The Sortase A Enzyme That Attaches Proteins to the Cell Wall of Bacillus anthracis Contains an Unusual Active Site Architecture*§

Received for publication, April 16, 2010, and in revised form, May 14, 2010. Published, JBC Papers in Press, May 19, 2010, DOI 10.1074/jbc.M110.135434

Ethan M. Weiner†1, Scott Robson†, Melanie Marohn‡, and Robert T. Clubb†1∥§‡

From the †Department of Chemistry and Biochemistry, §UCLA-DOE Institute of Genomics and Proteomics, and ¶Molecular Biology Institute, University of California, Los Angeles, California 90095-1570

The pathogen Bacillus anthracis uses the Sortase A (SrtA) enzyme to anchor proteins to its cell wall envelope during vegetative growth. To gain insight into the mechanism of protein attachment to the cell wall in B. anthracis we investigated the structure, backbone dynamics, and function of SrtA. The NMR structure of SrtA has been determined with a backbone coordinate precision of 0.40 ± 0.07 Å. SrtA possesses several novel features not previously observed in sortase enzymes including the presence of a structurally ordered amino terminus positioned within the active site and in contact with catalytically essential histidine residue (His126). We propose that this appendage, in combination with a unique flexible active site loop, mediates the recognition of lipid II, the second substrate to which proteins are attached during the anchoring reaction. pKa measurements indicate that His126 is uncharged at physiological pH compatible with the enzyme operating through a “reverse protonation” mechanism. Interestingly, NMR relaxation measurements and the results of a model building study suggest that SrtA recognizes the LPXTG sorting signal through a lock-in-key mechanism in contrast to the prototypical SrtA enzyme from Staphylococcus aureus.

Bacterial pathogens display proteins on their surface that enable them to evade the immune response of the host, adhere to sites of infection, acquire essential nutrients, and enter host cells (1). Gram-positive bacteria covalently attach proteins to the cell wall using sortase enzymes, a large family of membrane-associated transpeptidases (2–6). Proteins fated for cell wall attachment contain a C-terminal sorting signal that typically consists of a Leu-Pro-X-Thr-Gly motif (LPXTG, where X is any amino acid) followed by a hydrophobic segment and positively charged C-terminal amino acids. Many sortase enzymes catalyze a transpeptidation reaction that joins the threonine residue within the LPXTG motif to the free amino group within lipid II, a cell wall precursor (1). The protein is then displayed on the microbial surface when the lipid II-linked protein product is incorporated into the peptidoglycan by the transpeptidation and transglycosylation reactions of cell wall synthesis. Some members of the sortase enzyme family also assemble pili, hair-like proteinaceous structures that promote bacterial adhesion (7). These enzymes presumably function through a similar mechanism, but the transpeptidation reaction they catalyze polymerizes the protein subunits that construct the pilus. Because many clinically significant pathogens require a functioning sortase to be fully virulent, sortase enzymes are promising therapeutic targets for the development of novel antibiotics (8, 9).

Based on their primary sequences sortase enzymes can be classified into four subfamilies whose members have been shown experimentally to have distinct functions: Sortase A (SrtA)-,3 SrtB-, SrtC-, and SrtD-type enzymes (10, 11). SrtA-type enzymes are most closely related to the SrtA enzyme from Staphylococcus aureus (Sa-SrtA) and are “housekeeping” enzymes that anchor a large number of distinct proteins to the cell wall. They have attracted significant interest as potential drug targets because they are present in several clinically significant pathogens that exhibit attenuated virulence when their srtA gene is genetically eliminated (e.g. Staphylococcus aureus, Listeria monocytogenes, Streptococcus pyogenes, and Streptococcus pneumoniae among others). Other types of sortase enzymes have more specialized functions and process fewer protein substrates. SrtC-type enzymes are involved in pilin assembly, whereas the SrtB- and SrtD-type enzymes anchor proteins the cell wall involved in heme iron acquisition and sporation, respectively (5, 8).

NMR and crystal structures of several sortase enzymes have revealed that they adopt a common eight-stranded β-barrel fold that contains conserved active site residues (12–20). The mechanism of the sortase-catalyzed transpeptidation reaction is best understood for the Sa-SrtA enzyme. All sortase enzymes contain three conserved residues that when mutated in Sa-SrtA severely reduce enzymatic activity: His120, Cys194, and Arg197 (Sa-SrtA numbering) (21–23). Transpeptidation occurs

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3 and Tables S1 and S2.

The atomic coordinates and structure factors (code 2KW8) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

1 Supported by Ruth L. Kirschen National Research Service Award GM07185.
2 To whom correspondence should be addressed. Tel: 310-206-2334; Fax: 310-206-4749; E-mail: rclubb@mbi.ucla.edu.
3 The abbreviations used are: SrtA, Sortase A; m-Dap, meso-diaminopimelic acid; HISQC, heteronuclear single quantum coherence; abz-LPETG-DNP, o-aminobenzoyl-LPETG-2,4-dinitrophenyl; Ba-SrtA, B. anthracis Sortase A; Sa-SrtA, S. aureus SortA; Sp-SrtA, S. pyogenes SortA; MES, 4-morpholineethanesulfonic acid; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; r.m.s., root mean square; Cbz, carbobenzyloxy.

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JULY 23, 2010•VOLUME 285•NUMBER 30•23433

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through a ping-pong mechanism that is initiated when the thiol group of Cys\textsuperscript{184} within Sa-SrtA nucleophilically attacks the carbonyl carbon of the threonine residue within the sorting signal (24, 25). This forms a transient tetrahedral intermediate, which, upon breakage of the threonine-glycine peptide bond, rearranges into a more stable thioacyl enzyme-substrate linkage. Sa-SrtA then joins the terminal amine group within the pentaglycine branch of lipid II to the carbonyl carbon of the threonine, creating a second tetrahedral intermediate that is resolved into the lipid II-linked protein product. During catalysis the Cys\textsuperscript{184} thiol covalently attaches to the carbonyl carbon of the threonine residue within the LP\textsubscript{X}TG sorting signal. Arg\textsuperscript{197} is thought to stabilize the binding of the sorting signal by hydrogen bonding to its backbone (17, 26) and may also stabilize oxyanion transition states (20, 23). The function of His\textsuperscript{120} has not been clearly established, but it may act as a general acid that protonates the amide group of the glycine residue within the sorting signal as the scissile peptide bond is broken and/or it may deprotonate the amine group of the lipid II nucleophile (17, 23, 25).

*Bacillus anthracis* is a spore forming Gram-positive bacterium that causes lethal anthrax disease in humans. The high mortality rate of anthrax caused by the inhalation of aerosolized bacterial spores makes it a potential bioterrorism agent and has a mortality rate of anthrax caused by the inhalation of aerosolized *B. anthracis* spore. The high mortality rate of anthrax caused by the inhalation of aerosolized *B. anthracis* spore is due to the presence of a gene coding for a protein called anthrax lethal factor (LethA) which is involved in iron acquisition and anchors the protein onto the bacterial cell wall. The mechanism of anthrax lethal factor involves the formation of a protein-protein complex with the B subunit of the cholera toxin, which then binds to the cell surface receptor of the target cell, leading to the activation of the GTPase-activating protein (GAP) and the inhibition of the Rho GTPase, resulting in cytoskeletal rearrangement and cell death.

**EXPERIMENTAL PROCEDURES**

Protein Preparation—Four deletion mutants of the SrtA protein from *B. anthracis* were studied: Ba-SrtA\textsubscript{A23} (residues Lys\textsuperscript{24}–Lys\textsuperscript{210}), Ba-SrtA\textsubscript{A56} (residues Asp\textsuperscript{57}–Lys\textsuperscript{210}), Ba-SrtA\textsubscript{A246} (residues Asp\textsuperscript{65}–Lys\textsuperscript{210}), and Ba-SrtA\textsubscript{A74} (residues Asp\textsuperscript{75}–Lys\textsuperscript{210}). PET15b expression plasmids were used to produce the proteins in *Escherichia coli* BL21 (DE3) cells. Subcloning made use of PCR-amplified DNA from *B. anthracis* Sterne genomic DNA. \textsuperscript{13}C- and \textsuperscript{15}N-labeled proteins used in the NMR studies were produced by growing cells in M9 medium supplemented with \textsuperscript{15}NH\textsubscript{4}Cl or \textsuperscript{15}NH\textsubscript{4}Cl and \textsuperscript{13}C6 glucose, whereas standard Luria-Bertani broth was used to produce unlabeled proteins. All cell cultures were grown at 37 °C and induced for protein expression by adding isopropyl \textbeta-D-thiogalactoside to a final concentration of 1 mM when the cells reached an A\textsubscript{600} of ~0.6. Cells were harvested 4 h after induction by centrifugation at 6,000 × g and stored at −80 °C. Proteins were purified by resuspending the pellet in lysis buffer (50 mM NaPO\textsubscript{4}, pH 7.0, 300 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine). The cells were then lysed by sonication and the lysate was cleared by centrifugation at 13,000 × g for 40 min at 4 °C. After filtering the supernatant with a 0.45-μm filter it was incubated with TALON His-affinity resin (Clontech). The resin was then washed with lysis buffer containing 10 mM imidazole. The histidine tag was then removed from the protein by incubating with thrombin at 37 °C for 1 h and 15 min in cleavage buffer (20 mM Tris, pH 7.9, 150 mM NaCl, 2.5 mM CaCl\textsubscript{2}). The eluate was then further purified using a Sephacryl-100 gel filtration column equilibrated with either NMR buffer (10 mM MES, 20 mM BisTris, pH 6.0) or fluorescence resonance energy transfer assay buffer (20 mM HEPES, pH 7.5). Three samples of Ba-SrtA\textsubscript{A56} were studied by NMR and were dissolved in NMR buffer: 1) 4 mM [\textsuperscript{15}N]Ba-SrtA\textsubscript{A56} dissolved in NMR buffer containing 7% D\textsubscript{2}O; 2) 2.5 mM [\textsuperscript{15}N,\textsuperscript{13}C]Ba-SrtA\textsubscript{A56} dissolved in NMR buffer containing 7% D\textsubscript{2}O; and 3) 2.5 mM [\textsuperscript{15}N,\textsuperscript{13}C]Ba-SrtA\textsubscript{A56} dissolved in deuterated NMR buffer (obtained by lyophilization and redissolving in 99.999% D\textsubscript{2}O).

**NMR Spectroscopy and Structure Determination**—NMR spectra of Ba-SrtA\textsubscript{A56} were acquired at 298 K on Bruker Avance 500-, 600-, and 800-MHz spectrometers equipped with triple resonance cryogenic probes. NMR spectra were processed using NMRPipe (34) and analyzed using the PIP (35) and CARA (version 1.8.4) (36) software packages. Chemical shift assignments (\textsuperscript{1}H, \textsuperscript{13}C, \textsuperscript{15}N) were obtained by analyzing the following experiments: HNCA, HN(CA)CO, HNCO, HN(CA)CO, HNHA, HNHB, HBBH(CC)NH, CC(CC)NH, HCC-TOCSY, HCC-COSY, (HB)CB(GCGD-CE)HE, and (HB)CB(GCGD)HD (reviewed in Refs. 37 and 38). The majority of \phi and \psi dihedral angle restraints were obtained using the program TALOS+ (39). Additional backbone \phi angle restraints were obtained by analyzing HNHA spectra (40). Distance restraints were obtained from three-dimensional \textsuperscript{15}N- and \textsuperscript{13}C-edited NOESY spectra and a four-dimensional \textsuperscript{13}C,\textsuperscript{15}N-edited HMBC-NOESY-HSQC spectrum. \textsuperscript{1}D\textsubscript{NH} and \textsuperscript{1}D\textsubscript{CC} residual dipolar couplings were measured using protein samples partially aligned in PEG C12E5/hexanol, using two-dimensional \textsuperscript{15}N-coupled IPAP \textsuperscript{1}H-\textsuperscript{15}N HSQC and two-dimensional carbonyl-coupled \textsuperscript{1}H-\textsuperscript{15}N HSQC experiments, respectively.

NOE assignments were obtained automatically using the programs ATNOS and CANDID (41, 42). All of the NOE
assignments were then verified by manually inspecting the NOESY data. During this process additional NOE restraints were identified and included in subsequent structure calculations. Restraints for hydrogen bonds were implemented using the HBDB algorithm and identified by inspecting the NOESY data for characteristic patterns combined with deuterium exchange experiments (43). In the final set of calculations a total of 200 structures were generated, of which 73 had no NOE, dihedral angle, or scalar coupling violations greater than 0.5 Å, 5°, or 2 Hz, respectively. Of these, 40 structures with the lowest overall energy were chosen to represent the structure of SrtA_{56} and have been deposited in the protein data bank (accession code Protein Data Bank 2KW8).

Modeling of the Covalent Ba-SrtA_{56}-Sorting Signal Complex—The model was generated based on our recently determined structure of the S. aureus SrtA-LPAT* complex (17). The peptide in this structure is Cbz-LPAT*, where T* is (2R,3S)-3-amino-4-mercapto-2-butanol, and Cbz is a carboxbenzoxyl protecting group (44). The Ba-SrtA-LPAT* model was calculated using artificial intermolecular distance restraints between the peptide and the Ba-SrtA_{56} enzyme that were obtained by inspecting the intermolecular distance restraints experimentally identified for the Sa-SrtA-LPAT* complex. A total of 35 artificial intermolecular distance restraints were employed in simulated annealing calculations (supplemental Table S2). In addition, the set of restraints used to determine the structure of SrtA_{56} in its apo-state were employed without modification. A total of 200 structures of the complex were calculated, of which 45 exhibited no NOE, dihedral angle, or scalar coupling violations greater than 0.5 Å, 5°, or 2 Hz.

Backbone Dynamics of Ba-SrtA_{56} Determined from ^{15}N Relaxation Data—The ^{15}N relaxation data were collected on a Bruker Avance 600-MHz NMR spectrometer equipped with a triple resonance cryogenic probe. Data were analyzed using the program SPARKY (45) and included: ^{15}N longitudinal relaxation rates (R_1), transverse relaxation rates (R_2), and (^{1}H-^{15}N) heteronuclear NOEs. Complete R_1, R_2, and (^{1}H-^{15}N) NOE values were available for 94 of 150 backbone amides as well as the side chain amide of Trp^{171}. The average quantifiable R_1, R_2, and (^{1}H-^{15}N) NOE values for SrtA_{56} are 1.18 ± 0.06, 13.07 ± 0.21, and 0.81 ± 0.01 s⁻¹, respectively. Relaxation data were analyzed using programs kindly provided by Prof. Arthur G. Palmer III at Columbia University. The analysis procedure we used has been described previously (46, 47). Briefly, the program Pdbinertia was used to calculate the principal moments of inertia and yielded relative moments of 1.000:0.84:0.68. The program R2R1_tmn was used to calculate an approximate correlation time (τ_m) of 10.2 ± 0.4 ns using R_2/R_1 ratios. Only R_2/R_1 ratios that met the following criteria were used in this analysis: 1) they were within one standard deviation of the average, and 2) the residue had a (^{1}H-^{15}N) NOE value >0.6. This data were then inputted into the program Quadratic Diffusion (48, 49) indicating the isotropic model is statistically preferred for SrtA_{56} over the axially symmetric or anisotropic models of tumbling. The relaxation data were then interpreted using the Lipari-Szabo Model-free formalism (50) using the program FAST-Modelfree to iteratively run the program Modelfree 4.20 (51). Of the 107 amino acids that gave complete quantifiable relaxation information, data from 91 residues could be satisfactorily reproduced using the model-free approach. The data from the backbone amide nitrogen atoms of 59 residues could be fit using model 1 (S^2 only), 4 residues fit model 2 (S^2 and τ_m), 13 residues fit model 3 (S^2 and τ_e), 3 residues fit model 4 (S^2, τ_e, and τ_m), and 12 residues, in addition to the side chain indole Ne atom of Trp^{171} could be fit using model 5 (S^2, S^2, τ_e, and τ_m).

Enzyme Kinetics Measurements—Substrate cleavage reaction was performed as previously described (52). The cleavage of the substrate, o-aminobenzoyl-LPETG-2,4-dinitrophenyl (abz-LPETG-DNP), was monitored by excitation at 335 nm and recording emission at 420 nm on a SpectraMax M5 spectrophotometer (Molecular Devices). Assay conditions consisted of 20 mM HEPES, pH 7.5, and 10 μM enzyme. abz-LPETG-DNP peptide concentrations of 0, 5, 10, 20, 40, 80, 160, and 320 μM were used. Fluorescence was recorded for 10 h in 10-min increments. A standard curve was used to convert fluorescence units to rates and the steady state velocities were used to calculate K_m and k_cat as described previously (24).

Histidine Side Chain pK_a Measurements—^{15}N- and ^{13}C-labeled Ba-SrtA_{56} (0.5 mM) in buffer (10 mM MES, 20 mM Bis-Tris in 7% D_2O) was titrated using 0.2 M HCl or 0.2 M NaOH to pH 4.5–10. The chemical shift of the His1 atom from His^{126} and His^{177} were measured during the course of the titration by recording two-dimensional ^{1}H-^{13}C HSQC spectra. Chemical shifts recorded as a function of pH were fit to Equation 1,

\[
\delta_{obs} = \frac{\delta_{HA} + \delta_{A} \times 10^{pK_a-pK_H}}{1 + 10^{pK_a-pK_H}}
\]

where δ_{HA} and δ_A are the chemical shifts of the fully protonated and deprotonated states of the imidazolidone side chain respectively, and δ_{obs} is the observed chemical shift.

RESULTS

Structure of the Sortase A Enzyme from B. anthracis—The Sortase A enzyme from B. anthracis (Ba-SrtA) is 210 amino acids in length and consists of two parts, a non-polar N terminus that presumably embeds the protein in the membrane (residues Met^{1}-Gly^{29}) and a C-terminal catalytic region (residues Lys^{52}-Lys^{210}). Previously, a deletion mutant of Ba-SrtA that removes the transmembrane region (Ba-SrtA_{Δ23}, residues Lys^{24}-Lys^{210} of Ba-SrtA) was shown to have hydrolytic activity in vitro (31). To further delineate residues that form the structured catalytic domain of the protein we used NMR spectroscopy to study a uniformly^{15}N-labeled sample of Ba-SrtA_{Δ23}. Inspection of the ^{1}H-^{15}N HSQC spectra of [^{15}N]Ba-SrtA_{Δ23} revealed that a large portion of the polypeptide was disordered as the backbone amide correlations of ~30–40 amino acids exhibited narrow line widths and degenerate chemical shifts (data not shown). Because residues Lys^{24}–Val^{56} in Ba-SrtA share only limited primary sequence homology with other sortase enzymes (supplemental Fig. S3), we purified isotopically labeled proteins missing these residues (Ba-SrtA_{Δ56} residues Asp^{57}–Lys^{210} of Ba-SrtA). The NMR spectra of Ba-SrtA_{Δ56} are well resolved and have line widths that indicate that the majority of the protein is structured (Fig. 1a).

The structure of Ba-SrtA_{Δ56} was determined using multidimensional heteronuclear NMR and simulating annealing
Structure of B. anthracis SrtA Enzyme

Methods. A total of 2,812 experimental restraints were used to determine the structure, including: 2,177 inter-proton distance, 231 dihedral angle, 54 $^3\text{J}_{\text{HN}}$, 264 $^3\text{C}$ secondary shifts, and 116 residual dipolar coupling restraints. An ensemble containing 40 conformers representing the structure of the protein is shown in Fig. 2a. The conformers exhibit good covariant geometry and have no NOE, dihedral angle, or scalar coupling violations greater than 0.5 Å, 5°, or 2 Hz, respectively. The enzyme is structured from residues Ile61–Thr186 and Arg197–Val208, where H and β refer to helices and strands, respectively. Beginning at the N terminus, an extended segment (Ala58–Glu56) is positioned in the active site cleft and is then followed by a α-helix (H1, Leu66–Asn71). The barrel structure then begins with strand β1 (Gly81–Ile85), which is connected by a short hairpin that lies anti-parallel to strand β2 (Leu90–Leu95). A 3$_{10}$-helix (H2, Glu100–Ser105) then joins strand β2 to β3 (Ala107–Thr109), which lay in parallel. Helix H2 also forms a wall of the active site cleft. The chain then reverses direction so as to position strand β4 (Asn120–His126) in an anti-parallel orientation next to strand β3. An extended polypeptide segment containing a 3$_{10}$-helix (H3, Ile138–Ser146) then wraps around the enzyme to initiate strand β5 (Lys146–Asp154) on the opposite face of the protein. This strand pairs with β1 in an anti-parallel fashion and is separated by a short hairpin from residues in strand β6 (Asp154–Glu165), whose chain also is positioned in an opposite orientation. A long loop containing a 3$_{10}$-helix (H4, Trp171–Val173) then connects strands β6 and β7 (Glu181–Thr186). The β7 strand runs parallel with respect to the β4 strand and is followed by a structurally disordered loop that reverses the direction of the chain thereby enabling residues in strand β8 (Tyr197–Ala208) to hydrogen-bond with residues in strands β6 and β7 in an anti-parallel manner. Two adjacent β-bulges present within strands β6 (Thr199) and β8 (Val204) introduce a kink that enables extensive interactions between the chains and allows them to form opposing faces of the β-barrel structure. Residues His126, Cys187, and Arg196 are completely conserved in sortase enzymes and form the active site. They are located near the end of the sheet formed by strands β4, β7, and β8. Cys187 is situated at the C-terminal end of the β7 strand and is bracketed by side chains of His126 and Arg196 located on strands β4 and β8, respectively.

Unique Active Site Features: a Histidine Contacting N-terminal Extension and a Disordered β/β Loop—All sortases contain a conserved histidine residue whose mutation in Sa-SrtA inactivates the enzyme (21). In contrast to previously studied enzymes, in Ba-SrtA the histidine residue (His126) is contacted by an N-terminal extension positioned in the groove that separates helix H2 and the β7/β8 loop (Fig. 2b). The structured extension precedes strand β1 in the primary sequence and consists of residues Ile61–Val79. At its N terminus residues Ile61–Pro64 are positioned near His126. The chain then forms a short α-helix (H1) that packs against helix H2 before changing its direction to initiate strand β1. Contacts to His126 are extensive, with the methyl groups Ile61 partially encapsulating the imidazole ring (Fig. 2c). Ile61 methyl groups also contact the methyl
FIGURE 2. NMR solution structure of Ba-SrtA<sub>56</sub>. a, cross-eyes stereo image showing an ensemble of the 40 lowest energy structures of Ba-SrtA<sub>56</sub>. Residues Ile<sup>61</sup>–Lys<sup>210</sup> are shown. The majority of the protein is ordered and consists of residues Ile<sup>61</sup>–Thr<sup>186</sup> and Arg<sup>196</sup>–Lys<sup>210</sup> (colored blue). An active site loop connecting strands β<sub>7</sub> and β<sub>8</sub> is not defined by the NMR data and is structurally disordered (residues 187–195, colored red). The coordinates were superimposed by aligning the backbone N, Ca, and C atoms of Ser<sup>73</sup>–Thr<sup>186</sup> and Arg<sup>196</sup>–Lys<sup>210</sup>. b, ribbon drawing of the structure Ba-SrtA<sub>56</sub>. The structure on the left is in a similar orientation as shown in a, whereas the structure on the right has been rotated by 180°. The secondary structural elements are labeled and the conserved catalytic residues His<sup>126</sup>, Cys<sup>187</sup>, and Arg<sup>196</sup> are shown. The N-terminal extension that is unique to Ba-SrtA<sub>56</sub> is colored green. c, cross-eyed stereo image showing an expanded view of the enzyme active site and contacts to it that are made by residues in the N-terminal extension. This interaction is unique and has not been observed in previously determined structures of other sortase enzymes.
Structure of B. anthracis SrtA Enzyme

### TABLE 1

<table>
<thead>
<tr>
<th>Statistics for the NMR structure of Ba-SrtA_{566}</th>
<th>(Å)</th>
<th>(Å)</th>
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<tr>
<td>R.m.s. deviations from NOE-SrtA interproton distance restraints (Å) (2204)</td>
<td>0.050 ± 0.002</td>
<td>0.048</td>
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<tr>
<td>R.m.s. deviations from dihedral angle restraints (degrees)</td>
<td>0.489 ± 0.073</td>
<td>0.999</td>
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<tr>
<td>R.m.s. deviations from 3J_{HN−∗} coupling constants (Hz) (54)</td>
<td>0.834 ± 0.030</td>
<td>0.814</td>
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<tr>
<td>R.m.s deviations from secondary 13C shifts</td>
<td>1.450 ± 0.064</td>
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<tr>
<td>13C_{Cα} (ppm) (132)</td>
<td>1.323 ± 0.066</td>
<td>1.272</td>
</tr>
<tr>
<td>Residual dipolar coupling B-factors (%)</td>
<td>3.2 ± 0.3</td>
<td>3.9</td>
</tr>
<tr>
<td>DNCO (46)</td>
<td>22.3 ± 1.1</td>
<td>22.2</td>
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<tr>
<td>Deviations from idealized covalent geometry</td>
<td>0.0044 ± 0.0003</td>
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<td>Bonds (Å)</td>
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<tr>
<td>Angles (degrees)</td>
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<tr>
<td>Improper (degrees)</td>
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<tr>
<td>PROCHECK-NMR*</td>
<td>15.5 ± 2.0</td>
<td>14.4</td>
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<tr>
<td>Most favorable region (%)</td>
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<td>Generously allowed region (%)</td>
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<tr>
<td>Coordinate precision</td>
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<tr>
<td>Protein backbone (Å)</td>
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<tr>
<td>Protein heavy atoms (Å)</td>
<td>0.0131</td>
<td></td>
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</table>

*The notation of the NMR structures is as follows: (SA) represent an ensemble of the 40 best structures calculated by simulated annealing. (SÅ) is the average energy-minimized structure. The number of terms for each restraint is given in parentheses. None of the structures exhibited distance violations greater than 0.5 Å, dihedral angle violations greater than 5°, or constant coupling violations greater than 2 Hz.

groups of Leu\textsuperscript{107}, a residue believed to be important in recognizing the LPXTG substrate (17). Pro\textsuperscript{63} for the N-terminal extension also fits into the groove, making contacts with Leu\textsuperscript{104} in helix H2. The N-terminal tail, along with Leu\textsuperscript{103}, Leu\textsuperscript{104}, Met\textsuperscript{128}, and Leu\textsuperscript{134} enclose catalytic His\textsuperscript{126} in a hydrophobic pocket. This is unique, as the analogous active site histidine in other sortase enzymes is exposed to the solvent (Fig. 5, a–c). As shown in Fig. 1b, active site contacts to the N-terminal extension are well supported by the NOESY spectra. For example, unambiguous NOEs are observed between the Hy2 and Ha1 methyl groups of Ile\textsuperscript{61} and the He and Ha ring protons of His\textsuperscript{126}. In addition, NOEs are present between Glu\textsuperscript{68} and Leu\textsuperscript{104}, Val\textsuperscript{69} and Leu\textsuperscript{104}, Asn\textsuperscript{79} and Leu\textsuperscript{104}, Ala\textsuperscript{72} and Leu\textsuperscript{104}, as well as Ala\textsuperscript{72} and Ser\textsuperscript{105}.

Another unique feature of the Ba-SrtA structure is the presence of a mobile active site loop that connects strands β7 to β8 (the β7/β8 loop). The loop immediately follows Cys\textsuperscript{187} in the active site and has been postulated to form a surface that recognizes the second substrate of catalysis, lipid II (17, 20). In the \textsuperscript{1}H-\textsuperscript{15}N HSQC spectrum of Ba-SrtA\textsubscript{566} cross-peaks for residues Thr\textsuperscript{186}–Tyr\textsuperscript{199} are absent (Fig. 3b). Their signals are presumably broadened beyond detection because they undergo conformational rearrangements that occur on the micro- to millisecond time (i.e. they are broadened by exchange processes that are intermediate on the chemical shift time scale). The notation that the β7/β8 loop is mobile is consistent with the finding that resonances for Asn\textsuperscript{127} are also absent in the NMR data. This residue is located immediately following strand β4 near the base of the β7/β8 loop and its resonances are presumably broadened as a result of fluctuations in its magnetic environment that are caused by the movement of the loop (Fig. 3b). Flexibility in the β7/β8 loop is also supported by NMR relaxation measurements for the backbone nitrogen nuclei of Ba-SrtA described in detail below.

Backbone Dynamics of Ba-SrtA Revealed by \textsuperscript{15}N Relaxation Measurements—To gain insight into motions occurring within the enzyme we measured backbone \textsuperscript{1}H,\textsuperscript{15}N NOEs and \textsuperscript{15}N spin-lattice (R\textsubscript{1}) and spin-spin (R\textsubscript{2}) relaxation rates (supplemental Fig. S2). The data were then interpreted using the model-free formalism, which yields three parameters that...
describe the motion of the protein backbone: the general order parameter \((S^2)\), the effective correlation time for internal motions \((\tau_2)\), and \(R_{\text{ex}}\) \((50, 51)\) (supplemental Table S1). \(S^2\) describes the magnitude of fast picosecond time scale motions that the amide bond experiences and is characterized by the internal correlation time, \(\tau_2\). The value of \(S^2\) ranges from 0 to 1, with a value of 1 indicating that the bond is completely immobilized. \(R_{\text{ex}}\) is the chemical (conformational) exchange contribution to \(R_s\) and reports on slower micro- to millisecond time scale motions.

The \(S^2\) data indicate that the N-terminal extension exhibits elevated mobility (Fig. 3a). \(S^2\) parameters could be measured for backbone amides within all regions of the protein, with the notable exception of residues within the \(\beta7/\beta8\) loop whose signals as previously noted are absent from the NMR spectra. Consistent with the structure the \(S^2\) data reveals that residues forming the \(\beta\)-barrel beginning at strand \(\beta1\) and ending at strand \(\beta8\) are immobile (residues Ile61–Thr183 and Val198–Lys210) have an average \(S^2\) value of 0.85). However, the N-terminal extension that contacts the active site exhibits elevated mobility as compared with the body of the protein. Ser73 is the last highly ordered residue at the amino terminus of the protein, beginning at Ala72 immediately following helix H1 the \(S^2\) values become progressively smaller as the chain proceeds toward Ile64, the last structured residue in the tail. As numerous NOEs define the positioning of the N-terminal tail (Fig. 1b), this suggests that in solution the tail adopts two distinct conformational states, an ordered conformation observed in the NMR structure in which it contacts the active site and a less populated disordered mobile state in which it is dissociated from the body of the protein. The elevated mobility indicated by the relaxation data is presumably caused by averaging of these two states.

The \(R_{\text{ex}}\) data suggest that the N-terminal extension transiently binds to the active site. Several residues surrounding the active site have elevated \(R_{\text{ex}}\) values (Leu103, Ser105, Ala107, Lys131, Gly132, and Leu134, located in H2 or the \(\beta4/\beta3\) loop) (Fig. 3b, gray spheres). In the structure they are located within the surface that contacts the N-terminal appendage and their elevated \(R_{\text{ex}}\) values presumably report on the slow conformational changes associated with its binding and release. For some of the residues it is also possible that the \(R_{\text{ex}}\) values are caused by changes in the tautomeration state of the His126. The notion that the N-terminal extension can open and close over His126 is further supported by the observation of significant \(R_{\text{ex}}\) values for the backbone nitrogen atoms of Asp75, Lys76, Gln78, and Val79. These residues are located between helix H1 and strand \(\beta1\) and may undergo structural rearrangements that enable them to function as a hinge about which the N-terminal extension opens. This idea is substantiated by elevated \(R_{\text{ex}}\) values in residues Leu96, Lys98, and Ser98 within the loop that connects strand \(\beta4\) to helix H2. These residues are positioned immediately adjacent to the presumed hinge, and may be indirectly broadened by fluctuations in their magnetic environment caused by opening and closing of the extension.

The relaxation data indicate that Ba-SrtA uses a rigid pocket to interact with the LPXTG sorting signal. Structural and NMR relaxation studies of Sa-SrtA have shown that it adaptively recognizes the LPXTG sorting signal by closing and immobilizing its \(\beta6/\beta7\) loop over the substrate (12, 17, 19, 47). In contrast, residues in the analogous \(\beta6/\beta7\) loop in Ba-SrtA adopt a rigid conformation in the absence of the sorting signal as evidenced by \(S^2\) values for its residues that are on average 0.84 ± 0.05 (residues Val166–Asp180). Based on the NMR structure of the Sa-SrtA substrate complex (17), the side chain of Trp171 located in the central 310 helix of the loop can be expected to interact with the sorting signal. Interestingly, although the backbone of Trp171 is static (\(S^2\) of 0.921 ± 0.037), its side chain indole appears to be relatively flexible. This is evidenced by data for its indole Nε1 atom, which are best fit using slow and fast time scale order parameters (\(S^2\) of 0.507 ± 0.068 with \(S^2\) of 0.742 ± 0.041, and \(S^2\) of 0.683 ± 0.055). Similar order parameters have been observed for other surface-exposed tryptophan residues in E. coli RNase H (51). Presumably side chain motions caused by rotations about the \(\chi\) dihedral angles enable it to adjust its structure to productively contact the sorting signal.

Kinetic Measurements of Enzyme Activity—In vivo, Ba-SrtA anchors proteins to the cell wall by catalyzing a transpeptidation reaction that joins the threonine residue of the sorting signal to the free amino group of \(m\)-Dap. Previously, Schneewind and colleagues (31) demonstrated that purified Ba-SrtA\(_{A23}\) catalyzes the hydrolysis of a fluorogenic peptide containing the amino acid sequence LPETG. In this reaction the peptide mimics the sorting signal substrate and a water molecule replaces \(m\)-Dap as the nucleophile. However, kinetic parameters for the hydrolysis reaction were not reported. We therefore adapted this assay to quantitatively measure the steady state hydrolysis kinetics of an \(abz\)-LPETG-DNP (Fig. 4a). Ba-SrtA\(_{A56}\) cleaves this peptide with \(k_{\text{cat}}\) and \(K_m\) values of 4.0 \(\times 10^{-4} \pm 1 \times 10^{-3}\) min\(^{-1}\) and 38 ± 4 \(\mu\)M, respectively. Interestingly, our results indicate that in vitro Ba-SrtA\(_{A56}\) is unable to perform the transpeptidation reaction, because we were unable to detect transpeptidation products by mass spectrometry when Ba-SrtA\(_{A56}\) was incubated with \(abz\)-LPETG-DNP and up to a 100-fold molar excess of \(m\)-Dap (data not shown). This suggests that to be completely active, Ba-SrtA requires either the intact lipid II molecule as a substrate and/or additional cell wall components. A comparison of the activity of Ba-SrtA to other SrtA-type enzymes is provided in Table 2 and discussed later in the text.

To determine whether the presence of the N-terminal extension alters enzymatic activity of Ba-SrtA we studied a truncation mutant in which amino acids preceding helix H1 are removed, Ba-SrtA\(_{A64}\) (residues Asp65–Lys210 of Ba-SrtA). Ba-SrtA\(_{A64}\) is folded based on its \(^1\)H-\(^15\)N HSQC spectrum (supplemental Fig. S1). As compared with SrtA\(_{A54}\), it hydrolyzes the sorting signal with a modestly larger catalytic turnover \((k_{\text{cat}}\) is 20% larger, with a \(p\) value of 0.0015) and has a comparable \(K_m\) for the sorting signal (Table 2). We also attempted to study SrtA\(_{A74}\) (residues Asp75–Lys210 of Ba-SrtA), a truncation mutant that completely removes the N-terminal extension. This protein failed to express in E. coli, consistent with the idea that contacts from helix H1 to the body of the protein are needed to stabilize the structure of the enzyme. The importance of the active site histidine was confirmed by showing that a H126A mutant of Ba-SrtA\(_{A56}\) has no detectable hydrolytic
activity (data not shown). Combined, these data reveal that His\textsuperscript{126} is essential for the hydrolytic activity, although contacts to it from Ile\textsuperscript{61} within the N-terminal extension are dispensable. However, as the hydrolysis reaction only mimics the initial steps of transpeptidation, active site contacts from the N-terminal extension could still be important for downstream lipid II recognition events required to attach proteins to the cell surface.

\( \frac{pK_a}{2} \) Measurements Reveal that His\textsuperscript{126} Is Uncharged—Knowledge of the ionization state of residues within the active site is needed to understand the mechanism of catalysis, but thus far this information has only been reported for Sa-SrtA. We therefore used NMR spectroscopy to determine the \( pK_a \) values of the histidine residues within the catalytic domain of Ba-SrtA\textsubscript{A\textsubscript{364}}. These included His\textsuperscript{126}, located in the active site, and His\textsuperscript{177} located on the \( \beta6/\beta7 \) loop. A series of \( ^{1}H_{-}^{13}C \) HSQC spectra of [\( ^{13}C_{,}^{15}N \)]Ba-SrtA\textsubscript{A\textsubscript{364}} were collected at different pH values and the chemical shifts of the side chain histidine \( ^{1}He1_{-}^{13}Ce1 \) resonances were measured (Fig. 4b). Fitting the pH dependence of the chemical shift data reveals that His\textsuperscript{126} has a \( pK_a \) less than 5.5 ± 0.1 (a more precise measurement is not possible because Ba-SrtA was not stable at lower pH values needed to complete the titration curve). This clearly indicates that the His\textsuperscript{126} side chain within the active site is uncharged at physiological pH and therefore not a participant in an imidazolium-thiolate interaction with Cys\textsuperscript{187}. In contrast, the side chain of His\textsuperscript{177} located in the \( \beta6/\beta7 \) loop is fully protonated at physiological pH as it has a \( pK_a \) of 8.9 ± 0.1. This finding is compatible with the structure as the N\textsuperscript{61} and Ne2 atoms of His\textsuperscript{177} are positioned to interact with the side chain carboxyl group of Glu\textsuperscript{129} and the backbone carbonyl of Glu\textsuperscript{172}, respectively. These interactions may stabilize the conformation of the \( \beta6/\beta7 \) loop and partially explain why the loop adopts an ordered structure in the absence of the sorting signal.

**Model of the Sorting Signal Bound to Ba-SrtA**—Recently we determined the structure of Sa-SrtA covalently bound to an analog of the LPXTG sorting signal (17). Superposition of the apo-Ba-SrtA and the Sa-SrtA-sorting signal complex reveals that the substrate-contacting \( \beta6/\beta7 \) loop in each enzyme adopts a similar conformation (Fig. 5d). This is surprising as it suggests that the Ba-SrtA enzyme may contain a preformed binding pocket for the sorting signal in contrast to Sa-SrtA, which undergoes major structural and dynamics changes upon binding the peptide (17). To investigate how the static pocket in Ba-SrtA might recognize the sorting signal we modeled the structure of the Ba-SrtA-sorting signal complex using simulated annealing and a set of 35 artificial intermolecular peptide-protein distance restraints derived from the structure of the Sa-SrtA-sorting signal complex (listed under supplemental materials). The average energy minimized model of the complex reveals that minimal structural perturbations in the enzyme are required to bind the signal (Fig. 5, e and f). The peptide rests in a groove whose base is formed by residues in strands \( \beta4 \) and \( \beta7 \), and whose walls are formed by the \( \beta6/\beta7 \), \( \beta7/\beta8 \), \( \beta3/\beta4 \) and \( \beta2/\beta2 \) surface loops. The leucine methyl groups of the peptide are wedged between strand \( \beta8 \) and the \( \beta6/\beta7 \) loop, contacting the side chains of Val\textsuperscript{166}, Pro\textsuperscript{168}, Val\textsuperscript{173}, and Val\textsuperscript{174} on the \( \beta6/\beta7 \) loop, and Val\textsuperscript{198} on strand \( \beta8 \). The indole ring of Trp\textsuperscript{171} located in the \( \beta6/\beta7 \) loop rests on top of the signal, whereas the proline in the peptide forms a kink in the substrate that is contacted by residues Ala\textsuperscript{124} and Ile\textsuperscript{185} located in strands \( \beta4 \) and \( \beta7 \), respectively, as well as Val\textsuperscript{110} on the \( \beta3/\beta4 \) loop. Contacts to the remainder of the peptide cannot be reliably predicted from the model as the positioning of residues in the \( \beta7/\beta8 \) loop of Ba-SrtA that contact this portion of the substrate are poorly defined in the NMR struc-
**Structure of *B. anthracis* SrtA Enzyme**

A SrtA-type enzyme, a subfamily of the sortases that are most closely related to the SrtA enzyme from *S. aureus*. SrtA-type enzymes are believed to play a housekeeping role in the cell by anchoring a large number of distinct proteins that contain a LPXTG sorting signal (10). The structures of two other SrtA-type enzymes have been determined: *S. pyogenes* SrtA (Sp-SrtA) and *S. aureus* SrtA (Sa-SrtA) (12, 16, 17, 19). These proteins share only limited sequence homology with Ba-SrtA; Sa-SrtA and Sp-SrtA share 29 and 32% sequence identity with Ba-SrtA, respectively. In this study we have used a combination of NMR and enzyme kinetic measurements to investigate the structure, dynamics, and function of Ba-SrtA. Similar to previously characterized sortases, Ba-SrtA adopts a conserved β-barrel fold. However, there are substantial differences in both the structure and dynamics of its active site revealing significant mechanistic diversity.

The NMR structure of Ba-SrtA contains an N-terminal tail that forms numerous contacts to the active site histidine (His126). The tail is formed by residues that precede strand β1 and its positioning within the active site has not been previously observed in other sortase structures (12–20). If Ba-SrtA operates through a similar mechanism as the prototypical Sa-SrtA enzyme, during catalysis His126 may function as a general acid that protonates the amide group of the glycine residue within the sorting signal as the scissile peptide bond is broken and/or it may deprotonate the amine group of the lipid II nucleophile (17, 23, 25).

**DISCUSSION**

Sortase enzymes are promising targets for the development of new anti-infective agents as they are required for the virulence of a range of clinically significant pathogens (8, 9). The SrtA sortase from the pathogen *B. anthracis* (Ba-SrtA) is required for bacterial growth within a mouse macrophage-like cell line, suggesting that it plays a critical role in the early steps of inhalation anthrax disease progression in humans, the replication of germinated spores within lung alveolar macrophages (33). Based on its primary sequence Ba-SrtA is a sorting signal binding by other SrtA-type enzymes are discussed below.

**FIGURE 5. Comparison with other SrtA-type enzymes and the model of the Ba-SrtA-sorting signal complex.** The solvent accessible surfaces of SrtA-type enzymes are shown in panels a, Ba-SrtA<sub>H9252</sub>, b, Sa-SrtA-LPAT* (with LPAT substrate removed for clarity) (PDB code 2KID) (17); and c, Sp-SrtA (PDB code 3FN7) (16). The conserved active site residues histidine (yellow), cysteine (green), and arginine (blue) are highlighted. The LPXTG binding pocket (orange) is also highlighted. The N-terminal tail in Ba-SrtA<sub>H9004</sub> (red) prevents His126 from being solvent exposed. The grooves leading into the active site are labeled in panels b and c, d, an overlay of the structure of apo-SrtA<sub>H9004</sub> (blue) and the Sa-SrtA protein in the structure of the Sa-SrtA-LPAT* complex (green) (PDB code 2KID) (17). e, a ribbon drawing of the model of the Ba-SrtA-sorting signal complex. The solvent-accessible surface of the protein is semi-transparent and colored gray. f, expanded view of protein-peptide interactions in the model of the complex. The structure of the enzyme is shown in blue with residues interacting with the substrate labeled. The sorting signal peptide is colored by atom type and is shown in a stick representation. g, apo-Sa-SrtA (red) and Sa-SrtA-LPAT* (blue) demonstrate the induced fit model of LPXTG substrate recognition. h, apo-Ba-SrtA<sub>H9004</sub> (red) and Ba-SrtA<sub>H9004</sub>-LPAT* model (blue) demonstrate the lock-and-key binding model of LPXTG substrate recognition.
Structure of B. anthracis SrtA Enzyme

a different direction and it adopts a distinct structure. Unlike Ba-SrtA, the SrtC lid does not interact with the active site histidine, but instead occludes the binding site of the sorting signal.

The N-terminal tail in Ba-SrtA may be required for later steps in the transpeptidation reaction involving lipid II. We have shown that the tail can be removed without significantly altering the ability of Ba-SrtA to hydrolyze the sorting signal, a reaction that mimics only the first half of the transpeptidation reaction by replacing the lipid II nucleophile with water (24, 25). This suggests that contacts from the tail are not required to properly position the histidine for the first steps of the transpeptidation reaction. However, it is conceivable that the tail actually inhibits the hydrolysis reaction, but it unlashes from the enzyme active site at a rate that is sufficiently fast so as not to be rate-limiting. This possibility cannot be excluded, because similar to other sortase enzymes, the isolated Ba-SrtA enzyme exhibits slow reaction kinetics \textit{in vitro} (Table 2) and our $^{15}$N relaxation data show that residues within the tail exhibit elevated mobility that is compatible with a portion of the tail transiently unraveling from the enzyme (Fig. 3a).

It also possible that the N-terminal tail is involved in lipid II recognition. In the Sp-SrtA and Sa-SrtA structures two grooves lead into the active site (Fig. 5, b and c). The first groove binds to the LPXTG sorting signal and is formed by residues in strands $\beta 6$, $\beta 7$, and the $\beta 6/\beta 7$ loop (19, 53), whereas the second groove is located on the opposite side of the active site cysteine and is formed by residues in strands $\beta 4$ and $\beta 7$, helix H2, and the $\beta 7/\beta 8$ loop. The positioning of the second groove and the results of chemical shift perturbation studies has led to the suggestion that it forms the binding site for lipid II (16, 17, 20). Inspection of the Ba-SrtA structure reveals that the novel N-terminal extension masks the second groove in Ba-SrtA presumably affecting how lipid II is recognized (compare Fig. 5, a–c). \textit{In vitro} the isolated Ba-SrtA enzyme was unable to catalyze the transpeptidation reaction that joins the LPETG peptide to m-DAP (a component of lipid II in \textit{B. anthracis} that contains the amine to which the surface protein is attached) (31). This is in marked contrast to the Sa-SrtA and Sp-SrtA enzymes, which \textit{in vitro} catalyze the transpeptidation reaction that joins the sorting signal to the appropriate peptide mimics of lipid II (Gly$_3$ or Ala$_2$ in \textit{S. aureus} and \textit{S. pyogenes}, respectively) (16, 54). Thus it appears that \textit{in vitro}, Ba-SrtA requires additional protein components or larger portions of the lipid II molecule bearing m-DAP to successfully mediate transpeptidation.

The $pK_a$ of the catalytically essential His$^{126}$ side chain in Ba-SrtA may explain its reduced hydrolytic activity relative to the Sa-SrtA enzyme. Ba-SrtA hydrolyzes the LPXTG sorting signal 40 times slower than the Sa-SrtA enzyme (Table 2). In Sa-SrtA the side chains of the active site cysteine and histidine are predominantly uncharged (25, 55). McCafferty and colleagues (25) have proposed that catalysis in the Sa-SrtA enzyme occurs via a reverse protonation mechanism. In this model the vast majority of Sa-SrtA is inactive containing the cysteine in its thiolate form primed to nucelophilically attack the threonine carbonyl group of the sorting signal. In addition, the histidine is in its imidazolium form poised to protonate the nitrogen of the scissile peptide bond. Our $pK_a$ measurement of the active site histidine in Ba-SrtA indicates that like Sa-SrtA it is predominantly uncharged at physiological pH. Interestingly, its $pK_a$ value is smaller than the analogous histidine in Sa-SrtA; the $pK_a$ of His$^{126}$ in Ba-SrtA is less than $\sim 5.5$, whereas the $pK_a$ of His$^{120}$ in Sa-SrtA is $\sim 6.3–7.0$ (25, 55). The lower $pK_a$ may be caused by contacts from the side chain of Ile$^{64}$ on the N terminus, which partially encases the His$^{126}$ side chain within a hydrophobic pocket. If the reverse protonation mechanism is operative in Ba-SrtA we would anticipate a lower percentage of the Ba-SrtA enzyme to be in its active charged state as compared with Sa-SrtA. Although there are certainly other variables to consider when comparing the activities of the enzymes, such as possible differences in the substrate binding geometry in the active site, this may help explain the lower $k_{cat}$ of Ba-SrtA relative to Sa-SrtA.

Another surprising finding is that the active site loop in Ba-SrtA that connects strands $\beta 7$ and $\beta 8$ (the $\beta 7/\beta 8$ loop) is structurally disordered. This loop is positioned immediately adjacent to the N-terminal extension and has been proposed to bind to lipid II (17, 20). It was determined to be structurally disordered in Ba-SrtA because resonances for nearly all of its residues are broadened beyond detection, presumably because the loop undergoes large amplitude motions that occur on the micro- to millisecond time scales. In principle, the broadening could be caused by protein aggregation if the loop resides at a protein-protein aggregation interface, but this seems unlikely as $^{15}$N relaxation data indicates that Ba-SrtA is monomeric based on its measured molecular correlation time of 10.2 ± 0.4 ns. In all previously determined sortase structures the analogous $\beta 7/\beta 8$ loop is structurally ordered. A notable exception is the SrtB enzyme from \textit{B. anthracis} (Ba-SrtB), which is presumably flexible as many of its residues exhibit poorly defined electron density (18). Interestingly, both Ba-SrtA and Ba-SrtB presumably attach proteins to the free amino group within the m-DAP portion of lipid II, unlike other sortase enzymes of known structure that have rigid $\beta 7/\beta 8$ loops and attach proteins to structurally distinct lipid II molecules. This suggests that in \textit{B. anthracis} the attachment of proteins to the m-DAP moiety of lipid II is correlated with the presence of a flexible active site loop.

The \textit{B. anthracis} enzyme may recognize the sorting signal via a lock-and-key mechanism. Crystallographic and NMR studies of Sa-SrtA have shown that it binds to the LPXTG sorting signal through an induced fit mechanism in which signal binding nucleates the folding and immobilization of the $\beta 6/\beta 7$ loop (Fig. 5g) (12, 17, 19, 47). In contrast, the $\beta 6/\beta 7$ loop in apo-Ba-SrtA is immobile and appears to be in a conformation suited to interact with the sorting signal (Figs. 3a and 5h). To investigate this issue we generated a model of the Ba-SrtA-sorting complex based on the recently determined structure of the Sa-SrtA-sorting signal complex (17). This work revealed that apo-Ba-SrtA can bind to the sorting signal in a similar manner as the Sa-SrtA enzyme with only small perturbations in its structure (the backbone atoms of the protein in the model and the experimentally determined structure of Ba-SrtA can be superimposed with a
r.m.s. deviation of 0.52 Å). However, to elucidate the molecular basis of sorting signal binding the structure of the Ba-SrtA-peptide complex needs to be determined. An understanding of the binding site pocket and substrate recognition mechanism by SrtA enzymes may serve to be beneficial in the rational development of sortase inhibitors. We have recently discovered several small molecules that inhibit both the Ba-SrtA and Sa-SrtA enzymes with similar potency (52). The work reported here could therefore facilitate the further development of these molecules into useful anti-infective agents to treat infections caused by S. aureus, B. anthracis, and other Gram-positive pathogens.

Acknowledgments—We thank Robert Peterson for assistance with the NMR and members of the Clubb laboratory for useful discussions.

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