Structural and Mechanistic Basis for Drug Resistance Mutations in Altering the Specificity of CTX-M β-lactamases

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β-lactam antibiotics are the most often used class of antimicrobials. β-lactamases provide bacterial resistance by catalyzing the hydrolysis of the β-lactam ring to create an ineffective drug. Class A β-lactamases are serine active-site hydrolases that readily hydrolyze penicillins and older cephalosporins. Oxyimino-cephalosporins, such as cefotaxime and ceftazidime, however, are poor substrates for many class A enzymes and were introduced, in part, to circumvent β-lactamase-mediated resistance. In recent years, CTX-M β-lactamases have emerged as the most widespread extended spectrum β-lactamases (ESBLs) in Gram-negative bacteria. The CTX-M enzymes rapidly hydrolyze cefotaxime; however, ceftazidime, is poorly hydrolyzed. The P167S and D240G substitutions in CTX-M, however, have been identified in clinically resistant bacteria and increase the hydrolysis of ceftazidime by 10-fold. In addition, the N106S substitution is sometimes associated with the P167S and D240G substitutions and enhances resistance. X-ray crystallography and biochemical studies show that the P167S and D240G substitutions result in expansion of the active site to accommodate ceftazidime, thereby leading to increased hydrolysis. However, the substitutions also reduce enzyme stability and *in vivo* expression levels. N106S by itself decreases the hydrolysis of cefotaxime and ceftazidime but exhibits increased stability. In a N106S/D240G double mutant, the increase in stability and enzyme expression levels provided by N106S outweighs its negative catalytic effects, resulting in higher resistance levels to both cefotaxime and ceftazidime. Analysis of the X-ray structures of the N106S apo-enzyme and the cefotaxime complexes of S70G/N106S and S70G/N106S/D240G mutants, identified conformational changes in an active site loop that can account for alterations in enzyme stability and substrate specificity. In the mutants containing N106S, the Asn104 residue moves out of the active site due to the conformational change thereby causing a loss of an interaction with cefotaxime substrate, consistent with decreased catalytic activity. The re-orientation of the loop, however, also results in the relief of dihedral strain which is consistent with the increased stability resulting from the N106S mutation.