Structural Basis of $\beta$-Lactam Resistance in Methicillin-Resistant Strains of “Superbug” Revealed

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Some strains of the bacterium Staphylococcus aureus have developed resistance to multiple antibiotics and have emerged as major pathogens in hospitals worldwide. Resistance of these “superbugs” to the clinically important $\beta$-lactam class of antibiotics is mediated by a bacterial enzyme called penicillin-binding protein 2a (PBP2a). This enzyme catalyzes the formation of the bacterial cell wall, a process inhibited by $\beta$-lactam, thus weakening the bacterium, and ultimately killing it. The three-dimensional structure of PBP2a, determined by researchers at the University of British Columbia in Vancouver, Canada, reveals the structural basis of the resistance of S. aureus to $\beta$-lactam, and provides insights for the design of more effective inhibitors of the bacterium.

The introduction of penicillin in the 1940s quickly selected for resistant strains of the bacterium Staphylococcus aureus that produced penicillinase, an enzyme that hydrolyzes $\beta$-lactam antibiotics, the most commonly used line of defense against bacterial infections. To counter these resistant bacterial strains, methicillin, a semisynthetic penicillinase-resistant penicillin derivative, was subsequently introduced. Resistance to methicillin began to appear just one year after its introduction and has since spread to hospitals world-wide with alarmingly high prevalence of these strains, called methicillin-resistant strains of Staphylococcus aureus (MRSA), among clinical isolates in the United States and in a number of Asian and European countries. As MRSA strains are resistant to other classes of antibiotics, vancomycin has often been the treatment of choice against MRSA infections. But the appearance of vancomycin resistance in MRSA clinical isolates – first in Japan, and then in other countries, including the United States in recent months – underlines an urgent need for novel antibiotics.

$\beta$-lactams act as substrate analogs of bacterial enzymes called penicillin-binding proteins (PBPs) that catalyze the formation of peptide cross-links in the bacterial cell wall. By binding to PBPs, $\beta$-lactams irreversibly inhibit PBPs, resulting in a weakened cell wall and eventual cell death. Methicillin resistance in MRSA strains is due to the horizontal acquisition – from an unidentified species – of the mecA gene, which encodes PBP2a, a novel PBP distinct from the PBPs normally found in S. aureus. PBP2a is highly resistant to inhibition by all clinically used $\beta$-lactams and remains active to maintain cell wall synthesis at normally lethal $\beta$-lactam concentrations.

To identify the structural features of PBP2a that are responsible for S. aureus resistance, we determined the protein’s crystal structure. To obtain crystals of PBP2a, a soluble derivative was constructed by removing the N-terminal transmembrane anchor – which does not affect its interaction with $\beta$-lactams. The excellent facilities at NSLS beamline X8C allowed us to collect data to 1.8 angstrom resolution for crystals of the
apoenzyme (the enzyme with no inhibitor bound), and to 2.0 angstrom resolution for crystals of PBP2a bound to nitrocefin (a type of β-lactam). By comparing these structures, we noticed novel conformational changes at the active site that have not been observed in structures of β-lactam sensitive PBPs. Thus the active site of apoenzyme part of PBP2a is distorted relative to those of non-resistant PBPs.

Previous kinetic studies have shown that while the initial binding affinities of PBP2a for β-lactams are comparable to those of non-resistant PBPs, the subsequent acylation step, during which the PBP and β-lactam bind with an RCO- group (R being an organic group), is 1000 fold slower in PBP2a than in non-resistant PBPs. Acylation is key to the inhibition of PBPs by β-lactams, so the reduction of the acylation rate confers broad-spectrum resistance of S. aureus to antibiotics. But acylation is also key to the normal function of PBPs, so that the distorted active site of PBP2a provides a means of modulating the acylation rate to balance resistance while retaining activity.

Given that slow acylation is an intrinsic property of PBP2a, more effective inhibitors could be designed by improving their initial binding affinity to PBP2a. For example, novel forms of widely used antibiotics called cephalosporins provide a larger number of stabilizing interactions with and better shape complementarity to the narrow PBP2a active site groove, and thus show improved affinities for PBP2a. Optimization of cephalosporins and other inhibitors will be greatly facilitated by the structural information on the active site from our studies.

Figure 1. Structure of penicillin-binding protein of the bacterium Staphylococcus aureus (SauPBP2a*). The bilobed N-terminal (nPB) domain is colored orange with the N-terminal lobe (N-terminal extension) colored green. The transpeptidase domain is colored blue with the position of the active site indicated by the red nitrocefin adduct (shown in stick rendering). The secondary structure elements of the transpeptidase domain were labeled in accordance with the labeling scheme used for R6 PBP2x. The N- and C-termini are labeled N and C, respectively. Shown to the left of the ribbon representation is a linear representation of the domain structure of SauPBP2a* with residue numbers shown in parentheses.

Figure 2. Superposition of the active site region from the native penicillin-binding protein of the bacterium Staphylococcus aureus (SauPBP2a*) and SauPBP2a* acylated with nitrocefin, shown in yellow and blue, respectively. Nitrocefin (purple) is shown in thin stick rendering. Covalent binding of nitrocefin to SauPBP2a* requires conformational changes at strand β3 and at the N-terminus of helix α2.