

Abstract

Mycobacterium tuberculosis (*M. tb*), the causative agent for tuberculosis (TB), infected 8.6 million and killed 1.3 million people in 2012 (WHO). TB is most prevalent in countries with a high incidence of infectious diseases, such as HIV, due to weakened immune systems. TB mainly affects the lungs, but can also affect other systems. When a host organism is infected with TB, the *Mycobacteria* in the lungs multiply, often resulting in pneumonia, chest pain, and prolonged coughing. In response, host macrophages, a part of the natural immune system, engulf the *Mycobacteria* and oxidize bacterial cell proteins in an attempt to destroy it. To protect itself against this attack, the bacterial thioreductase system, consisting of the redox protein thioredoxin reductase (TrxR) and the thioredoxin proteins TrxA, TrxB, and TrxC, gives electrons back to the oxidized proteins. As this system works to maintain cellular redox homeostasis, finding ways to stop it might provide a new method for treating people with TB. TrxA and TrxC have similar structures, thus it can be hypothesized that their functions are similar. Comparing binding sites between the proteins can provide insight if TrxA can react with TrxR similarly to TrxC. By modeling TrxA and TrxC with 3D printing technology, the Messmer SMART Team (Students Modeling A Research Topic) can compare the structures of the thioredoxins which may lead to new strategies for curing or preventing TB.

Introduction

About 1/3 of the world's population is infected with TB, an airborne disease. If a person with active TB goes untreated, they will infect approximately 10 to 15 people a year.¹ TB usually infects the lungs, but can attack almost any part of the body. In many countries, tuberculosis is becoming increasingly problematic due to weakened immune systems from co-infection with HIV and drug-resistant due to incomplete treatment.

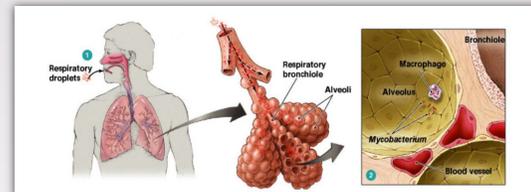


Figure 1. Route tuberculosis takes to enter the body through the respiratory system and into the alveoli.²

As a result, researchers are hoping to stop TB by learning more about TrxA and TrxC, 2 of 3 thioredoxin proteins that TB uses to protect itself from attacks by a host's immune system. Thioredoxin proteins are responsible for helping maintain cellular redox homeostasis within the bacterial cell (see Figure 3a,b). TrxA's function is unknown; however, it is believed to be similar to the function of TrxC. If this is true, TB can possibly be stopped by inhibiting these proteins from functioning.

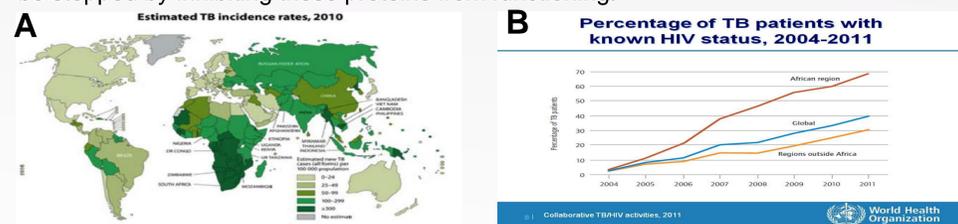


Figure 2. A) Worldwide TB Incidence Rates.¹ B) TB Co-Infection with HIV.³

TB is the leading killer of people living with HIV. A person with HIV is about 20 to 30 times more likely to develop active TB due to HIV weakening the immune system. Figure 2a and 2b show that Africa and Asia are the two biggest continents with TB and HIV as overlapping diseases.

Mechanism

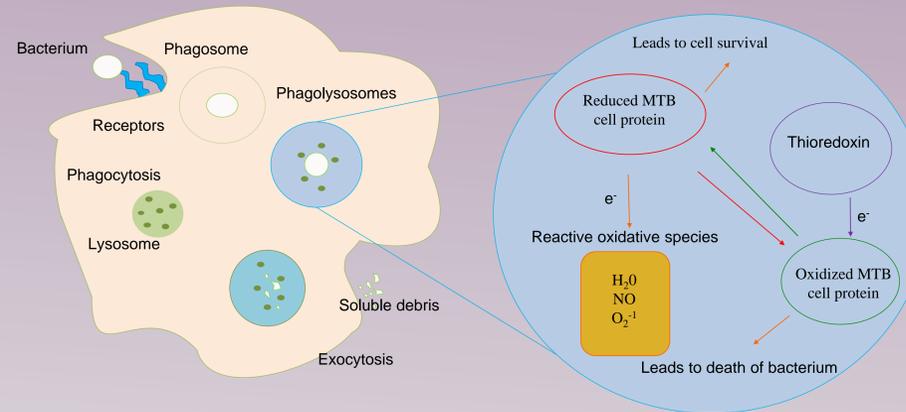


Figure 3. A) Alveolar macrophage engulfing *M. tb*. B) Phagolysosome in macrophage with thioredoxin reducing oxidized *M. tb* cellular proteins.

Phagocytosis is one of the processes of removing bacteria from the host. The fusion of the lysosome in the macrophage forms a phagolysosome, leading to the destruction of the bacterium. *M. tb* is able to resist this process and survive in the phagolysosome.

The phagolysosome is a difficult place to survive as it contains low amounts of oxygen and reactive oxidative species. The reactive oxidative species in the macrophage phagolysosome oxidize the bacterial cell proteins, normally leading to bacterial cell death. Reduction of the bacterial cell proteins by a redox cycle, involving thioredoxins, allows the bacteria to survive, allowing for the progression of the disease. TrxC is known to be involved in this redox cycle. The related TrxA has not been confirmed to behave similar to TrxC. Identifying the TrxA structure, scientists can find the function of TrxA and leverage this information to develop new TB therapies.

The Structure of TrxA

In order for a protein to have a defined structure and function it must be well folded. Previous work indicates that TrxA was not well folded and thus was thought to be non-functional.⁴ Each crosspeak (dot) in this Nuclear Magnetic Resonance (NMR), shown below, represents the chemical environment of an atom in TrxA. If the dots were coalesced into the center of the spectrum, the protein would not be structured. In the data shown in Figure 4, the well-spread cross peaks indicate that the protein is well-folded and so it can be hypothesized that TrxA has a defined structure and function.

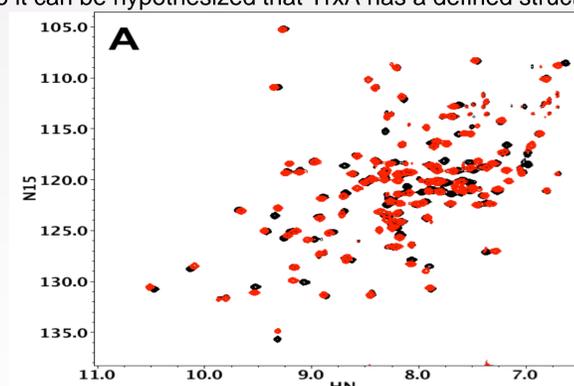


Figure 4. ¹H-¹⁵N Heteronuclear single quantum coherence spectroscopy (HSQC) for TrxA in reduced (red) and oxidized (black) forms.⁵

Model

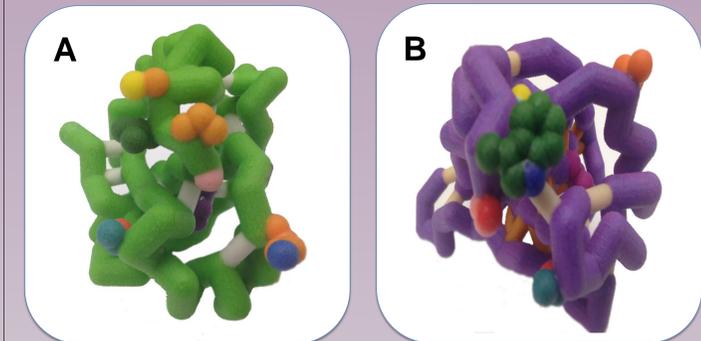


Figure 5. A) TrxA model highlighting amino acids predicted to be involved with binding interactions to TrxR.⁵ B) TrxC model highlighting amino acids experimentally verified to be involved in TrxR Binding.⁶

TrxA may have a similar function due to its structural similarities with TrxC, however, the location of the amino acids is different, suggesting a different function. For example, both proteins have three amino acids that form a plane, but the size of the planes are different between the two proteins. Also, TrxA Cys30 and the corresponding cysteine from TrxC (Cys37) point in opposite directions, showing the difference in folding. The highlighted amino acids on TrxC are from experimental data providing evidence exhibiting binding to TrxR.⁶ The amino acids highlighted on TrxA were chosen based on sequence similarity to TrxC. TrxC gives electrons to the oxidized bacteria cell proteins by binding with TrxR but due to these structural differences, TrxA may not bind to TrxR in the same way.

Conclusion

TB is increasingly becoming a world health issue due to its increasing drug resistance. HIV coinfection exacerbates the problem. New therapies are required to combat this global pandemic. In order to come up with new strategies, we need to know more about how TB behaves in its host. Part of this is understanding how the thioredoxin system protects the bacterium in the phagolysosome. If we know how that works, we could develop therapies targeting the thioredoxin system. By targeting the thioredoxin system, drugs would allow our bodies natural defenses to fight the *Mycobacteria*. TrxA is well-folded suggesting that it has a definite function, which is currently under investigation. Structural characterization of TrxA will allow for a better understanding of the thioredoxin system.

References

1. World Health Organization, Global Tuberculosis Control 2010
2. Fig. 1: http://www2.bakersfieldcollege.edu/bio16/22_Resppictures.htm
3. World Health Organization, <http://www.who.int/hiv/topics/tb/data/en/>
4. Akif, M. et al. *Journal of Bacteriology*, 2008, 190, 7087.
5. Daniel Sem Lab, Concordia University Wisconsin, unpublished.
6. Olson, A.L., et. al, *Proteins: Structure, Function, and Bioinformatics*. 2013, 81, 675.