acetyl group.

Implications for Antibiotic Resistance and Drug Design—Our structural studies of dalfopristin binding to VatD demonstrate how this semisynthetic derivative of streptogramin A is accommodated by the enzyme active site despite the presence of a large additional substituent on the pyrrolidine ring and a significant conformational change that accompanies this structural modification of the antibiotic. This interpretation is consistent with results of steady-state kinetic analyses (28) showing that the specificity constant \( k_{cat}/K_m \) for dalfopristin acetylation by VatD is only 7-fold lower than the equivalent parameter determined using VM as a substrate. To the best of our knowledge, no equivalent comparison has been made for the binding of dalfopristin and VM to the peptidyltransferase center of the 50S ribosomal subunit. However, \textit{in vitro} and animal-model studies of dalfopristin inhibition of the growth of \textit{S. aureus} suggest that the ribosomal target is similarly insensitive to these changes in the antibiotic structure (36).

\textit{vat} genes (including \textit{vatD}) are found on transposable genetic elements, where they are sometimes associated with \textit{erm} genes that confer parallel resistance to streptogramin B-class compounds (37). What then is the potential for the development of new VM or dalfopristin derivatives that are insensitive to inactivation by Vats? The fact that we observe no contacts involving amino acid residues of VatD and atoms of the additional sulfonyl triethylamine group of dalfopristin suggests that further modification of this part of the antibiotic structure is unlikely to yield compounds that are insensitive to inactivation by Vats. Because atoms C-21–C-24 of both dalfopristin and VM (11) form close main-chain contacts with residues Gly-79, Ala-80, and Asn-81 of VatD, incorporation of additional steric bulk to this part of the antibiotic structures would be expected to preclude their binding to the enzyme. However, it is difficult to imagine how this might be achieved, other than by engineering of the VM biosynthetic pathway (38), and the proximity of

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FIG. 2—continued

The AcCoA-VatD complex showing amino acid residues that form contacts (< 4 Å) with the substrate. Atoms and secondary structural elements are colored as in c, and asterisks indicate residues associated with a symmetry-related subunit of the trimer. The figure was produced using MOLSCRIPT (40), Raster3D (41), and ISIS DRAW v2.4 (MDL Information Systems Inc.).
these atoms to the critical O-18 hydroxy group suggests that such compounds may no longer function as peptidyltransferase inhibitors. A more promising strategy may be to introduce structural modifications of the N-25–O-27 amide group of Pro-103 of VatD. Because this amino acid residue is absolutely conserved in enterococcal and staphylococcal Vats, attachment of additional functional groups to O-27 could yield novel compounds that are refractory to acetylation side-chain atoms of Pro-103 could yield novel compounds that are refractory to acetylation by the enzymes of these important pathogens. However, in the absence of structural insights into the binding of VM or dalfopristin and VM, it is not currently possible to predict whether such compounds would retain antibiotic activity.

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Structure of VatD-Dalfopristin Complex

these atoms to the critical O-18 hydroxy group suggests that such compounds may no longer function as peptidyltransferase inhibitors. A more promising strategy may be to introduce structural modifications of the N-25–O-27 amide group of Pro-103 of VatD. Because this amino acid residue is absolutely conserved in enterococcal and staphylococcal Vats, attachment of additional functional groups to O-27 could yield novel compounds that are refractory to acetylation side-chain atoms of Pro-103 could yield novel compounds that are refractory to acetylation by the enzymes of these important pathogens. However, in the absence of structural insights into the binding of VM or dalfopristin and VM, it is not currently possible to predict whether such compounds would retain antibiotic activity.

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Structural Basis of Synercid® (Quinupristin-Dalfopristin) Resistance in Gram-positive Bacterial Pathogens
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