Modulation of Membrane-Bound Glutathione Transferase Activity by Phospholipids Including Cardiolipin

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Received August 26, 2010; accepted November 15, 2010; published online November 22, 2010

Membrane-bound glutathione transferases (MGST1) distributed mostly in liver microsomal and mitochondrial membranes are activated by the thiol modification. In the present study, the effect of phospholipids on MGST1 activity was investigated using purified enzyme. When MGST1 was mixed with liposomes of cardiolipin (CL), phosphatidylcholine (PC), phosphatidylserine (PS), or phosphatidylethanolamine (PE), its activity was increased in a magnitude which was dependent on the anionic property of lipids in the order of CL>PS>PE>PC, indicating that MGST1 activity is enhanced by surrounding anionic lipids. Although MGST1 was activated by the thiol alkylation with N-ethylmaleimide (NEM), the activation was suppressed in the presence of anionic phospholipids as clearly observed in the presence of CL. Similarly, the activation of MGST1 by diamide or diamide plus glutathione through disulfide-bond formation was also disturbed in the presence of CL. Suppression of NEM-derived MGST1 activation by CL was lost when MGST1 was incubated with CL in the presence of the detergent Triton X-100. These results indicate that reactivity (stability) of the thiol in MGST1 is affected by surrounding lipids, namely CL which prevents MGST1 activation by thiol modification. Since CL is a mitochondria specific lipid located in the inner membrane, it was suggested that function of mitochondrial MGST1 could be regulated by interaction with CL.

Key words glutathione transferase; mitochondria; cardiolipin; N-ethylmaleimide; thiol modification

Membrane-bound glutathione transferase (MGST1) was originally found in rat liver microsome. MGST1 is a detoxification enzyme which is not limited to catalyze the glutathione conjugation with various electrophiles but also acts as glutathione peroxidase against byproducts of oxidative stress1,2) and is now classified as MAPEG (membrane associated proteins in eicosanoid and glutathione metabolism) superfamily.3) MGST1, distinct from soluble type of glutathione transferases (GSTs), is a homotrimer with sole thiol (Cys49) per each subunit and its activity is dependent on a reactivity of the thiol.4,5) The enzyme activity is markedly increased by the thiol modification as seen by covalent binding,6,7) a mixed-disulfide bond formation,8) a disulfide-linked dimer formation in vitro9) and by oxidative stress in vivo.10,11)

Recently we found that MGST1 is distributed in rat liver mitochondrial outer and inner membranes, and contributes to the mitochondrial permeability transition (MPT) pore opening and cytochrome c release from mitochondria.12,13) That is, mitochondrial MGST1 is activated by oxidative stress through a thiol modification such as a mixed-disulfide bond formation or oxidation to sulfenic acid leading to MPT pore opening. In addition, we observed that MGST1 in the inner membrane is inhibited by cyclosporin A (CsA) and bongkrekic acid (BKA) which are MPT inhibitors.14) Many lines of evidence show that cyclophilin D (CypD) in the mitochondrial matrix and adenine nucleotide translocator (ANT) in the inner membrane are MPT regulator proteins, in which CsA binds to CypD whereas BKA/ADP bind to ANT resulting in inhibition of the MPT.15—18) Thus it was suggested that mitochondrial inner membrane-bound MGST1 be able to interact with ANT and/or CypD, and thereby contributing to MPT pore regulation. We also mentioned that MGST1 inhibition by CsA or ADP was lost in the presence of the detergent Triton X-100.14) Therefore, it was assumed that MGST1 activity may also be regulated by surrounding lipids.

In the present study we investigated the effect of various phospholipids including cardiolipin (CL) on MGST1 activity using purified enzyme. CL is a mitochondria specific lipid which plays an active role in several mitochondrial dependent steps for apoptotic cell death.19) Data presented here indicate that among phospholipids used, CL shows the highest increase of MGST1 activity and disturbs MGST1 activation by modification of the thiol, suggesting that mitochondrial CL interacts to the thiol moiety of MGST1 resulting in altering MGST1 function.

MATERIALS AND METHODS

Materials Glutathione (GSH), Triton X-100, cardiolipin (from bovine brain, CL) and CM Sepharose CL 6B were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Phosphatidylcholine (from Egg yolk, PC), phosphatidylethanolamine (PE), N-ethylmaleimide (NEM) and benzamidine (Bz) were from Nacalai Tesque (Kyoto, Japan). Chloroform, phoshoatidylserine (from bovine spinal cord, PS), 1-chloro-2,4-dinitrobenzene (CDNB) and cholesterol standard were obtained from Wako Pure Chemicals (Osaka, Japan). Hydroxyapatite and Bio-Beads SM-2 were from Bio-Rad Laboratories (Richmond, CA, U.S.A.). All other reagents were of analytical grade.

Purified MGST1 MGST1 purified from rat liver microsomes according to the method of Morgenstern and DePierre with slight modification was used. Protein concentration of purified MGST1 was determined by the method of Peterson.

Preparation of Liposomes As phospholipids, cardiolipin (CL), phosphatidylcholine (PC), phosphatidylserine (PC), and phosphatidylethanolamine (PE) were used. Each lipid (12 mg) was dissolved in 1 ml of chloroform/methanol

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(1:1, v/v) and the solvent was removed by spraying nitrogen gas. The lipid film thus obtained was suspended with 112 μl of Triton X-100 and 0.01 mM potassium phosphate buffer (pH 7.4) in total volume of 3 ml, followed by shaking on a shaker-incubator for 2 h at room temperature. To remove Triton X-100, hydrophobic beads (Bio-Beads SM-2) were added into the solution followed by further incubation for 2 h and the bead was removed with special filter paper or centrifugation. The liposomes thus obtained were used in all experiments.

Effect of Various Lipids on MGST1 Activity Purified MGST1 (3—8 μg/25 μl) were incubated with liposome (10—60 μl) in 0.01 mM potassium phosphate buffer (pH 7.4) in total volume of 200 μl and then GST activity was measured by the method of Habig et al. using 1 mM CDNB and 5 mM GSH as substrates. When necessary, 1 mM NEM was added into the reaction mixture and incubated for 2 min, then GST activity was determined. In place of NEM, diamide (1 mM) or diamide (1 mM) plus GSH (0.25 mM) was also incubated with MGST1.

Effect of Triton X-100 on CL-Induced Enhancement of MGST1 Activity Cardiolipin liposome (10 μl) and purified MGST1 were mixed in 0.01 mM potassium phosphate buffer (pH 7.4) in the presence or absence of Triton X-100 (10 μl) in total volume of 200 μl and then GST activity was measured. When 1 mM NEM was added, GST activity was measured after 2 min incubation at room temperature.

Preparation of Mitochondrial Inner and Outer Membrane Rat liver mitochondrial inner (IMM) and outer (OMM) membranes were isolated by previous osmotic method. Protein concentration of each membrane fraction was determined by the method of Lowry et al. using bovine albumin as a standard.

RESULTS

Figure 1 indicates a concentration dependent effect of each lipid on MGST1 activity. When CL was incubated with MGST1, GST activity was increased and reached to a maximum level (500% of control) at 0.2 mg/ml and then gradually decreased, followed by return to control level at 1.2 mg/ml. PS increased the MGST1 activity to 380% at 0.5 mg/ml and still showed 300% of control at 1.2 mg/ml. PC indicated moderate increase in MGST1 activity in accordance to the increase of PC concentration (300% of control at 1.2 mg/ml) whereas PE showed weak increase of the enzyme activity indicating 200% of control at more than 0.2 mg/ml. Cholesterol did not change the MGST1 activity. Thus the order of the increase of MGST1 activity was dependent on the anionic property of lipids, CL>PS>PE>PC, indicating that MGST1 activity is enhanced by surrounding anionic lipids.

Since CL caused the most increase of MGST1 activity among phospholipids used, its kinetic parameters were determined (Fig. 2). When K_m and V_max values were calculated from Lineweaver–Burk plots (inserted figures), it was found that K_m value for GSH was increased in the presence of CL (2 mM in control, 10 mM by CL) whereas the K_m for CDNB was decreased by CL (0.05 mM in control and 0.01 mM in CL). On the other hand, V_max values for GSH and CDNB were markedly increased by adding CL (GSH, 1.33 μmol/mg/min in control and 13.3 μmol/mg/min by CL; CDNB, 1.04 μmol/mg/min in control and 5.6 μmol/mg/min by CL).

Thus the decrease of K_m value by CL for CDNB suggests that the affinity of MGST1 for CDNB, a hydrophobic substrate, was increased by adding of CL, probably due to the change of conformation of MGST1 by CL.

To confirm the effect of CL further, we examined whether reactivity of the thiol of MGST1 is altered by phospholipids. Figure 3 shows the effect of SH alkylating reagent NEM on MGST1 activity in the presence or absence of lipids. MGST1 activity was increased 10- to 14-folds by NEM in the absence of lipid whereas NEM-derived increase of MGST1 activity was altered by lipids. MGST1 activity was not increased by NEM in the presence of any concentrations (0.2—1.2 mg/ml) of CL (Fig. 3A). PS suppressed it dose-dependently and the increase of MGST1 activity by NEM was not observed at 1.2 mg/ml (Fig. 3B). On the other hand, MGST1 activity in the presence of NEM was further enhanced by increasing concentrations of PC and Cho (Fig. 3C). These results indicate that reactivity of the thiol in MGST1 is affected by surrounding lipids where anionic lipids suppressed MGST1 activation by alkylation of the thiol with NEM.

Furthermore, we examined the effect of diamide or diamide plus GSH on MGST1 activity in the presence or absence of CL, because the former can activate MGST1 by disulfide-linked MGST1 dimer formation and the later by a mixed disulfide (MGST1-S-SG) bond formation. As shown in Fig. 4, MGST1 activity was increased to 730% of control by diamide plus GSH and to 670% by diamide alone.
in the absence of lipids whereas the activation by diamide or diamide plus GSH was not observed in the presence of CL (170% of control). In the case of PC, MGST1 activity was increased to 300% by PC alone and further increased to 600% and 670% by diamide and diamide plus GSH, respectively. These results confirmed that the thiol-mediated activation of MGST1 is suppressed by anionic lipid CL, not by neutral lipid PC.

Figure 5 indicates the effect of various concentrations of detergent Triton X-100 on CL-induced increase of MGST1 activity. The increase of MGST1 activity by 0.3 mg/ml of CL (460% of control) was suppressed by 0.5% of Triton X-100 (310%) and almost lost at 1% of Triton X-100 (Fig. 5A). Although NEM could not increase MGST1 activity in the presence of CL (150% of control), MGST1 activity was increased to 900% of control by NEM in co-existence with CL and Triton X-100 (Fig. 5B), indicating that MGST1 interacts with CL by which MGST1 is activated and the MGST1-CL interaction is lost by Triton X-100 resulting in recovery of NEM-derived activation.

Since CL is present in mitochondrial inner membrane and MGST1 resides both in the inner and outer membranes, it was expected that MGST1 in the inner membrane may interact with CL, thereby NEM-derived activation of MGST1 may be decreased. As shown in Fig. 6, when mitochondrial inner (IMM) and outer (OMM) membranes were incubated...
with NEM, the former MGST1 activity was increased to 260% whereas MGST1 activity in the OMM was increased to 480%. Repeated experiments confirmed that the increase by NEM of MGST1 activity in the IMM was less than that of OMM, suggesting that the thiol moiety in MGST1 in the IMM may interact with CL which leads to limit thiol reactivity in the enzyme.

DISCUSSION

Our previous report showed that MGST1 activity in the mitochondrial inner membrane (IMM) is inhibited by MPT inhibitors in which the inhibitory action of MPT inhibitors is lost in the presence of detergent Triton X-100, suggesting that MGST1 in the inner membrane interacts with MPT pore regulators such as CypD/ANT through membrane lipids.¹⁴ We examined in the present study the effect of various lipids on purified MGST1. Our results showed that MGST1 activity was increased by anionic phospholipids, especially by CL, and MGST1 activation by the modification of thiols with NEM, diamide or diamide plus GSH was not observed in the presence of CL. These findings suggest that CL interacts with thiol moiety in MGST1 by which a reactivity of the thiol to NEM, diamide or diamide plus GSH is disturbed and the CL-MGST1 interaction is dissociated with Triton X-100 resulting in recovery of the thiol-mediated activation of MGST1. We also observed a dose dependent modification of MGST1 activity where MGST1 activity was markedly increased by CL at lower concentrations and slightly at higher concentrations (Figs. 1, 3A). It has been evidenced that CL binds to ANT at lysine residues in a graded manner including tight binding and reversible binding.¹⁹ In consideration that MGST1 is a basic protein with an isoelectric point of 10.1 and Cys49 of MGST1 is located in the cytosolic domain in which 5 lysine residues are involved,⁴,²⁴ it is presumed that at low concentrations CL may bind to a critical lysine resulting in conformational change of MGST1 accompanied by its activation whereas at higher concentrations CL binds to more lysine residues by which the reactivity of the thiol is reduced, resulting in decrease in NEM-derived activation of MGST1. In contrast, PC enhanced NEM-derived activation of MGST1 (Fig. 1). It may reflect a stabilization of Cys49 by non-anionic lipid, PC although further studies are needed to confirm it.

CL is a mitochondria specific anionic phospholipid located in the inner membrane, which plays an essential role in the organization of mitochondrial electron-transport complexes involved in the generation of energy and in the mitochondria dependent steps of apoptosis including cytochrome c release from mitochondria.¹⁹,²⁵ Many lines of evidence supported the presence of MPT regulators such as ANT and CypD in the outer and inner membranes of mitochondrial contact sites.²⁶,²⁷ Our previous reports also suggest that MGST1 is present in the inner membrane/contact sites and interacts with ANT/CypD.¹²—¹⁴ It is therefore presumed that the thiol moiety of MGST1 in the inner membrane (contact sites) may be directed to the inner membrane side, not to the cytosolic side, and interacts with CL located in the inner membrane, thereby the activation of MGST1 in the inner membrane through the thiol modulation is limited. This was supported with our observation that when the inner and outer mitochondrial membranes were incubated with NEM, the increase in MGST1 activity in the inner membrane was lower than that of MGST1 in the outer membrane (Fig. 6). In addition, we noticed that various oxidants which activate MGST1 in the outer membrane through thiol modulations showed less or no activation of the inner membrane MGST1 (unpublished data). It reflects that reactivity of the thiol in MGST1 against oxidative stress may be changed by CL-MGST1 interaction.

It is currently a well known fact that oxidation of mitochondrial membrane protein thiols is involved in MPT. ANT, a regulator protein of MPT pore, has 3 thiols including critical thiol (Cys⁵⁶) which is oxidized by oxidative stress resulting in the MPT pore opening.²⁸ ANT can bind 6 mol of CL where CL-ANT interaction alters the alkylation of ANT-Cys⁵⁶ suggesting that an eventual dissociation or weakening in the cardiolipin/ANT interaction changes the lability of critical mitochondrial membrane thiol residues. Several investigations also showed the activation of ANT function by acidic phospholipids where CL was found the most effective.¹⁰,²⁹,³⁰ It is therefore conceivable that reactivity of the thiol in MGST1 is also changed through interaction of MGST1 with CL as observed by ANT-CL interaction.

In conclusion, the reactivity of the thiol in MGST1 is modified by interacting with CL resulting in the increase of MGST1 activity or in inhibition of thiol-mediated activation of MGST1. It is suggested that in mitochondria MGST1 activity including MGST1 mediated MPT pore regulation is affected by an interaction with CL.

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