People Sulfur Because of A Disulfide Bond
The Role of Thioredoxin in Tuberculosis

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Tuberculosis, a disease caused by the bacterium, Mycobacterium tuberculosis (M.tb) affects about one-third of the world’s population, killing 2 million people each year. The bacteria reside in macrophages of the respiratory tract of infected individuals. Macrophages are a type of immune cell whose function is to engulf and kill foreign substances such as bacteria that invade the body. Macrophages do this by bleaching, or oxidizing, bacterial cell proteins, rendering the bacterial cell susceptible to cell death. To protect against these lethal oxidative attacks by macrophages, two bacterial cell proteins, Thioredoxin C (TrxC) and Thioredoxin Reductase (TrxR), function to reduce the oxidized proteins, thus stabilizing them and enabling the survival of the bacteria. To accomplish this protective reduction and maintain redox homeostasis in the bacterial cell, TrxC donates electrons to the oxidized bacterial cell proteins, becoming oxidized in the process. In order to continue to donate electrons to protect the cell, TrxR itself must now gain electrons (be reduced). TrxR is the protein that donates electrons to oxidized TrxC converting it back to the reduced form, continuing the redox cycle. NADPH then reduces the oxidized TrxR with its electrons stored in a tightly bound FAD. To accomplish this redox cycle, TrxC binds to TrxR through a disulfide bond, and stabilized by a hydrophobic pocket on TrxC that fits into a crevice on TrxR. If this reaction can be prevented, the protective redox cycle of TrxC/TrxR could be stopped thus leading to cell death of the Mycobacterium tuberculosis, preventing many deaths.

Abstract

Role of Thioredoxin In Bacterium Cell Survival

Redox Cycle and Formation of Critical Disulfide Bond between TrxC and TrxR

To protect itself from oxidative attack by host macrophages and avoid cell death, Thioredoxin (TrxC) reduces the oxidized cellular proteins and, in turn, is itself oxidized. To continue the redox process, the reducing power of TrxC must be restored via a redox pathway involving Thioredoxin Reductase (TrxR) and the cofactors FADH₂ and NADPH. The figure to the right shows the steps in this redox pathway and how TrxC goes from the oxidized state, to the reduced state becoming HS-TrxC-SH. The formation of a disulfide bond between TrxR and TrxC is crucial to this redox pathway and to the survival of the bacterium.

Identification of Amino Acids of TrxC and TrxR Complex by NMR

Fig. 4a: Each cross peak corresponds to a backbone amide (NH) for an amino acid in TrxC and has been overlaid for TrxC alone (blue), or complexed with TrxR NADPH cofactor (green).
- Only two amino acids, Cys 139 of TrxC and Cys 350 of TrxR, have cross peaks that disappear showing that these two cysteines form the critical disulfide bond in the TrxC-TrxR complex (data not shown).
- A group of amino acids disappear, due to a process called “exchange broadening,” suggesting that these amino acids are undergoing a conformational change and are no longer in the same position. The disappearance of the cross peaks shows which amino acids form the hydrophobic interface between TrxC and TrxR.

A Physical model of TrxC and TrxR Complex

Fig. 5 - Shows TrxC (orchid) bonded to TrxR (cyan). The blue portions of the figure are the amino acids of the hydrophobic pocket that form a crevice on TrxC that TrxR fits into. In order for TrxR to bind to TrxC, as described in Fig. 3, a disulfide bond (yellow) must form between Cys 139 of TrxR and Cys 350 of TrxC. Preventing this bond from forming could save millions from Tuberculosis.

Conclusion

Two million people die each year from tuberculosis, mostly in third world countries. TB infects mainly respiratory tissues. Alveolar macrophages try to kill the bacterium by oxidizing bacterial cell proteins but the bacterium is able to fight back and prevent cell death by reducing these cell proteins back to their original state via the oxidation of the protein Thioredoxin. To continue to provide protection for the bacterium, TrxR must be reduced (gain electrons) in a redox cycle involving the formation of a complex between Thioredoxin and Thioredoxin Reductase and NADPH cofactors involving a disulfide bond between the two proteins. This bond allows TrxR to donate electrons to TrxC which then donates electrons to the oxidized TB cell proteins. This bond is crucial to the survival of the bacterium. If this bond can be prevented, the oxidized TB cell proteins can be stopped from being reduced thus leading to the death of the TB bacterium. In conclusion, if we can prevent this critical disulfide bond from forming, then we can save the lives of millions who are infected by the bacterium.