R61 D,D-peptidase Complexed with Helen-1, a Species-Specific Cephalosporin: One "Hel"-en of an Antibiotic?

Messmer SMART Team: Sonia Sosa-Gonzalez, Michaun Cobb, Ngozi Osademe, Isioma Osademe, Merari Marin, Jhordy Rios Llamosa, Brigitte Rios Llamosa and Kasaundra Jones

Teacher Advisors: Ms. Carol Johnson and Ms. Meg Garland
Mentor: Nicholas R. Silvaggi, Ph.D., Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee

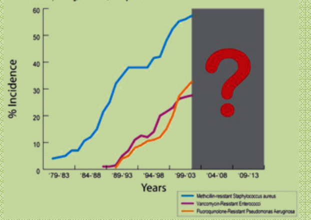
Abstract

Although antibiotics like penicillin save lives, antibiotic-resistant bacteria is a growing issue. According to Purdom (2007), over 70% of infections acquired by hospital patients post admission, are resistant to at least one prescribed antibiotic. Penicillin, a β-lactam antibiotic, treats bacterial infections caused by bacteria producing toxins within a host. Many pathogenic bacteria need a peptidoglycan cell wall for normal functionality. Enzymes in the cell membrane help form this cell wall by cross-linking peptidoglycan units. β-lactam antibiotics hinder bacterial cell wall biosynthesis by competing with the peptide substrate for the active site in these enzymes. While not the main enzyme used to produce bacterial cell walls, R61 DD-peptidase, a cytoplasmic enzyme, is easily crystallized to show bacterial enzyme chemistry. The active site of R61 consists of amino acid residues Ser62, Lys65, Tyr159, Arg285, Thr299, and Thr301. The Messmer SMART Team chose to design a model of R61 complexed with Helen-1, a species-specific β-lactam, highlighting the functionality and chemistry of the active site amino acids and their interaction with the beta-lactam. Understanding the structure and function of the active site of penicillin binding proteins, like R61, could lead to new, species-specific antibiotics that could prevent antibiotic resistance in bacteria.

Bacterial Resistance

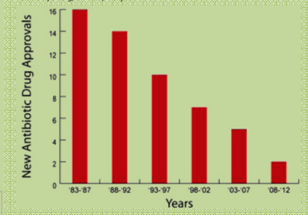
Rapid Increase in Antibacterial-Resistant Strains

Source: Spellberg, CID 2004, Modified

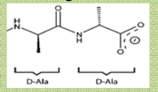


Dramatic Decrease in Antibiotic Drug Approvals

Source: Spellberg, CID 2004, Modified

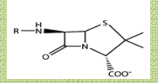


- Bacterial resistance is a serious problem because, not only are new, antibiotic-resistant strains emerging, the development of new antibiotics has slowed to a trickle.
- β-Lactam antibiotics, primarily drugs in the penicillin and cephalosporin classes, have been our primary defense against infections since World War II.
- β-Lactams work because the 4-membered lactam ring mimics the D-Ala-D-Ala portion of the cell wall building blocks.



Cell Wall Substrate

- Mimicking allows β-lactams to compete with the normal cell well substrate for binding to active sites of cell wall-building enzymes, called penicillin binding proteins or PBP's.



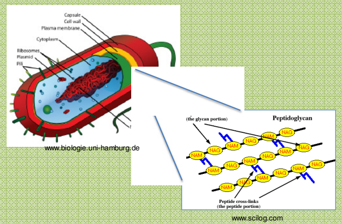
Beta-Lactam

- β-lactamases, enzymes produced by certain bacteria, are accountable for the bacteria's resistance to β-lactam antibiotics.
- β -lactamases most likely evolved as chemicals interfere among bacteria.

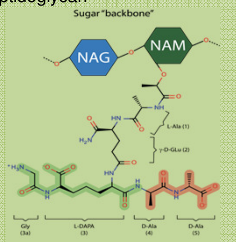
Current Research

Because β-lactams closely match the chemical structure of the normal cell wall substrate, researchers hypothesize that they should bind better to the enzymes, making them a more effective antibiotic than the current "general" antibiotics. Such **species-specific,** peptidoglycan- mimetic β-lactams could also be less prone to developing antibiotic resistance and might minimize side effects by killing only the pathogen causing the infection.

Bacteria Cell Wall Biosynthesis and Inhibition



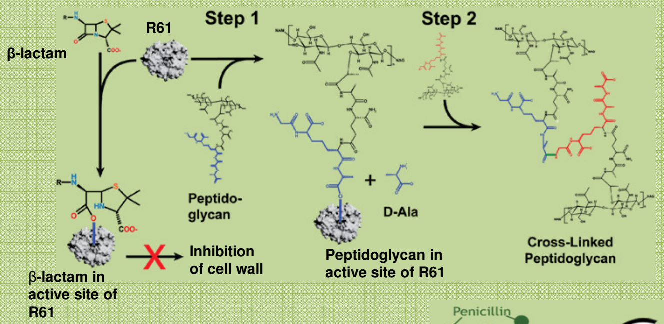
- For some Bacteria, a bacterial cell wall is essential for survival
- The cell wall is formed by cross-linked peptidoglycan strands.



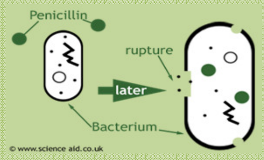
Bacterial Cell Wall Structure (Gram +)

- Peptidoglycans have a sugar backbone and a "linker" peptide that allows adjacent strands to be cross-linked.

Cell Wall Biosynthesis

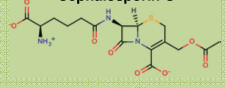


- Beta-lactam antibiotics like penicillin, fit into the active site of penicillin binding proteins (PBPs).
- The final cross-linking step of the bacterial wall synthesis is stopped.
- The cell wall structure is weakened and the cell ruptures.

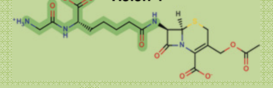


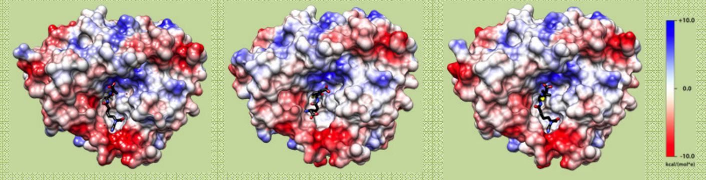
Substrate competition for the active site

Cephalosporin C



Helen-1

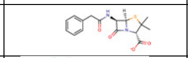
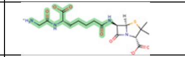
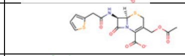
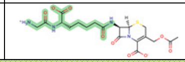




R61 with part of the cell wall substrate (left), cephalosporin C (center), or the species-specific Helen-1 (right) bound in the active site. Species specific antibiotics fit better in the active site of PBPs.

Reactivity of Generic vs. Species-Specific Beta-lactams with R61.

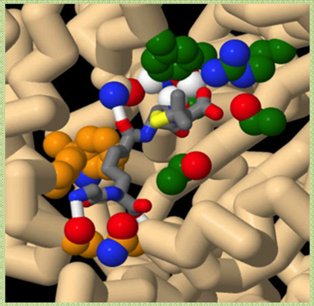
Measurement of Rate Constants for Inactivation of Penicillin-Binding Proteins(PBP) by β-Lactams[n]

PBP	Structure	$k_i (s^{-1} M^{-1})$
Benzylpenicillin		1.37×10^4
Helen-2		1.5×10^7
Cephalothin		1.4×10^3
Helen-1		5.6×10^5

[n] Taken from ref. 3

- This table shows the rate at which different beta-lactam antibiotics react with R61.
- The species-specific penicillin (Helen-2 in table) and cephalosporin (Helen-1, in table) are roughly1000 times and100 times, respectively, faster reacting, than their generic counterparts, benzylpenicillin and cephalothin.
- The species-specific side chain allows the drugs to bind better to the enzyme.
- A next step will be to investigate if this result extends to the larger cell wall-building enzymes in living cells.

Helen- 1 Covalently Bound to Active Site of R61



Structure based on 1pwg.pdb

This Jmol image of the bacterial enzyme R61 shows Helen-1 bound to the active site. Ser62, Thr301, Lys65, Tyr159, Arg285 and Thr299 are all amino acids in the active site. Helen-1, a species-specific cephalosporin, contains a 4-membered lactam ring that is under stress, which helps it react with the enzyme. Before R61 reacts with the Helen-1, Lys65 takes a hydrogen from Ser62. When this happens, the oxygen on Ser62 attacks the carbonyl carbon on the beta lactam ring, breaking the ring, and covalently bonding with Helen-1.

Conclusion

Currently, β-lactam antibiotics are a standard measure to treat bacterial infections. However, they need to be more distinct in terms of what bacteria they are trying to annihilate. The Messmer SMART Team designed a model of R61 complexed with Helen-1 to help understand how such antibiotics interact with penicillin-binding proteins. The model demonstrates that the specific antibiotic binds better to R61 because it makes more hydrogen bonds and salt bridges with the enzyme. Utilization of this information may aid researchers in the development of the next generation of antibiotics.

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