In 2011, nearly 9 million people became sick with tuberculosis, of which 1.4 million died worldwide, according to the World Health Organization. Tuberculosis (TB) is an infectious, airborne disease caused by the pathogenic bacterium, Mycobacterium tuberculosis. This bacterium primarily attacks the lungs and is often fatal if not treated promptly. 3-dehydroquinate dehydratase (DHQase) is an enzyme that catalyzes the third step of the shikimate pathway, which is essential to M. tuberculosis. The shikimate pathway is a precursor to the aromatic amino acids phenylalanine, tyrosine, and tryptophan. Inhibition of DHQase will block the shikimate pathway and the TB bacteria will die. Inhibitors can be used for drug development to treat tuberculosis, especially people affected by multidrug-resistant strains, called MDR-TB. Since DHQase is absent in human cells, the drug will only affect bacteria cells, where the enzyme is inactive until substrate binds to its active site. 3-dehydroquinase, a natural ligand, and six inhibitors can interact with DHQase. Effectively inhibiting this enzyme would render tuberculosis harmless. DHQase has a flexible catalytic loop at residues 18-24. Arg19 and Tyr24 are the two key conserved residues. The ligand binding induces closure of the loop through its interaction with the side-chain atoms of loop residues: Tyr19 and Arg24. DHQase may hold the key to saving the lives of those infected by MDR-TB. The Brookfield Central High School SMART Team (Students Modeling a Research Topic) created physical models of DHQase dodecamer and monomer to show active-site binding molecules and inhibitors using 3-D modeling printing technology.

Inhibitors for DHQase

There are seven compounds that are known to bind to the active site of DHQase. One of these is a ligand, which reacts with the enzyme, under normal conditions, to produce the reactants for the next step in the shikimate pathway. The other six compounds are competitive inhibitors of which five will be discussed, namely inhibitors 2, 3, and 6 (shown in figure 6). When these inhibitors bind to the active site, the ligand, 3-dehydroquinase, is not able to bind to DHQase. Inhibitors 3, 5, and 6 are variations of inhibitor 2. One important factor that determines the potency of the inhibitors is flexibility. Flexibility refers to the ease with which the inhibitor binds to the active site. Inhibitor 5 is the most flexible, followed by inhibitors 6, 3, and 2.

Inhibition of DHQase Activity

Figure 7 below shows the effect on 3-dehydroquinic acid in a solution of Inhibitor 7 as well as the ligand. Rate of production of 3-dehydroquinate in μM per second along with the concentration of 3-dehydroquinate in μM when in a solution of inhibitor 7 of concentration 0 – 300 μM. The graph shows that Inhibitor 7 acts as a competitive inhibitor of DHQase, as increasing the concentration of inhibitor 7 decreases the amount of 3-dehydroquininate produced.

References


Inhalation Times: DHQase and the Shikimate Pathway of Mycobacterium tuberculosis

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Abstract

The Shikimate pathway which consists of seven enzymes that catalyze separate steps and produce chorismate.

Figure 3: The Shikimate pathway which consists of seven enzymes that catalyze separate steps and produce chorismate.

DHQase

Figure 4 shows 3-dehydroquinate dehydratase, or dehydroquinase (DHQase), an enzyme that is essential to the synthesis of aromatic hydrocarbons. It does so by catalyzing the third step in the Shikimate pathway, as denoted by the reaction shown in Fig. 3. However, this pathway is not present in human cells, and the presence of this pathway and enzyme allow M. tuberculosis to thrive. The key characteristics of DHQase:

- Conserved residues Arg19 and Tyr24 are located on a flexible loop.
- Arg19 and Tyr24 are essential for substrate binding, catalysis, and DHQase inhibitor design.

Figure 4: Modes of DHQase A. Dodecamer, B. Monomer, C. Active Site

Pathophysiology

The disease may not manifest itself with evident symptoms and may remain latent until the illness becomes fatal; however, if treated in due time, TB is curable. Symptoms include chronic cough, fatigue, night sweats, and shortness of breath. This bacterium primarily targets the lungs (Fig. 2B), but the disease can be spread to any organ of the body through the bloodstream. Because TB is an airborne disease, it is extremely contagious. Each infected person transmits the bacteria to approximately 10 to 15 others each year (WHO TB Report, 2012).

Shikimate Pathway

In Mycobacterium tuberculosis, the Shikimate pathway is critical to the survival of the bacteria. The diagram of the Shikimate pathway below (Figure 3) consists of seven enzymes that catalyze separate steps of the pathway. They convert erythrose-4-phosphate and phosphoenol pyruvate to produce chorismate, an essential precursor of the aromatic compounds that produce the essential amino acids typtophan, phenylalanine, and tyrosine. DHQase is the enzyme that catalyzes the third step in the Shikimate pathway. DHQase catalyzes the irreversible conversion of 3-dehydroquinine into 3-dehydroshikimate and water. In the first step of the DHQase reaction, a conserved tyrosine residue removes the pro-S proton to form the enolate intermediate. At the same time, an asparagine residue

Figure 5: Enzyme, substrate, and inhibitor interaction in competitive inhibition.

A. Enzyme and substrate prepare to bind
B. Enzyme binds substrate
C. Enzyme has acted on substrate
D. Substrate cannot bind because inhibitor is already bound to enzyme.

Competitive Inhibition

Competitive inhibitors structurally mirror the natural substrate and compete for binding with the active site of an enzyme. If the inhibitor binds to the enzyme, the substrate itself is prevented from binding to the enzyme, and the enzyme is blocked from performing its normal function.

Figure 6: Competitive inhibitors 2, 3, and 6 for DHQase

Inhibition of DHQase Activity

Figure 7 shows the effect on 3-dehydroquinic acid in a solution of Inhibitor 7 as well as the ligand. Rate of production of 3-dehydroquinate in μM per second along with the concentration of 3-dehydroquinate in μM when in a solution of inhibitor 7 of concentration 0 – 300 μM. The graph shows that Inhibitor 7 acts as a competitive inhibitor of DHQase, as increasing the concentration of inhibitor 7 decreases the amount of 3-dehydroquininate produced.

Biological Significance

Tuberculosis is a common disease that is prevalent in third-world countries, but is spreading to more developed areas. Although less common, multidrug-resistant tuberculosis (MDR-TB) is a more lethal strain that affects 630,000 people worldwide. Attempts to treat MDR-TB have met with little to no success. In the future, there is hope to find a suitable inhibitor for DHQase in order to develop drugs that effectively treat MDR-TB.

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