Review

The role and characteristic of antioxidant for redox homeostasis control system in Mycobacterium tuberculosis

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Abstract

At the time macrophages phagocyte against Mycobacterium tuberculosis entry, it occurs respiratory burst, and become the initial formation of reactive oxygen species (ROS). Reactive oxygen species such as nitric oxide and oxygen radicals kill the mycobacterium bacteria. However, Mycobacterium tuberculosis has an antioxidant that plays a role in redox homeostasis system which protects itself from the attack of oxygen radicals and nitric oxide. We intend to examine the various roles and characteristics of antioxidant for redox homeaostasis control system in Mycobacterium tuberculosis. We have got conclusion there are some antioxidants which protects Mycobacterium tuberculosis from oxidative damage such as KatG, SOD, Mycothiol and thioredoxin.

Keywords: Reactive oxygen species, mycobacterium tuberculosis.

INTRODUCTION

Tuberculosis (TB) is the leading cause of mortality worldwide due to a single bacterial infection. *Mycobacterium tuberculosis* is one of the most devastating pathogens, which infects 8 million people annually, and is responsible for an estimated 2 million deaths per year (WHO, 2010). According to WHO, in 2009 Indonesia ranks fifth after India, China, South Africa, Nigeria in the number of patients in the world after India (1,600,000 people of TB patients), China (1,500,000 people of TB patients), South Africa (590.000 people TB patients), Nigeria (550,000 TB patients) and Indonesia as much as the TB patient 520.000 (WHO, 2010), there are estimated 2020 population TB attacks the 1 billion with 70 million deaths, if not done controlling (Soemantri et al., 2007). Therefore, WHO have a program "The Stop TB Global Plan Partnership's to Stop TB, 2006-2015" as a strategy for control of tuberculosis and to the Millennium Development Goals (MDGs) the decrease in the number of TB sufferers occurs up to 2015 (WHO, 2010).

Primary exposure to M. tuberculosis will lead to either immediate development of active TB or a latent asymptomatic disease state, which occurs in most cases. Approximately 10% of healthy immunocompetent individuals who are latently infected will develope active TB within their lifetimes. However, immunocompromised

individuals are more susceptible to active TB development. For instance, in HIV–positive individuals, this risk is as high as 90%. India, China, Indonesia, South Africa and Nigeria have the highest absolute numbers of cases. The African region has the highest incidence rate per capita (363 per 100,000 population). Overall, 60% of all TB cases occur in Africa and South East Asia, and there is an alarming trend of co-infection with HIV in regions mentioned above. In addition to high incidence rates, these areas are associated with extreme poverty, malnutrition, poor hygiene, and other environmental factors that lead to immunosuppression (WHO, 2008). Therefore, the high incidence of TB in these areas and also in AIDS patients has led to an increased spread of TB.

The virulence of *Mycobacterium tuberculosis* is dependent upon the establishment of an infection within human macrophages. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced by macrophages as part of their antimicrobial response. The production of ROS is initiated by NADPH oxidase, which catalyzes the reduction of molecular oxygen to superoxide (O_2^-). Superoxide can then be converted to H_2O_2 and hydroxyl radical (Miller and Britigan, 1997). In addition, nitric oxide (NO) produced by inducible nitric

oxide synthase (iNOS) can combine with superoxide to generate additional products with enhanced toxicity, such as peroxynitrite (ONOO2) (Bogdan et al., 2000).

Despite the toxic effects of ROS and RNS, M. tuberculosis can survive and grow within macrophages. Several M. tuberculosis gene products have been associated with the detoxification of ROS and RNS. KatG is a catalase peroxidase (Rouse et al., 1996) that protects M. tuberculosis from killing by hydrogen peroxide (Manca et al., 1999). KatG also has peroxynitritase activity (Wengenack et al., 1999). The alkylhydroperoxide reductase AhpC is capable of catalyzing the breakdown of peroxynitrite (Bryk et al., 2000). Lipoarabinomannan can scavenge potentially toxic oxygen free radicals (Chan et al., 1991). M. tuberculosis also produces two superoxide dismutase (SOD) proteins, SodA and SodC. The enzymatic function of SOD is to convert O_2^- into molecular oxygen and hydrogen peroxide. This activity removes the toxic effects of O_2^- and prevents the formation of higher H_2O_2 levels by other reactions (Teixiera et al., 1998) and the synergism of ROS with RNS (Liochev and Fridovich, 1994; Teixiera et al., 1998). SodA is an Mn,Fe SOD and is one of the major extracellular proteins in M. tuberculosis (Harth and Howitz, 1999; Zhang et al., 1991). SodC is a Cu,Zn SOD and is produced at much lower levels by M. tuberculosis. However, SodC contains a lipoprotein binding motif that may mediate its attachment to the outer membrane of the bacteria. Immunogold electron microscopy has detected SodC at the periphery of M. tuberculosis (Wu et al., 1998). The peripheral location of the Cu,Zn SOD suggests that it may protect the surface of M. tuberculosis against extracellular superoxide generated by host cells. Because of the location of SodC on the surface of the bacteria, we examined the role of the Cu,Zn SOD in protecting M. tuberculosis from exogenous sources of ROS. A mutation in the sodC gene was constructed, and the M. tuberculosis mutant was used to assess the importance of SodC in protecting M. tuberculosis from the toxic effects of superoxide or a combination of superoxide and nitric oxide generated in vitro.

The redox control system in M. tuberculosis is a complex and dynamic network of regulatory systems, consisting of transcription factors, mycothiol [a low-molecular-weight (LMW) thiol], thiol-disulfide oxidoreductases (thioredoxins) (den Hengst and Buttner, 2008), and other features. Mechanisms by which these responses are regulated in M. tuberculosis are significantly different from those observed in other bacteria (Sherman et al., 1995). For instance, in Escherichia coli, the redox-sensitive transcriptional regulator OxyR is responsible for production of enzymes that are involved in defense against oxidative stress (Christman et al., 1989). oxyR is conserved in several mycobacterial species including M. leprae and M. tuberculosis. In M. tuberculosis, oxyR is a pseudogene,

which is inactivated by multiple mutations, deletions or frameshifts (Deretic et al., 1995).

The interaction between the intracellular pathogen Mycobacterium tuberculosis and mononuclear phagocytes typically leads to induction of host adaptive immune responses, which control infection without sterilizing the host (Flynn and Chan, 2001; North and Jung, 2004). Survival of M. tuberculosis to expression of adaptive immunity is presumably associated with entry into a "dormant" state, in which tubercle bacilli exhibit no or slow growth and low metabolic activity. As a result, a latent infection is established. When immunity fails to control infection, tubercle bacilli exit dormancy, resume growth and cause disease. Thus, adaptation to the conditions found inside the phagocytic cell is key to the survival and persistence of M. tuberculosis in vivo.

In this paper, we report review of role and characteristic of antioxidant for redox homeostatis control system in Mycobacterium tuberculosis.

Superoxide Dismutase role in Mycobacterium tuberculosis

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. They are initial components of the cellular defense against reactive oxygen intermediates (ROI) resulting from univalent reduction of oxygen, and they contribute to the survival of bacterial pathogens such as Shigella flexneri (Franzon et al., 1990), Campylobacter jejuni (Pesci et al., 1994), Salmonella enteric serovar Typhimurium (Fang et al., 1999; Farrant et al., 1997; Tsolis et al., 1995), Yersinia enterocolitica (Roggenkamp et al., 1997), and Neisseria meningitidis (Wilks et al., 1996).

Mycobacterium tuberculosis produces a tetrameric iron-cofactored SOD (FeSOD or SodA) encoded by the sodA gene (Cooper et al., 1995; Zhang et al., 1991) and a copper and zinc SOD (CuZnSOD or SodC) encoded by the sodC gene (Wu et al., 1998). FeSOD is among the major extracellular proteins released by M. tuberculosis during growth (Andersen et al., 1991). It is exported in an active form via a signal peptide-independent pathway that has not been fully characterized (Harts and Horwits, 1999; Zhang et al., 1999). The CuZnSOD possesses a putative signal peptide and is localized to the periphery of M. tuberculosis (Wu et al., 1998). It has been hypothesized that the presence of SODs at the periphery of M. tuberculosis and in the extracellular milieu could protect bacteria from superoxides generated exogeneously, e.g., by host phagocytes (Harts and Howits, 1999; Wu et al., 1998; Zhang et al., 1991).

The killing of M. tuberculosis by host-activated phagocytic cells is mediated to some extent by ROI along with reactive nitrogen intermediates (Adams et al., 1997; Chan et al., 1992; Lau et al., 1998; MacMiking et al.,

1997). The contribution of mycobacterial CuZnSOD to the defense of bacteria against oxidative killing. Cu,Zn SOD of M. tuberculosis is protective against the toxic effects of superoxide generated by hypoxanthine/xanthine oxidase and contributes to resistance to killing by oxidative products generated by activated macrophages. Recently it was reported that Cu,Zn SOD mutants of M. tuberculosis and of BCG are only moderately sensitive to superoxide generated in vitro using the superoxide generating agent menadione or plumbagin and that the BCG sodC mutant was unaffected in activated murine bone marrow macrophages or in guinea pig tissues (Dusurget et al, 2001).

The sodC mutant appears to have greater sensitivity to superoxide that is generated extracellularly using hypoxanthine/xanthine oxidase. Both plumbagin and menadione are known to increase intracellular levels of superoxide (Archibald and Duong, 1986; Farr et al, 1985; Kim et al 2001). If the Cu,Zn SOD protects the surface of the bacteria from externally generated superoxide, the influence of a sodC mutation is expected to be more pronounced when an extracellular superoxide-generating agent such as hypoxanthine/xanthine oxidase is used. The contribution of the Cu,Zn SOD to survival of M. tuberculosis in macrophages is dependent upon the quantity of ROS generated. In the present study reported that the sodC mutant was sensitive to killing by peritoneal macrophages from normal mice, and this sensitivity was abolished in peritoneal macrophages from gp91phox2/2 mice. These results indicate that Cu,Zn SOD contributes to the resistance of M. tuberculosis to phagocyte-derived oxidative burst products. Inactivation by macrophages of the sodC mutant corresponded with the initial detection of superoxide production in these cells. From the kinetics of inactivation of the sodC mutant in macrophages, it appears that the initial interaction with superoxide is most critical in determining whether the mutant bac In concert with the phagocyte respiratory burst, macrophages generate toxic NOS. Synergism between the NADPH oxidase and iNOS pathways generate products with enhanced toxicity. Peroxynitrite as well as other synergistic intermediates formed by the reaction of ROS with nitric oxide have potent antimicrobial activity (Nathan and Shiloh, 2000; Pacelli et al., 1995). M. tuberculosis was resistant to a combination of superoxide and nitric oxide when generated in vitro. Thus the virulent M. tuberculosis is relatively resistant to peroxynitrite (Yu et al., 1999).

The sodC mutant was, however, extremely sensitive in vitro to a combination of superoxide and nitric oxide. Despite this sensitivity in vitro, inactivation of the sodC mutant was unchanged in iNOS-deficient macrophages compared to normal macrophages. We also cultured macrophages from wild-type mice in medium deficient inL-arginine, which prevents the production of RNS, and did not observe a change in virulence of the sodC mutant (data not shown). Therefore, it appears that the produc-tion of RNS does not contribute to killing of the sodC mutant during the in vitro macrophage assay. This corresponds with our observations that the production of NO by the M. tuberculosis-infected macrophages does not occur until after killing of the sodC mutant has subsided and that there is no additional inactivation of the mutant with the appearance of NO at 24 h. It is clear that the simultaneous addition of exogenous superoxide and nitric oxide has different consequences for the sodC mutant than exposure to these products by macrophages cultured in vitro. Presumably, the kinetics for production of superoxide and nitric oxide in vivo may be different from those in our macrophage system, as activation of both the NADPH oxidase and the iNOS is dependent on the complex cytokine response generated by M. tuberculosis infection.

Cu,Zn SOD genes have been detected in a number of other bacteria, including Haemophilus (San Mateo et al., 1999), Neisseria (Wilks et al., 1998), Escherichia (Gort et al., 1999), Legionella (St John and Steinman, 1996), and Salmonella (Canvin et al., 1996). In some pathogenic bacteria, such as Neisseria meningitidis (Wilks et al., 1998), Salmonella enterica serovar Typhimurium (De Grooce et al., 1997; Farrant et al., 1997), and Haemophilus ducreyi (San Mateo et al., 1999), the Cu,Zn SOD has been associated with virulence. In fact, virulent strains of Salmonella produce two distinct Cu,Zn SODs, each of which contributes to the virulence of Salmonella (Fang et al., 1999). In other pathogenic bacteria, an association of the Cu,Zn SOD with virulence is unclear. In the swine pathogen Actinobacillus pleuropneumoniae, a sodC mutant was not attenuated after intratracheal infection (Sheehan et al, 2000), and one of two studies using a sodC mutant of Brucella abortus found no attenuation of the mutant (Latimer et al., 1992; Tatum et al., 1992). This suggests that the role of Cu,Zn SOD during infection may depend upon a variety of factors, including the infecting organism, host, route of acquisition, and site of infection. In addition to the Cu,Zn SOD, M. tuberculosis carries an Mn,Fe SOD, SodA. Interestingly, SodA is one of the major secreted proteins of M. tuberculosis (Zhang et al., 1999). The disproportionate production of SodA versus SodC on SOD activity gels, making it impossible to visualize SodC activity in thismanner. Although SodC is produced in much smaller amounts than SodA, the phenotype of the M. tuberculosis sodC mutant was very dramatic in the in vitro assays. Thus, despite the presence of large amounts of SodA, Cu,Zn SOD is essential for protecting M. tuberculosis from the toxic effects of superoxide. The Cu,Zn SOD may serve another function for mycobacteria in addition to protecting the bacteria from ROS generated by activated macrophages. The sodC gene was detected in 14 out of 15 mycobacterial species, which include rapid growers and nonpathogenic species. This suggests that the sodC gene product may play a role in detoxifying superoxide during growth of bacteria outside the host.

Although we detected no defect in growth of the sodC mutant during culture in 7H9 broth, the Cu,Zn SOD may protect the bacteria from endogenously generated superoxide under specialized conditions.

Gort et al. have suggested that the Cu,Zn SOD of Escherichia coli plays a role in protecting the bacteria from endogenously produced superoxide generated during specific phases in the growth cycle, such as the transition into stationary phase (Gort et al., 1999). Cu,Zn SOD may fulfill a similar role in mycobacteria. This report demonstrates that Cu,Zn SOD of M. tuberculosis is protective against extracellular superoxide and against a combination of superoxide and nitric oxide. Furthermore, sodC mutant M. tuberculosis has increased sensitivity to hydrogen peroxide compared to the isogenic parental strain (data not shown). These results support the hypothesis that the Cu,Zn SOD protects M. tuberculosis from extracellular sources of ROS. However, despite the contribution of SodC to survival in macrophages in vitro, a preliminary study using low-dose aerosol infection suggests that the sodC gene is not essential for survival of M. tuberculosis in the lungs of mice during early stages of infection (data not shown). In this initial study, no difference in bacterial numbers between parental M. tuberculosis and the sodC mutant was observed in the lung up to 60 days postinfection. At 60 days, a slight difference in organism burden in the lungs was noted. This may indicate that in the absence of SodC, SodA is capable of protecting the bacteria from superoxide generated during the early stages of pulmonary infection. While performing the macrophage assays, we observed that sodC mutant M. tuberculosis was sensitive to killing by macrophages only when the macrophages were activated with IFN-g. In nonactivated macrophages, which produce a less vigorous oxidative burst, the phenotype of the sodC mutant was lost. This suggests that a major role for the Cu,Zn SOD is to protect M. tuberculosis from large quantities of toxic reactive products produced by activated macrophages. During the early stages of infection, the lower levels of respiratory burst products generated by nonactivated macrophages may not require the presence of Cu,Zn SOD. Future experiments will evaluate the contribution of Cu,Zn SOD to M. tuberculosis infections in a long-term in vivo study.

Catalase-peroxidase (katG) role in Mycobacterium tuberculosis

All living organisms, except anaerobic bacteria, require protection against the damaging effects of reactive oxygen species. The M. tuberculosis KatG is a component of the oxidative defense system of the bacteria and functions primarily as a catalase to remove hydrogen peroxide. Such catalase activity is regarded as one of the virulence factors for M. tuberculosis (Middlebrook, 1954), because this reaction detoxifies

reactive oxygen species generated by macrophages and facilitates the bacterial survival. Strains of M. tuberculosis lacking the katG gene are heavily attenuated in animal models (Pym et al., 2002). On the other hand, M. tuberculosis KatG functions as a peroxidase, oxidizing INH to its active form. KatG is thought to be the only enzyme in M. tuberculosis that is capable of generating isoniazid susceptibility (Zhang et al., 1992). It has been shown that most INH resistance in clinical isolates results from blocking prodrug activation through mutation in the katG gene that alters or diminishes catalase-peroxidase activity (Musser, 1995).

M. tuberculosis KatG is an 80 kDa bifunctional enzyme exhibiting a high catalase activity and a peroxidase activity with broad specificity (Heyme et al., 1993) (Welinder, 1992a) (Zhang et al., 1992). It is capable of using a single active site to catalyze both the catalase and peroxidase reactions and thereby remove hydrogen peroxides from the biological system efficiently. It is believed that the catalase function of KatG is essential for the protection of the bacteria against oxygen free radicals within the macrophage (Manca et al., 1999).

KatGs differ from classic catalases in many respects. They have little sequence homology with typical catalases. In contrast to classic catalases which show a broad pH activity (range from pH 5.5 to 10.0), KatGs show maximum activity in a narrow pH range (6.0-6.5) and are not inhibited by typical catalase inhibitors (Nadler, 1986). The catalase activity of KatG is greatly reduced by dialysis against low concentration hydrogen peroxide, while dialysis against hydrogen peroxide had no effect on the classic catalase (Nadler 1986). A covalent bond between the active site tryptophan, tyrosine, and methionine residues was found to present in the H. marisomortui KatG, Synechocystis KatG, B. pseudomallei KatG, and M. tuberculosis KatG (Yamada et al.,2002; Jakopitsch et al., 2003a). Thus, the Trp-Tyr-Met adduct may be a common feature to all catalase-peroxidase. The integrity of the covalent adduct correlates to catalase activity but not the peroxidase activity of KatGs (Jakopitsch et al., 2003a). Studies had been shown that upon exchange of the active site tryptophan, the catalase activity was Properties of MtbKatG M. tuberculosis

Catalase-peroxidase (KatG) of Mycobacterium tuberculosis is a bifunctional heme enzyme that has been shown to play an important role in the activation of a first line drug, isoniazid (INH), used in the treatment of tuberculosis infection. Mutations in the katG gene have been found to be associated with INH resistance. The most commonly encountered mutation is the Ser315Thr point mutation M. tuberculosis Catalase-Peroxidase (KatG). Classification of Peroxidases Based on the sequence similarity, KatGs have been classified into the class I peroxidase superfamily (Welinder 1992b). Peroxidases are ubiquitous oxidative heme-containing enzymes that utilize hydrogen peroxide to oxidize a wide

variety of organic and inorganic compounds. They have been implicated in diverse biological processes that include cell wall synthesis and degradation, stress response, signaling during oxidative stress, and removal of xenobiotics (Rodriguez-Lopez 2001). The plant peroxidase superfamily includes peroxidases of prokaryotic origin, secreted fungal and secreted plant peroxidases. The plant peroxidase superfamily is further classified into three families, class I, class II, and class III (Dunford 1999). Class I peroxidases consist of peroxidases of prokaryotic origin, including plant peroxidases, e.g. horseradish peroxidase (HRP), ascorbate peroxidase (APX), fungal peroxidases, e.g. cytochrome c peroxidase (CCP), and bacterial catalase-peroxidases (KatGs). Class I peroxidases contain no carbohydrate, no cysteine bridge ions, and no signal peptide for secretion. Class II peroxidases include the secretory fungal peroxidases such as lignin peroxidase (LiP), manganese peroxidase (MnP) (Sundaramoorthy 1994), and Arthromyces ramosus peroxidase (ARP). Class II peroxidases contain an amino terminal signal peptide sequence for the secretion through the endoplasmic reticulum (Welinder 1992a). Class III peroxidases are the secretory plant peroxidases that are targeted for the secretory pathways (Welinder 1992b). All members of the three classes catalyze the reduction of hydrogen peroxide.

KatGs are homodimeric proteins with monomers being twice as large as other members of class I peroxidase e.g. CCP or APX, which is ascribed to gene duplication (Welinder 1992a). Their N-terminal domain binds a heme and shows high sequence homology to yeast cytochrome c peroxidase (Duroux and Welinder 2003). The C-terminal domain has lost its heme and has a much lower homology to cytochrome c peroxidase. In contrast to other members of the class I family, KatGs exhibit a high catalase activity comparable to monofunctional catalase, despite having low sequence similarity with typical monofunctional catalase (Murthy et al., 1981).

Mycothiol role in Mycobacterium tuberculosis

Mycobacteria are devoid of GSH, and instead they produce mycothiol (MSH) (1-D- myo-inositol-2-(N-acetyl-L-cysteinyl) amido-2-deoxy-D-glucopyranoside (Newton et al., 1993). MSH is a pseudo-disaccharide and contains a functional cysteine group (Newton et al., 1993, Sakuda et al., 1994, Spies and Steenkamp, 1994). Several functions have been attributed to MSH beside its reducing characteristics. The primary role of MSH is to maintain the intracellular redox homeostasis. As such, it acts as an electron acceptor/donor and serves as a cofactor in detoxification reactions for alkylating agents, free radicals and xenobiotics. It functions as a line of defense against ROIs (Ung and Av-Gay, 2006), stores cysteines, and detoxifies antibiotics (Steffek et al., 2003,

Rawat and Av-Gay, 2007). The MSH biosynthesis pathway has been well characterized (Newton and Fahey, 2002, Newton et al., 2006). Another thiol present in actinomycetes is ergothioneine (ESH). ESH is a betaine of 2-thiol-L-histidine (Genghof and Vandamme, 1964). Unlike MSH, ESH has been detected in plants, fungi, animals and bacteria, although only fungi and actinomycetes are able to synthesize this thiol (Genghof, 1970). The amount of ESH present in actinomycetes is ten-fold lower than that of MSH and its exact function in these bacteria is still unknown (Fahey, 2001). As mentioned earlier, the antioxidant properties of both MSH and GSH are due to the presence of a functional reduced cysteine. MSH and GSH undergo oxidation to the disulfide form mycothione (MSSM) and GSSG respectively. The disulfide compounds are converted back to their reduced form by specific disulfide reductases such as mycothione reductase in the case of MSH (Newton and Fahey, 2002, Patel and Blanchard, 1999) and glutathione reductase in GSH (Karplus and Schulz, 1987).

Thioredoxins role in Mycobacterium tuberculosis

One of the major ubiquitous disulfide reductases responsible for maintaining proteins in their reduced state are thioredoxins. They function in presence of thioredoxin reductases constituting a thioredoxin system. Thioredoxins are small (8-14 kDa), heat-resistant proteins. They have multiple functions and they are involved in a plethora of cellular regulatory and metabolic pathways (Jeffery, 1999). Thioredoxins are characterized by the presence of a dithiol/disulfide active site CXXC (Holmgren, 1985). They are ubiquitously distributed and highly conserved in all organisms from Archea to humans (Eklund et al., 1991). Rapid and reversible thiol-disulfide exchange reactions control protein function via the redox state of structural or catalytic SH groups. Oxidation of a critical SH group will lead to changes in structural and functional changes in proteins. Therefore, thioredoxins have a wide range of functions in cellular physiology and pathological conditions by contributing to the maintenance of the redox state of protein thiols (Holmgren, 1985). Oxidized thioredoxins are reduced by thioredoxin reductases (TrxR) in a NADPH-dependent reaction (Holmgren, 1985). Thioredoxin reductases are a large family of FAD-containing enzymes. The enzymatic mechanism involves the transfer of reducing equivalent from NADPH to a disulfide bond in the enzyme within the highly conserved sequence CATC via FAD. Subsequently, these electrons are transferred to a final acceptor via thioredoxins.

Thiol-disulfide switching is essential for maintenance of cellular proteins in their reduced form during oxidative conditions. Thiol-disulfide exchange reactions via redox active disulfides are efficient for electron transport and

are used in mechanisms of essential enzymes such as ribonucleotide reductase required to provide deoxyribonucleotides for DNA synthesis and sulfate reductases essential for sulfur containing metabolite homeostasis (Black et al., 1960; Holmgren, 1985). The oxidation-reduction function of the thiol/disulfide oxidureductase activity of thioredoxins depends on two major determinants, the pKa values of the cysteine residues in the CXXC active site motif, and the standard-state redox potential. Generally Trxs have the lowest redox potential as opposed to other members of the oxidoreductase family and thus are the most reducing members of this family as well (Akif et al., 2008). For instance, the redox potential of TrxA is -270mV, while the redox potential of glutaredoxins that are other members of this family range from -233 to -198 mV (Aslund et al., 1997).

The M. tuberculosis genome encodes three thioredoxin proteins (TrxA, TrxB1 and TrxC) and it also bears a single copy of thioredoxin reductase TrxB2 (TrxR) (Cole et al., 1998). Interestingly, only in pathogenic mycobacteria, genes encoding TrxC and TrxR are found at the same locus overlapping in one nucleotide (Wieles et al., 1995) has been reported that only TrxB1 and TrxC are reduced by TrxR and display oxidoreductase activity (Akif et al., 2008) by proficiently reducing disulfide bonds in the classic substrate model insulin (Holmgren, 1979). This suggests that TrxB1 and TrxC are the major cellular thioredoxins in M. tuberculosis (Akif et al., 2008). However, it appears that M. tuberculosis mutants missing any of the trx ORFs are not drastically impaired for survival in vitro, and only trxB2 turns out as an essential gene (Sassetti et al., 2003). The mechanisms by which M. tuberculosis resist oxidative killing elicited by mononuclear phagocytes are not yet clearly defined. Several mechanism have been proposed, one of which is the scavenging of free radicals produced by activated macrophages upon infection (Shinnick et al., 1995). As mentioned earlier, M. tuberculosis is devoid of GSH and synthesizes MSH (Cole et al., 1998, Rawat and Av-Gay, 2007). The involvement of thioredoxins in protection against oxidative stress and maintenance of intracellular thiol homeostasis in prokaryotes suggests that TrxC and TrxR of M. tuberculosis are most likely carrying out the same functions (Holmgren, 2000, Prinz et al., 1997, Holmgren, 1989). The system comprising TrxR and TrxC is one of the most well studied thioredoxin systems in M. tuberculosis. It has been shown to be involved in several detoxification pathways such as reducing dinitrobenzene, and hydroperoxides (Akif et al., 2008; Zhang et al., 1999). This thioredoxin system contributes to pathogen's defense against ROIs in two ways (Manca et al., 1999; Jaeger et al., 2004). First by reducing alkyl hydroperoxide (ahpC), and keeping it catalytically active (Jaeger et al., 2004). Additionally, thioredoxin system serves as in efficient detoxification pathway against hydroperoxides and peroxynitrite once

the thiol peroxidase Tpx complements the system (Jaeger et al., 2004). Tpx is part of an oxidative stress defense system that uses electrons donated by TrxC and TrxR to reduce alkyl hydroperoxides (Jaeger et al., 2004). Tpx contains a redox-active intrasubunit disulfide bond, which undergoes reversible oxidation/reduction. Due to the fact that both TrxC and Tpx are consistently detectable as a major spot in proteomic analyses of M. tuberculosis cell-free filtrate (Rosenkrands et al., 2000), it is predicted that detoxification of ROIs and RNIs are predominantly mediated by the second pathway (Jaeger et al., 2004)

CONCLUSION

In conclusion, we have established that M. tuberculosis Cu,Zn SOD is required for resistance to exogenous superoxide-dependent cytotoxicity, including the products of activated macrophages. Further work will address the role of the Cu,Zn SOD during infection within the host and how the SOD activity derived from sodC relates to the Mn,Fe SOD in M. tuberculosis. KatG is a catalase peroxidase that protects M. tuberculosis from killing by hydrogen peroxide. Thioredoxins have a wide range of functions in cellular physiology and pathological conditions by contributing to the maintenance of the redox state of protein thiols.

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