The Growing Problem of Antibiotic Resistance: How Kanamycin Nucleotidyl Transferase (KNTase) Inactivates the Antibiotic Kanamycin in Drug Resistant Bacteria

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The Problem of Antibiotic Resistance
Kanamycin is a member of the aminoglycoside family of antibiotics that include such antibiotics as gentamicin, streptomycin, and neomycin, which are used to fight life-threatening diseases. A problem in that when bacteria develop a resistance to one of the members of the group of aminoglycoside antibiotics, they tend to also develop resistance to other antibiotics in this family.

Normally, kanamycin kills bacteria by irreversibly binding to the bacterial ribosomes, subsequently inhibiting the translation of mRNA and the production of proteins. The ribosome is the place where the mRNA code is read and amino acids are assembled in the chain and ordered dictated by the code to produce proteins. When kanamycin binds to the ribosomes, the bacterial cell cannot produce proteins. The result is that the cell dies. However, some bacteria can produce an enzyme called kanamycin nucleotidyltransferase, or KNTase. This enzyme binds to the kanamycin and prevents it from binding to the bacterial ribosomes so that the antibiotic can no longer kill the bacterial cells.

Unfortunately, one of the big problems facing today's health officials is the development of antibiotic-resistant strains of Bacteria. Some disease causing bacteria are already resistant to two or more different antibiotics. One reason for the overuse of antibiotics is that beneficiaries are being prescribed for viral infections, such as the common cold, but antibiotics have no effect on viruses. Another cause is that antibiotics are being used in cattle feed. In fact, more than 40% of the antibiotics produced in the United States are mixed with animal feed to promote growth (Levy 5). In addition, antibiotics are commonly sprayed on grain to control or prevent bacterial infections. When antibiotics are used in cattle feed or crop sprays, it provides a low dose of antibiotics over a long period of time, which is the perfect recipe to create resistance. The beneficiaries select for the bacteria with the resistance to the killing of antibiotics that are sensitive to the antibiotic. As a result, the resistant population increases, while the population of bacteria that is susceptible to the antibiotic decreases. An understanding of the molecular mechanisms of drug resistance is important for the development of new and effective antibiotics and the development of inhibitors that can prevent the resistance of antibiotics and once again destroy drug-resistant bacteria.

The KNTase Model
For our project we researched and modeled Kanamycin Nucleotidyltransferase (KNTase), the enzyme produced by bacteria enabling them to be resistant to the antibiotic kanamycin. We used the model from the Brookhaven Protein Database, I Kan and 1KYN. I Kan was the first structural analysis, from a paper published in 1990 (Tanil, 1990). 1KYN contains the data for the alpha carbon backbone of KNTase. I KYN was published in 1995 (Owen, 1995). I KYN is a more high-resolution model with side chains of both the CSP and the kntase molecular active sites.

KNTase works by binding with kanamycin molecules. It does this by cleaving an ATP molecule (adenosine triphosphate) and binding the neomycin phosphate and the ATP to the active sites of the enzyme, hence the name kanamycin nucleotidyltransferase. The amino acid in the active site is negatively charged which helps the molecule to gain the positively charged kanamycin. The molecule is an ideal system for the study of protein stability and folding as it is a small molecule with only a single amino acid. The modeler of the neomycin phosphate and ATP was done from the Brookhaven Protein Database.

Thermostability
KNTase has two main forms that can function at higher temperatures. Normally, enzymes have a very narrow temperature range in which they can function. The first thermostable mutant of KNTase was isolated from the thermostable bacteria Bacillus steinertii, which was found in hot springs. The structure of the KNTase from these bacteria differs by only one amino acid from the KNTase that was first isolated from Staphylococcus aureus. The original KNTase molecule that we used in our project had two functional, arginine to arginine mutations at position 8 and one threonine to tyrosine mutation at position 130. These two mutations increased the thermostability of the KNTase model up to 12 degrees Celsius. This double mutations KNTase can sustain reactions at temperatures as hot as 90 degrees Celsius. Although many subsequent studies were conducted to investigate why these mutations and changes cause thermostability, the exact mechanism is still unknown.

Fig. 1 - A sketch of the KNTase model showing the active site where the ATP and the neomycin phosphate are located. The blue is the active site, the brown is the ATP, and the yellow is the neomycin phosphate.

Fig. 2 - The two proteins shown are the active site of KNTase and the neomycin phosphate.

Fig. 3 - The Thermostable Mutant The grase is in the position 130 mutations and the yellow is in the position KNTase in each of the structures.

Fig. 4 - TheThermostable Mutant The grase is in the position 130 mutations and the yellow is in the position KNTase in each of the structures.

Fig. 5 - The structural details of the KNTase molecule. The cyan is the active site, the green is the ATP, and the yellow is the neomycin phosphate.

Fig. 6 - The structural details of the KNTase molecule. The cyan is the active site, the green is the ATP, and the yellow is the neomycin phosphate.