Structures of Two Kinetic Intermediates Reveal Species-Specificity of Penicillin-Binding Proteins

M.A. McDonough[1], J.W. Anderson[2], N.R. Silvaggi[1], R.F. Pratt[2], J.R. Knox[1], and J.A. Kelly[1]

[1]Department of Molecular and Cell Biology and Institute for Materials Science, University of Connecticut; [2] Department of Chemistry, Wesleyan University

β-lactam antibiotics are the most commonly used line of defense against bacterial infections, but the efficacy of antibiotics is threatened by the constant emergence of resistant bacteria. Scientists from the University of Connecticut in Storrs, and Wesleyan University in Middletown, Connecticut, have obtained the first crystallographic structures of complexes involving a peptide substrate and a penicillin-binding protein, which is the target of β-lactams. This research provides structural and mechanistic information to aid drug design efforts aimed at fighting β-lactam-resistant infections.

Authors (from left): Michael A. McDonough, Judith A. Kelly, and Nicholas R. Silvaggi

Most bacteria synthesize a rigid peptidoglycan cell wall that provides the characteristic cell shape and prevents destruction by osmotic lysis. Peptidoglycan is a polymer of carbohydrate units with branching peptide chains that usually terminate in D-alanyl-D-alanine. The final step in cell wall biosynthesis is a chemical reaction forming peptide bridges between neighboring peptidoglycan strands. The reaction is catalyzed by penicillin-binding proteins (PBPs), which also catalyze a carboxypeptidase reaction (**Figure 1**) that helps to maintain the proper degree of cell wall cross-linking.

β-lactam antibiotics are the most commonly used line of defense against bacterial infections. These antibiotics inhibit PBPs because they mimic the D-alanyl-D-alanine portion of peptidoglycan and form very long-lived covalent complexes, so that growing bacteria are unable to cross-link their cell walls and are vulnerable to cell lysis. Our research provides structural and mechanistic information to aid drug design efforts aimed at fighting β-lactam resistant infections.

By using x-rays at beamline X12C, we have obtained the first crystallographic structures of kinetic intermediates involving a peptide substrate and a PBP, the D-alanyl-D-alanine carboxypeptidase/transpeptidase from the bacterium Streptomyces R61. This enzyme is a model for membrane-bound PBPs that do the majority of cell wall synthesis.

Two members of our team, Rex F. Pratt and John W. Anderson, have recently identified a tetrapeptide substrate (**Figure 2**) that proved to be the most specific substrate yet for the R61 enzyme. This tetrapeptide corresponds exactly to a portion of Streptomyces peptidoglycan.

By soaking chemically cross-linked, inactive R61 crystals in a solution containing the tetrapeptide substrate, we were able to trap the noncovalent enzyme-substrate (ES) complex at 1.9 angstrom resolution. By performing the same experiment using active enzyme crystals, we determined the structure of the enzyme-products (EPs) complex with a 1.25 angstrom resolution.

With the ES structure (**Figure 3A**), we showed that the catalytic oxygen of the active serine, S62, is positioned 2.8 angstroms from the carbonyl carbon of the scissile peptide bond. Numerous noncovalent interactions

Beamline
X12C

Funding
State of Connecticut Critical Technologies Program in Drug Design; Connecticut Innovations, Inc.; National Institutes of Health; Pfizer

Publication
M.A. McDonough, J.W. Anderson, N.R. Silvaggi, R.F. Pratt, J.R. Knox, J.A. Kelly, "Structures of Two Kinetic Intermediates Reveal Species Specificity of Penicillin-Binding Proteins," *Journal of Molecular Biology,* 322, 111-122 (2002).

Contact information
Judith A. Kelly, Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT

Email: Judith.Kelly@uconn.edu

between the enzyme and the tetrapeptide substrate explain the extraordinary specificity of this substrate. Indeed, the part of the substrate that changes from one species to another is bound tightly in a subsite of the active site. Presumably, PBPs of other species have analogous subsites complementary to their peptidoglycan. These species-specificity subsites could be exploited for species-specific antibiotics.

Figure 1. The carboxypeptidase reaction of the R61 DD-peptidase, where E is free enzyme, S is the substrate, ES is a non-covalent complex, ES* is a covalent complex, EP is a non-covalent complex between the enzyme and the hydrolyzed peptide, and P represents the free peptide product.

The EPs structure (**Figure 3B**) highlights conformational changes of both the tripeptide product and active site residues that ultimately lead to the ejection of products from the active site. The terminal D-alanine rotates 110 degrees about the nitrogen-alpha carbon bond, placing the carboxyl group (COOH) in a relatively hydrophobic environment, while the methyl (CH_3) side chain is moved into the plane of a hydrogen bond involving a conserved asparagine residue, weakening this interaction. Further, the main chain of threonine 301 is forced into a highly strained conformation. All these factors are likely to contribute to the destabilization of product binding.

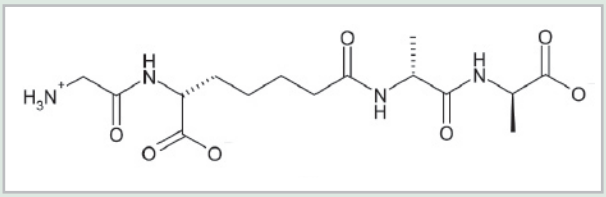

Figure 2. Tetrapeptide substrate that proved to be the most specific substrate yet for the R61 enzyme.

The two structures (ES and EPs) reveal detailed structural information about kinetic intermediates, providing insight into the catalytic mechanism of PBPs and critical information for future antibiotic design efforts.

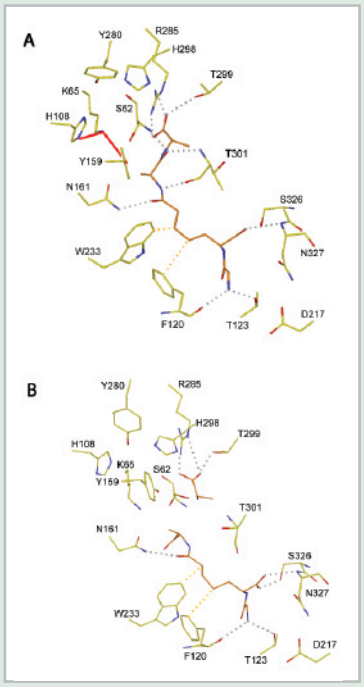

Figure 3. (A) The ES complex. Active site residues are yellow, the tetrapeptide substrate is orange. Hydrogen bonds are shown as gray dotted lines, hydrophobic interactions as yellow dotted lines, and the inactivating cross-links as solid red lines. (B) The EPs complex. Both the tripeptide product and free D-alanine are orange. The figure was produced by using XTALVIEW and Raster3D.