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Brief Article

Structural Basis for the Interaction of Lactivicins with Serine β-Lactamases†

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Lactivicin (LTV) is a natural non-β-lactam antibiotic that inhibits penicillin-binding proteins and serine β-lactamases. A crystal structure of a BS3−LTV complex reveals that, as for its reaction with PBPs, LTV reacts with the nucleophilic serine and that cycloserine and lactone rings of LTV are opened. This structure, together with reported structures of PBP1b with lactivicins, provides a basis for developing improved lactivicin-based γ-lactam antibiotics.

Introduction

The β-lactam antibiotics remain among the most important antibiotics in clinical use. Their antibacterial action is mediated via inhibition of the transpeptidase activity of penicillin-binding proteins (PBPs[a]), which play roles in bacterial cell wall biosynthesis (Figure 1).[1,2]

All clinically used PBP inhibitors contain a β-lactam ring and are based on natural products, including the penicillins and cephalosporins, and react covalently with PBPs. Of all the identified naturally occurring PBP inhibitors, only lactivicin (LTV) does not contain a β-lactam ring. Instead, LTV contains a linked cycloserine and γ-lactone ring structure.[3,4]

The β-lactam antibiotics react with a nucleophilic serine at the active site of PBPs to form stable acyl-enzyme complexes. It is proposed that β-lactams are suited to the inhibition of PBPs not only because they mimic the substrate binding conformation[5] but because the ring strain inherent in the four-membered ring makes them suitably activated acylating agents and hinders reversible formation of the β-lactam.[6,7] The transpeptidase reaction is inhibited because the presence of a β-lactam-derived acyl-enzyme complex blocks approach to the acyl-enzyme (Figure 1).

In contrast to β-lactams, simple γ-lactam analogues can undergo reversible reaction with nucleophilic enzymes after acyl-enzyme formation.[6,8−10] The unusual bicyclic γ-lactam/γ-lactone ring structure of LTV is proposed to enable formation of a stable enzyme complex of a γ-lactam-based inhibitor. Initial opening of the cycloserine ring by the nucleophilic serine is followed by lactone opening to give an acyl-enzyme in which retro-lactam formation is disfavored (Figure 1e).[11,12] LTV and a more potent analogue, phenoxyacetyllactivicin (PLTV), have been reported to be active against a range of bacteria[4,13,14] including penicillin-resistant strains of *Streptococcus pneumoniae*.[12] Structures of complexes of lactivicins with PBP1b[12] reveal that PBP inhibition by LTV/PLTV involves opening of cycloserine and γ-lactone rings. In the PBP1b complexes the ring-derived atoms of LTV and PLTV show a clear structural convergence with those derived from an analogous cephalosporin (cefotaxime) complex,[15] suggesting that the lactivicin and cephalosporin nuclei are related in function.[12]

An important mechanism of resistance to β-lactam antibiotics is via β-lactamases, one set of which employs a nucleophilic serine and operates via nucleophilic catalysis closely related to PBP transpeptidase catalysis. An essential difference between PBPs and serine β-lactamases is that the acyl-enzyme complex formed in β-lactamases undergoes rapid hydrolysis, whereas hydrolysis is much slower in complexes between PBPs and β-lactam antibiotics.

LTV and some synthetic γ-lactam antibiotics can, at least sometimes, be susceptible to β-lactamase hydrolysis.[16] Extensive structure−activity relationship studies have shown that it is possible to reduce the susceptibility of β-lactams to β-lactamase action by appropriate substitution; hence, it may be possible to improve the biomedicinal properties of the lactivicins. Here we report structural analyses on the interaction of LTV and PLTV with the class A serine β-lactamase from *Bacillus licheniformis* BS3. The results reveal that lactivicins react with serine β-lactamases in a closely related manner compared to that in which they react with PBPs and should be useful in studies directed at the development of improved lactivicins that are less susceptible to β-lactamases.

Results and Discussion

We began by investigating whether nondenaturing ESI-MS could be used to detect stable complexes between lactivicins and the BS3 β-lactamase. We did not observe a complex between BS3 and LTV at a 1:1 ratio; when an excess of LTV was used, the quality of the spectra was poor. In contrast, a relatively stable BS3−PLTV complex formed quickly (Figure 2). Time-course analysis indicated that the BS3−PLTV complex was stable for ∼10 min (Supporting Information Figure 1). Although the lifetime of the BS3−PLTV complex is less than that of the PBP1b−PLTV complex, the observation of the

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[a]Abbreviations: PBP, penicillin-binding protein; ESI-MS, electrospray ionization mass spectrometry.

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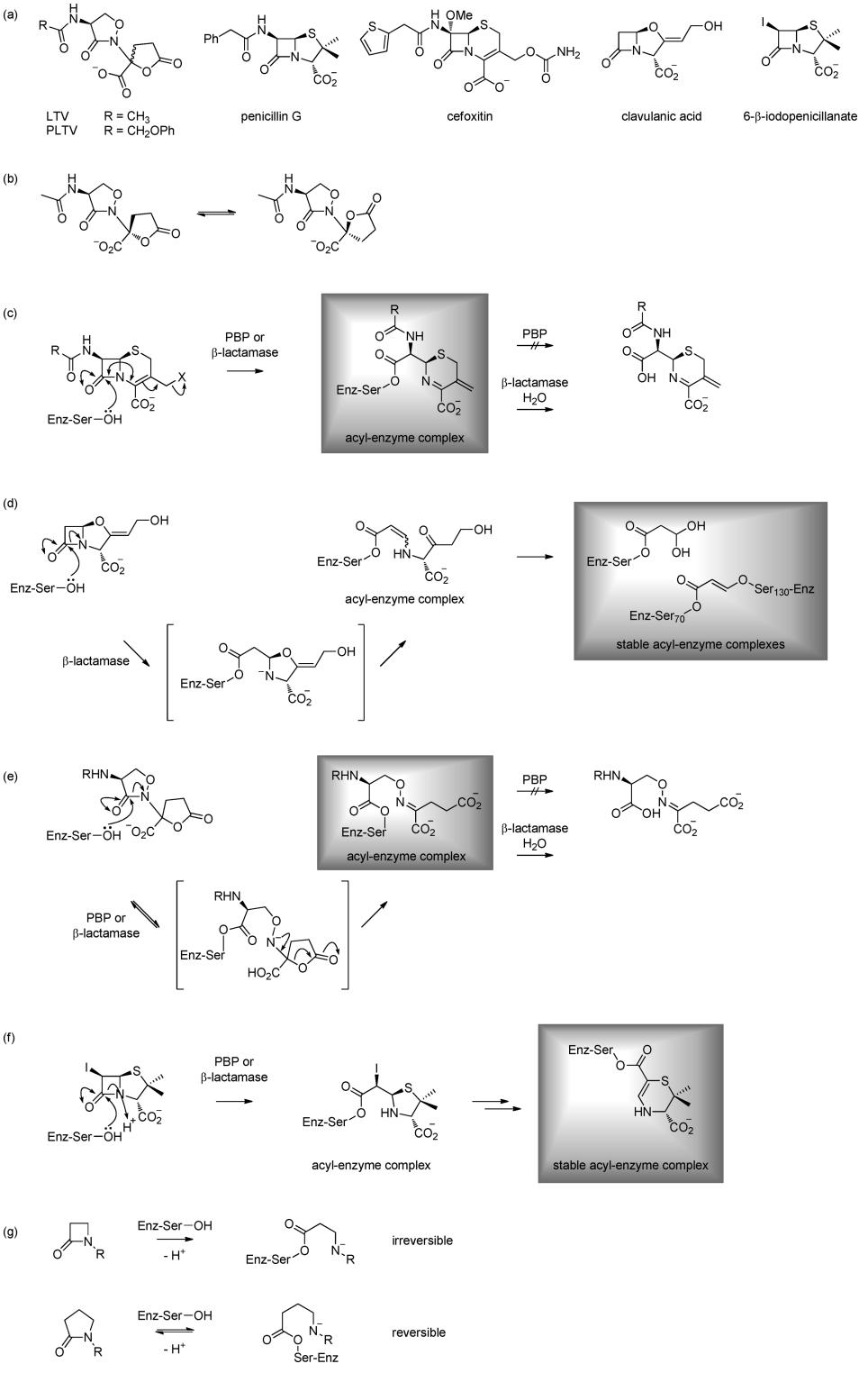

Figure 1. Structures and mechanisms of action of β-lactams and lactivicins: (a) structures of lactivicins and β-lactam antibiotics; (b) epimerization in LTV; (c) mechanism of action of cephalosporin antibiotics showing formation of the hydrolytically stable acyl-enzyme complex; (d) outline mechanism of inactivation of β-lactamases by clavulanic acid; (e) mechanism of action of lactivicins; (f) mechanism of action of 6-β-iodopenicillanate; (g) β-lactams react irreversibly with serine enzymes, whereas γ-lactams react reversibly.

BS3−PLTV complex is likely significant because class A β-lactamases are efficient catalysts.[17]

Work with serine proteases has shown that, whereas β-lactam inhibitors react irreversibly, at least some simple γ-lactams react reversibly.[6,7,10,18,19] In contrast, lactivicins bind irreversibly with PBPs.[15] To investigate the mechanism of reaction of lactivicins with serine β-lactamases and in particular to address the question of whether they react similarly to the manner reported for PBP1b,[11,12] we initiated crystallographic studies using the BS3 β-lactamase. Like other class A β-lactamases, BS3 contains two domains, with the active site at their interface. The active site of Class A β-lactamases contain three motifs: Ser−X−X−Lys, which carries the Ser nucleophile, Ser−X−Asn, positioned in the loop between α4 and α5, and Lys−Thr/Ser−Gly, located on the

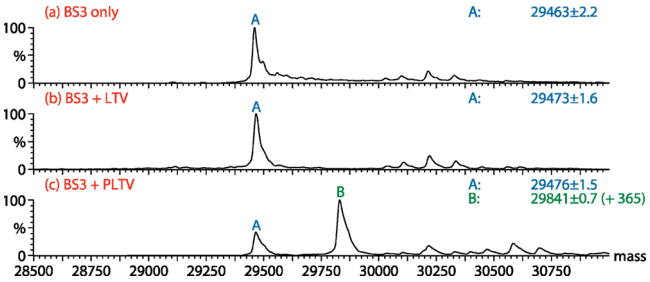

Figure 2. Deconvoluted ESI-MS spectra under nondenaturing conditions (pH 7.0) showing complexes formed between BS3 and lactivicins: (a) BS3 only (10 μM); (b) BS3 (10 μM) + LTV (10 μM); (c) BS3 (10 μM) + PLTV (10 μM). Spectra were obtained after 2 min at 25 °C: peak A, BS3; peak B, BS3−PLTV complex; molecular masses LTV 272 Da and PLTV 364 Da; sample cone voltage 80 V. Mass shifts relative to peak A are indicated in parentheses after peak B masses.

mobile β3 region. The Ser70−Lys73 tetrad (which includes the nucleophilic Ser70) is present at the center of the active site. One face of the active site is defined by the Ser130−X−Asp132 loop and the opposite side by the Lys234−Gly236 triad. These motifs are linked by the "Ω-loop" (Phe165−Glu171).[20−22]

The crystal structure of the BS3−LTV acyl-enzyme complex was solved to 2.1 Å resolution (Figure 3a,b). As with other reported BS3 crystal structures,[22] there are two molecules in each asymmetric unit that were refined independently. The electron density in the BS3−LTV crystal is well-defined. LTV was observed in only one of the monomers, and the following discussion relates to that monomer only.

The structure of the BS3−LTV complex is similar to that of free BS3 (the rms deviation between the equivalent backbone atoms is 0.25 Å) and of the BS3−6-β-iodopenicillanate (BIP) complex (rms deviation between equivalent backbone atoms: 0.18 Å). The conformational change in the Ω-loop is less than that observed for the reported complex with cefoxitin, a cephamycin (the rms deviation between the equivalent backbone atoms of the Phe165−Glu171 residues in the cephamycin complex is 0.49 Å).[22] As in the case of the complex of LTV with PBP1b,[12] the L-cycloserinyl and γ-lactone rings are open. LTV is positioned to form hydrogen bonds with the hydroxyl group of Ser130, with the backbone NH groups of the oxyanion hole-forming residues (Ser70, Ala237) and with the residues (Thr235 and Arg244) that interact with the β-lactam carboxylate. Further hydrogen bonds occur with Ala237, Asn104, Tyr105, and Asn132; the last is conserved in class A β-lactamases.[23] The Val103−Tyr105 loop, which is located at the entrance of the catalytic cleft and which is involved in substrate binding, appears to have moved slightly in the LTV complex compared to its position in the apo protein so that Tyr105 can hydrogen bond with the carboxylate derived from the opening of the γ-lactone ring of LTV. This results in a slight closing of the entrance to the catalytic cleft relative to the apo enzyme (Supporting Information Figure 2).

Comparison of the BS3−LTV complex with the PBP1b−LTV complex reveals striking similarities (Figure 3c,d). The amino acid residues in the active sites of the two enzymes are well conserved. Six of the seven hydrogen bonds that LTV forms with PBP1b are conserved in the complex between LTV and BS3 (the hydrogen bond between LTV and $Thr654_{PBP1b}$ is not present in the BS3 complex, in which $Thr654_{PBP1b}$ is replaced by Ala237). LTV forms three additional hydrogen bonds in the BS3 complex: with Tyr105 (an analogous hydrogen bond is observed in the PBP1b−PLTV complex with $Tyr498_{PBP1b}$; no hydrogen bond with $Tyr498_{PBP1b}$ is observed in the PBP1b−LTV complex); with Asn104; and with Arg244 (the homologous residues in PBP1b are $Met497_{PBP1b}$ and $Met661_{PBP1b}$, respectively, and are not close enough in the crystal structure to form interactions with LTV).

We then compared the BS3−LTV complex with structures of class A β-lactamases and the inhibitor clavulanic acid (Figure 3e,f).[24−29] In the reaction between clavulanic acid and β-lactamases both β-lactam and oxazoline rings are opened (Figure 1d).

In contrast with the LTV/PLTV complexes, those with clavulanic acid do not appear, on the basis of crystallographic analyses, to form many protein−ligand interactions (Figure 3e). Nonetheless, some of the acyl-enzyme complexes formed between clavulanic acid and β-lactamases are stable; they contain an aldehyde or (protonated) imine functional group β to the carbonyl of the acyl-enzyme complexes which is proposed to act as a sink for the "hydrolytic" water molecule, so stabilizing the ester.[27] In the case of LTV/PLTV we have no evidence for hydrolysis of the assigned imine in the acyl-enzyme complex. Further, the acyl-enzyme of the LTV/PLTV complexes is bound in the oxyanion hole; thus, rotation of the carbonyl out of the oxyanion hole cannot account for the acyl-enzyme stability of the LTV/PLTV−BS3 complexes, as proposed for some β-lactam inhibitors of serine proteases.[8,9] The BS3−LTV acyl-enzyme complex might be stabilized, at least in part, by an interaction with the rearranged LTV acetyl group and Asn-104/Asn-132. Overall, deacylation is apparently perturbed in a way somewhat similar to that observed for the complex formed between BS3 and 6-β-iodopenicillinate (BIP)[30] (Figure 1f, Figure 3e,f).

Conclusions

Overall the results described here reveal that lactivicins bind to serine β-lactamases in a very similar manner to that in which they react with PBPs. Notably the results demonstrate that although the reactions of lactivicins and clavulanic acid with β-lactamases involve opening of the β-lactam and adjacent rings, the stability of the resultant acyl-enzyme complexes likely involves different contributions from steric and other factors. In the case of clavulanic acid acyl-enzyme hydrolysis is proposed to be slowed by the provision of an alternative pathway for reaction of potentially "hydroytic" water molecules.[27] However, it appears that the stability of the LTV/PLTV complexes with respect to hydrolysis results primarily from steric blocking of the approach of water molecules in a productive manner to the ester carbonyl, together with interactions with residues that may enable acid/base catalysis. It may be that the longer lifetime of the PBP1b−PLTV complex relative to the BS3−PLTV complex reflects better steric blocking of hydrolysis in the former case. The described structure may be useful in modifying the core lactivicin nucleus to make better PBP/β-lactamase inhibitors.

Experimental Section

Synthesis of LTV and PLTV. LTV and PLTV were synthesized according to a reported procedure.[12]

BS3 Cocrystallization Experiments. The expression and purification of the BS3 enzyme were performed as described previously.[31] Crystals of the BS3−LTV adduct were obtained by cocrystallization. Crystals were grown at 20 °C using the hanging drop vapor diffusion method, with drops containing

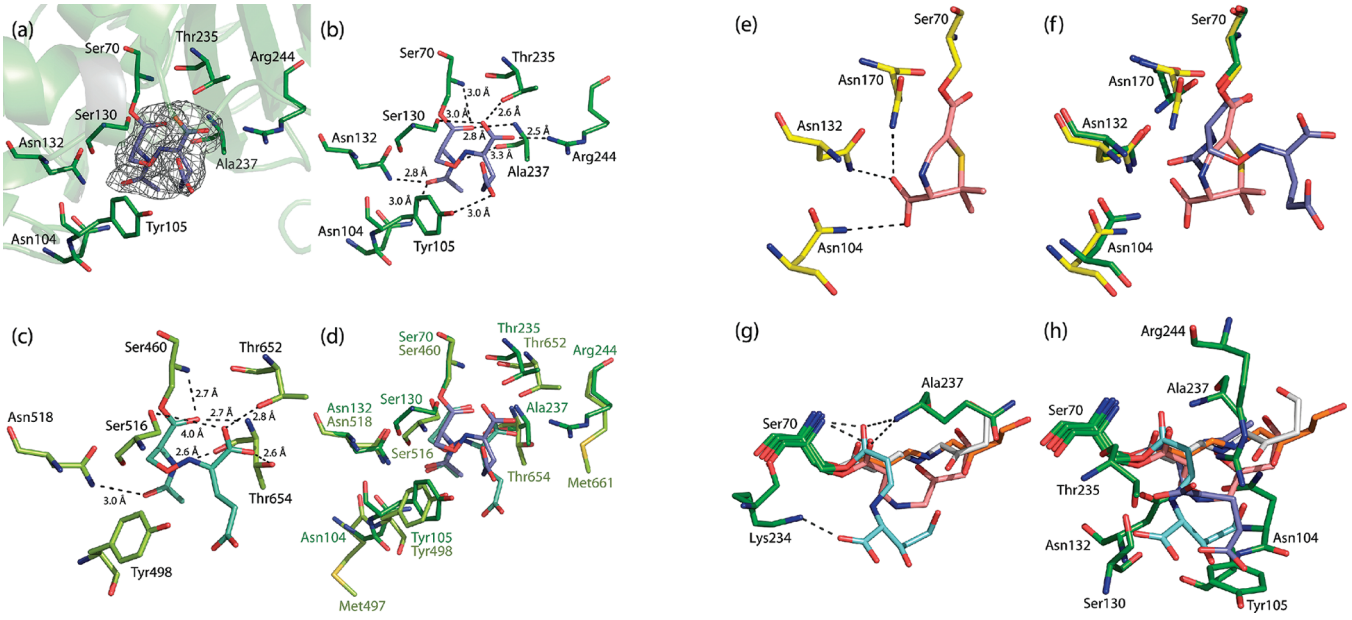

Figure 3. Views from structures of the BS3−LTV complex and comparison with related structures. (a, b) Views from the structure of the BS3−LTV complex (see shaded structure in Figure 1e). Residues involved in hydrogen bonding are in green; LTV is in blue. (a) $F_o - F_c$ map (gray) contoured at 3.0σ, generated around LTV, before the inclusion of LTV in the model and (b) the nine hydrogen bonds (gray dashed lines) between LTV and BS3. (c) View from the structure of the PBP1b−LTV complex, showing seven hydrogen bonds and (d) comparison with the BS3−LTV complex. Active site residues involved in hydrogen bonds are in yellow (PBP1b) and green (BS3). LTV is in turquoise (PBP1b) and blue (BS3). (e) View from the crystal structure of the BS3−BIP complex, showing hydrogen bonds with Asn104, Asn132, and Asn170 (see shaded structure in Figure 1f), and (f) comparison with the BS3−LTV complex. Active site residues are in yellow (BS3−BIP complex) and green (BS3−LTV complex). BIP is in pink; LTV is in blue. (g, h) Comparison of *cis*-/*trans*-enamine complexes resulting from clavulanic acid and LTV complexes with class A β-lactamases. (g) Overlay of crystal structures of β-lactamase−clavulanic acid complexes (see Figure 1d; note both *cis*- and *trans*-enamines have been observed): *cis*-enamine intermediate in complex with *Staphylococcus aureus* PC1 (light blue, PDB code 1BLC); decarboxylated *trans*-enamine intermediate in complex with *S. aureus* PC1 (pink, PDB code 1BLC); decarboxylated *trans*-enamine intermediate in complex with *Klebsiella pneumoniae* SHV-1 (orange, PDB code 2A49); decarboxylated *trans*-enamine intermediate in complex with *Mycobacterium tuberculosis* TB (white, PDB code 3CG5); enzyme residues are in green; interactions with *S. aureus* PC1 intermediates are shown by dotted lines. (h) Comparison of β-lactamase−clavulanic acid complexes with the BS3−LTV complex (LTV is in dark blue; interactions between LTV and BS3 are shown by dotted lines).

2.5 μL of a protein solution (38 mg mL^{-1}), 0.2 μL of LTV (0.1 M), 2 μL of PEG 6000 (8%) in sodium citrate buffer (100 mM, pH 3.4), and equilibrated against 1 mL of the latter solution at 20 °C. A fresh solution of LTV was added 15 min before data collection.

Crystallographic Data Collection, Structure Determination, and Refinement. Data were collected at 100 K on an ADSC Q315r CCD detector at a wavelength of 0.9797 Å on beamline BM30A at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). X-ray analyses were carried out at 100 K after transferring crystals into cryoprotectant containing glycerol (30% v/v) and ammonium sulfate (2.4 M). Intensities were indexed and integrated using MOSFLM.[32] Data were scaled with SCALA of the CCP4 program suite.[32] Refinement used REFMAC,[33] TLS,[34] and COOT.[35] The structure of the BS3−LTV complex was refined to 2.1 Å (Supporting Information Table 1).

Nondenaturing Protein Electrospray Ionization Mass Spectrometry. BS3 was desalted into buffer (ammonium acetate, 15 mM, pH 7.0) using a spin desalting column equilibrated with buffer (Micro Bio-Spin 6; Bio-Rad, Hercules, CA). The concentration was adjusted to 50 μM by dilution. Ligands were dissolved in water at 100 mM, then diluted to 50 μM in buffer.

Automated Nanoelectrospray Ionization Mass Spectrometry. ESI-MS experiments used a Q-Tof Micro mass spectrometer (Micromass, Altrincham, U.K.) interfaced with a NanoMate HD chip-based nanoelectrospray source (Advion Biosciences, Ithaca, NY). The instrument was equipped with a standard Z-spray source block. Clusters of $Na_{(n+1)}I_n$ (1 mg mL^{-1} NaI in 50:50 water/isopropanol) were used for calibration. Typical instrument settings were: spraying voltage 1.70 kV; sample cone voltage 80 V; source temperature 40 °C; acquisition time 15 s; scan time 0.5 s; acquisition range m/z 500−5000. The pressure at the interface between the atmospheric source and the high vacuum region was 6.60 mbar (measured with the roughing pump Pirani gauge) by throttling the pumping line using an Edwards Speedivalve.

Protein and ligand were diluted with buffer in a 96-well plate (final concentrations 10 μM). Aliquots were delivered to the mass spectrometer via the NanoMate (estimated flow rate 100 nL min^{-1}) at intervals of 1, 2, 5, 10, and 20 min after mixing (25 °C).

Spectra were analyzed using MassLynx (Waters, Milford, MA). Raw data were processed by smoothing (mean method; smooth windows 10; number of smooths 2), subtracting the background (polynomial order 15; % below curve 10; tolerance 0.01), and centering peaks (minimum peak width at half-height 2; % centroid top 80).

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Supporting Information Available: MS data for the reaction between BS3 and PLTV, figure showing the movement of the Val103−Tyr105 loop in BS3, and crystallographic data collection and refinement statistics. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sauvage, E.; Kerff, F.; Terrak, M.; Ayala, J. A.; Charlier, P. The Penicillin-Binding Proteins: Structure and Role in Peptidoglycan Biosynthesis. *FEMS Microbiol. Rev.* **2008**, *32*, 234–258.

(2) Zapun, A.; Contreras-Martel, C.; Vernet, T. Penicillin-Binding Proteins and beta-Lactam Resistance. *FEMS Microbiol. Rev.* **2008**, *32*, 361–385.

(3) Harada, S.; Tsubotani, S.; Hida, T.; Ono, H.; Okazaki, H. Structure of Lactivicin, an Antibiotic Having a New Nucleus and Similar Biological Activities to beta-Lactam Antibiotics. *Tetrahedron Lett.* **1986**, *27*, 6229–6232.

(4) Harada, S.; Tsubotani, S.; Hida, T.; Koyama, K.; Kondo, M.; Ono, H. Chemistry of a New Antibiotic—Lactivicin. *Tetrahedron* **1988**, *44*, 6589–6606.

(5) Tipper, D. J.; Strominger, J. L. Mechanism of Action of Penicillins: A Proposal Based on Their Structural Similarity to Acyl-D-alanyl-D-alanine. *Proc. Natl. Acad. Sci. U.S.A.* **1965**, *54*, 1133–1141.

(6) Westwood, N. J.; Claridge, T. D. W.; Edwards, P. N.; Schofield, C. J. Reversible Acylation of Elastase by gamma-Lactam Analogues of beta-Lactam inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2973–2978.

(7) Imming, P.; Klar, B.; Dix, D. Hydrolytic Stability Versus Ring Size in Lactams: Implications for the Development of Lactam Antibiotics and Other Serine Protease Inhibitors. *J. Med. Chem.* **2000**, *43*, 4328–4331.

(8) Wilmouth, R. C.; Westwood, N. J.; Anderson, K.; Brownlee, W.; Claridge, T. D. W.; Clifton, I. J.; Pritchard, G. J.; Aplin, R. T.; Schofield, C. J. Inhibition of Elastase by *N*-Sulfonylaryl beta-Lactams: Anatomy of a Stable Acyl-Enzyme Complex. *Biochemistry* **1998**, *37*, 17506–17513.

(9) Wilmouth, R. C.; Kassamally, S.; Westwood, N. J.; Sheppard, R. J.; Claridge, T. D. W.; Aplin, R. T.; Wright, P. A.; Pritchard, G. J.; Schofield, C. J. Mechanistic Insights into the Inhibition of Serine Proteases by Monocyclic Lactams. *Biochemistry* **1999**, *38*, 7989–7998.

(10) Wright, P. A.; Wilmouth, R. C.; Clifton, I. J.; Schofield, C. J. "pH-Jump" Crystallographic Analyses of gamma-Lactam Porcine Pancreatic Elastase Complexes. *Biochem. J.* **2000**, *351* (Part 2), 335–340.

(11) Baldwin, J. E.; Lynch, G. P.; Pitlik, J. Gamma-Lactam Analogues of beta-Lactam Antibiotics. *J. Antibiot.* **1991**, *44*, 1–24.

(12) Macheboeuf, P.; Fischer, D. S.; Brown, T., Jr.; Zervosen, A.; Luxen, A.; Joris, B.; Dessen, A.; Schofield, C. J. Structural and Mechanistic Basis of Penicillin-Binding Protein Inhibition by Lactivicins. *Nat. Chem. Biol.* **2007**, *3*, 565–569.

(13) Nozaki, Y.; Katayama, N.; Ono, H.; Tsubotani, S.; Harada, S.; Okazaki, H.; Nakao, Y. Binding of a Non-beta-Lactam Antibiotic to Penicillin-Binding Proteins. *Nature* **1987**, *325*, 179–180.

(14) Tamura, N.; Matsushita, Y.; Kawano, Y.; Yoshioka, K. Synthesis and Antibacterial Activity of Lactivicin Derivatives. *Chem. Pharm. Bull.* **1990**, *38*, 116–122.

(15) Macheboeuf, P.; Di Guilmi, A. M.; Job, V.; Vernet, T.; Dideberg, O.; Dessen, A. Active Site Restructuring Regulates Ligand Recognition in Class A Penicillin-Binding Proteins. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 577–582.

(16) Nozaki, Y.; Katayama, N.; Harada, S.; Ono, H.; Okazaki, H. Lactivicin, a Naturally-Occurring Non-beta-Lactam Antibiotic Having beta-Lactam-like Action. Biological Activities and Mode of Action. *J. Antibiot.* **1989**, *42*, 84–93.

(17) Fisher, J. F.; Meroueh, S. O.; Mobashery, S. Bacterial Resistance to beta-Lactam Antibiotics: Compelling Opportunism, Compelling Opportunity. *Chem. Rev.* **2005**, *105*, 395–424.

(18) Wilmouth, R. C.; Clifton, I. J.; Robinson, C. V.; Roach, P. L.; Aplin, R. T.; Westwood, N. J.; Hajdu, J.; Schofield, C. J. Structure of a Specific Acyl-Enzyme Complex formed between beta-Casomorphin-7 and Porcine Pancreatic Elastase. *Nat. Struct. Biol.* **1997**, *4*, 456–462.

(19) Wilmouth, R. C. Structural Studies on the Mechanism and Inhibition of Elastase. D.Phil. Thesis, University of Oxford, Oxford, U.K., **1998**.

(20) Knox, J. R.; Moews, P. C. beta-Lactamase of *Bacillus licheniformis* 749/C. Refinement at 2 Å Resolution and Analysis of Hydration. *J. Mol. Biol.* **1991**, *220*, 435–455.

(21) Leszczynski, J. F.; Rose, G. D. Loops in Globular Proteins. A Novel Category of Secondary Structure. *Science* **1986**, *234*, 849–855.

(22) Fonze, E.; Vanhove, M.; Dive, G.; Sauvage, E.; Frere, J. M.; Charlier, P. Crystal Structures of the *Bacillus licheniformis* BS3 Class A beta-Lactamase and of the Acyl-Enzyme Adduct Formed with Cefoxitin. *Biochemistry* **2002**, *41*, 1877–1885.

(23) Hermann, J. C.; Hensen, C.; Ridder, L.; Mulholland, A. J.; Holtje, H. D. Mechanisms of Antibiotic Resistance: QM/MM Modeling of the Acylation Reaction of a Class A beta-Lactamase with Benzylpenicillin. *J. Am. Chem. Soc.* **2005**, *127*, 4454–4465.

(24) Chen, C. C. H.; Herzberg, O. Inhibition of beta-Lactamase by Clavulanate: Trapped Intermediates in Cryocrystallographic Studies. *J. Mol. Biol.* **1992**, *224*, 1103–1113.

(25) Padayatti, P. S.; Helfand, M. S.; Totir, M. A.; Carey, M. P.; Carey, P. R.; Bonomo, R. A.; van den Akker, F. High Resolution Crystal Structures of the trans-Enamine Intermediates Formed by Sulbactam and Clavulanic Acid and E166A SHV-1 beta-Lactamase. *J. Biol. Chem.* **2005**, *280*, 34900–34907.

(26) Tremblay, L. W.; Hugonnet, J. E.; Blanchard, J. S. Structure of the Covalent Adduct formed between *Mycobacterium tuberculosis* beta-Lactamase and Clavulanate. *Biochemistry* **2008**, *47*, 5312–5316.

(27) Brown, R. P.; Aplin, R. T.; Schofield, C. J. Inhibition of TEM-2 beta-Lactamase from *Escherichia coli* by Clavulanic Acid: Observation of Intermediates by Electrospray Ionization Mass Spectrometry. *Biochemistry* **1996**, *35*, 12421–12432.

(28) Sulton, D.; Pagan-Rodriguez, D.; Zhou, X.; Liu, Y. D.; Hujer, A. M.; Bethel, C. R.; Helfand, M. S.; Thomson, J. M.; Anderson, V. E.; Buynak, J. D.; Ng, L. M.; Bonomo, R. A. Clavulanic Acid Inactivation of SHV-1 and the Inhibitor-Resistant S130G SHV-1 beta-Lactamase. Insights into the Mechanism of Inhibition. *J. Biol. Chem.* **2005**, *280*, 35528–35536.

(29) Hugonnet, J. E.; Blanchard, J. S. Irreversible Inhibition of the *Mycobacterium tuberculosis* beta-Lactamase by Clavulanate. *Biochemistry* **2007**, *46*, 11998–12004.

(30) Sauvage, E.; Zervosen, A.; Dive, G.; Herman, R.; Amoroso, A.; Joris, B.; Fonze, E.; Pratt, R. F.; Luxen, A.; Charlier, P.; Kerff, F. Structural Basis of the Inhibition of Class beta-Lactamases and Penicillin-Binding Proteins by 6-beta-Iodopenicillanate. *J. Am. Chem. Soc.* **2009**, *131*, 15262–15269.

(31) Ledent, P.; Duez, C.; Vanhove, M.; Lejeune, A.; Fonze, E.; Charlier, P.; Rhazi-Filali, F.; Thamm, I.; Guillaume, G.; Samyn, B.; Devreese, B.; Van Beeumen, J.; Lamotte-Brasseur, J.; Frere, J.-M. Unexpected Influence of a C-Terminal-Fused His-Tag on the Processing of an Enzyme and on the Kinetic and Folding Parameters. *FEBS Lett.* **1997**, *413*, 194–196.

(32) The CCP4 suite: programs for protein crystallography. *Acta Crystallogr., Sect. D.: Biol. Crystallogr.* **1994**, *50*, 760−763.

(33) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. Refinement of Macromolecular Structures by the Maximum-Likelihood Method. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1997**, *53*, 240–255.

(34) Painter, J.; Merritt, E. A. Optimal Description of a Protein Structure in Terms of Multiple Groups Undergoing TLS motion. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2006**, *62*, 439–450.

(35) Emsley, P.; Cowtan, K. Coot: Model-Building Tools for Molecular Graphics. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2004**, *60*, 2126–2132.