Structure, Function, and Inhibition of b-lactamases

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Introduction

β-lactamase is the major enzyme that encodes resistance to β-lactam antibiotics, including penicillins and cephalosporins. We are investigating the structures of two major classes of β-lactamase enzymes, AmpC and TEM-1, in both WT and mutant forms and in both apo- and complexed structures to investigate their mechanism. We are also investigating novel inhibitors of these enzymes that have shown promise in reversing the resistance encoded by the enzymes and have shown efficacy in cell culture.

Methods and Materials

TEM-1 and AmpC are crystallized out of hanging drops of 1.2-1.8 M phosphate buffer, at 7.5-9.0 pH. Crystals grow at room temperature in 3 d to 1 mo. Crystals are frozen by typical cryogenic techniques. Data collection typically involves collecting between 90° and 180° of diffraction, swinging between 0.5° and 1° oscillations. Depending on how well the crystals diffract and whether a low- and high-resolution scan are necessary, data collection times vary from 1 to 3 h.

Results

We have determined approximately 20 structures of AmpC and approximately 10 structures of TEM-1 in multiple forms (mutants, different complexes). These results have led to a number of publications, all of which acknowledge the APS [1-12].

Discussion

These structural studies will allow us to improve these inhibitors in a structure-based design cycle. Several of our enzymes diffract to genuine atomic resolution (up to 0.85 Å), and it is clear that such data could be collected only at a third-generation synchrotron like the APS.

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