Active-Site Protonation States in an Acyl-Enzyme Intermediate of a Class A β-Lactamase

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Antimicrobial resistance is a threat to the effective treatment of a growing number of infectious diseases. One worrisome example of antimicrobial resistance is the production of enzymes that hydrolyze β-lactam-containing antibiotics. These β-lactamases have been classified into four distinct groups (A-D). Class B β-lactamases are metalloenzymes that use a water molecule bound to a zinc ion to hydrolyze the β-lactam ring. Classes A, C and D enzymes utilize a catalytic serine amino acid residue to open the β-lactam ring by formation and release of an acyl-enzyme intermediate. Toho-1 β-lactamase is a class A β-lactamase composed of 262 amino acid residues arranged in two highly conserved domains (α/β and α). The active site cavity is formed at the domain interface and the conserved residues Ser 70, Lys 73, Ser 130, and Glu 166 play a role in the catalytic mechanism. We have used neutron and X-ray crystallography to directly visualize the protonation states of these key residues in an acyl-enzyme intermediate with a selection of antibiotics. Our data provides new insights on hydrogen bonding patterns in the active site and several mechanistic details on the hydrolysis of β-lactam antibiotics.