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Sortase from *Staphylococcus aureus* Does Not Contain a Thiolate-Imidazolium Ion Pair in Its Active Site*

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Many surface proteins are anchored to the cell wall by the action of sortase enzymes, a recently discovered family of cysteine transpeptidases. As the surface proteins of human pathogens are frequently required for virulence, the sortase-mediated anchoring reaction represents a potential target for new anti-infective agents. It has been suggested that the sortase from *Staphylococcus aureus* (SrtA), may use a similar catalytic strategy as the papain cysteine proteases, holding its Cys^{184} side chain in an active configuration through a thiolate-imidazolium ion interaction with residue His^{120}. To investigate the mechanism of transpeptidation, we have synthesized a peptidyl-vinyl sulfone substrate mimic that irreversibly inhibits SrtA. Through the study of the pH dependence of SrtA inhibition and NMR, we have estimated the pK_as of the active site thiol (Cys^{184}) and imidazole (His^{120}) to be ~9.4 and 7.0, respectively. These measurements are inconsistent with the existence of a thiolate-imidazolium ion pair and suggest a general base catalysis mechanism during transpeptidation.

Gram-positive bacteria infect humans through an array of surface-associated proteins that promote bacterial adhesion, resistance to phagocytic killing, and host cell invasion. Many surface proteins are covalently anchored to the cell wall by the action of sortase enzymes, a family of novel transpeptidases (reviewed in Refs. 1–5). The SrtA protein from *Staphylococcus aureus* is the most extensively characterized sortase enzyme (6) and anchors surface proteins that contain a C-terminal sorting signal consisting of a conserved LPXTG motif, a hydrophobic domain, and a tail of mostly positively charged residues (7, 8). SrtA and related proteins may be excellent targets for new broad-spectrum anti-infective agents, because sortase-like enzymes and the LPXTG signal are universally conserved in Gram-positive bacteria (5, 9), and sortase (−) strains of *S. aureus* (10–12), *Listeria monocytogenes* (13, 14), and *Streptococcus gordonii* (15) display defects in their virulence.

The structure of SrtA revealed a novel protein fold and localized the active site to a hydrophobic surface depression that contains two highly conserved and enzymatically important residues, His^{120} and Cys^{184} (SrtA numbering) (16–18). This catalytic dyad is reminiscent of the active sites of the papain cysteine proteases (Cys^{25}-His^{159}-Asn^{175}, papain numbering) (19, 20), suggesting that they are mechanistically related (21). In this model of SrtA function, the imidazole ring of His^{120} promotes the formation of the Cys^{184} thiolate, which then nucleophilically attacks the carbonyl carbon at the scissile Thr-Gly peptide bond in the LPXTG motif (22). After covalent linkage via a thioacyl bond to the threonine carbonyl group, the incoming amine of the cell wall precursor lipid II (23, 24) may then be deprotonated by His^{120} for attack on the covalent intermediate (16, 18).

Previous work left unresolved how His^{120} activates Cys^{184}. In the papain cysteine proteases, the cysteine side chain is held in an active configuration through a thiolate-imidazolium ion interaction with the histidine (25). However, in the NMR structure of SrtA solved in the absence of its substrates, the side chains of Cys^{184} and His^{120} do not interact, arguing against the presence of an ion pair (16). To resolve this issue, and as a first step toward the design of a therapeutically useful anti-infective agent, we have synthesized a peptidyl-vinyl sulfone substrate mimic that inhibits SrtA. The pH dependence of SrtA inhibition and NMR studies preclude the presence of an ion pair in the active site, because His^{120} and Cys^{184} have pK_a values of 7.0, and ~9.4, respectively.

EXPERIMENTAL PROCEDURES

Reagents—Residues 60–206 of wild-type sortase ($SrtA_{\Delta N59}$) and a single amino acid mutant of the protein containing a cysteine to alanine substitution at position 184 ($^{C184A}SrtA_{\Delta N59}$) were overexpressed from plasmids pSRTA and pHTT45, respectively (16, 18). The expression, uniform isotopic labeling (where applicable), and purification of $SrtA_{\Delta N59}$ and $^{C184A}SrtA_{\Delta N59}$ have been described previously (16). The fluorescent substrate peptide *d*-QALPETGEE-*e* (where *d* is dabcyl (4-([4-(dimethylamino)phenyl]azo)-benzoyl-) and *e* is EDANS ([(2-aminoethyl)-amino]naphthlene-1-sulfonyl-)) was purchased from Synpep (Dublin, CA) and purified by HPLC.[1] Reagents for the synthesis of the vinyl sulfone inhibitor were purchased from Aldrich.

Synthesis of Vinyl Sulfone Inhibitor—The vinyl sulfone inhibitor was synthesized using solution phase methodology (see Fig. 1). The Leu-Pro-Ala tripeptide was synthesized by standard amino acid coupling chemistry using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) and 4-(dimethylamino)pyridine (DMAP), starting with the carbobenzyloxy-protected amino acid Cbz-Leu-OH and *N-tert*-butoxycarbonyl (*N-t*-Boc) methyl esters of alanine and proline. L-Threonine was fully protected as the *N-t*-Boc-threonine methyl ester with the alcohol protected as the t-butyldiphenylsilyl ether (**2**) in three steps. The ester was reduced with diisobutylaluminum hydride (DIBAL-H) to the aldehyde, which was immediately reacted without purification with diethyl phenylsulfonylmethylphosphonate (**3**) to give the desired vinyl sulfone functionality (**4**) in good yield. Removal of the Boc group with trifluoroacetic acid followed by coupling the amine with the Cbz-pro-

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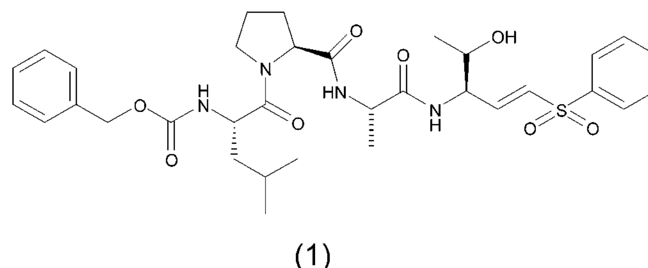
[1] The abbreviations used are: HPLC, high pressure liquid chromatography; *N-t*-Boc, *N-tert*-butoxycarbonyl; Cbz, benzyloxycarbonyl.

A.



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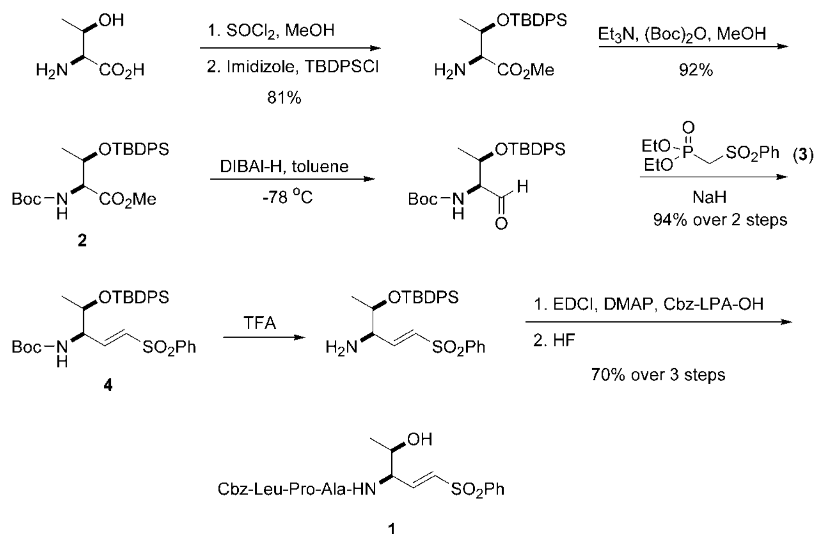

FIG. 1. *A*, molecular structure of the peptidyl-vinyl sulfone inhibitor Cbz-Leu-Pro-Ala-Thr-SO_2Ph. *B*, strategy for synthesis of vinyl sulfone inhibitor. L-Threonine was fully protected as the *N-t*-Boc-threonine methyl ester, with the alcohol protected as the *t*-butyldiphenylsilyl ether (**2**), in three steps. The ester was reduced with diisobutylaluminum hydride (*DIBAL-H*) to the aldehyde, which was immediately reacted without purification with diethyl phenylsulfonylmethylphosphonate (**3**) to give the desired vinyl sulfone functionality (**4**). Removal of the Boc group with trifluoroacetic acid (*TFA*) followed by coupling the amine with the Cbz-protected tripeptide (Cbz-Leu-Pro-Ala) gave the desired tetrapeptide. Removal of the *t*-butyldiphenylsilyl group with HF gave the vinyl sulfone inhibitor (**1**). *OTBDPS*, *t*-butyldiphenylsilyl; *TBDPSC1*, *t*-butylchlorodiphenylsilane; *DMAP*, 4-(dimethylamino)pyridine; *EDCI*, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride.

tected tripeptide gave the desired tetrapeptide. Removal of the *t*-butyldiphenylsilyl group with HF gave the vinyl sulfone inhibitor (**1**). The inhibitor was purified by silica gel chromatography and the structure confirmed by ^{1}H and ^{13}C NMR spectroscopy and FAB+ (fast atom bombardment) mass spectrometry.

Enzyme Assays—Fluorescent measurements of SrtA activity in the presence of the vinyl sulfone inhibitor were performed in 96-well microtiter plates as described previously (26). Inhibition reactions contained 5 μM SrtA and vinyl sulfone inhibitor (100, 300, or 600 μM) in buffer R (50 mM Tris, pH 3.5–10, 150 mM NaCl, 5 mM glycine, 5 mM $CaCl_2$). *d*-QALPETGEE-*e* was dissolved in dimethyl sulfoxide and added to the reaction at a final concentration of 25 μM, for a total reaction volume of 200 μl. The final concentration of Me_2SO in the reaction was kept below 5%. The reactions were incubated for 12 h at 37 °C with gentle mixing in a GENios multiwell fluorimeter (Tecan, Durham, NC) (360 nm excitation filter, 465 nm emission filter). Fluorescence emission was recorded at 10-min intervals and blanked against a reference solution containing SrtA in buffer R. All enzyme assays were performed in triplicate, resulting in ~450 measurements for each inhibitor concentration assayed. The inhibition parameters K_i and k_i were solved simultaneously for each inhibitor concentration using the equation,

$$[P]_t = [E]\,([S]K_i/[I]K_m)\,(k_{cat}/k_i)\,[1 - e^{-kit}/\{1 + K_i/[I](1 + [S]/[K_m])\}] \quad \text{(Eq. 1)}$$

where $[P]_t$ is the concentration of product at time t, $[E]$ is the total concentration of SrtA, and [S] and [I] are the total concentrations of the fluorogenic substrate and inhibitor, respectively (27). K_m and k_{cat} (the Michaelis and first-order rate constants for the uninhibited SrtA transpeptidation reaction, respectively) were determined independently for each pH value (data not shown). Curves were fit using the program Sigmaplot2000 (SPSS version 6.0).

HPLC Analysis of Inhibitor Modification of SrtA—Thirty microliters of SrtA (95–100 μM) in buffer I (50 mM Tris-HCl, 150 mM NaCl, and 5 mM $CaCl_2$) were adjusted to the desired pH with 1 M HCl or 1 M NaOH as needed and were incubated with a 20-fold molar excess of the vinyl sulfone inhibitor for 20 h at 37 °C with gentle agitation. The reaction was stopped by adding 500 μl of buffer A (0.1% trifluoroacetic acid in water), and the products were separated by reverse phase HPLC using a C18 column (Waters, Milford, MA) with the application of a gradient of 25–50% buffer B (90% acetonitrile, 10% H_2O, 0.1% trifluoroacetic acid).

NMR Spectroscopy—NMR experiments were carried out at 308 K on a Bruker DRX500 spectrometer equipped with a triple resonance probe using ^{15}N- and ^{13}C-labeled SrtA$_{\Delta N59}$ and ^{C184A}SrtA$_{\Delta N59}$ proteins (0.5 mM) in buffer N (50 mM Tris-HCl (pH 6.2), 100 mM NaCl, 20 mM $CaCl_2$, 3 mM dithiothreitol, and 7% D_2O). The chemical shifts of wild-type SrtA$_{\Delta N59}$ have been reported previously, and the resonances of the single histidine side chain (His^{120}) in the ^{C184A}SrtA$_{\Delta N59}$ mutant were readily assigned by reference to these data. For the pK_a measurements, a series of two-dimensional ^{1}H-^{13}C heteronuclear single quantum correlation spectra were recorded to monitor the pH dependence of the ^{1}H-δ2-^{13}C-δ2 and ^{1}H-ε1-^{13}C-ε1 resonances of the His^{120} side chain (spectra recorded at pH values of 4.5–10). Chemical shifts in the NMR spectra were plotted as a function of pH and fit to the following equation,

$$\delta_{obs} = (\delta_{HA} + \delta_A \times 10^{pH - pK_a})/(1 + 10^{pH - pK_a}) \quad \text{(Eq. 2)}$$

where δ_{HA} and δ_A are the chemical shifts of the fully protonated and deprotonated forms of the ionizable group, and δ_{obs} is the observed chemical shift (28, 29). The data were fit using SigmaPlot2000.

RESULTS AND DISCUSSION

To characterize the active site His^{120}-Cys^{184} dyad, we synthesized an irreversible inhibitor that consisted of the substrate recognition motif of SrtA (-Leu-Pro-*X*-Thr-Gly-, where *X*

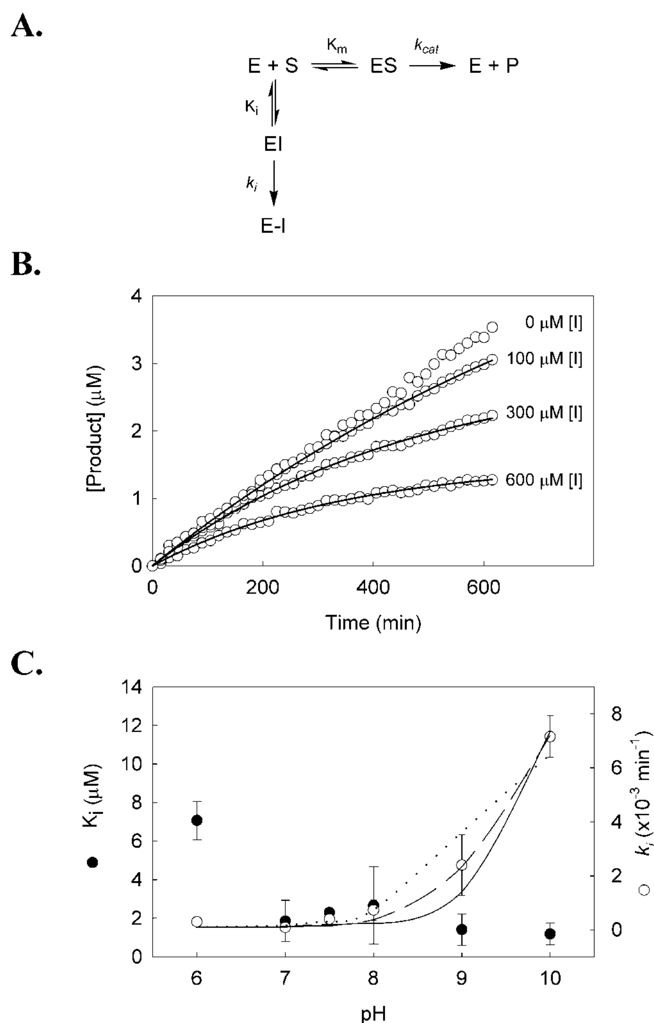

FIG. 2. *A*, mechanism of vinyl sulfone inhibition. In this mechanism, sortase (*E*) catalyzes the transpeptidation of its substrate (*S*) with the kinetic constants K_m and k_{cat}. The irreversible vinyl sulfone inhibitor (*I*) binds to sortase to form a Michaelis complex with affinity constant K_i. The rate of covalent attachment of the inhibitor via the vinyl sulfone moiety is described by the first-order rate constant k_i. *B*, progress curves of vinyl sulfone inhibition of SrtA. The curves were fit to Equation 1 to simultaneously determine the inhibition constants K_i and k_i (as described under "Experimental Procedures"). SrtA catalyzed the transpeptidation of the *d*-QALPETGEE-*e* substrate with a K_m of 3.6×10^{-6} M and a k_{cat} of 7.4×10^{-5} s^{-1}. These values are comparable with previously published values ($K_m = 1.6 \times 10^{-5}$ M; $k_{cat} = 2.3 \times 10^{-5}$ s^{-1}) determined under similar assay conditions (17). *C*, pH dependence of k_i and K_i for the vinyl sulfone inhibitor. The k_i (*open circles*) and K_i (*solid circles*) values were determined from progress curves of the inhibition reaction at each pH. The curves are plots of Equation 3, fixing the pK_a values at 9 (*dotted line*), 9.5 (*dashed line*), and 10 (*solid line*).

is any amino acid) but replaced the scissile Thr-Gly amide bond with a vinyl sulfone group (C = C-SO_2Ph) (Fig. 1*A*). Alanine was placed at the *X* position to facilitate the synthesis, and the vinyl sulfone group was used because it has previously been shown to covalently modify the active site thiol in cysteine proteases (30–34). This is relevant because mutagenesis studies have demonstrated the catalytic importance of SrtA residue Cys[184] (18), and several sulfhydryl-directed reagents block the activity of SrtA *in vitro* (17, 18).

The efficacy of the vinyl sulfone compound was tested *in vitro* by determining how it altered the SrtA-catalyzed hydrolysis of an internally quenched fluorescent substrate analogue (*d*-Gln-Ala-Leu-Pro-Glu-Thr-Gly-Glu-Glu-*e*). Hydrolysis progress curves were generated by monitoring the increase in fluorescence that accompanies the cleavage of the substrate (18, 26).

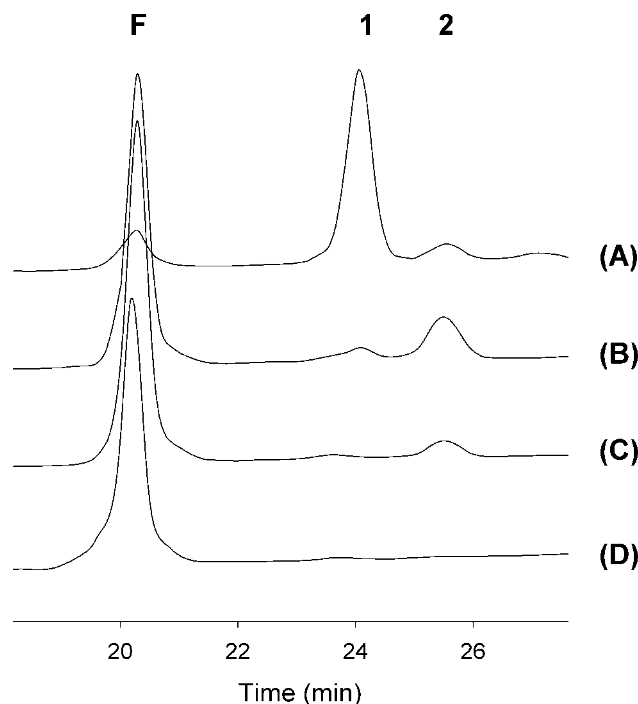

FIG. 3. **HPLC elution profiles for the separation of SrtA and the SrtA-inhibitor complex.** The peaks representing free SrtA (*F*) and the SrtA-inhibitor covalent complex (*1* and *2*) are labeled. *A–C*, SrtA$_{\Delta59}$ with inhibitor at pH 6 (*A*), pH 7 (*B*), and pH 9 (*C*). Reactions contained 95–100 μM SrtA and 20-fold molar excess of the vinyl sulfone inhibitor, and were incubated for 20 h at 37 °C. *D*, SrtA$_{\Delta59}$ pre-incubated with a 2-fold molar excess of spin label HO-225 prior to incubation with the vinyl sulfone inhibitor, pH 9.

The curves were consistent with the vinyl sulfone compound acting as an irreversible inhibitor of SrtA (Fig. 2*A*) (35). Typical progress curves of the reaction in the presence of 100, 300, and 600 μM inhibitor are shown in Fig. 2*B*. Fits of these data to Equation 1 by non-linear regression analysis determined the first-order rate constant of inactivation (k_i) and the dissociation constant of inhibitor binding (K_i). At pH 7, the K_i of the inhibitor is 9×10^{-6} M, which is comparable with the measured K_m of SrtA of 3×10^{-6} M for the fluorogenic substrate analogue (data not shown). The rate constant of inactivation (k_i) of the vinyl sulfone inhibitor is 4×10^{-4} min^{-1}. To date, only two other irreversible SrtA inhibitors have been characterized. Both contain the Cbz-Leu-Pro-Ala-Thr sorting signal mimic of the vinyl sulfone compound but utilize different reactive groups (diazomethane and chloromethane) (26). The first-order rate constants of the peptidyl-diazomethane (5.8×10^{-3} min^{-1}) and -chloromethane (1.1×10^{-2} min^{-1}) SrtA inhibitors are larger than the vinyl sulfone k_i by ~10- and 20-fold, respectively (26). This difference in reactivity is consistent with the higher electrophilicity of chloro- and diazomethane reactive groups and has been observed in inhibition studies of other cysteine proteases (36). For example, diazomethane and chloromethane inhibitors of human cathepsin L have second-order rate constants up to 100-fold higher than vinyl sulfone inhibitors (30, 36). As the transpeptidation reaction of SrtA is extremely poor *in vitro* (k_{cat}/K_m = 20.6 M·s^{-1}) it probably does not reflect the efficiency of the enzyme *in vivo*, where it must complete the anchoring of surface proteins within the doubling time of the bacterium. The development of a quantitative *in vivo* assay for inhibition will be necessary to evaluate the efficacy of peptidyl inhibitors of sortase as anti-infective agents.

Because the vinyl sulfone warhead of the peptide inhibitor is expected to be most reactive toward cysteine thiolates, and

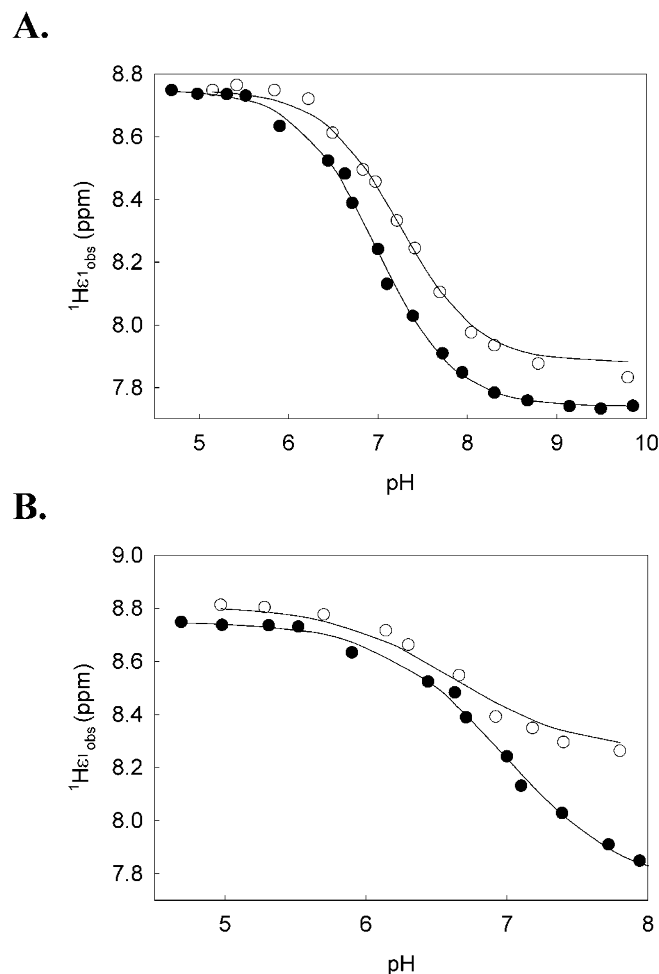

FIG. 4. *A*, pH titration of His[120]. Plot of the chemical shift changes in the ^{1}H-ε1 resonance of His[120] as a function of pH. The titrations were performed in the presence of 20 mM calcium, and the data were fit to Equation 2 to obtain pK_a values of 7.0 and 7.3 for wild-type SrtA$_{\Delta N59}$ (*solid circles*) and ^{C184A}SrtA$_{\Delta N59}$ (*open circles*), respectively. Analysis of the heteronuclear single quantum correlation NMR spectra over the same pH range (data not shown) indicates that the protein remains folded throughout the titration study. *B*, effect of calcium on the pK_a of His[120]. The *solid* and *open circles* are titration data obtained for the wild-type SrtA$_{\Delta N59}$ in the presence (*solid circles*) and absence (*open circles*) of 20 mM $CaCl_2$. Fits to Equation 2 reveal the pK_a of His[120] in the absence of calcium to be 6.6.

Cys[184] is the only cysteine in SrtA, we measured the pH dependence of inactivation to determine the pK_a of Cys[184]. The *in vitro* hydrolysis reaction was repeated at the three inhibitor concentrations over the pH range of 3.5–10, and the inhibition parameters K_i and k_i were determined by curve fitting. As shown in Fig. 2*C*, k_i increases dramatically above pH 8, whereas the K_i value is not significantly affected. To estimate the pK_a of Cys[184], a modified Henderson-Hasselbach equation (37),

$$k_i = (k_{\min} + (k_{\max} - k_{\min}))/(1 + 10^{\mathrm{p}K_a - \mathrm{pH}}) \quad \text{(Eq. 3)}$$

(where $k_{\min}$ and $k_{\max}$ are the minimum and maximum observed first-order inhibition constants) was modeled for the inhibitor data by fixing $k_{\min}$ (4×10^{-4} min^{-1} at pH 6) and solving for $k_{\max}$ over a range of pK_a values. Plots of Equation 3 for pK_a values of 9 (*dotted line*), 9.5 (*dashed line*), and 10 (*solid line*) are shown in Fig. 2*C*. The estimated the pK_a of Cys[184] is ~9.4.

To confirm that the pH-dependent inhibition of SrtA results from the covalent modification of Cys[184], we tracked the modification reaction by chromatography. The SrtA protein was incubated with a 20-fold molar excess of inhibitor for 20 h, and the reaction products were separated by HPLC. Fig. 3, *A–C* shows the results of incubating SrtA with the inhibitor at pH 9, 7, and 6, respectively. Two inhibitor-modified SrtA species elute on a C18 reverse phase HPLC column (*peaks 1* and *2*), and mass spectrometry indicates that both peaks contain SrtA covalently modified by a single inhibitor molecule. At pH values of 6 and 7, little modification occurs within 20 h with a slight excess of species 2 being produced. However, at pH 9.0, nearly all of the SrtA protein is converted to species 1 (Fig. 3*A*). An analysis of the pH dependence of modification indicates that the production of species 1 is most efficient at pH values nearing 9, whereas maximal production of the minor species 2 occurs at pH ~7 (data not shown). Because the enzyme is maximally inhibited by the vinyl sulfone compound at high pH values (Fig. 2*C*), where species 1 is almost exclusively present, the data strongly suggest that species 1 corresponds to the inhibitor-SrtA complex that forms during the inactivation. This hypothesis was substantiated by thiolating Cys[184] with a 2-fold excess of nitroxide spin label HO-225 ((1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl)-methanethiosulfonate) (38) and testing for its ability to react with the vinyl sulfone inhibitor at pH 9. As shown in Fig. 3*D*, preincubation with HO-225 prevented the formation of both species 1 and 2. These data are consistent with Cys[184] being the primary and most reactive site for modification by the vinyl sulfone at pH values above 8.

To investigate whether an imidazolium ion is present in the active site of SrtA, NMR was used to determine the pK_a of the His[120] side chain. Because the chemical shifts of atoms within the imidazole are expected to be sensitive to the ionization state of the side chain, a series of ^{1}H-^{13}C heteronuclear single quantum correlation spectra were recorded using a ^{13}C- and ^{15}N-enriched sample of SrtA, and the chemical shifts of the ^{1}H-δ2 and ^{1}H-ε1 atoms of His[120] were recorded at pH values between 4.5 and 10. Fig. 4 shows a plot of the chemical shift of the ^{1}H-ε1 atom as a function of pH (a similar curve was obtained for the ^{1}H-δ2 atom). A fit of the titration data to Equation 2 (see "Experimental Procedures") indicates that the His[120] side chain has a pK_a of 7.0 ± 0.1 in the presence of 20 mM $CaCl_2$. Because Ca^{2+} binding near the active site has been shown to enhance the *in vitro* activity of SrtA 8-fold (16), we repeated the pH titration experiment in calcium-free buffer (buffer N with no calcium) to determine the effects of Ca^{2+} on the ionization state of the active site His[120] residue. No significant changes in the pK_a of His[120] were detected in the absence of calcium (pK_a = 6.6 ± 0.1) (Fig. 4*B*). The data indicate that at physiological pH, the imidazole side chain is only partially protonated and that calcium does not stimulate the activity of SrtA by altering the ionization state of His[120]. Ca^{2+} binding may stabilize substrate binding or the fold of the protein.

If a thiolate-imidazolium ion pair exists in the active site of SrtA, one would expect the thiolate of Cys[184] to perturb the ionization of His[120] (*i.e.* alter its pK_a). We ascertained the effect of Cys[184] on His[120] by repeating the NMR-pH titration experiment using the mutant ^{C184A}SrtA$_{\Delta N59}$. The results of this titration (Fig. 4*A*, *open circles*) were fit to Equation 2 to obtain a pK_a of 7.3 ± 0.1. The similarly measured pK_a values for His[120] in the wild-type and mutant SrtA proteins suggest that its ionization state is independent of the Cys[184] side chain, a finding that is inconsistent with the existence of an ion pair between His[120] and Cys[184].

A thiolate-imidazolium ion pair between the side chains of His[120] and Cys[184] would enhance the reactivity of the thiol toward electrophiles at weakly acidic and neutral pH (36) because the imidazole group would act to polarize the thiol. In this scenario, it is expected that the pK_a value of the cysteine

thiol would be more acidic and the histidine imidazole would be more basic than their normal values of 7.85 and 6.0, respectively (39). For example, the anomalous pK_a values of 3.3 (for the Cys^{25} thiol) and 8.5 (for the His^{159} imidazolium) support the existence of a thiolate-imidazolium ion pair in papain (40, 41). In SrtA, however, our investigation argues against the use of a thiolate-imidazolium ion pair in the reaction mechanism.

The pH dependence of modification of Cys^{184} by the vinyl sulfone inhibitor (Fig. 2*C*) shows a dramatic increase in the first-order rate of inactivation as the pH is raised from 7.5 to 10 (at pH 10 the inhibitor is 20 times more reactive than at pH 7.5). Because the inhibitor is expected to be more reactive toward a thiolate ion, the pH dependence of inhibition can be attributed to the deprotonation of Cys^{184}. These data argue against the presence of an ion pair in the active site, because the pK_a of Cys^{184} is estimated to be ~9.4.

NMR studies of the His^{120} side chain have measured its pK_a at 7.0 in the wild-type protein, which is inconsistent with the presence of an imidazolium cation at neutral pH. Moreover, our finding that the removal of the Cys^{184} side chain (^{C184A}SrtA$_{\Delta N59}$) has only a modest effect on the ionization state of His^{120} side chain argues against the presence of an ion pair, because in the papain system the pK_a of His^{159} is lowered by 4.5 pH units upon the methylthiolation of the Cys^{25} (25).

Although the thiolate-imidazolium ion pair is a common catalytic entity of cysteine proteases, it is not universal (42, 43). The absence of an ion pair in SrtA suggests its catalytic mechanism may be similar to the viral 3C proteases (picornains), a structurally and mechanistically distinct group of cysteine proteases that perform general base catalysis (reviewed in Refs. 36 and 44). The crystal structures of the picornains of hepatitis A (45), rhinovirus (46), and poliovirus (47) show a similarity of three-dimensional structures and catalytic mechanisms to the serine proteases of the trypsin/chymotrypsin family and may be evolutionarily related (36, 43, 44). The pH-dependent alkylation of the active site cysteine of poliovirus protease 3C with iodoacetamide has measured its pK_a at 8.86 (42), which is similar to the estimated pK_a of the SrtA thiol. This is consistent with a reaction mechanism in which the cysteine nucleophile is uncharged at physiological pH, and the histidine functions as a general base.

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