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Antimycin: the big bad chain blocker

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Abbreviations
ETC – electron transport chain
CoQ – coenzyme Q or ubiquinone
DHE - dihydroethiedene

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Abstract

Antimycin is a potent electron transport chain (ETC) inhibitor. It inhibits the flow of electrons through complex III of the ETC by blocking the passage of electrons from cytochrome b to cytochrome c. All species that depend on mitochondrial respiration are very sensitive to antimycin and undergo toxic effects when they are exposed. Antimycin is widely used in research to study cellular respiration because of its potent ability to block the ETC. In addition to mitochondrial respiratory inhibition it has been shown to directly inhibit Bcl-2 related proteins. It is a carbon based, ring-containing compound that also contains oxygen and nitrogen. Antimyin is soluble in organic solvents and insoluble in water. Due to its mode of action it is very lethal to all organisms that rely on mitochondrial respiration.

Introduction

Leben and Keitt first isolated antimycin in 1945 at the University of Wisconsin. It was discovered as a white contaminant that inhibited the growth of *Venturia inequalis*. The researchers were able to isolate the unknown substance, succeed in growing it in pure culture and demonstrate its inhibitory activity. They noted the antibiotic was very effective against fungi and they named the new compound antimycin [1]. Antimycin is most prominently produced by various species of *Streptomyces* [2]. Early studies of antimycin were hampered by low yields. The greatest difficulty was purification and obtaining enough of it to study [1]. Since these early times antimycin has become a commonly used inhibitor of mitochondrial respiration. It primarily functions by blocking the flow of electrons in complex III of the electron transport chain (ETC). This paper will review the basic chemical and structural properties of antimycin, and provide an overview of its functional properties.

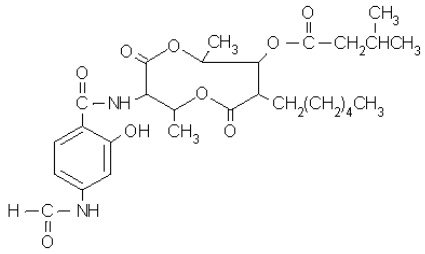
Chemical Properties

Early analysis of antimycin revealed that the composition was 61% carbon, 7% hydrogen, 5% nitrogen and 27% oxygen by weight. This data led to the molecular formula of antimycin $C_{28}H_{40}O_9N_2$ [1]. The molecular weight of antimycin has been reported a s 506 g and 493 g [1]. It has a melting point of 139-140^{o}C. Antimycin is very sensitive to alkaline environments. Exposure to an environment of pH 9.3 resulted in a loss of activity in 3 days at room temperature, while exposure to pH 11 stripped antimycin of all activity in 1 hour [1]. Dried antimycin crystals or acetone solutions are stable for as long as 18 months [1]. Antimycin is readily soluble in acetone and

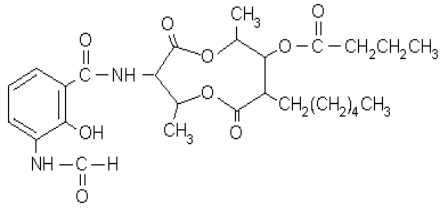
chloroform (100 mg/mL), moderately soluble in organic solvents such as methanol and benzene (~70 mg/mL), and nearly insoluble in water and HCl (less than 5%). An interesting characteristic of antimycin that can be used for identification and quantification is its ability to fluoresce in alcohol containing solutions [3].

Structure

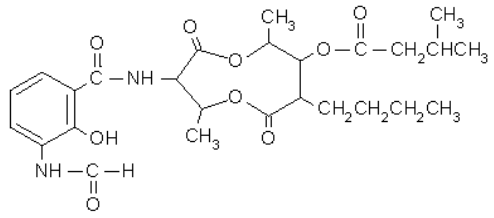
The antimycin family consists of four co-crystallizable compounds recognized as A_1, A_2, A_3 and A_4. A_1 and A_3 are the most prominent forms of antimycin. Each form of antimycin shares a common basic structure consisting of an acyl and alkyl-substituted, nine member dilactone ring that is linked *via* amide bond to 3-formamidosalicyclic acid [3]. The structures of A_1, A_2 and A_3 are shown in Figure 1. The major overall structure is conserved in each form. The variation can be observed in the addition, subtraction and length of the side chains.



antimycin A_1



antimycin A_2



antimycin A_3

Figure 1. The basic chemical structure of antimycin A_1, A_2, and A_3 [4].

Mechanism of ETC inhibition

The most well-known and widely utilized function of antimycin by researchers is its ability to inhibit of the flow of electrons in complex III of the ETC. Specifically, antimycin disrupts the electron flow from cytochrome b to cytochrome c in complex III. It accomplishes this by competitively binding to cytochrome b. When antimycin is bound to cytochrome b it displaces coenzyme Q (CoQ), which is the primary electron carrier in the ETC. The presence of antimycin covers the proper binding site for CoQ preventing any interaction between CoQ and cytochrome b. This disruption makes it impossible for CoQ to pass its electrons to cytochrome b [5]. Since this bond cannot be formed and the electrons cannot pass any further, the chain is blocked.

The blockage of the ETC creates a build up and backflow of electrons that begin to leak at complex II and I. As the electrons leak they are able to form products that are toxic to the cell. One example of this is the reduction of oxygen to superoxide. Superoxide can be converted into H_2O_2, which is toxic to the cell, by superoxide dismutase. Under normal conditions catalase is able to detoxify the cell by converting H_2O_2 into water and oxygen. However, when antimycin is present the cell will leak more electrons and create more toxic products than the cell can manage. In this case the amount of H_2O_2 builds up to toxic levels that exceeds the cell's survival capabilities and lead to cell death.

In the Dr. Douglas Spitz lab we have shown that treating cells with antimycin produces a significant increase in superoxide production. This was done using MB231 cells and dihydroethidiene (DHE) staining, which is a reliable, specific indicator of

superoxide. We have observed a five to seven-fold increase in DHE mean florescence in the antimycin treated cells as shown in Figure 2.

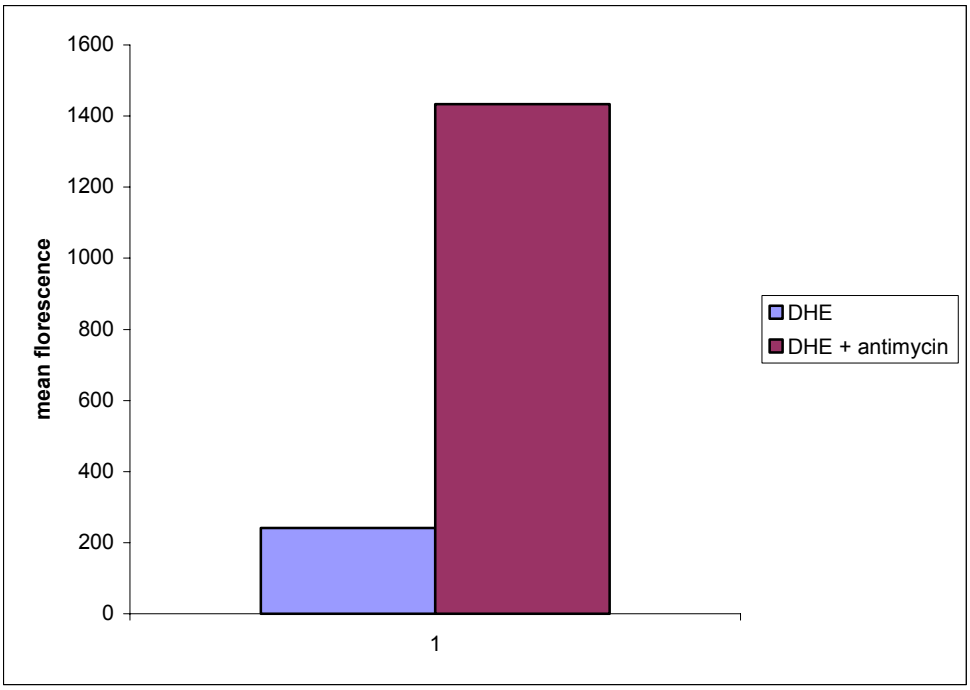


Figure 2. DHE staining results of MB231 cells treated with and without antimycin. Antimycin induces a seven-fold increase of superoxide.

The inability of CoQ to bind cytochrome b was demonstrated using modeled density in the native complex III structure by Zhang et al. Density corresponding to CoQ was lost in the presence of antimycin crystals [5]. Since antimycin blocks the binding of CoQ, it inhibits any electrons from passing from cytochrome b to cytochrome c. This model has been supported by fast kinetic experiments. Fast kinetics has demonstrated that the reduction of cytochrome c by CoQ (ubiquinol) and succinate is inhibited by antimycin [6]. In purified preparations it has been shown that equal molar concentrations of antimycin and cytochrome b inhibit the reduction of cytochrome c by approximately 90% [6].

Bcl-2 inhibition

Another function of antimycin has recently been reported by Tzung *et al*. They have been able to demonstrate that antimycin can directly inhibit the activity of Bcl-2 related proteins, specifically Bcl-x_L. The mechanism of action is similar to that of the blockage of the ETC. Again it competitively binds to the target area inhibiting normal interactions. In this case it has been shown to bind to the hydrophobic groove of Bcl-x_L, which can be seen in Figure 3 [7]. The hydrophobic groove is created by domains BH1, BH2 and BH3. The presence of antimycin disrupts the pore forming ability of Bcl-x_L, which cause mitochondrial swelling. This discovery is noteworthy because it demonstrates that a non-peptide ligand can directly inhibit the function of Bcl-2 related proteins [7].

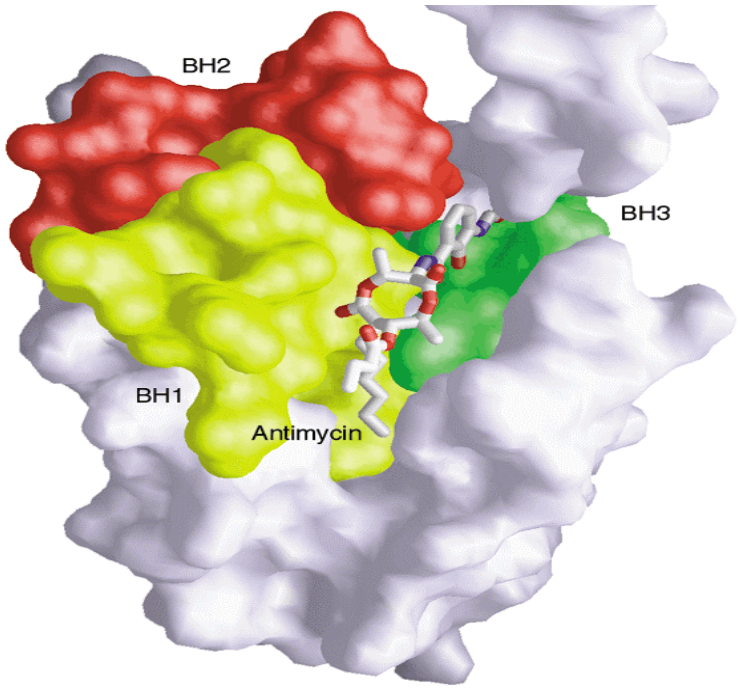


Figure 3. Antimycin, represented by a stick model, binds to the hydrophobic BH1, BH2 and BH3 domains of Bcl-x_L. This interaction inhibits the function of Bcl-x_L, a Bcl-2 related protein [7].

Toxicity

Anitmycin was originally identified because of its antifungal properties. As mentioned previously antimycin is toxic to all organisms that rely on cellular respiration. This list ranges from yeast to humans. Antimycin can produce toxic effects by It can be fatal if it is inhaled, in contact with the skin or swallowed [8].

Toxicity of antimycin via oral administration [8]

Animal	**LD_{50} (mg/kg)**
Rat	28
Dog	5
Rabbit	10
Guinea pig	1.8
Chicken	160
Duck	2.9

Summary

Antimycin is a powerful inhibitor of the ETC. It blocks the flow of electrons from cytochrome b to cytochrome c in complex III of the ETC. Early on antimycin was very difficult to isolate and to obtain in large amounts. Today, it is commonly used in research to study the effects of the inhibition of cellular respiration. It has also been shown that antimycin is able to inhibit Bcl-2 related proteins through competitive binging. Antimycin exists in four different forms that can be isolated. A1 and A3 are the most commonly found forms. Most antimycin used in research in a mixture of the different forms, which all possess the same a function. Due to its catastrophic effect on celluar respiration, antimycin is extremely toxic to all organisms that rely on respiration.

It is a vital biological research tool today and perhaps in the future even more functions of antimycin will be uncovered.

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