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Published on Web 02/27/2004

Vinyl Sulfones: Inhibitors of SrtA, a Transpeptidase Required for Cell Wall Protein Anchoring and Virulence in *Staphylococcus aureus*

Brenda A. Frankel, Matthew Bentley, Ryan G. Kruger, and Dewey G. McCafferty*

Johnson Research Foundation and the Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6059

Received October 13, 2003; E-mail: deweym@mail.med.upenn.edu

During pathogenesis, Gram-positive bacteria utilize surface protein virulence factors such as the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) to aid the initiation and propagation of infection through adherence to host endothelial tissue and immune system evasion.[1] These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycan through a general sorting mechanism catalyzed by a superfamily of membrane-associated transpeptidases termed *sortases*.[2,3] Disruption of surface protein anchoring correlates with a dramatic decrease in pathogenicity of Gram-positive bacteria.[4,5] Selective inhibition of sortase activity therefore constitutes a new potential avenue of antivirulence chemotherapy for combating bacterial infection, especially those resistant to front-line antimicrobials.[6]

In *Staphylococcus aureus*, the sortase SrtA isoform is responsible for the covalent attachment of numerous virulence and colonization-associated proteins to the bacterial cell wall peptidoglycan.[4,7] SrtA utilizes two substrates: branched Lipid II and secreted proteins containing a highly conserved C-terminal LPXTG sequence (Figure 1).[3,8] SrtA simultaneously cleaves the Thr-Gly bond of the LPXTG-containing protein and forms a new amide bond with the nucleophilic amino group of the Gly_5 portion of branched Lipid II, which is subsequently polymerized into mature peptidoglycan.[9]

Comparative sequence analysis of sortase superfamily members revealed absolutely conserved cysteine and histidine residues (C184 and H120 in SrtA).[2,10] Subsequent chemical modification, site-directed mutagenesis, NMR titration, and inhibitor studies have provided supporting evidence for the participation of C184 in catalysis as an active-site nucleophile with H120 serving as a general base.[11,12] Chemical trapping experiments and bisubstrate kinetic studies collectively suggest that the C184 nucleophile, facilitated by H120, attacks the Thr-Gly peptide bond of substrate proteins, resulting in the formation of an enzyme-acyl intermediate.[11,13] This intermediate is subsequently resolved by transfer of the surface protein to branched Lipid II.[9]

Intriguingly, we observed that broad specificity cysteine hydrolase inhibitors such as leupeptin, E-64, chymostatin, and antipain were ineffective against SrtA at concentrations up to 1 mM (data not shown). Vinyl sulfones, on the other hand, represent a new class of small molecule electrophilic inactivators of cysteine proteinases.[14] Members of this inhibitor class act by electrophilic capture of Cys nucleophiles via 1,4-conjugate addition, forming a stable thioether adduct that irreversibly inactivates the enzyme. We therefore tested several commercially available small-molecule vinyl sulfones for their ability to inhibit SrtA activity in vitro (Table 1) using an HPLC-based activity assay.[15]

Initial measurement of vinyl sulfone IC_{50} values provided an indication of the appropriate range of inhibitor concentrations for examining the time and concentration dependence of inhibition (Figure 2). Fitting the resultant curves to a single-exponential function allowed the calculation of the observed rate constants for inactivation (k_{obs}), which were then plotted as an inverse function of inhibitor concentration (Figure 2 inset) to determine k_{inact}/K_i, the second-order rate constant for inactivation (Table 1).

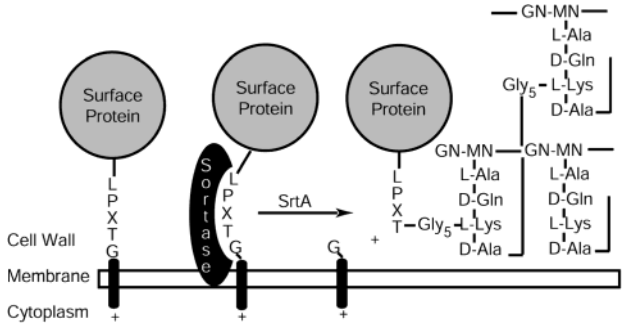

Figure 1. Reaction catalyzed by the *S. aureus* SrtA transpeptidase.

Table 1. Inhibition Parameters of Vinyl Sulfones for SrtA

inhibitor[a]	IC_{50} (mM)	k_{inact}/K_i (M^{-1} min^{-1})	MIC (mg/mL)
phenyl vinyl sulfone	0.736	20.1	>1
3,3,3-trifluoro-1-(phenylsulfonyl)-1-propene	0.190	90.0	0.075
methyl vinyl sulfone	6.24	3.08	0.6
ethyl vinyl sulfone	4.71	3.60	1
divinyl sulfone	1.06	4.74	1
cis-1,2-bis(phenylsulfonyl)ethylene	1.13	n.d.[b]	n.d.
phenyl *trans*-styryl sulfone	no inhibition		n.d.

[a] All compounds were purchased from Aldrich and used without further purification. Stock solutions were prepared fresh before each assay in 100% DMSO. [b] n.d., not determined. See Supporting Information for experimental details.

SrtA treated with vinyl sulfones exhibited irreversible time-dependent inhibition with values for k_{inact}/K_i, ranging from 3 to 90 M^{-1} min^{-1} (Table 1; Figure 2). [16] Of the inhibitors examined, 3,3,3-trifluoro-1-(phenylsulfonyl)-1-propene proved most potent, with an IC_{50} value of 190 μM and a second-order inactivation constant of 90.0 M^{-1} min^{-1}. Since this value was markedly increased over that of the closely related phenyl vinyl sulfone (PVS), we attribute this 5-fold difference to the increased electrophilicity of the trifluoromethyl-substituted compound. Further inspection revealed an apparent single step inactivation mechanism (i.e., collisional inactivation) for PVS, as indicated by a double reciprocal plot that intersects the origin. As anticipated, however, the remainder of the compounds appeared to exhibit more complicated inhibition kinetics suggestive of a multistep inactivation mechanism. For PVS, MALDI-TOF and ESI MS/MS sequencing revealed a single modification (168.05 Da) specifically localized to C184, consistent with the formation of a PVS−SrtA covalent adduct (see Supporting Information).

10.1021/ja0390294 CCC: $27.50

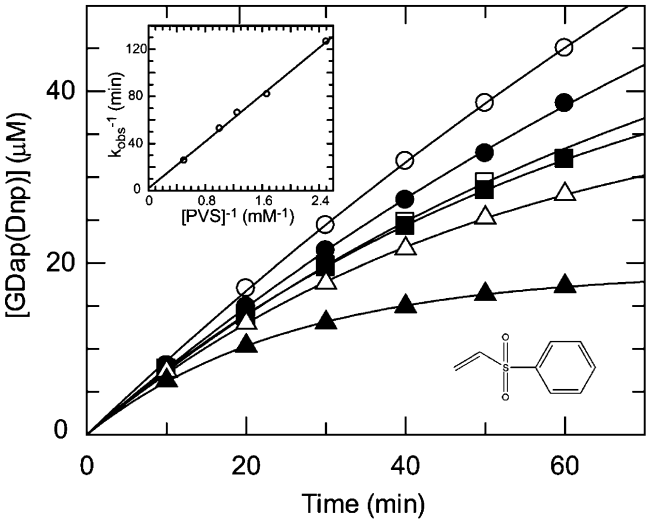

Figure 2. Representative inhibition data for PVS. Observed rate constants for inactivation were calculated from the progress curves shown at 0 (○), 0.4 (●), 0.6 (□), 0.8 (■), 1.0 (△), and 2.0 mM (▲) PVS, and k_{inact}/K_i was calculated from the linear relationship between k_{obs}^{-1} and $[PVS]^{-1}$ (inset).

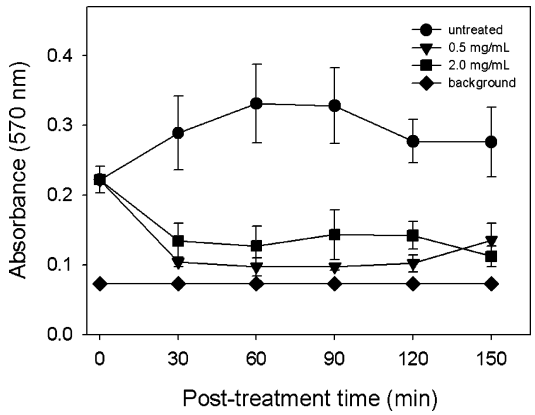

Figure 3. Phenyl vinyl sulfone inhibits SrtA-mediated *S. aureus* adhesion to fibronectin via fibronectin-binding protein.

Cell membrane anchored SrtA catalyzes the covalent anchoring of adhesins such as fibronectin- and fibrinogen-binding proteins that take advantage of low nanomolar binding affinity for fibronectin or fibrinogen to facilitate attachment to host tissues.[6] We hypothesized that vinyl sulfones should inhibit SrtA activity in vivo and in turn reduce fibronectin-binding protein surface display. To test this hypothesis, we employed an assay in which cell adhesion to fibronectin-coated plates was quantified by measuring the absorbance following staining with crystal violet. In these experiments, we employed concentrations of PVS below the MIC to rule out effects on fibronectin binding due to inhibition of cell growth. As expected, treatment of *S. aureus* Newman strain with PVS significantly reduced the capacity of the bacteria to adhere to fibronectin-coated surfaces (Figure 3).[17] It is important to note that the onset and magnitude of inhibition of fibronectin binding in *S. aureus* treated with PVS is comparable to the behavior of untreated *srtA*$^-$ deletion strains.[4] This result supports the notion that phenyl vinyl sulfone is an effective inhibitor of SrtA activity in vivo.

Collectively, these data highlight the potential of small molecule vinyl sulfones for the treatment of *S. aureus* infections via inhibition of SrtA activity. In contrast to other promiscuous irreversible inhibitors, vinyl sulfones hold promise for in vivo efficacy. Additional studies are underway to incorporate vinyl sulfone privileged structures within isoform-selective sortase inhibitors.

Acknowledgment. We thank Gang Xiao, Christine Busch, and Chao-Xing Yuan at the UPenn Proteomics Facility. This research was generously supported by National Institutes of Health NIH Grants AI46611 and GM65539. Brenda Frankel is the recipient of an NSF Predoctoral Fellowhip.

Supporting Information Available: Experimental protocols, ESI MS/MS sequencing, and progress curve analysis for vinyl sulfone inhibitors (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0390294