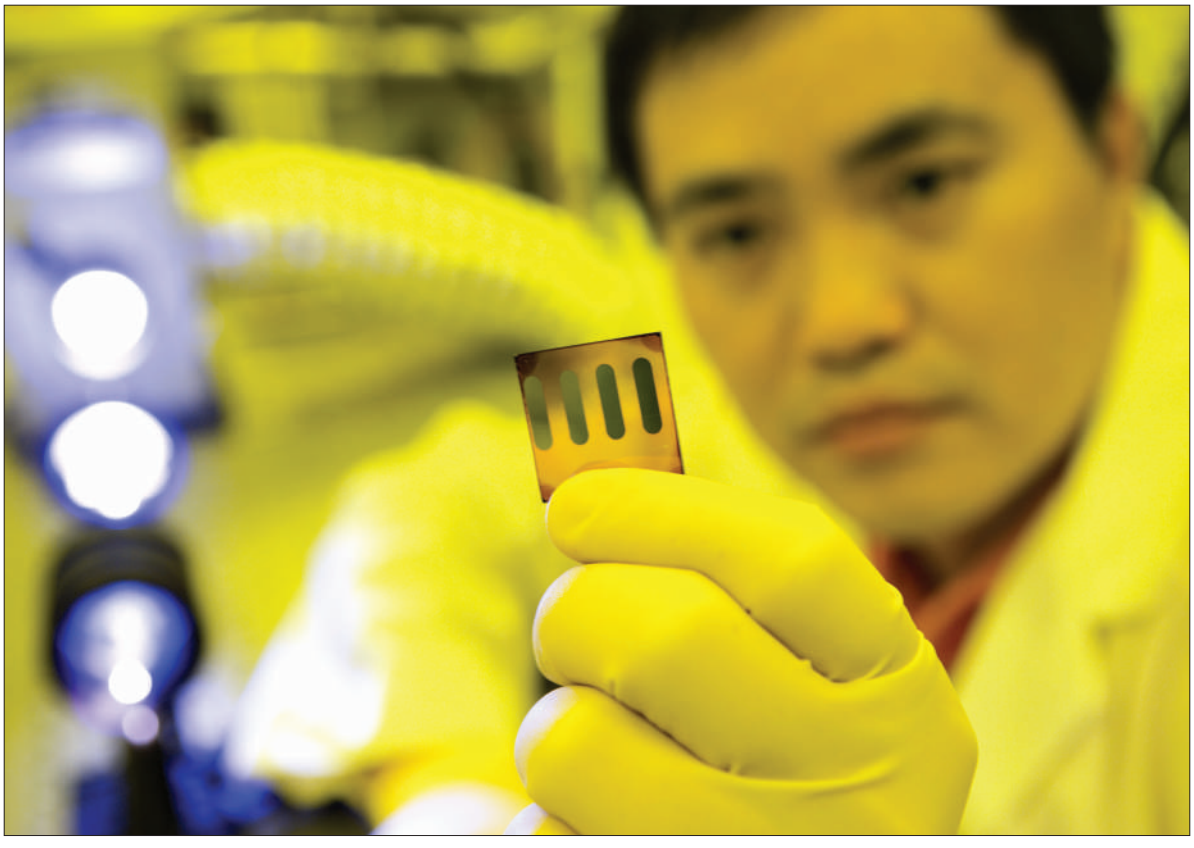

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Anti Mycobacterial Activity of Actinomycetes Producing Mycothiol

M. Mohamed Mahroop Raja, A. Raja, M. Mohamed Imran and E. Vani Ugin
Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli-620 020, Tamil Nadu, India

Corresponding Author: M. Mohamed Mahroop Raja, Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli-620 020, Tamil Nadu, India

ABSTRACT

Mycothiol (MSH or AcCys-GlcN-Ins) is an unusual thiol compound found in the Actinobacteria including Actinomycetes and Mycobacterium. The enzymes involved in mycothiol biosynthesis are of interest as potential targets for new drugs directed against Mycobacterium tuberculosis. In this review, it is focused on MSH-dependent enzymes that utilize MSH for their activity either as a cofactor or as a substrate indicate that MSH plays a key role in protecting cells against a variety of challenges and suggest that enzymes of MSH metabolism could be possible targets for development of new drugs and vaccines directed against Mycobacterium tuberculosis and other infectious Actinomycetes.

Key words: Mycothiol, Mycobacterium, MSH, actinomycetes, drug

INTRODUCTION

Mycothiol[2-(N-acetylcysteinyl)amido-2-deoxy-alpha-D-glucopyranosyl-(1-->1)-myo-inositol] (MSH) has recently have been identified as a major thiol in a number of Actinomycetes (Aharonowitz *et al*., 1995). Low molecular-weight thiols play a key role in maintaining a reducing environment in the cell, which is necessary for regular metabolic activities to occur. These thiols thus represent a major biological adaptation that is important for the survival of organisms under various endogenous and exogenous stresses. In eukaryotes and gram-negative bacteria, the much-studied tripeptide Glutathione (GSH) is the dominant thiol. GSH plays a major role in protecting the cell against oxygen toxicity (Anderson, 1998) by removing reactive oxygen species that may result from atmospheric oxygen and basal metabolic activities in aerobic organisms.

Another thiol present in Actinomycetes, Ergothioneine (ESH), is a betaine of 2-thiol-L-histidine. Unlike MSH, ESH has been detected in plants, fungi, animals and bacteria. However, 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. Like GSH, MSH has a functional cysteine moiety but instead of the two amino acids, glycine and glutamic acid, present in GSH, there are two sugar moieties, inositol and N-glucosamine. The glycosidic link between glucosamine and inositol is comparable in strength to the amide bond connecting the acetyl group to the cysteine and the second amide bond connecting the cysteine to the glucosamine (Fig. 1). This stability in the bonds may have played a role in the evolution of MSH as the major intracellular thiol in actinomycetes (Fahey, 2001). Moreover, certain actinomycetes such as Mycobacteria are known for their complex cell walls consisting of polysaccharides, lipopolysaccharides and complex fatty acids. The preponderance of glucosamine and in particular inositol, in the Actinomycete cell wall may have

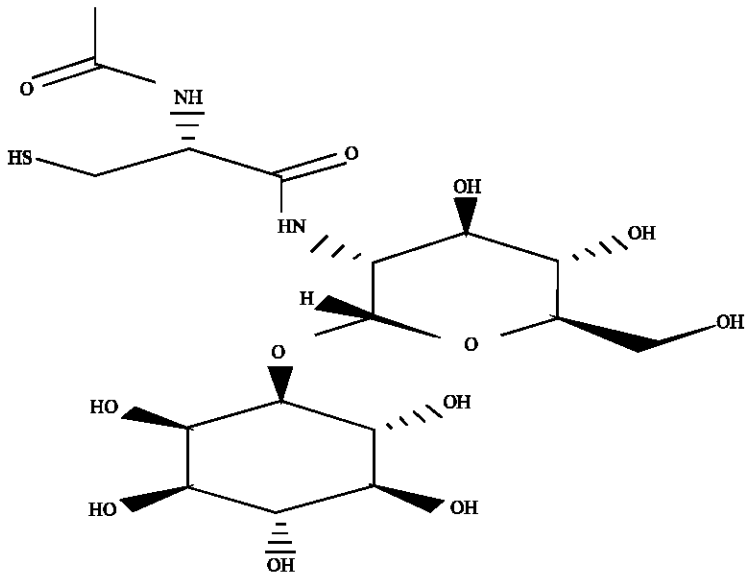


Fig. 1: Structure of mycothiol

favoured the use of a 'sugar' thiol (MSH) for redox control over the peptide (GSH) thiol. Actinomycetes in MSH are potential targets for drugs directed against Mycobacteria. New antibiotics that are active against resistant bacteria (Raja *et al.,* 2010) have lived on earth for several billion years. During this time, they encountered in nature a wide range naturally occurring antibiotics. To survive, bacteria developed antibiotic resistance mechanism (Hoskeri *et al.*, 2010).

FUNCTIONS OF THIOL

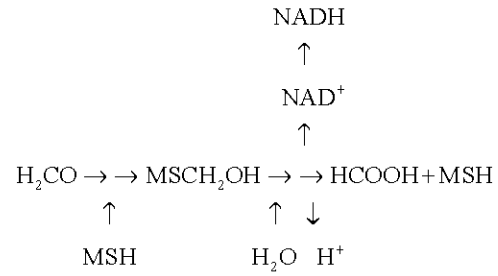
One of the major functions of thiols is to serve as a storage form of cysteine, because cysteine tends to autooxidize in a matter of minutes, readily forming toxic peroxy radicals and hydrogen peroxide. MSH undergoes copper catalyzed auto-oxidation 30-fold more slowly than cysteine and 7-fold more slowly than GSH. This important difference implies that the ability to cope with oxidative stress is much higher in actinomycetes. In terms of chemical reactivity, MSH is an inherently poor nucleophile compared with ovothiol and GSH (Spies and Steenkamp, 1994). The physical parameters of mycothiol such as the thiol pKa and the stability of the free radical form have not been elucidated, although the redox potential of mycothiol is expected to be similar to that of glutathione.

In this review which focus on MSH-dependent enzymes that utilize MSH for their activity either as a cofactor or as a substrate which summarizes the current knowledge about MSH metabolism. As most studies on MSH metabolism have been performed on Mycobacteria, the majority of the information is drawn from Mycobacterial research.

IN VIVO AND *IN VITRO* REACTION OF MYCOTHIOL ON MYCOBACTERIAL CELL

All organisms growing in an aerobic environment produce one or more low molecular mass thiol (s) as a major metabolite. In the majority of eukaryotes, Gluthathione (GSH) is the principal antioxidant thiol in gram negative bacteria. In prokaryotes and certain eukaryotes, Gluthathione

(GSH), Trypanothione (TSH) and Thioredoxin (Trx), maintains the cellular redox homeostasis. Most Actinomycetes produce mycothiol (MSH) as their principal low molecular mass thiol (1D-myo-inosityl-2-(N-acetyl-L-cysteinyl)-amido-2-deoxy-alpha-D-glucopyranoside). Present knowledge indicates (Eq. 1 and 2) that the redox cycling of Mycothiol (MSH) to mycothiol disulfide (MSSM) is very critical for the *in vivo* and *in vitro* survival of mycobacteria (Newton and Fahey, 2002) Since MSH is only present in Actinomycetes and plays an important protective role, all the enzymes involved in its maintenance are potential drug targets (Newton *et al.*, 2008).



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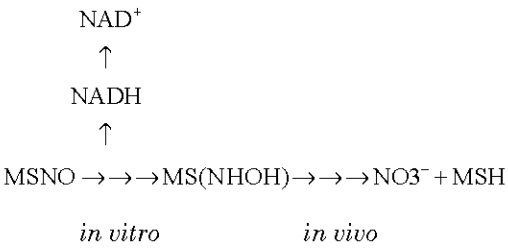

(2)

Eq. 1 and 2: The formaldehyde dehydrogenase reaction of MscR. The nitrosothiol reductase reaction of MscR, indicating that MSH sulphinamide is formed *in vitro* and that MSNO is eventually converted to nitrate and MSH *in vivo* through unknown reactions.

METABOLIC REACTION OF MYCOTHIOL

The biosynthetic pathway of MSH has been elucidated in the formation of Nacetylglucosaminylinositol is catalyzed by the gene product of mshA. N-acetylglucosaminylinositol is then deacetylated by MSH deacetylase, encoded by MshB, to yield glucosaminylinositol (Buchmeier *et al.*, 2003). The coupling of cysteine to the 20 amine of this pseudodisaccharide is catalyzed by an MSH ligase, encoded by mshC. The final step is the N-acetylation of cysteinylglucosaminylinositol to yield MSH, catalyzed by mycothiol synthase, the product of mshD (Koledin *et al.*, 2002). Streptomyces coelicolor mutants in the four genes involved in MSH biosynthesis have been isolated and recently an mshD mutant of Amycolatopsis mediterranei has been reported (Chen *et al.*, 2005). S1 mapping analysis has demonstrated that three of these genes, mshA, mshC, mshD, are induced under osmotic challenge in Streptomyces coelicolor (Park *et al.*, 2006).

Biosynthesis of mycothiol catalyzed by MshA, MshB, MshC, MshD. Degradation reactions to scavenge mycothiol for cysteine in times of nutrient starvation. Protective reactions catalyzed by: Mycothiol Amidase (Mca) and putative mycothiol-S-tranferases involved in the detoxification of xenobiotic agents; putative thiol transferases, nitrosothiol reductase (MscR) and thiol peroxidases

or peroxiredoxins involved in oxidative and nitrosative stress protection and Mycothiol Reductase (Mtr), which maintains mycothiol in a reduced form within the Mycobacterial cell. Metabolic reactions catalyzed by enzymes such as maleylpyruvate isomerase requiring mycothiol as a cofactor for growth on diverse carbon sources. More recently demonstrated that the GSH-independent gentisate pathway in actinomycetes requires MSH as a cofactor for the catalysis of maleylpyruvate to fumarylpyruvate by a maleylpyruvate isomerase, encoded by ncg l2918 (Feng *et al*., 2006).

CONCLUSION

In this review, it is focused conclusively to require MSH for activity. These enzymes play important roles in the Actinomycetes, as they maintain the redox balance within the cell and protect the cell against nitrosative stress and toxins (Rawat and Av-Gay, 2007). In addition, the role of MSH in growth-supporting biodegradative metabolism is the beginning to be elucidated with the identification of MSH-dependent enzymes such as maleylpyruvate isomerase, whether MSH participates in the novel enzymatic activities, such as the MSH dependent amidase, that require MSH as a cofactor. Because MSH or enzymes involved in MSH biosynthesis and metabolism are not present in mammals and given the emerging importance of MSH in Mycobacteria, MSH and reactions involving MSH are potential targets for drugs directed against Mycobacteria.

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