**Metabolic Pathways and Cellular Molecules in *Mycobacterium tuberculosis* that are Used for Heme Utilization**

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 **Abstract**

Since iron is an important mineral used for cellular function in *Mycobacterium tuberculosis* (Mtb), it is crucial for the bacterium to find ways to utilize heme in human hosts in order to continue survival. Iron is not only crucial for growth in Mtb, but it also plays important roles in human cellular processes and is stored in several forms of proteins in the human body such as lactoferrin, ferritin, transferrin, and heme (Abbaspour, N). Evidence showed that the abundance of these molecules inside the human host is a potential factor for whether a pathogen will be infectious such as a highly infectious disease like Tuberculosis. The study shows that Mtb have multiple pathways and proteins that are used for acquiring iron from heme such as the DppA protein that binds substrates located inside the Dpp transporter, and pathways that are inclusive to albumin, also independent heme uptake pathways. Furthermore, two outer membrane proteins named PPE36, and PPE62 are also needed for iron acquisition. The complexity of multiple ways pathogens acquire heme can show that Mtb uses different options of heme acquisition based on the environmental factors of the human host. This may be helpful for future medicines combating Mtb, by keeping the hosts body under ideal conditions that will inhibit Mtb from using these pathways thus slowing down infection or stopping colonization**.**

**Introduction**

Iron is used for several important mechanisms in the human body such as ion transportation, blood production, oxygen transport, and a building block of some proteins and enzymes. Although Iron is abundant on Earth, its highly oxidizable state makes it unstable and insoluble for outside consumption. Therefore, humans must obtain iron through diet and store it in complex forms to protect from damaging factors, usually enclosed in proteins. Most of the percentage of iron in the body is bound to a protein called hemoglobin, which is responsible for oxygen transportation throughout the blood, ferratin, a protein primarily used for iron storage, and transferrin, another form that is used for iron transportation when iron levels are too elevated. Microbes that rely on human hosts like *Mycobacterium tuberculosis* also rely on iron for growth and cellular processes. Because of its importance, the body is known to restrict iron in case of infection from pathogens. A balance of iron in the body is needed to counteract Tuberculosis as well as slow down infection. Too much iron result in increased risk of infection and treatment failure while too little will result in a resilient form of *Mycobacterium Tuberculosis.* When iron is limited Mtb will secrete siderophores that are carboxymycobactins. These molecules are highly attracted to iron. To further its opportunity to acquire iron, it will also begin to utilize heme. These mechanisms along with ABC transporters used for iron utilization from ferric carboxymycobactins show how important iron mechanism are for Mtb infections. This article highlights important factors such as Heme-binding proteins, dipeptide permease, cell permeability, and the presence of albumin and showed how these factors are required for iron mechanisms and the infection of Tuberculosis.

**Recent Progress**

 The study showed that heme-binding proteins called PPE36 and PPE62 are involved and needed for Mtb to be able to acquire iron from heme. These proteins sit on the outside of the cell surface and lack the common amino acid sequence of other heme-binding proteins. A mutant called ML2411, lacking the PPE36 gene was used and grown in an environment of hemoglobin and heme as the only source of iron. The results showed that the mutant ML2411 did not exhibit growth proving that without PPE36, hemoglobin and heme utilization is not possible. Therefore, indicating the importance of PPE proteins for Mtb.

 Another important protein that was shown to be involved in heme-binding is DppA. This heart shaped protein has a protein core consisting of a tetrapeptide with high electron density. “Despite the compact appearance, the DppA tertiary structure is built by two globular and slightly offset halves that fold onto each other with perfect complementary like shells of a clam.” (Mitra pg 4, p 12). The spring like structure achieved by an alpha helix as the opening allows the DppA to remain closed and always prepared for binding. The binding site of Dppa was visualized by modeling using crystallography. The study further indicated that when only heme is available, DppA is crucial for heme-binding and heme uptake. This is helped by its unique structure. To show this essentialness, the amino acid sequence of the tetrapeptide inside the protein core was changed by removing iron residues and replacing them with alanine. This resulted in a decrease in the ability to bind in DppA.

 Furthermore, the study showed that the Dpp system is involved in the uptake of heme. This was done by monitoring the growth of the Dpp mutant and the regular strain in a medium that included 1 µM of ferric citrate and the gradual increase of hemin concentration. The mutant reached a toxic level of hemin after deleting the dpp gene showing that Mtb with dpp have a resistance to heme, indicating that the Dpp transporter is an uptake system.

 It was also showed that the Dpp transporter system is crucial for heme utilization in *Mycobacterium tuberculosis*. The Dpp transporter have multiple components involved in heme utilization. The study showed the importance of the Dpp transporter for heme utilization by working with an avirulant strain of Mtb and deleting the Dpp operon. This process involved homologous recombination and after the deletion, the mutant was named ML2436. The strain was able to grow in a medium rich in ammonium ferric citrate, however it was not able to grow in a medium that was rich in hemin and hemoglobin from humans. According to the study the requirement of the Dpp system in Mtb was showed to be present for virulence in macrophages.

 It is important to point out that peptides can interfere with the Dpp system’s roles of heme utilization. the strain was grown in two different iron rich environments, one being Ferric citrate and the other being human heme. Tryptone was added for peptides in both mediums, and the observation was no decrease in growth in the medium in ferric citrate, however, a decrease in growth was indicated in the medium rich in heme. This inhibition was overcome by adding more Dpp operons thus boosting the growth back to regular levels. Albumin is another component that can interfere with heme uptake by the dpp transporter in Mtb. The mutants ppe36 and dpp was used and could not grow when bull albumin was absent. However, when Albumin was present, the two mutants were able to grow as nearly as much as a wildtype strain of Mtb and showed that albumin can allow for growth without the essential conditions needed for PPE36 and the Dpp system by an unclear mechanism.

**Discussion**

 The results from the study were a clear indicator on how important iron is and the multiple survival techniques that Mtb has evolved to acquire heme in humans. The significance of the heme acquisition in Mtb explains why there are different ways for Mtb to acquire heme. This is logical as the human host will try to limit its source of iron. Therefore, the pathogen had to develop proteins and transporting systems to counteract the iron poor environments, thus proving that based on the environment of Mtb, it will act accordingly. The question remains unclear on how albumin is to successfully allow for heme acquisition for PPE36 and Dpp systems and what mechanisms are involved.

**References**

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