Role of MGST1 in reactive intermediate-induced injury

Courtney S Schaffert

MGST1 STRUCTURE AND FUNCTION

MGST1 is an approximately 17 kDa membrane bound glutathione s-transferase. It is found in the highest
quantity in the liver; however it is also present in other tissues in smaller amounts. It is found primarily in the microsomes (3% of microsomal protein) and the inner and outer mitochondrial membranes (5% of mitochondrial membrane protein) of cells, where the majority of reactive intermediates are produced. It exists in membranes as a homotrimer which can bind three molecules of GSH. However, only one molecule is modified to a thiolate anion, and this molecule binding site has a much higher affinity than the other two sites.

MGST1 has two enzymatic activities; one as a GST, and the other as a glutathione dependent peroxidase (GPX). The function of MGST1 in both organelles is thought to be the neutralization of lipid peroxides and conjugation of other reactive intermediates to glutathione. Based on these two enzymatic activities, MGST1 has been shown to have three substrate binding sites. One site binds GSH on the cytosolic face of each monomer. A second site binds electrophilic substances in a hydrophobic pocket, also on the cytosolic face. This site may partially overlap with the glutathione thiolate anion binding site. This site contains cysteine (the only cysteine per monomer) which acts as an oxidative and chemical stress sensor. Because the binding pocket for electrophiles is hydrophobic, MGST1 is uniquely suited among the GSTs to detoxify more hydrophobic reactive intermediates, as often occurs during drug metabolism. The third site is the phospholipid/fatty acid binding site that may bind these hydrophobic lipid peroxides through an opening in the tertiary structure of the trimer, rather than binding through the cytosolic face. This site also overlaps the GSH binding site, but is separate from the electrophilic site.

The majority of research has been performed on MGST1 purified from liver microsomes. These studies have identified that MGST1 exists as a trimeric complex where three monomers interact to give the active enzyme. Modification of the thiol group of cysteine induces a conformational change and increased activation, and at increasing protein concentration, the trimers aggregate into higher molecular weight complexes that have even further increased activity as measured by GST assay. Multiple studies have also shown that the MGST1 trimer is activated by various chemical treatments that appear to modify the cysteine. These modifications include alkylation, formation of disulfide bonds (either mixed disulfides with glutathione, or protein dimer formation), or sulfenic acid modification. An additional mechanism of activation is proteolysis at lysine. In fact, a liver enzyme, hepsin, has been implicated in this mode of activation. It is postulated that proteolysis and cysteine modification induce a conformational change that induces the GSH that is bound to be converted to the thiolate anion more readily than what occurs under basal levels.

**PROTECTIVE EFFECTS OF MGST1**

A variety of reactive intermediates can induce activation of MGST1 in vitro and in vivo, but the physiological function of MGST1 is still unclear. It has also been noted that transfection of cytochrome P450 2E1 (CYP2E1), a significant generator of ROS, in a hepatoma cell line (HepG2) induced increased expression and activation of catalase, GSTQ (cytosolic GST) and MGST1. Incubation of these cells with increasing concentrations of H2O2 showed that they had better viability and were better able to remove the H2O2 when compared with vector controls. The CYP2E1 transfected cells also showed higher viability compared to controls when incubated with 4-HNE (a lipid peroxidation product) or the free radical generators, menadione or antimycin A. Resistance to ROS-induced injury in this cell line was attributed to the increased expression of the previously mentioned antioxidant response proteins; although it is unclear if similar effects would be observed if only MGST1 were overexpressed.

To help specifically determine the role of MGST1 in protection against oxidative stress, a series of studies was performed using a breast cancer cell line, MCF7, stably transfected either with the sense strand of MGST1 or its anti-sense negative control. Transfection yielded a clone that expressed MGST1 at 0.2-0.5 μg/mg total cellular protein. This level is approximately a tenth of what is expressed in the liver, but is comparable to other extracellular tissue expression levels. This cell line was originally designed to determine the contribution of MGST1 to anti-cancer drug resistance, but was used in subsequent studies to test the ability of MGST1 to protect the cells from reactive intermediates. Treatment of the MGST1 transfected cells with either lipophilic hydroperoxide (cumene hydroperoxide, CuOOH), or hydrophilic H2O2 revealed that they were more resistant to cell death (by MITT, LDH, and colony forming efficiency assays) than the cells transfected with the antisense vector. CuOOH is a substrate for MGST1 and is a target for its GPX activity, while H2O2 is not a direct substrate. So MGST1 in this system has both direct and indirect effects on hydroperoxide induced toxicity. Measurement of hydroperoxide levels in these cell lines revealed a significant drop only in the MGST1 containing cell line. These results are consistent with transient MGST1 transfection studies by Maeda et al.

Subsequent studies revealed that not only did MGST1 lower hydroperoxide levels, it lowered lipid peroxidation. Treatment of MGST1 transfected cells with CuOOH and tert-Butyl hydroperoxide (BuOOH) resulted in fewer lipid peroxidation products than in antisense controls. Notably, in the absence of any treatments, the MGST1 transfected cells exhibited less lipid peroxidation than their control counterparts. Vitamin E added to the MGST1 transfected cells mediated protection against cytotoxicity induced by the hydroperoxides, but was ineffective in contributing to the MGST1 mediated suppression of the cytotoxic effects of 4-HNE (lipid peroxidation product) or cisplatin. This suggests that these cytotoxic agents may be neutralized by the GST activity of MGST1 rather than its GPX activity. This is consistent with the previous findings that MGST1 neutralizes 4-HNE through its GST activity. The study by Johansson et al. also examined the effect of hydroperox-
ides on mitochondrial function in the control and MGST1 transfected cells. Excessive ROS has been shown to induce mitochondrial dysfunction, MPT and cell death\cite{13,31} and MGST1 makes up a sizable amount of the mitochondrial outer membrane protein, possibly as a defense mechanism against lipid peroxidation and reactive oxygen species generated by normal mitochondrial function\cite{27}. This study examined mitochondrial respiration in the control and MGST1 transfected cells after treatment with CuOOH\cite{27}. They found that in control cells, treatment with CuOOH suppressed both phosphorylating respiration and uncoupled respiration, while overexpression of MGST1 protected against the CuOOH induced suppression. The effect of MGST1 on mitochondrial Ca++ loading and release was also examined after CuOOH treatment, since high levels of Ca++, especially under conditions of oxidative stress, can damage the mitochondria, inducing mitochondrial permeability transition (MPT). MPT is identified as a key event in some forms of apoptosis and necrosis\cite{30}. CuOOH treatment lowered the amount of Ca++ required to induce MPT in the control cells. However, MGST1 overexpression had a protective effect on the mitochondria, as an increased amount of Ca++ was required to induce MPT in these cells after CuOOH treatment. These authors attribute this to the mitochondrial population of MGST1 (hereafter, referred to as mtMGST1), but it cannot be ruled out that the microsomal population of MGST1 is also actively detoxifying the CuOOH, since these experiments were performed in plasma membrane permeabilized cells. Further studies need to be performed on mitochondria from these cells to discern the role of mtMGST1 concerning oxidative stress induced mitochondrial dysfunction. In addition, while overexpression of MGST1 has protective effects in this study, the levels in the MGST1 transfected cells are only one tenth of what occurs normally in the liver. Therefore this cell line may not be appropriate for studying the deleterious effects of mtMGST1 that contribute to oxidative stress induced liver injury as described below.

**DELETERIOUS EFFECTS OF MGST1**

Until recently, examination of MGST1 has focused on the microsomal population. From these studies, it has been suggested that MGST1 plays an essential role in the protection of cells from reactive intermediates\cite{13,25,27,28}. Up to this point, virtually nothing was known about the mitochondrial population of MGST1 concerning its activation and function. Mitochondria are a significant producer of ROS, and oxidative stress has been shown to deplete mitochondrial glutathione and induce both MPT and the release of cytochrome C\cite{31,32,33}. This mitochondrial dysfunction leads to apoptosis and necrosis. Since mtMGST1 is localized in the inner and outer mitochondrial membranes, it is most likely activated under these conditions of oxidative stress, similar to the microsomal population. In fact, early animal studies showed that administration of galactosamine, carbon tetrachloride, or a combination of galactosamine and lipopolysaccharide (GalN/LPS) induced liver injury that was characterized by damaged mitochondria, depleted mitochondrial GSH, increased ROS and lipid peroxidation and apoptosis, and increased mtMGST1 activity\cite{35,37}. Preliminary studies indicated that mtMGST1 was activated by ROS, so using the GalN/LPS animal model of liver injury, Lee et al\cite{32} set out to examine specifically the activation and function of mtMGST1. GalN/LPS induced activation of mtMGST1, which was reduced by dithiothreitol. Immunoblotting of mitochondrial extracts showed the formation of S-S linked mtMGST1 dimers, as well as glutathionylated mtMGST1 (mixed S-S with GSH). In contrast, mitochondrial extracts from control rat livers showed no modified mtMGST1. This is consistent with previous studies which showed that modification of cys49 in purified MGST1 by disulfide and mixed disulfide formation induced activation\cite{17,23}. In addition, mitochondria from the GalN/LPS treated rats exhibited cytochrome C release, indicative of MPT and mitochondrial dysfunction\cite{32}. Incubation of mitochondria with anti-MGST1 antibodies inhibited the cytochrome C release, suggesting the involvement of activated mtMGST1 in ROS-induced mitochondrial dysfunction and MPT pore formation.

Complementary *in vitro* studies using mitochondria from control rats treated with diame (a thiol oxidizing agent) or diamide with GSH showed that treatment induced dimerization or glutathionylation of mtMGST1 through cys49. These treatments increased mtMGST1 activity, and induced mitochondrial swelling and cytochrome C release similar to the GalN/LPS treatment mentioned above\cite{32}. Addition of cyclosporin A (CsA) or bongkrekic acid (BKA) (two inhibitors of MPT pore formation) inhibited mtMGST1 activation, mitochondrial swelling and cytochrome C release. Based on the *in vivo* and *in vitro* studies, the authors concluded that activation of mtMGST1 through modification of cys49 contributed to MPT pore formation and mitochondrial dysfunction.

Subsequent studies delved further into the role of mtMGST1 activation and MPT pore formation. Hossain et al\cite{38} investigated the effect of the ROS generator gallic acid (GA), MPT inhibitors and GST inhibitors on activation of mtMGST1 and MPT. Incubation of rat liver mitochondria with GA induced significant activation of mtMGST1, which was blocked by antioxidant enzymes and singlet oxygen quenchers. Activation of mtMGST1 was also inhibited by GST inhibitors and CsA. However, when mitochondrial swelling was examined, GST inhibitors inhibited the swelling induced by GA, whereas addition of MPT inhibitors was largely ineffective. This suggested that GA induced non-classical or unregulated MPT, and that mtMGST1 activation was involved. Previous studies have indicated that protein misfolding induces aggregation and pore formation in a Ca++ independent manner that is insensitive to CsA. In fact, there is evidence that triterpenoids like GA interact with mitochondrial proteins, resulting in oxidation of their thiol residues and leading to formation of higher molecular weight aggregates, which then form a CsA insensitive MPT pore\cite{39,42}. It was noted that GA treatment induced thiol oxidation (sulfenic acid) of cys49 of
mgST1, instead of disulfide formation as observed previously with diamide and diamide + GS1[42]. In addition, treatment of mitochondria with GA induced formation of high molecular weight protein aggregates containing mtMGST1, consistent with previous studies showing protein aggregation is involved in the non-classical MPT[46,49]. It is also noteworthy that examination of the inner and outer membrane populations of mtMGST1 showed that only the outer membrane population was affected by GA. Based on these results, the authors concluded that oxidation of the outer membrane mtMGST1 population by GA leads to its activation, protein aggregation and induction of non-classical MPT, suggesting a novel function for mtMGST1 involving mitochondrial dysfunction and cell death.

Further studies examined the contributions of the inner and outer membrane populations of mtMGST1 to MPT[46]. Using rat liver mitochondria under basal conditions, this group examined the effect of both MPT inhibitors and GST inhibitors on mtMGST1 activation. They observed that in the absence of nonionic detergent, MPT inhibitors CsA, BKA, ADP and ATP were all effective in decreasing basal mtMGST1 activity. However, addition of detergent ameliorated this effect. Incubation of mitochondria with GST inhibitors decreased mtMGST1 activity regardless of addition of detergent. These results indicated that while the GST inhibitors directly affected the mtMGST1 activity, the MPT inhibitors affected mtMGST1 activity indirectly, possibly by affecting interaction of mtMGST1 with other proteins like cyclophilin D (CypD) and the adenine nucleotide translocator (ANT), which are known to be involved in MPT. Further studies using inner mitochondrial and outer mitochondrial membrane fractions showed that MPT inhibitors were effective in reducing mtMGST1 activity only in the inner membrane component, and this inhibition was lost after addition of detergent. Subsequent experiments with cytosolic GST and microsomal MGST1 showed that the MPT inhibitors had no effect on their activity, further suggesting that the contribution of inner membrane mtMGST1 to oxidative stress-induced MPT resides in its ability to interact with specific proteins involved in regulating MPT (CypD and ANT). This may be due to activation induced (cys49 modification) conformational changes in the protein, that allow interaction with other MPT regulating proteins. Collectively, the studies using mtMGST1 have shown that it is activated by oxidant-induced modification of cys49, which induces a conformational change. This path to activation is similar to what has been observed for the microsomal population of MGST1. However, activation of mtMGST1 by reactive intermediates induces protein aggregation, MPT and mitochondrial dysfunction, either through non-classical or classical pathways which results in apoptosis and necrosis. Interestingly, it is unclear if activated mtMGST1 is able to actually detoxify reactive intermediates while contributing to MPT. It is also unclear if ROS-induced depletion of mitochondrial GSH could induce or contribute to mtMGST1 participation in mitochondrial dysfunction.

ETHANOL-INDUCED CHANGES IN MGST1

It has been previously shown that chronic alcoholism induces liver oxidative stress that is in great part produced by the mitochondria[46]. Ethanol-induced production of ROS is detrimental to the mitochondria, leading to glutathione depletion and mitochondrial dysfunction, including MPT and cytochrome C release. Initial studies have found that MGST1 from the livers of ethanol-fed rats is modified by a product of ethanol metabolism, the malondialdehyde-acetaldehyde (MAA) adduct[46-47]. Examination of both the mitochondrial and microsomal fractions from ethanol-fed rat livers and their pair-fed controls show that while ethanol feeding induces activation of both microsomal MGST1 and mtMGST1, the mitochondrial population has a higher activity, and is more highly modified with MAA[48]. These results suggest that the mtMGST1 may be involved in ethanol-induced mitochondrial dysfunction. Studies are in progress to further characterize both the ethanol-induced modification and the contribution of modified MGST1 to ethanol-induced liver injury.

CONCLUSION

This review focuses on the function of MGST1 in situations of oxidative stress. Extensive studies on this protein have identified its monomeric and trimeric structures, its three substrate binding sites, its ability to be activated by oxidation or alkylation of the lone cysteine (cys49) in each subunit, and its ability to detoxify reactive intermediates by either its GST activity or its GPX activity. Additional studies have also indicated that activation of the enzyme alters its conformation. Therefore, this enzyme has been implicated as part of a complex method of defense by cells in response to reactive intermediates to protect them from cell death. However, these studies focused only on the microsomal population of this enzyme. Recent studies examining mtMGST1 indicate that this population is activated by oxidative stress in a manner similar to that reported for the microsomal enzyme. mtMGST1 also undergoes cys49 modification, leading to increased GST activity and activation-induced conformational changes. However, in the mitochondria, oxidant-induced mtMGST1 activation induces or increases its association with other mitochondrial proteins, resulting in MPT pore formation, cytochrome C release and induction of apoptotic and/or necrotic pathways. From these collective studies it is apparent, depending on the context and MGST1 population involved, that MGST1 activation can either exhibit a protective or toxic effect on the liver (and possibly other tissues). However, much more work is necessary to evaluate not only the individual contributions of these two populations to oxidative stress-induced liver injury, but also how the effects of the activation of these two populations combine to help determine cell survival.
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