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The Structure of Sortase B, a Cysteine Transpeptidase that Tethers Surface Protein to the *Staphylococcus aureus* Cell Wall

Yinong Zong,[1] Sarkis K. Mazmanian,[2] Olaf Schneewind,[2] and Sthanam V.L. Narayana[1,*]
[1]Center for Biophysical Sciences and Engineering
School of Optometry
University of Alabama at Birmingham
Birmingham, Alabama 35294
[2]Committee on Microbiology
University of Chicago
Chicago, Illinois 60637

Summary

Many surface proteins of Gram-positive bacteria, which play important roles during the pathogenesis of human infections, are anchored to the cell wall envelope by a mechanism requiring sortases. Sortase B, a cysteine transpeptidase from *Staphylococcus aureus*, cleaves the C-terminal sorting signal of IsdC at the NPQTN motif and tethers the polypeptide to the pentaglycine cell wall cross-bridge. During catalysis, the active site cysteine of sortase and the cleaved substrate form an acyl intermediate, which is then resolved by the amino group of pentaglycine cross-bridges. We report here the crystal structures of $SrtB_{\Delta N30}$ in complex with two active site inhibitors, MTSET and E64, and with the cell wall substrate analog tripleglycine. These structures reveal, for the first time, the active site disposition and the unique Cys-Arg catalytic machinery of the cysteine transpeptidase, and they also provide useful information for the future design of anti-infective agents against sortases.

Introduction

The cell wall envelope of Gram-positive bacteria can be viewed as a surface organelle, containing anchored proteins that promote adherence to host tissues and enable bacterial escape from the innate immune response (Navarre and Schneewind, 1999). Those surface proteins are first synthesized in the bacterial cytoplasm, then transported across the cytoplasm membrane, and finally covalently linked to the peptidoglycan of the cell wall. Sortases, a class of membrane-anchored enzymes, are responsible for the anchoring of the surface proteins to the cell wall (Mazmanian et al., 2001; Novick, 2000). In *Staphylococcus aureus*, sortase A (SrtA) recognizes and cleaves the surface protein precursor molecules at the LPXTG motif. Among the LPXTG motif-bearing proteins already identified are many bacterial adhesion proteins, which are also called MSCRAMMs (microbial surface component-recognizing adhesive matrix molecules) such as Spa, FnbA, FnbB, ClfA, ClfB, Cna, SdrC, SdrD, SdrE, and Pls. Bacteria which fail to display MSCRAMMs lose their virulence (Foster and Hook, 1998; Patti et al., 1994). Not surprisingly then, bacterial strains in which the sortase gene had been inactivated or mutated could not display their surface proteins properly and therefore were defective in the pathogenesis of animal infections (Bierne et al., 2002; Bolken et al., 2001; Mazmanian et al., 2000, 2002). As more and more pathogens become resistant to antibiotics, inhibition of surface proteins on the cell wall may offer a novel strategy against the Gram-positive bacterial infections (Cossart and Jonquieres, 2000).

In most cases, Gram-positive bacterial genomes encode more than one sortase gene (Pallen et al., 2001), and it has been suggested that different sortases function to anchor different classes of surface proteins (Ton-That et al., 2001). *Staphylococcus aureus* harbors two sortases, sortase A (SrtA) and sortase B (SrtB), which exhibit about 45% primary sequence homology (Figure 1A). The newly identified SrtB anchors IsdC, a polypeptide involved in heme-iron transport (Mazmanian et al., 2002, 2003). Ion acquisition from the living environment is critical for bacterial survival and pathogenesis. While SrtA anchors surface proteins involved in adherence and immune evasion, SrtB seems to be dedicated to the essential process of iron acquisition during bacterial infection. SrtB cleaves the protein precursor molecule at the NPQTN motif, and it shows no cross-activity with SrtA (Mazmanian et al., 2002). The structural basis for the substrate specificity of the two *Staphylococcus aureus* sortases remains to be elucidated.

Because of a single conserved cysteine residue that was implicated in their transpeptidation reaction in vitro (Ton-That et al., 2002), sortases were thought to be cysteine transpeptidases. Such transpeptidation was abrogated by the addition of MTSET (2-(Trimethylammonium)-ethyl-methanethiosulfonate), a cysteine protease inhibitor (Akabas et al., 1992), confirming the role of the conserved single cysteine as the active nucleophile. In the case of SrtB, the enzyme first breaks the peptide bond between T and N of the NPQTN sorting motif to form a tetrahedral acyl intermediate. The amino group of pentaglycine cross-bridges tethered to the lipid II peptidoglycan precursor molecules is thought to function as a nucleophile, resolving the acyl intermediate and generating an amide bond between the surface protein and lipid II, with subsequent incorporation of this intermediate into the cell wall envelope (Perry et al., 2002). This process could well be analogous to the reaction catalyzed by serine DD-transpeptidases or penicillin binding proteins (PBPs) (Grandchamps et al., 1995; Rhazi et al., 2003). But the exact catalytic mechanisms of sortases, including the identity of catalytic residues added to the nucleophilic cysteine and of the structural correlates responsible for substrate binding and its specificity, are not known.

Although extensive biochemical studies of SrtA have been performed in vivo and in vitro (Perry et al., 2002; Ton-That et al., 2000), structural characterization of this transpeptidase has been limited to NMR spectroscopy, and the location and disposition of its active site have remained a matter of speculation (Ilangovan et al., 2001). The active site's catalytic function was attributed to single cysteine and histidine residues, known to be con-

*Correspondence: narayana@uab.edu

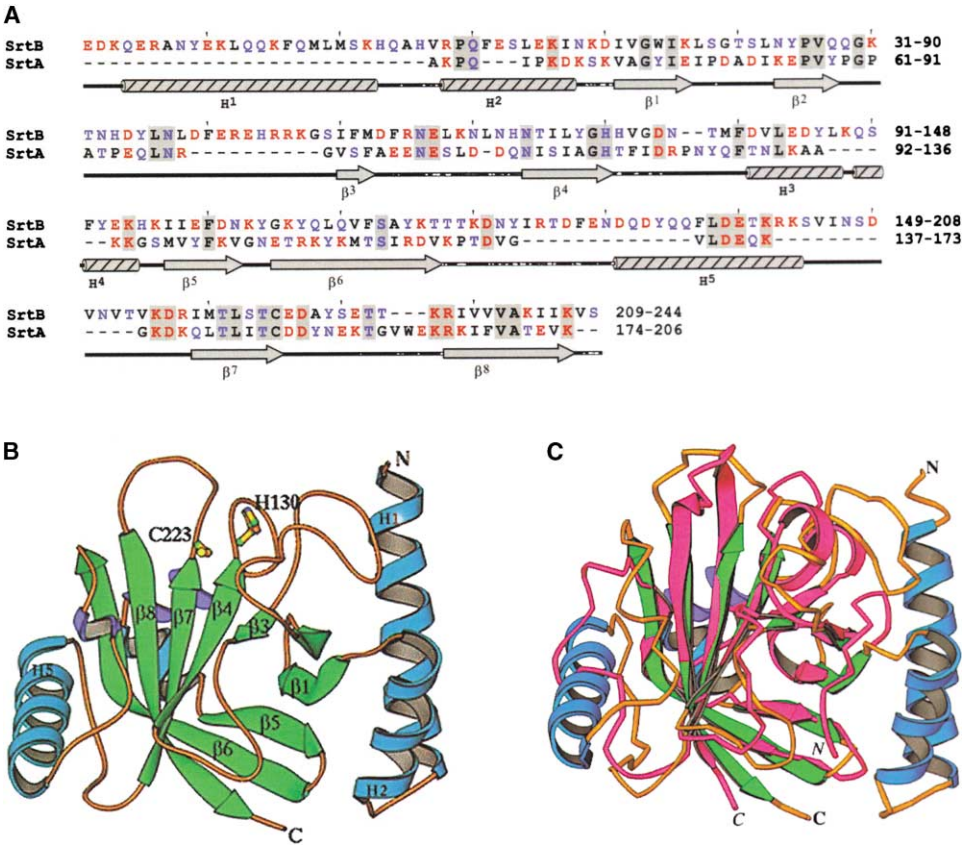

Figure 1. Overall Structure of *S. aureus* $SrtB_{\Delta N30}$

(A) The primary sequence alignment of SrtA and SrtB utilizing the crystal structure of $SrtB_{\Delta N30}$. The secondary structure elements of $SrtB_{\Delta N30}$ are represented as arrows for β strands and cylinders for helices. The residue numbers show the starts and ends of $SrtA_{\Delta N59}$ and $SrtB_{\Delta N30}$, respectively.

(B) Ribbon representation of $SrtB_{\Delta N30}$, in which individual β strands (green) and α helices (blue and cyan) are labeled. Putative catalytic Cys and His residues are shown as ball-and-stick models.

(C) Superimposition of $SrtB_{\Delta N30}$ and one of the $SrtA_{\Delta N59}$ NMR models. The NMR $SrtA_{\Delta N59}$ backbone ribbons are shown in red, and $SrtB_{\Delta N30}$ is in other colors. The N-terminal two-helix bundle and helices H4 and H5 observed in the $SrtB_{\Delta N30}$ crystal structure were not observed in $SrtA_{\Delta N59}$ NMR structure.

served in all the sortases of all Gram-positive bacteria, and presumably positioned in its near vicinity. The NMR structures of $SrtA_{\Delta N59}$ suggested that the conserved Cys184 and His120 residues, along with Asn98, displayed a spatial arrangement almost identical to that observed for the catalytic triad in the cysteine protease papain (Ilangovan et al., 2001). Thus, Cys184, His120, and Asn98 were assumed to form a putative catalytic triad for SrtA (Ton-That et al., 2002). However, the thiol group of Cys184 was observed to point away from the imidazole group of His120, and the distance between them was too large to form a typical thiolate-imidazolium ion pair without necessitating substantial main chain conformational changes. Moreover, the imidazole ring of His120, as a member of a hydrophobic cluster and having limited interactions with Cys184, is unlikely to have direct interactions with the peptide substrates as a general acid/base during the catalysis. Hence, confined by the $SrtA_{\Delta N59}$ NMR structure to the concept that sortases utilize the Cys-His-Asn catalytic triad seen in classic cysteine protease, one could not define the active site of SrtA and the catalytic mechanism for sortases.

In this report we present the crystal structures of SrtB in complex with two inhibitors and one substrate and reveal that the catalytic triad in sortases is distinct from that of classical cysteine proteases. Even though the transpeptidation reactions catalyzed by *Staph aureus* SrtA and SrtB enzymes are similar, they recognize almost identical yet subtly different anchoring motif in substrates. In addition to presenting the novel folding of these enzymes, the crystal structures reported here, for the first time, reveal the unique Cys-Arg catalytic dyad involved in the transpeptidation reaction and provide a clear picture of the catalytic machinery of this enzyme and of its class.

Results and Discussion

Crystallization and Structure Determination

Although sortases are membrane-anchored proteins, recombinant SrtB, known as $SrtB_{\Delta N30}$, created by deleting the N-terminal transmembrane segment, remains fully functional (Mazmanian et al., 2002). MTSET, commonly used for probing active and accessible thiol groups in proteins, is an effective inhibitor for both SrtA

Table 1. Crystallographic Statistics

Data Collection							
Data Set	Wavelength (Å)	Resolution (Å)	Total	Unique	Completeness	$<I/\sigma\ (I)>$	R_m[a] (%)
$C222_1$ (E64)	0.9791	2.7	309,446	10,290	99.4%	12.3	5.9
$C222_1$ (MTSET)	1.5418	2.5	164,067	6,905	96.8%	7.1	11.1
C2 (MTSET)	1.5418	1.7	408,147	20,145	97.4%	19.8	5.2
Phasing Statistics							
	Se Sites	FOM (Before/After DM)					
$C222_1$ (E64)	5	0.34/0.78					
Refinement Statistics			Rmsd				
	Water	R[b] (%)	R_{free}[c] (%)	Bonds (Å)	Angle (°)	B_{ave} (Å^2)	
$C222_1$ (E64)	25	22.5	27.5	0.0086	1.236	29.8	
$C222_1$ (MTSET)	27	19.1	27.4	0.0063	1.147	35.8	
C2 (MTSET)	291	18.3	22.8	0.0047	1.110	20.2	

[a] $R_m = \Sigma||I_O| - |I_{Ave}||/\Sigma|I_{Ave}|$, where I_O is the individual measurement of a reflection and I_{Ave} is the average intensity for symmetry-related reflections.
[b] $R = \Sigma|F_{Obs} - F_{Cal}|/\Sigma F_{Obs}$.
[c] $R_{free} = \Sigma_{test}(|F_{Obs}| - |F_{Cal}|)^2/\Sigma_{test}|F_{Obs}|^2$, where Σ_{test} are 10% reflections being randomly selected as a test set.

and SrtB with binding constants in the 20 μM range (Mazmanian et al., 2002; Ton-That et al., 2000). $SrtB_{\Delta N30}$ treated with MTSET was crystallized in two space groups, $C222_1$ and C2. When incubated with E64 ([n-(l-3-trans-carboxyoxirane-2-carbonyl)-l-leucyl]-amido (4-guanido)butane), an inhibitor that reacts with nucleophilic cysteine residue in cysteine proteases, $SrtB_{\Delta N30}$ was crystallized in the $C222_1$ space group. A $C222_1$ $SrtB_{\Delta N30}$-MTSET complex crystal was soaked with a triple glycine (Gly_3) peptide. Molecular replacement trials using $SrtA_{\Delta N59}$ NMR structures (1IJA) as the searching model failed. To determine their initial structure using the single anomalous scattering technique, we grew $C222_1$ crystals of $SrtB_{\Delta N30}$ complexed with MTSET in which the methionine residues were substituted with Selenomethionine. Subsequently, other $SrtB_{\Delta N30}$ complex crystal structures were determined by molecular replacement methods. The crystallographic data collection and model refinement statistics are presented in Table 1.

Overall Structure of $SrtB_{\Delta N30}$

The structures of $SrtB_{\Delta N30}$ in the two different space groups are essentially identical, except for small variations in the connecting loop regions. The overall structure of $SrtB_{\Delta N30}$ consists of a unique eight-stranded β-barrel core structure, and a two-helix bundle subdomain at the N-terminal end (Figure 1B). The topology of the β-barrel is identical to that observed in $SrtA_{\Delta N59}$ NMR structures, and there structures superimpose with an rms deviation of 1.25 Å for 84 Cα atoms (Figure 1C). However, the $SrtB_{\Delta N30}$ crystal structure differs from $SrtA_{\Delta N59}$ NMR structures by the insertion of a four-turn α helix and one-turn 3_{10} helix between the β6 and β7 strands, and a two-turn 3_{10} helix and one-turn α helix between the β4 and β5 strands (Figure 1D). The N-terminal of $SrtB_{\Delta N30}$ forms two well-defined α helices. The H1 helix is directly linked to the transmembrane segment of SrtB and the H2 helix is packed against the main body of the enzyme. The two helices may help to project the enzyme into extracellular space. The external side of the β-barrel forms a concave region, and the critical cysteine residue, Cys223, is located at the tip of the β7 strand directed toward the concave pocket.

$SrtB_{\Delta N30}$ Complexed with MTSET

The 2-(trimethylammonium) ethyl thiol group of MTSET is linked to the thiol group of Cys223 via a covalent bond, suggesting that the nucleophilic thiol of Cys233 is readily accessible in the resting state (Figure 2A). This finding provides the first structural evidence that SrtB functions as a cysteine protease. In classic cysteine proteases, the catalytic cysteine residue is usually found at the N-terminal end of a helix bordering the substrate binding cleft (Grzonka et al., 2001; Turk et al., 1997, 1998). Characteristically, the Cys223 of $SrtB_{\Delta N30}$ is located at the C-terminal end of a β strand, a situation similar to that observed in interleukin-1β converting enzyme (ICE), an unconventional cysteine protease in monocytes (Wilson et al., 1994). However, ICE is an α/β protein with a modified Rossman fold and its catalytic Cys/His residues are present at the easily accessible β sheet tip, whereas $SrtB_{\Delta N30}$ and $SrtA_{\Delta N59}$ have Cys/His residues anchored on the concave side of a β sheet, suggesting that sortases represent an unconventional group of cysteine proteases characterized by a novel β-barrel fold.

$SrtB_{\Delta N30}$ Complexed with E64

The active site of $SrtB_{\Delta N30}$ can be further defined by the crystal structure of the SrtB ΔN30-E64 complex (Figure 2B). With the presence of clear electron density in the active site, E64 exhibits two possible conformations for its terminal guanidino group. The E64 C_2 (numbering according to the caricain-E64 structure [1MEG] [Katerelos et al., 1996]) atom is covalently linked to Cys223 Sγ. However, its carboxyl group $(C_1OO)^-$ is in hydrogen-bonding distance to the guanidinium group of Arg233, which might function as a stabilizing agent for $SrtB_{\Delta N30}$, just as an "oxyanion hole" and a catalytic histidine residue did for the E64 carboxyl group in the papain-E64

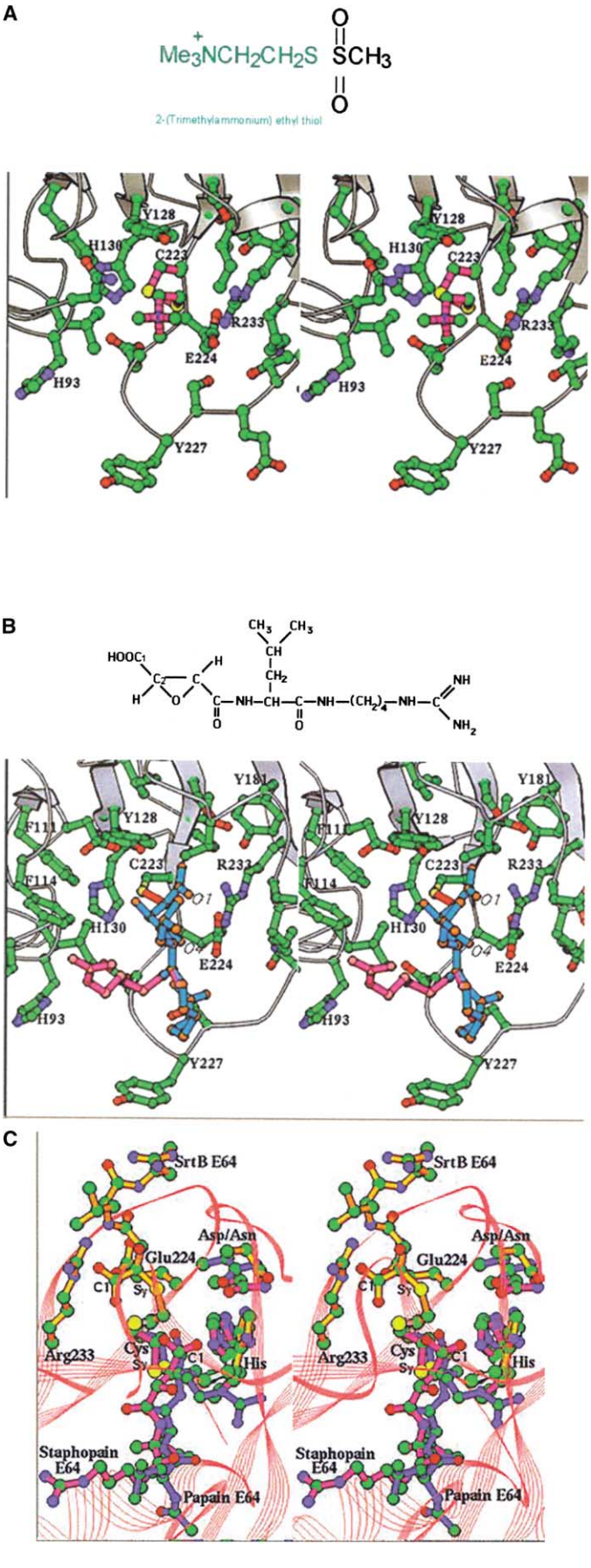

Figure 2. The Active Site Disposition of $SrtB_{\Delta N30}$ Complexed with Two Inhibitors

(A) The MTSET molecule (upper panel) is shown in two colors, with its cyan part linked to $SrtB_{\Delta N30}$. In the lower panel, MTSET (purple bonds) is covalently linked to Cys223 Sγ and it can be seen that Cys223 Sγ is pointing away from the His130 imidazole ring.

(B) Stereo view of E64-bound active site of $SrtB\Delta N_{30}$. The E-64 molecule representation is shown in the upper panel. In the lower panel, the Sγ of Cys223 is covalently linked to the C_2 atom of E64, and the guanidino group of E64 has two conformations, which are represented in two colors, purple and cyan.

(C) Spatial disposition of the papain, staphopain, and $Srtb_{\Delta N30}$ catalytic residues and the respective active site-bound inhibitor E64 molecules. The stereo view of $SrtB_{\Delta N30}$-E64 is superimposed on the papain-E64 and staphopain-E64 complexes by aligning the $SrtB_{\Delta N30}$ Cys223-His130-Asp225 residues with the catalytic Cys/His/Asn triad residues of papain and staphopain inhibitor complex crystal structures. In all three, the E64 inhibitor is covalently linked to the respective Cys residue Sγ atom. Atoms are colored according to their type (carbons in green, oxygens in red, nitrogens in blue, and sulfurs in yellow). The bonds for the side chain residues and the respective inhibitor molecules are colored identically: gold bonds for $SrtB_{\Delta N30}$, red for papain, and blue for staphopain. The thin lines in the background represent the backbone of $SrtB_{\Delta N30}$. For comparison, we have also superimposed the $SrtA_{\Delta N59}$ Cys183 and His120 residues (gray bonds) with the corresponding C223 and His130 residues of $SrtB_{\Delta N30}$.

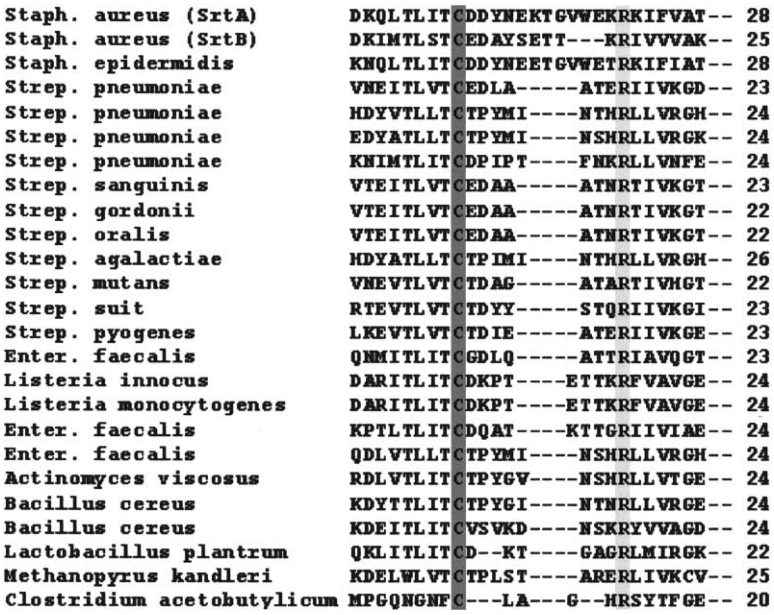

Figure 3. Sequence Alignment around Active Sites of Sortases from Different Gram-Positive Bacteria

The active Cys and Arg are conserved across all species, and though gap length between the Cys and Arg may vary, the relative disposition of Cys and Arg remains constant. The alignment was run by ClustalW 1.82 of EMBL-EMI (Thompson et al., 1994).

(1PPP) and staphopain-E64 (1CV8) crystal structures (Hofmann et al., 1993; Varughese et al., 1989; Yamamoto et al., 1991). Assuming that the $SrtB_{\Delta N30}$-E64 complex resembles the acyl-enzyme intermediate and that a general acid/base is needed for the acylation and deacylation of substrates during catalysis (Harrison et al., 1997; Matsumoto et al., 1999), the observed distance of 7.0 Å between the side chain of His130 and the covalently bound E64 C_2 atom makes it impossible for the His130 to participate in the cleavage of the NPQTN peptide substrate. For the purposes of comparison, we superimposed Cys223, His130, and Asn97 of the $SrtB_{\Delta N30}$ crystal structure on the catalytic triad residues of papain and staphopain, along with the respective covalently bound E64 molecules (Figure 2B). Although its Cys-His-Asn triad residues are in a similar spatial disposition to those of papain and staphopain, the $SrtB_{\Delta N30}$-E64 complex displays the covalently bound inhibitor directed away from the His130 residue, suggesting that SrtB may employ a catalytic apparatus other than the traditional Cys-His-Asn/Asp triad of cysteine proteases.

The nearest ionizable side chain to Cys223, other than the imidazolium group of His130, is the guanidino group of Arg233 (5.1 Å to Cys233 Sγ and 3.1 Å to E64 C2). It is conceivable that the guanidino group is free to move closer to Cys223 Sγ or to the incoming substrate without disrupting the overall architecture of the active site. Other ionizable groups in proximity to Cys223 are Tyr128 (7.6 Å), His93 (9.2 Å), and Arg115 (11.5 Å). Interestingly, Arg233 is conserved in all sortases of Gram-positive bacteria (Figure 3), and similar spatial arrangements for Cys-Arg have been observed in the $SrtA_{\Delta N59}$ NMR structures. Since the loop joining the Cys-Arg pair could vary in length and shape, we can reasonably assume that the Cys-Arg configuration is common among sortases. Although the His130 residue in $SrtB_{\Delta N30}$ is unlikely to participate directly in the catalytic process, it could play an important role in maintaining the electrostatic environment of the active site. It has been suggested that in cysteine proteases the nucleophilic character of the active site cysteine residue is not assured by the simple presence of the Cys^{-}-His^{+} ion pair, but requires the surrounding charged or polar clusters that potentiate the catalytic competence of the Cys (Pinitglang et al., 1994, 1996). It is interesting to note that the side chains of Glu224 and Asn180 are in hydrogen-bonding distance to the guanidinium group of Arg233 and so each could conceivably form a catalytic triad with Cys223-Arg233. However, neither Glu224 nor Asn180 is conserved among sortases.

$SrtB_{\Delta N30}$ Complexed with Gly_3 Peptide

After the peptide cleavage/acylation step, sortases use a second substrate, Gly_5, one of the cross-bridges of peptidoglycan, as a nucleophile to resolve the acyl-enzyme intermediate. The transpeptidation reaction is dominant if Gly_5 or Gly_3 is present (Ton-That et al., 2000). This peptide transfer is similar to that seen in the catalysis by DD-transpeptidase of the peptide cross-linking reaction involved in bacterial cell wall peptidoglycan assembly (Fonze et al., 1999; Grandchamps et al., 1995; Rhazi et al., 2003). When incubated with the nucleophile Gly_3, SrtA catalyzes exclusively the transpeptidation reaction with a K_m of 16.48 μM and a K_{cat} of 2.27×10^5 (1/s), and the incorporation of Gly_3 is the rate-limiting step in the reaction (Ton-That et al., 2000). Anticipating a well-defined binding site for the second substrate Gly_3 close to the sortase catalytic apparatus, we soaked Gly_3 peptide into the $SrtB_{\Delta N30}$-MTSET crystals. Clear electron density for Gly_3 was seen in 2Fo-Fc and Fo-Fc (Figure 4A) maps far from His130, and the N-terminal amino group of Gly_3 was observed to be about 5.2 Å to the guanidinium group of Arg233, about 3.5 Å to the S atom of the covalently linked MTSET and about 4.5 Å to the Sγ of Cys223 (Figure 4B). Gly_3 is held in position by a backbone hydrogen bond between the substrate and a residue in the loop region between Cys223 and Arg233. During deacylation, a general base is needed to de-

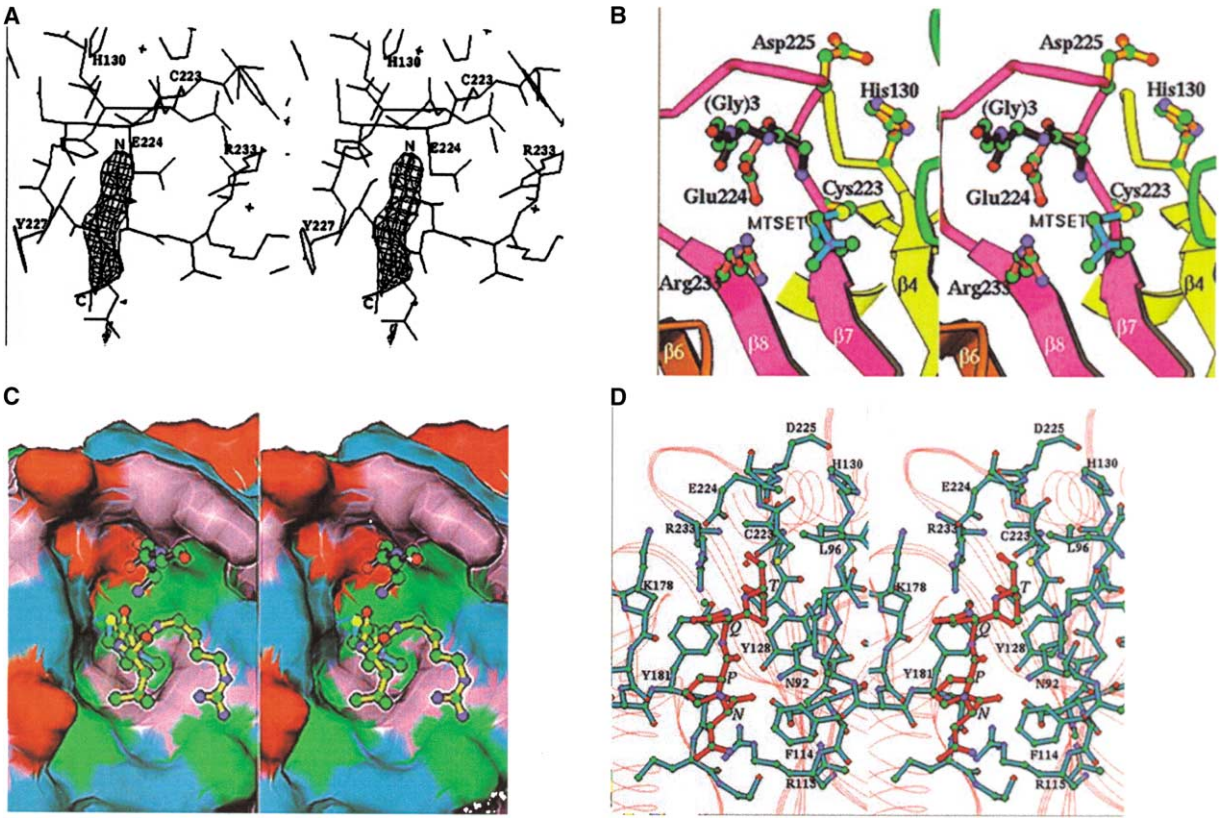

Figure 4. $(Gly)_3$-Bound $SrtB_{\Delta N30}$ and a Molecular Model of $SrtB\Delta N_{30}$-NPQT Complex

(A) Stereo view of the difference electron density (plotted at 2.5σ) in a (Fo-Fc) map to 3.0 Å resolution utilizing the calculated phases without the contribution of $(Gly)_3$.

(B) Stereo view of the peptide substrate $(Gly)_3$-bound $SrtB_{\Delta N30}$ active site. The inhibitor MTSET is covalently linked to Cys223. $(Gly)_3$ (black bonds) is partially held in position by the loop joining the β7 and β8 strands, and its N-terminal NH_2 group is in proximity to the guanidinium group of Arg233 and Cys223 thiolate.

(C) Surface representation of $SrtB_{\Delta N30}$ with $(Gly)_3$, E64, and MTSET bound in the active site.

(D) Ball-and-stick representation of the modeled NPQT-enzyme intermediate. The carbonyl carbon of Thr is covalently linked to $S\gamma$ of C223, and the carbonyl oxygen is pointing toward the guanidinium group of Arg233. The rest of the peptide, in extended conformation, is stabilized by two backbone hydrogen bonds and hydrophobic interactions of the Pro residue in the NPQT motif.

protonate the N-terminal amino group of Gly_3 or Gly_5, and the guanidinium group of Arg233 is suitably poised for this role. Interestingly, the position of Gly_3 overlaps with the guanidinium group of E64 in one of the two conformations seen for this inhibitor and with a conserved water molecule observed in all of the inhibitor complex crystal structures of $SrtB_{\Delta N30}$.

Molecular Modeling of $SrtB_{\Delta N30}$ with NPQTN Peptide

We suggest that the catalytic active site of $SrtB_{\Delta N30}$ is located on a β sheet with the catalytic residues anchored onto the respective β strands and that it has walls constituted of three loops (Figures 4B and 4C). The large loop (residue 175–185) linking the β7 and β8 strands seems flexible and can readily undergo conformational changes to accommodate polypeptide substrate. Using the carboxylate group of covalently linked E64 stabilized by the guanidinium group of Arg233 as a guide, we have modeled the substrate NPQT peptide, covalently linked to Cys223 residue, by pointing the acylated Thr carbonyl toward the Arg233 side chain of the N1 atom (Figure 4D). The side chain of Thr can fit into a pocket formed by the side chains of Tyr128, Phe111, Leu96, and Arg233. With the Thr residue position fixed, we have built an extended conformation for the remaining peptide, where the side chain of Gln points into the solvent. A peptide accepting two hydrogen bonds to its backbone anchors the proline residue in a hydrophobic pocket formed by the Phe114, Ile182, and Tyr181 residues of the enzyme. This model may help us to understand the substrate binding mode for sortase B; however, the crystal structures of $SrtA_{\Delta N59}$-LPXTG and $SrtB_{\Delta N30}$-NPQTN will be needed to explain the ability of *S. aureus* sortases to distinguish between closely related sorting signal motifs.

Conclusion

Crystal structure of SrtB of *S. aureus* complexed with two inhibitors and one peptide substrate clearly revealed the active site disposition of cysteine transpeptidases, which is unlike that of any other proteases/transpeptidases known till now. The active site residues are anchored to the neighboring rigid β strands, and in the

absence of conventional "oxyanion hole," the side chains of catalytic residues exhibit flexibility to accommodate and stabilize the acyl-enzyme intermediates. Recent pK_a measurements for SrtA Cys184 and His120 residues by Connolly et al. (2003) confirmed our finding that the conserved His residue in bacterial sortases cannot be the required catalytic partner in transpeptidation. We have identified an Arg residue in close proximity to catalytic Cys that could assist in enzyme function. Based on the predicted structural homology and primary sequence alignments, we suggest that sortases from all across Gram-positive bacteria may share similar structural motifs responsible for the peptide cleavage and transfer to the cell surface peptidoglycan.

Experimental Procedures

Crystallization and Data Collection

The method for expression and purification of recombinant $SrtB_{\Delta N30}$ protein has been previously described (Mazmanian et al., 2002). The SeMet protein of $SrtB_{\Delta N30}$, in which methionines were substituted with Seleno-methionines, was produced in the standard way (Ramakrishnan and Biou, 1997). $SrtB_{\Delta N30}$ was incubated for a few hours with either E64 or MTSET before setting up drops for crystallization by hanging drop vapor diffusion method. The $SrtB_{\Delta N30}$-E64 crystals belong to the $C222_1$ space group and have unit cell dimensions of a = 57.3, b = 66.6, c = 101.8 (Å), diffracted to 2.7 Å. Assuming one molecule in the asymmetric unit, the estimated solvent content is about 42%. The $SrtB_{\Delta N30}$-MTSET crystals are in the $C222_1$ space group and have unit cell dimensions of a = 57.7, b = 67.2, c = 102.6 (Å), diffracted to 2.5 Å. They have one molecule in the asymmetric unit and the estimated solvent content is about 43%. The C2 crystals of $SrtB_{\Delta N30}$-MTSET, with unit cell dimensions of a = 73.5, b = 61.6, c = 45.7 (Å) and $\beta = 91.58^\circ$, are diffracted to 1.7Å. With one molecule in the asymmetric unit, the estimated solvent content is about 32%. The three-wavelength Se-MAD phasing data on one $SrtB_{\Delta N30}$-E64 crystal were collected at 100 K on the 19 BM beamline at APS. Diffraction data on $SrtB_{\Delta N30}$-MTSET crystals were collected with in-house X-ray source. All the diffraction data were processed with the help of the HKL2000 or the DENZO-SCALEPACK program (Otwinowski and Minor, 1997).

Phasing Method and Refinement

Due to radiation damage, we used only one data set collected at the peak wavelength of Se to determine the structure of the $SrtB_{\Delta 30}$-E64 complex. Five selenium sites were found using the CNS program (Brunger et al., 1998). The electron density map became interpretable after density modifications using CNS. The molecular model of SrtBΔ30 was built with program O (Jones et al., 1991). The $C222_1$ and C_2 crystal structures of SrtBΔN30-MTSET were solved by molecular replacement using the $SrtB_{\Delta N30}$-E64 crystal structure as the starting model. All the structures were refined with CNS. The crystallographic statistics are shown in Table 1.

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Accession Numbers

Coordinates for the SrtBΔN30-MTSET, $SrtB_{\Delta N30}$-E64, and $SrtB_{\Delta N30}$-MTSET-$(Gly)_3$ have been deposited in the Protein Data Bank, with accession codes 1QWZ, 1QX6, and 1QXA respectively.