

Co-opting the Cell Wall in Fighting Methicillin-Resistant *Staphylococcus aureus*: Potent Inhibition of PBP 2a by Two Anti-MRSA β -Lactam Antibiotics

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The second generation of penicillins (including methicillin) was introduced to the clinic in 1959, and by 1961 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) had emerged.¹ β -Lactam antibiotics were the standard treatment for staphylococcal infections prior to the emergence of MRSA. MRSA is now a global health threat,² and community-acquired MRSA has become a significant challenge in the clinic.²

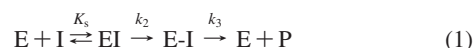
β -Lactams, which include penicillins, cephalosporins, and carbapenems, target the transpeptidase activity of penicillin-binding proteins (PBPs) involved in cell wall biosynthesis. The molecular basis for resistance to β -lactams in MRSA is complex and a full discussion is outside of the scope of this report; however, the key component is the presence in MRSA isolates of a gene, *mecA*, absent in methicillin susceptible strains, which encodes a novel PBP (PBP 2a).^{3–5} Whereas other *S. aureus* PBP transpeptidases are susceptible to inhibition by β -lactams, the strains that possess PBP 2a are able to perform the critical cell wall cross-linking reaction even in the presence of β -lactam antibiotics.^{3,6,7} PBP 2a escapes inhibition by β -lactams because they fail to readily gain access to the active site of this enzyme.

The crystal structure of PBP 2a reveals it to have a closed active site.⁸ This is a paradox, as the enzyme must bind to the peptidoglycan to carry out the cross-linking reaction. We previously disclosed that the two strands of peptidoglycan occupy in excess of 1000 Å³ of volume,⁹ hence the X-ray structure does not reveal how peptidoglycan could bind the active site. We presented evidence that interactions of PBP 2a with the peptidoglycan at an allosteric site trigger a conformational change that leads to accessibility to the active site, an event that should play a critical role in the physiological function of this important enzyme.^{10,11}

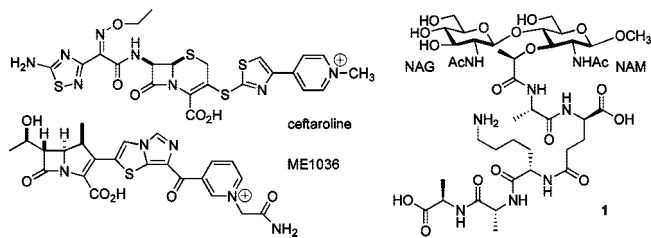
In this report we characterize the mode of action of two new anti-MRSA β -lactam antibiotics from Cerexa, Inc., ceftaroline (CPT) a cephalosporin and ME1036 (ME) a carbapenem, which are currently undergoing clinical trials. Both compounds are broad-spectrum antibiotics, but their activities against MRSA and multidrug resistant streptococci are especially noteworthy. In contrast to the commercially available β -lactam antibiotics, CPT and ME are exquisite inhibitors of PBP 2a of MRSA.

The backbone of the peptidoglycan is made up of repeating units of *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM). A peptide stem—terminating with two D-Ala residues and typically a pentapeptide—is appended to the NAM unit. Compound **1** is a synthetic surrogate for the repeat unit of the peptidoglycan, whose synthesis was reported recently.¹² The penultimate D-Ala residue of the peptidoglycan acylates an active site serine in PBP 2a resulting in an acyl–enzyme species, with concomitant departure of the terminal D-Ala as the leaving group. This acyl–enzyme species reacts with the second strand of peptidoglycan in the course of the cross-linking reaction. A similar two-step process is invoked for inhibition of transpeptidases by β -lactam antibiotics (eq 1). The β -lactam antibiotic mimics the terminal acyl-D-Ala-D-Ala of the

peptidoglycan and forms a noncovalent complex (EI) with the enzyme, which leads to the acylation of the active site serine (E-I). The acyl–enzyme species enjoys extreme longevity, hence it inhibits the PBP irreversibly. In some instances, the E–I complex undergoes deacylation, but the rate of such deacylation is sufficiently slow that the bacterium is deprived of the enzyme function and dies.^{7,13}



As indicated earlier, PBP 2a is not effectively inhibited by the commercially available β -lactams. This is reflected by dissociation constants (K_S) in the high micromolar range as shown for nitrocefin (a cephalosporin) and imipenem (a carbapenem) (Table 1), among others.⁷ The acylation proceeds slowly (k_2), and the acyl–enzyme species in each case does undergo deacylation, albeit very slowly. The second-order rate constants for the encounter between PBP 2a and these two antibiotics (k_2/K_S) are $<20 \text{ M}^{-1} \text{ s}^{-1}$, indicating a very inefficient reaction, as has been observed with other β -lactams.⁷ In contrast, CPT and ME exhibit K_S values as much as 3 orders of magnitude smaller and somewhat larger k_2 , compared to nitrocefin and imipenem. The k_2/K_S for these antibiotics was $>10^4 \text{ M}^{-1} \text{ s}^{-1}$, indicating efficient formation of the inhibitory acyl–enzyme intermediate. Additionally, we were not able to detect any deacylation of the CPT or ME acyl–enzyme species, while monitoring for four days. Previously it was reported that upon acylation by antibiotics PBP 2a precipitates,¹³ or alternatively an oligomerization of the acylated protein could explain the sheltering of the acyl–enzyme species to impart longevity. Neither of these two possibilities is applicable in the cases of CPT or ME binding to PBP 2a, as detailed in Supporting Information. These antibiotics bind with high affinity to PBP 2a and inhibit its activity, thus disrupting cell wall biosynthesis.



Compound **1** is a minimal surrogate for the bacterial cell wall. The lysine in *S. aureus* cell wall is modified on the side chain by a pentaglycyl moiety. We intentionally left the pentaglycyl moiety out of the structure, so it would not serve as a substrate for the enzyme. We have documented that compound **1** binds in a saturable manner to the allosteric site in PBP 2a with the dissociation constant of $1.2 \pm 0.2 \text{ mM}$.¹⁰ As the amounts of compound **1** increases in the assay mixture, the values for k_2 increase and the values for K_S decrease (Table 1). The consequence is enhancement of k_2/K_S in

Table 1. Kinetic Parameters for Interactions of Antibiotics with PBP 2a in the Presence and Absence of Compound 1.

antibiotic	1 (mM)	k_2 (s ⁻¹) × 10 ³	K_s (nM)	k_2/K_s (M ⁻¹ s ⁻¹)	k_3 (s ⁻¹) × 10 ⁶
Nitrocefin ^a	0	3.7 ± 0.3	(190 ± 25) × 10 ³	19 ± 3	7.2 ± 0.1
	0.5	15 ± 2	(135 ± 12) × 10 ³	105 ± 15	35 ± 10
	2.0	35 ± 4	(70 ± 14) × 10 ³	515 ± 100	60 ± 4
Ceftaroline ^b	0	8 ± 0.3	340 ± 40	(2.4 ± 0.1) × 10 ⁴	— ^c
	0.5	10 ± 0.3	210 ± 10	(4.7 ± 0.1) × 10 ⁴	
	2.0	13 ± 0.4	200 ± 10	(6.5 ± 0.1) × 10 ⁴	
Imipenem ^a	0	1.7 ± 0.1	(603 ± 93) × 10 ³	2.8 ± 0.4	3.3 ± 0.3
	0.5	12 ± 1	(270 ± 30) × 10 ³	44 ± 1	40 ±
	2.0	17 ± 2	(230 ± 32) × 10 ³	74 ± 2	
ME1036 ^d	0	8 ± 0.2	250 ± 20	(3.2 ± 0.1) × 10 ⁴	— ^c
	0.5	15 ± 1	215 ± 15	(7.0 ± 0.1) × 10 ⁴	
	2.0	18 ± 1	170 ± 10	(1.1 ± 0.1) × 10 ⁵	

^a The kinetic values for nitrocefin and imipenem in the presence and absence of compound 1 have been reported earlier.⁷ ^b K_i of 330 ± 40 nM and IC₅₀ of 300 ± 40 nM were evaluated for CPT. ^c No deacylation noted during 96 h of monitoring. ^d K_i of 260 ± 10 nM and IC₅₀ of 260 ± 10 nM were evaluated for ME.

the presence of increasing amounts of the cell wall surrogate. These observations are consistent with interactions of the cell wall surrogate at the allosteric site to stimulate the opening of the active site. We see this trend consistently for all four β -lactam antibiotics.

It is evident that the cell wall surrogate enhances the ability of the antibiotics to inhibit PBP 2a. It is likely that in the course of active bacterial growth, CPT and ME are able to co-opt the allosteric site, thus facilitating subsequent interaction of the inhibitors at the transpeptidase active site of PBP 2a, since k_2/K_s approaches the clinically useful value of 10⁴–10⁵ M⁻¹ s⁻¹ under conditions that the allosteric site is triggered. We hasten to add that a direct competition experiment between the cell wall surrogate and the antibiotics was not performed.

Binding of the cell wall at the allosteric site entails a conformational change that opens up the active site.¹⁰ We document by circular dichroism (CD) spectra of PBP 2a in the presence of CPT and ME that a similar conformational change is discernible (Supporting Information). The change in the helicity of the protein as measured from the CD minima at 208 and 222 nm were monitored as a function of time, from which the observed first-order rate constants (k_{obs}) of 0.015 ± 0.004 and 0.006 ± 0.001 s⁻¹, respectively for antibiotics CPT and ME, were evaluated. We note that these values are comparable to the k_2 values for CPT and ME in the absence of the cell wall surrogate (Table 1), hence the conformational change is a likely precursor to the active site acylation, an event that these antibiotics would appear to be able to trigger by themselves. This process is rapid with CPT and ME, and not seen with typical known β -lactam antibiotics.⁷ Hence, these antibiotics both trigger the allosteric site by themselves as well as work in concert with cell wall in inhibiting PBP 2a.

Ceftaroline and ME1036 are broad-spectrum anti-MRSA antibiotics.^{14,15} We have confirmed the antimicrobial activities of CPT and ME with strains from our own collection (Supporting Information), including *S. aureus* isolates ATCC 29213 (Clinical and Laboratory Standards Institute (CLSI) susceptible standard), vancomycin-resistant MRSA clinical isolates VRS1 and VRS2, and linezolid-resistant MRSA isolates NRS119 and NRS120. Linezolid and vancomycin are commonly used in treatment of MRSA infections, although documented resistance to these agents is worrisome. CPT and ME were exquisitely active against these

problematic strains with minimum inhibitory concentrations (MIC) of 0.25–2 $\mu\text{g/mL}$ (Supporting Information).

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Supporting Information Available: Experimental procedures of susceptibility testing, kinetics, and circular dichroism. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Braaten, D. *Nat. Med.* **2007**, *13*, 522–523. (b) Lowy, F. D. *Nat. Med.* **2007**, *13*, 1418–1420.
- (2) Jarvis, W. R.; Schlosser, J.; Chinn, R. Y.; Tweeten, S.; Jackson, M. *Am. J. Infect. Control* **2007**, *35*, 631–637. Zetola, N.; Francis, J. S.; Nuermberger, E. L.; Bishai, W. R. *Lancet Infect. Dis.* **2005**, *5*, 275–286.
- (3) Fuda, C. C. S.; Fisher, J. F.; Mobashery, S. *Cell. Mol. Life Sci.* **2005**, *62*, 2617–2633.
- (4) Kajita, E.; Okano, J. T.; Bodine, E. N.; Layne, S. P.; Blower, S. *Nat. Rev. Microbiol.* **2007**, *5*, 700–709.
- (5) Mwangi, M. M.; Wu, S. W.; Zhou, Y.; Sieradzki, K.; de Lencastre, H.; Richardson, P.; Bruce, D.; Rubin, E.; Myers, E.; Siggia, E. D.; Tomasz, A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 9451–9456.
- (6) Fisher, J. F.; Meroueh, S. O.; Mobashery, S. *Chem. Rev.* **2005**, *105*, 395–424.
- (7) Fuda, C.; Suvorov, M.; Vakulenko, S. B.; Mobashery, S. *J. Biol. Chem.* **2004**, *279*, 40802–40806.
- (8) Lim, D.; Strynadka, N. C. *J. Nat. Struct. Biol.* **2002**, *9*, 870–876.
- (9) Lee, W.; McDonough, M. A.; Kotra, L. P.; Li, Z.-H.; Silvaggi, N. R.; Takeda, Y.; Kelly, J. A.; Mobashery, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 1427–1431.
- (10) Fuda, C.; Heseck, D.; Lee, M.; Morio, K.-I.; Nowak, T.; Mobashery, S. *J. Am. Chem. Soc.* **2005**, *127*, 2056–2057.
- (11) Fuda, C.; Heseck, D.; Lee, M.; Heilmayer, W.; Novak, R.; Vakulenko, S. B.; Mobashery, S. *J. Biol. Chem.* **2006**, *281*, 10035–10041.
- (12) Heseck, D.; Suvorov, M.; Morio, K.-I.; Lee, M.; Brown, S.; Vakulenko, S. B.; Mobashery, S. *J. Org. Chem.* **2004**, *69*, 778–784.
- (13) Graves-Woodward, K.; Pratt, R. F. *Biochem. J.* **1998**, *332*, 755–761.
- (14) Kurazono, M.; Ida, T.; Yamada, K.; Hirai, Y.; Maruyama, T.; Shitara, E.; Yonezawa, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 2831–2837.
- (15) Sader, H. S.; Fritsche, T. R.; Kaniga, K.; Ge, Y.; Jones, R. N. *Antimicrob. Agents Chemother.* **2005**, *49*, 3501–3512.

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