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 (His\(^{126}\)). 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. pK\(_a\) measurements indicate that His\(^{126}\) 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)-, 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 sporulation, 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: His\(^{220}\), Cys\(^{194}\), and Arg\(^{197}\) (Sa-SrtA numbering) (21–23). Transpeptidation occurs within the LP motif to the free amino group within lipid II.
Structure of B. anthracis SrtA Enzyme

through a ping-pong mechanism that is initiated when the thiol group of Cys\(^{184}\) 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\(^{184}\) thiol covalently attaches to the carbonyl carbon of the threonine residue within the LP\(_{234}\)XG sorting signal. Arg\(^{197}\) is thought to stabilize the binding of the sorting signal by hydrogen bonding to its backbone (17, 26) and may also stabilize oxycyan transition states (20, 23). The function of His\(^{229}\) 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 driven the search for new therapeutics to treat and prevent infections caused by this microbe (27). *B. anthracis* encodes three sortase enzymes: Ba-SrtA, Ba-SrtB, and Ba-SrtC. The Ba-SrtB enzyme is involved in iron acquisition and anchors the heme-binding IsdC protein to the cell wall (28). Ba-SrtA, which is a SrtA-type enzyme, anchors two proteins required for proper spore formation (29, 30). The Ba-SrtA enzyme is a SrtA-type sortase that attaches 7 proteins to the cell wall by joining the threonyl of the C-terminal LP\(_{234}\)XTG sorting signal to the amine group of meso-diaminopimelic acid (\(m\)-Dap) within lipid II (31, 32). Ba-SrtA is a potential target for new therapeutics as it is required for *B. anthracis* survival and replication within macrophages (33), a presumed early step in the development of inhalation anthrax.

The molecular basis of Ba-SrtA function is not well understood because it shares only limited sequence identity with previously characterized SrtA-type enzymes; the structures of the Sa-SrtA and Sp-SrtA enzymes have been determined and share 29 and 32% sequence identity with Ba-SrtA, respectively (12, 16, 17, 19). Here we report studies of the structure, dynamics, and function of Ba-SrtA. Unlike previously studied sortase enzymes, Ba-SrtA contains several unique active site features that include the presence of an N-terminal extension that contacts the catalytically essential histidine and a large structurally disordered active site loop. Moreover, in contrast to Sa-SrtA, the sorting signal binding pocket in Ba-SrtA is ordered and rigid in the apo-state and therefore presumably only needs to undergo minimal structural changes to recognize the sorting signal. The mechanistic implications of these large differences are discussed.

**EXPERIMENTAL PROCEDURES**

* Protein Preparation—* Four deletion mutants of the SrtA protein from *B. anthracis* were studied: Ba-SrtA\(_{1-22}\) (residues Lys\(^{24}\)--Lys\(^{210}\), Ba-SrtA\(_{126}\) (residues Asp\(^{57}\)--Lys\(^{210}\), Ba-SrtA\(_{156}\) (residues Asp\(^{65}\)--Lys\(^{210}\)), and Ba-SrtA\(_{274}\) (residues Asp\(^{75}\)--Lys\(^{210}\)). 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. \(^{13}\)C- and \(^{15}\)N-labeled proteins used in the NMR studies were produced by growing cells in M9 medium supplemented with \(^{15}\)NH\(_4\)Cl or \(^{15}\)NH\(_4\)Cl and \(^{13}\)C\(_6\) 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 \(\beta\)-d-thiogalactoside to a final concentration of 1 mM when the cells reached an \(A\)\(_{600}\) of ~0.6. Cells were harvested 4 h after induction by centrifugation at 6,000 \(\times\) g and stored at −80 °C. Proteins were purified by resuspending the pellet in lysis buffer (50 mM NaPO\(_4\)_\(_{4}\), pH 7.0, 300 mM NaCl, 2 mM phenylmethanesulfonyl fluoride, 2 mM benzamidine). The cells were then lysed by sonication and the lysate was cleared by centrifugation at 13,000 \(\times\) 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\(_2\)). The eluate was then further purified using a Sephacryl-100 gel filtration column equilibrated with either NMR buffer (10 mM MES, 20 mM NaCl, 2 mM NaPO\(_4\)_\(_{4}\), pH 7.0) or fluorescence resonance energy transfer assay buffer (20 mM HEPES, pH 7.5). Three samples of Ba-SrtA\(_{156}\) were studied by NMR and were dissolved in NMR buffer: 1) 4 mM \(^{15}\)N-Ba-SrtA\(_{156}\) dissolved in NMR buffer containing 7% D\(_2\)O; 2) 2.5 mM \(^{15}\)N,\(^{13}\)C-Ba-SrtA\(_{156}\) dissolved in NMR buffer containing 7% D\(_2\)O; and 3) 2.5 mM \(^{15}\)N,\(^{13}\)C-Ba-SrtA\(_{156}\) dissolved in deuterated NMR buffer (obtained by lyophilization and redissolving in 99.999% D\(_2\)O).

* NMR Spectroscopy and Structure Determination—* NMR spectra of Ba-SrtA\(_{156}\) 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 PIPP (35) and CARA (version 1.8.4) (36) software packages. Chemical shift assignments (\(^1\)H, \(^13\)C, \(^15\)N) were obtained by analyzing the following experiments: HNCA, HNCA(CO)NH, HCNO, HC(NH)CA(CO), HNNH, HNNB, HBHA(CO)NH, CC(CO)NH, HCCCH-TOCSY, HCCCH-COSY, (HB)CB(CGCD)-HE, and (HB)CB(CGCD)-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 analyzingHNHA spectra (40). Distance restraints were obtained from three-dimensional \(^1\)N- and \(^1\)H-edited NOESY spectra and a four-dimensional \(^1\)C,\(^15\)N-edited HMQCN-OEHSY-HSOCO spectrum. \(^1\)D\(_{\text{NH}}\) and \(^1\)D\(_{\text{CC}}\) residual dipolar couplings were measured using protein samples partially aligned in PEG C12ES/hexanol, using two-dimensional \(^1\)N-coupled IPAP \(^1\)H-\(^1\)N HSQC and two-dimensional carbonyl-coupled \(^1\)H-\(^15\)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-aminoo-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 yield relative moments of 1.000:0.84:0.68. The program R2R1-tm was used to calculate an approximate correlation time \(\tau_m\) 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 \(\tau_s\)), 13 residues fit model 3 (\(S^2\) and \(\tau_e\)), 3 residues fit model 4 (\(S^2\), \(\tau_s\), and \(\tau_e\)), and 12 residues, in addition to the side chain indole Ne atom of Trp^{171} could be fit using model 5 (\(S^2\), \(S^2\), \(S^2\), \(\tau_s\), and \(\tau_e\)).

**Enzyme Kinetics Measurements**—Substrate cleavage reaction was performed as previously described (52). The cleavage of the substrate, o-amino-n-benzoyl-LPETG-2,4-dinitrophenyl (abz-LPETG-DNP), was monitored by excitation at 335 nm and recording emission at 420 nm on a SpectraMax M5 spectrofluorometer (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 pKₐ Measurements**—^{15}N- and ^{13}C-labeled Ba-SrtA_{56} (0.5 mM) in buffer (20 mM Bis-Tris in 7% D₂O) was titrated using 0.2 mM HCl or 0.2 mM NaOH to pH 4.5–10. The chemical shift of the His177 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,

\[ \delta_{obs} = \left( \delta_{1H} + \delta_{15N} \times \frac{10^{pH-pK_a}}{1 + 10^{pH-pK_a}} \right) \]

where \(\delta_{1H}\) and \(\delta_{15N}\) are the chemical shifts of the fully protonated and deprotonated states of the imidizolium side chain respectively, and \(\delta_{obs}\) 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 Met¹–Gly²3) and a C-terminal catalytic region (residues Lys²³–Lys⁴⁰). Previously, a deletion mutant of Ba-SrtA that removes the transmembrane region (Ba-SrtA_{56}, residues Lys²³–Lys⁴⁰ 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_{56}. Inspection of the \(^{1}H\)–^{15}N HSQC spectra of \(^{15}N\)Ba-SrtA_{56} 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 Lys²³–Val⁵⁶ 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_{56} residues Asp⁵⁷–Lys⁴⁰ of Ba-SrtA). The NMR spectra of Ba-SrtA_{56} are well resolved and have line widths that indicate that the majority of the protein is structured (Fig. 1a).

The structure of Ba-SrtA_{56} was determined using multidimensional heteronuclear NMR and simulating annealing
Structure of B. anthracis SrtA Enzyme

FIGURE 1. NMR Spectra of Ba-SrtA<sub>356</sub>. A, 1H-15N HSQC spectrum. The amino acid assignment for each cross-peak is indicated. Cross-peaks for residues Thr<sup>196</sup>-Try<sup>197</sup> and Asn<sup>127</sup> are broadened beyond detection. An asterisk indicates five cross-peaks that could not be assigned. # indicates cross-peaks that are folded into the spectrum that originate from side chain nitrogen atoms.

b, selected panels showing proton-proton NOEs between the N-terminal extension and the side chain of His<sup>126</sup> and other active site residues. The panels are taken from a three-dimensional 13C-edited NOESY-HSQC spectrum of Ba-SrtA<sub>356</sub> dissolved in deuterated buffer. The identity of the proton from Ba-SrtA<sub>356</sub> that gives rise to the set of NOEs and its chemical shift are shown at the top and bottom of each panel, respectively. On the left side of each cross-peak the proton within the active site that is proximal to the N-terminal extension is indicated. For clarity only protons within the active site are labeled.

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<sup>H</sup>N<sub>α</sub>, 264 13C 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 covalent geometry and have no NOE, dihedral angle, or scalar coupling violations greater than 0.5 Å, 5°, or 2 Hz, respectively. The geometry and have no NOE, dihedral angle, or scalar coupling violations greater than 0.5 Å, 5°, or 2 Hz, respectively. The ensemble contains 40 conformers representing the structure of the protein and enables extensive interactions between the chains and allows them to form opposing faces of the β-barrel structure. Residues His<sup>126</sup>, Cys<sup>187</sup>, and Arg<sup>196</sup> 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. Cys<sup>187</sup> is situated at the C-terminal end of the β7 strand and is bracketed by side chains of His<sup>126</sup> and Arg<sup>196</sup> located on strands β4 and β8, respectively.

Unique Active Site Features: a Histidine Contacting N-terminal Extension and a Disordered β7/β8 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 (His<sup>126</sup>) 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 Ile<sup>61</sup>-Val<sup>79</sup>. At its N terminus residues Ile<sup>61</sup>-Pro<sup>64</sup> are positioned near His<sup>126</sup>. The chain then forms a short α-helix (H1) that packs against helix H2 before changing its direction to initiate strand β1. Contacts to His<sup>126</sup> are extensive, with the methyl groups Ile<sup>61</sup> partially encapsulating the imidazole ring (Fig. 2c). Ile<sup>61</sup> 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 β7 and β8 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.
groups of Leu103, a residue believed to be important in recognizing the LPXTG substrate (17). Pro63 for the N-terminal extension also fits into the groove, making contacts with Leu103, Leu104, Val79, Leu95, Lys96, Ser98, Ser105, Ala107, Lys131, Gly132, Leu144, Val166, and Thr167. The figure also shows residues within the β7/β8 loop that presumably exhibit large amplitude motions because their backbone amide resonances could not be assigned (black spheres).

![FIGURE 3. NMR relaxation data that defines the mobility of Ba-SrtA. a, a graph showing the general order parameter (S^2) of the backbone nitrogen atoms as a function of residue number. The right panel shows data for the side chain Ne1 atom of Trp171. Secondary structural elements are shown above the figure, with the semi-flexible N-terminal extension shaded. Data for the β7/β8 loop is absent because many of the resonances for these residues are broadened beyond detection presumably because they are mobile. b, ribbon drawing of the structure of Ba-SrtA showing the location of backbone amide atoms in the structure that have significant 15N relaxation rates. Residues with 15N relaxation rates greater than 2.0 Hz (gray spheres) include residues: Asp75, Lys76, Gln78, Val80, Leu95, Lys96, Ser98, Ser105, Ala107, Lys131, Gly132, Leu144, Val166, and Thr167. The figure also shows residues within the β7/β8 loop that presumably exhibit large amplitude motions because their backbone amide resonances could not be assigned (black spheres).](image-url)
describe the motion of the protein backbone: the general order parameter ($S^2$), the effective correlation time for internal motions ($\tau_s$), and $R_{ex}$ ($50, 51$) (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, $\tau_s$. The value of $S^2$ ranges from 0 to 1, with a value of 1 indicating that the bond is completely immobilized. $R_{ex}$ 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 $\beta\beta$ 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 Ile$^{64}$–Thr$^{183}$ and Val$^{198}$–Lys$^{210}$ 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. Ser$^{73}$ is the last highly ordered residue at the amino terminus of the protein, beginning at Ala$^{72}$ immediately following helix H1 the $S^2$ values become progressively smaller as the chain proceeds to the surface that contacts the N-terminal appendage and their elevation is also possible that the changes associated with its binding and release. For some of the residues it is also possible that the amide bond experiences and is characterized by the relaxation data is presumably caused by averaging of these two states.

The $R_{ex}$ data suggest that the N-terminal extension transiently binds to the active site. Several residues surrounding the active site have elevated $R_{ex}$ values (Leu$^{103}$, Ser$^{105}$, Ala$^{107}$, Lys$^{131}$, Gly$^{132}$, and Leu$^{134}$, 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 $R_{ex}$ 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 $R_{ex}$ values are caused by changes in the tautomeration state of the His$^{126}$. The notion that the N-terminal extension can open and close over His$^{126}$ is further supported by the observation of significant $R_{ex}$ values for the backbone nitrogen atoms of Asp$^{75}$, Lys$^{76}$, Gln$^{78}$, and Val$^{79}$. 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 $R_{ex}$ values in residues Leu$^{90}$, Lys$^{96}$, and Ser$^{98}$ 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 Val$^{166}$–Asp$^{180}$). Based on the NMR structure of the Sa-SrtA substrate complex (17), the side chain of Trp$^{171}$ located in the central $\beta10$ helix of the loop can be expected to interact with the sorting signal. Interestingly, although the backbone of Trp$^{171}$ 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 $S^2_p$ of 0.742 ± 0.041, and $S^2_s$ 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 χ 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, Schnee- weid and colleagues (31) demonstrated that purified Ba-SrtA$\Delta_{223}$ 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$\Delta_{56}$ cleaves this peptide with $k_{cat}$ and $K_m$ values of $4.0 \times 10^{-4} \pm 1 \times 10^{-3} \text{ min}^{-1}$ and 38 ± 4 μM, respectively. Interestingly, our results indicate that in vitro Ba-SrtA$\Delta_{56}$ is unable to perform the transpeptidation reaction, because we were unable to detect transpeptidation products by mass spectrometry when Ba-SrtA$\Delta_{56}$ 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$\Delta_{64}$ (residues Asp$^{65}$–Lys$^{210}$ of Ba-SrtA). Ba-SrtA$\Delta_{64}$ is folded based on its $^1$H–$^15$N HSQC spectrum (supplemental Fig. S1). As compared with SrtA$\Delta_{54}$, it hydrolyzes the sorting signal with a modestly larger catalytic turnover ($k_{cat}$ 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$\Delta_{74}$ (residues Asp$^{75}$–Lys$^{210}$ 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$\Delta_{56}$ has no detectable hydrolytic activity.
activity (data not shown). Combined, these data reveal that His126 is essential for the hydrolytic activity, although contacts to it from Ile61 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.

**pK_a Measurements Reveal that His126 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_A56. These included His126, located in the active site, and His177 located on the β6/β7 loop. A series of 1H,13C HSQC spectra of [13C,15N]Ba-SrtA_A56 were collected at different pH values and the chemical shifts of the side chain histidine 1Hε1.13Cε1 resonances were measured (Fig. 4b). Fitting the pH dependence of the chemical shift data reveals that His126 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 His126 side chain within the active site is uncharged at physiological pH and therefore not a participant in an imidazolium-thiolate interaction with Cys187. In contrast, the side chain of His177 located in the β6/β7 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 Nδ1 and Ne2 atoms of His177 are positioned to interact with the side chain carboxyl group of Glu129 and the backbone carbonyl of Glu172, respectively. These interactions may stabilize the conformation of the β6/β7 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 β6/β7 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 sorting signal (Fig. 5, e and f). The peptide rests in a groove whose base is formed by residues in strands β4 and β7, and whose walls are formed by the β6/β7, β7/β8, β3/β4 and β2/H2 surface loops. The leucine methyl groups of the peptide are wedged between strand β8 and the β6/β7 loop, contacting the side chains of Val166, Pro168, Val173, and Val174 on the β6/β7 loop, and Val198 on strand β8. The indole ring of Trp171 located in the β6/β7 loop rests on top of the signal, whereas the proline in the peptide forms a kink in the substrate that is contacted by residues Ala124 and Ile185 located in strands β4 and β7, respectively, as well as Val110 on the β3/β4 loop. Contacts to the remainder of the peptide cannot be reliably predicted from the model as the positioning of residues in the β7/β8 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). Interestingly, in all other sortase enzymes studied to date the analogous active site histidine is exposed to the solvent and there is great structural variability in residues that precede strand β1 (Fig. 5, a–c). Notably, the Sp-SrtA enzyme also contains a structured N-terminal extension, but unlike Ba-SrtA it is positioned distal to the active site (16). This diversity is consistent with a multiple sequence alignment, which reveals that this region in the primary sequence is poorly conserved in SrtA-type enzymes (supplemental Fig. S3, green box). Interestingly, the SrtC-type sortase enzymes that catalyze pilin assembly contain an N-terminal extension that precedes the β-barrel (13, 15). However, this extension acts as “lid” that approaches the active site from

ture of the apo-form of the enzyme (Fig. 2a). The implications of the model with respect to the mechanism of sorting signal binding by other SrtA-type enzymes are discussed below.

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
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 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 unriaveling 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 β6, β7, and the β6/β7 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 β4 and β7, helix H2, and the β7/β8 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). *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 *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 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 *S. aureus* and *S. pyogenes*, respectively) (16, 54). Thus it appears that in *vitro*, Ba-SrtA requires additional protein components or larger portions of the lipid II molecule bearing m-DAP to successfully mediate transpeptidation.

The pKₐ 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 nucleophilically 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 pKₐ measurement of the active site histidine in Ba-SrtA indicates that like Sa-SrtA it is predominantly uncharged at physiological pH. Interestingly, its pKₐ value is smaller than the analogous histidine in Sa-SrtA; the pKₐ of His$^{126}$ in Ba-SrtA is less than ~5.5, whereas the pKₐ of His$^{126}$ in Sa-SrtA is ~6.3–7.0 (25, 55). The lower pKₐ 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 β7 and β8 (the β7/β8 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 N$^{15}$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 β7/β8 loop is structurally ordered. A notable exception is the SrtB enzyme from *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 attack proteins to the free amino group within the m-DAP portion of lipid II, unlike other sortase enzymes of known structure that have rigid β7/β8 loops and attach proteins to structurally distinct lipid II molecules. This suggests that in *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 *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 β6/β7 loop (Fig. 5g) (12, 17, 19, 47). In contrast, the β6/β7 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
REFERENCES

1. Navarre, W. W., and Schneewind, O. (1999) Microbiol. Mol. Biol. Rev. 63, 174–229
2. Mazmanian, S. K., Ton-That, H., and Schneewind, O. (2001) Mol. Microbiol. 40, 1049–1057
3. Ton-That, H., Marraffini, L. A., and Schneewind, O. (2004) Biochim. Biophys. Acta 1694, 269–278
4. Paterson, G. K., and Mitchell, T. J. (2004) Trends Microbiol. 12, 89–95
5. Marraffini, L. A., Dedent, A. C., and Schneewind, O. (2006) Microbiol. Mol. Biol. Rev. 70, 192–221
6. Desvaux, M., Dumas, E., Chafsey, I., and Hebraud, M. (2006) FEMS Microbiol. Lett. 256, 1–15
7. Mandlik, A., Swierczynski, A., Das, A., and Ton-That, H. (2008) Trends Microbiol. 16, 33–40
8. Maresso, A. W., and Schneewind, O. (2008) Pharmacol. Rev. 60, 128–141
9. Suree, N., Jung, M. E., and Clubb, R. T. (2007) Mini. Rev. Med. Chem. 7, 991–1000
10. Comfort, D., and Clubb, R. T. (2004)
11. Suree, N., Jung, M. E., and Clubb, R. T. (2009) J. Biol. Chem. 284, 23129–23139
12. Neiers, F., Madhurantakam, C., Fällker, S., Manzano, C., Dessen, A., Normark, S., Henriques-Normark, B., and Achour, A. (2009) J. Mol. Biol. 393, 704–716
13. Race, P. R., Bentley, M. L., Melvin, J. A., Crow, A., Hughes, R. K., Smith, W. D., Sessions, R. B., Kehoe, M. A., McCafferty, D. G., and Banfield, M. J. (2009) J. Biol. Chem. 284, 6929–6933
14. Suree, N., Liew, C. K., Villareal, V. A., Thieu, W., Fadeev, E. A., Clemens, J. J., Jung, M. E., and Clubb, R. T. (2009) J. Biol. Chem. 284, 24465–24477
15. Zhang, R., Wu, R., Joachimiak, G., Mazmanian, S. K., Missiakas, D. M., Gornicki, P., Schneewind, O., and Joachimiak, A. (2004) Structure 12, 1147–1156
16. Zong, Y., Rice, T. W., Ton-That, H., Schneewind, O., and Narayan, S. V. (2004) J. Biol. Chem. 279, 31383–31389
17. Zong, Y., Mazmanian, S. K., Schneewind, O., and Narayan, S. V. (2004) Structure 12, 105–112
18. Ton-That, H., Mazmanian, S. K., Alksne, L., and Schneewind, O. (2002) J. Biol. Chem. 277, 7447–7452
19. Marraffini, L. A., Ton-That, H., Zong, Y., Narayan, S. V., and Schneewind, O. (2004) J. Biol. Chem. 279, 37763–37770
20. Frankel, B. A., Tong, Y., Bentley, M. L., Fitzgerald, M. C., and McCafferty, D. G. (2007) Biochemistry 46, 7269–7278
21. Huang, X., Aulabaugh, A., Ding, W., Kapoor, B., Alksne, L., Tabei, K., and Elstad, G. (2003) Biochemistry 42, 11307–11315
22. Bentley, M. L., Lamb, E. C., and McCafferty, D. G. (2008) J. Biol. Chem. 283, 14762–14771
23. Inglesby, T. V., Henderson, D. A., Bartlett, J. G., Ascher, M. S., Etzen, E., Friedlander, A. M., Hauer, J., McDade, J., Osterholm, M. T., O’Toole, T., Parker, G.,Perl, T. M., Russell, P. K., and Tonat, K. (1999) JAMA 281, 1735–1745
24. Naier, S. R., Ton-That, H., Jung, M. E., and Clubb, R. T. (2009) J. Biol. Chem. 284, 221–226
25. Connolly, K. M., Smith, B. T., Pilpa, R., Ilangovan, U., Jung, M. E., and Clubb, R. T. (2003) J. Biol. Chem. 278, 34061–34065
26. Marraffini, L. A., and Schneewind, O. (2006) Mol. Microbiol. 62, 1402–1417
27. Marraffini, L. A., and Schneewind, O. (2007) J. Biol. Chem. 282, 6924–6933
28. Suree, N., Liew, C. K., Villareal, V. A., Thieu, W., Fadeev, E. A., Clemens, J. J., Jung, M. E., and Clubb, R. T. (2009) J. Biol. Chem. 284, 24465–24477
29. Marraffini, L. A., and Schneewind, O. (2005) J. Biol. Chem. 280, 4646–4655
30. Budzik, J. M., Oh, S. Y., and Schneewind, O. (2008) J. Biol. Chem. 283, 36676–36686
31. Zink, S. D., and Burns, D. L. (2005) Infect. Immun. 73, 5222–5228
32. Delaglio, F., Grzesieki, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) J. Biomol. NMR 6, 277–293
33. Ranganathan, D. S., and Schneewind, O. (2008) J. Mol. Biol. 382, 213–223
34. Vuister, G. W., and Bax, A. (1999) J. Am. Chem. Soc. 121, 7772–7777
35. Hermann, T., Güntert, P., and Wüthrich, K. (2002) J. Biol. Chem. 277, 171–189
36. Hermann, T., Güntert, P., and Wüthrich, K. (2002) J. Mol. Biol. 319, 209–227
37. Ranganathan, D. S., and Schneewind, O. (2008) J. Mol. Biol. 382, 213–223
38. Suree, N., Jung, M. E., and Clubb, R. T. (2009) J. Biol. Chem. 284, 31591–31600
39. Naier, S. R., Ton-That, H., Jung, M. E., and Clubb, R. T. (2006) J. Biol. Chem. 281, 1817–1826
40. Brüschweiler, R., Liao, X., and Wright, P. E. (1995) Science 268, 886–889
41. Lee, L. K., Rance, M., Chazin, W. I., and Palmer, A. G. (2003) J. Biomol. NMR 9, 287–298
42. Lipari, G., and Szabo, A. (1982) J. Am. Chem. Soc. 104, 4546–4559
43. Mandel, A. M., Akke, M., and Palmer, A. G. (1993) J. Mol. Biol. 246, 144–163
44. Suree, N., Liew, C. K., Thieu, W., Marohn, M., Damoiseaux, R., Chan, A., Jung, M. E., and Clubb, R. T. (2009) Bioorg Med. Chem. 17, 7174–7185
45. Liew, C. K., Smith, B. T., Pilpa, R., Suree, N., Ilangovan, U., Connolly, K. M., Jung, M. E., and Clubb, R. T. (2004) FEBS Lett. 571, 221–226
46. Ton-That, H., Mazmanian, S. K., Faull, K. F., and Schneewind, O. (2000) J. Biol. Chem. 275, 9876–9881
47. Connolly, K. M., Smith, B. T., Pilpa, R., Ilangovan, U., Jung, M. E., and Clubb, R. T. (2003) J. Biol. Chem. 278, 34061–34065

Structure of B. anthracis SrtA Enzyme

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.