



Letter

Glyceraldehyde 3-phosphate dehydrogenase converts methylmercury to its sulfur adduct with lowered toxicity through sulfane sulfur atoms on Cys247

Yumi Abiko^{1,*}, Eiko Yoshida^{2,*} and Yoshito Kumagai¹

¹Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

²Graduate School of Comprehensive Human Sciences, University of Tsukuba,
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

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ABSTRACT — Methylmercury (MeHg) reacts with nucleophilic sulfur species to form sulfur adducts, such as the low-toxic metabolite dimethylmercury sulfide [(MeHg)₂S]. We found that protein-bound persulfides interact with MeHg to form (MeHg)₂S and identified glutathione S-transferase pi 1 as a S-sulfhydrated protein involved in (MeHg)₂S formation. Although glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a house-keeping protein abundantly expressed in various tissues, has been reported to undergo S-sulfhydration in the presence of sulfur donors or cystathionine γ-lyase, the biological significance of this post-translational modification is poorly understood. In this study, we investigated the possible interaction between GAPDH and MeHg to form (MeHg)₂S. High-performance liquid chromatography/atomic absorption spectrophotometry revealed that (MeHg)₂S was formed during the reaction of MeHg with a model of cysteine persulfide and GAPDH following incubation of the protein with NaHS. After reacting with NaHS, GAPDH C152S and C156S mutants transformed MeHg into (MeHg)₂S, whereas formation of the sulfur adduct was not observed for the C247S mutant, suggesting that Cys247 is critical for conversion of MeHg to (MeHg)₂S. These results suggest that the sulfane sulfur on Cys247 of GAPDH plays a protective role in reducing MeHg toxicity.

Key words: Methylmercury, Dimethylmercury sulfide, Persulfide, GAPDH

INTRODUCTION

Methylmercury (MeHg) readily binds nucleophilic sulfur species to form MeHg adducts because of its electrophilicity (Clarkson, 1997). Per/polysulfide species have lower pK_a values and thus have higher nucleophilicity than the corresponding monosulfides, such as cysteine (Cys) and glutathione. We found that hydrogen sulfide and per/polysulfides such as hydrogen persulfide, glutathione per/polysulfide, and protein-bound per/polysulfides react with MeHg to form dimethylmercury sulfide [(MeHg)₂S] (Yoshida *et al.*, 2011; Abiko

et al., 2015). This sulfur adduct has nominal *in vitro* and *in vivo* toxicity when compared with that of MeHg (Yoshida *et al.*, 2011; Abiko *et al.*, 2015). We showed previously that several proteins in mice liver cytosol mediate transformation of MeHg to (MeHg)₂S and identified glutathione S-transferase pi 1 (GSTP1) as a S-sulfhydrated protein that reacts with MeHg to yield (MeHg)₂S (Abiko *et al.*, 2015). Several reports have indicated an abundance of S-sulfhydrated proteins in cells (Abiko *et al.*, 2015; Ida *et al.*, 2014; Akaike *et al.*, 2017). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a house-keeping protein expressed abundantly in a variety of tissues,

Correspondence: Yoshito Kumagai (E-mail: yk-em-tu@md.tsukuba.ac.jp)

*These authors equally contributed to this work.

and the Cys residues are sulfhydrated in the presence of cystathionine γ -lyase (CSE), which produces hydrogen sulfide and Cys persulfide (CysSSH), under physiological conditions (Mustafa *et al.*, 2009; Akaike *et al.*, 2017). Human and mouse GAPDH have three redox-sensitive Cys residues, and Cys152, Cys156, and Cys247 in human GAPDH all undergo *S*-sulfhydration (Mustafa *et al.*, 2009; Jarosz *et al.*, 2015). The biological function(s) of these GAPDH-bound persulfides are unknown. Thus, we hypothesized that the sulfane sulfur atoms of Cys residues in GAPDH may function as sacrificial sulfurs, thereby forming (MeHg)₂S from MeHg. In this report, we investigated the potential formation of (MeHg)₂S *in vitro* through the interaction between *S*-sulfhydrated GAPDH and MeHg. Using high-performance liquid chromatography/atomic absorption spectrophotometry (HPLC/AAS) to detect Hg compounds, we detected (MeHg)₂S formation during incubation of recombinant human GAPDH (hGAPDH) with MeHg.

MATERIALS AND METHODS

Materials

MeHgCl and NaHS were purchased from Sigma-Aldrich (St Louis, MO, USA) and Strem Chemicals (Newburyport, MA, USA), respectively. (MeHg)₂S was synthesized as described previously (Yoshida *et al.*, 2011). All other reagents used were of the highest grade available. *S*-Methoxycarbonyl penicillamine disulfide (MCPD) was kindly gifted by Christopher L. Bianco, Johns Hopkins University.

Preparation of hGAPDH

Wild-type (WT) hGAPDH and its site-specific mutants (C152S, C156S, and C247S) were prepared by an *Escherichia coli* overexpression system, as described in Miura *et al.* (2011). After purification, the protein concentrations were determined by the Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA).

The three-dimensional structure of hGAPDH was taken from the RCSB protein data bank (PDB ID: 1U8F) (Jenkins and Tanner, 2006) and visualized using Mol* (Sehnal *et al.*, 2021).

Reaction of MeHg with persulfides

We added 2-mercaptoethanol and dithiothreitol to cleave S–S bonds in purified hGAPDH that readily oxidized during the purification process (Miura *et al.*, 2011). Thus, WT hGAPDH and mutants in 25 mM Tris-HCl (pH 7.5) were incubated with NaHS in a 1:2 molar ratio at 25°C for 1 hr to form persulfides (Steudel, 2003; Mustafa *et al.*, 2009), and excess NaHS was removed by using

an Econo-Pac 10DG column (Bio-Rad). Protein sample concentrations were determined using the Bio-Rad protein assay kit. The *S*-sulfhydrated hGAPDH (0.27 nmol as a monomer) was incubated with MeHg (1.62 nmol) at 25°C for 30 min. Trichloroacetic acid (final concentration of 10%) was added to stop the reaction, followed by centrifugation at 9,000 × g for 5 min. An aliquot (80 μ L) of the supernatant was mixed with 200 μ L of 1 M Tris-HCl (pH 8.0), and 20 μ L of the sample was analyzed by HPLC/AAS.

MCPD (10 nmol) was incubated with MeHg (20 nmol) in 20 mM Tris-HCl (pH 7.5) at 25°C for 30 min. An aliquot (20 μ L) of the reaction mixture was analyzed by HPLC/AAS.

High-performance liquid chromatography/atomic absorption spectrophotometry

MeHg and (MeHg)₂S were measured as previously described (Yoshida *et al.*, 2011). Briefly, compounds were separated by an HPLC system (Shimadzu, Kyoto, Japan) equipped with a Zorbax Eclipse XDB-C₁₈ column (50 mm long, 2.1 mm i.d., 5 μ m particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was 10% (v/v) methanol-0.1% (v/v) formic acid at a flow rate of 0.5 mL/min. The mercury concentrations of the eluate were determined by AAS (MA-3000; Nihon Instruments, Osaka, Japan). Representative data are shown.

RESULTS AND DISCUSSION

Our previous study showed that (MeHg)₂S was formed during incubation of MeHg and GSTP1, indicating that persulfides on Cys residues contributed to the formation of (MeHg)₂S (Abiko *et al.*, 2015). To confirm that MeHg reacts with CysSSH in proteins, we used MCPD, which transforms to *N*-methoxycarbonyl penicillamine persulfide in buffer at pH 7.4 (Fig. 1A) (Artaud and Galardon, 2014; Bianco *et al.*, 2016). As expected, (MeHg)₂S was observed during incubation of MeHg with MCPD using the HPLC/AAS assay (Fig. 1B).

Incubation of prepared recombinant *S*-sulfhydrated hGAPDH (see Materials and Methods) with MeHg yielded a Hg-containing peak with the same retention time as (MeHg)₂S (0.049 \pm 0.025 nmol) in HPLC/AAS analysis, indicating the formation of (MeHg)₂S from this mixture (Fig. 2A and 2B). HPLC/AAS analysis of the hGAPDH Cys-to-Ser mutants revealed that (MeHg)₂S production decreased for the C152S (0.032 \pm 0.017 nmol, 66% \pm 36% of WT) and C156S (0.033 \pm 0.010 nmol, 67% \pm 17% of WT) mutants (Fig. 2C–D), whereas (MeHg)₂S was not detected in the mixture including the hGAP-

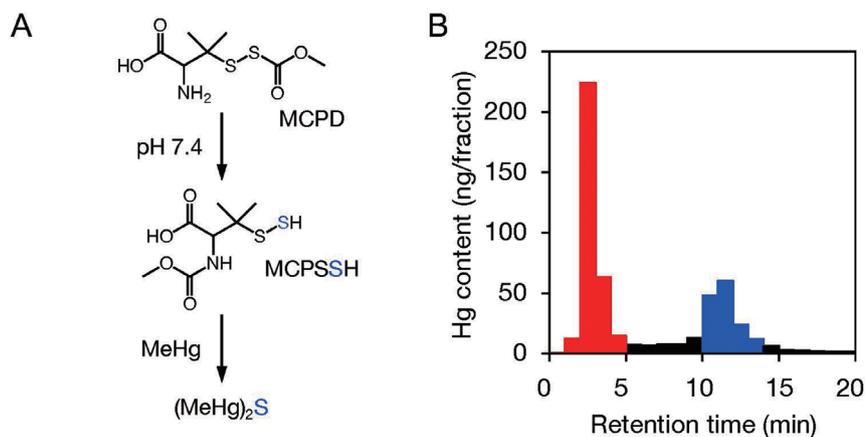
(MeHg)₂S formation during reaction of GAPDH and MeHg

Fig. 1. Formation of (MeHg)₂S during incubation of MCPD with MeHg. (A) *S*-Methoxycarbonyl penicillamine disulfide (MCPD) is transformed into *N*-methoxycarbonyl penicillamine persulfide (MCPSSH) at neutral pH and sulfane sulfur atom in MCPSSH reacts with MeHg, producing (MeHg)₂S. (B) Red and blue bars indicate methylmercury (MeHg) and bismethylmercury sulfide [(MeHg)₂S], respectively. MeHg was incubated with MCPD, and MeHg and (MeHg)₂S were analyzed by HPLC/AAS. Representative data are shown.

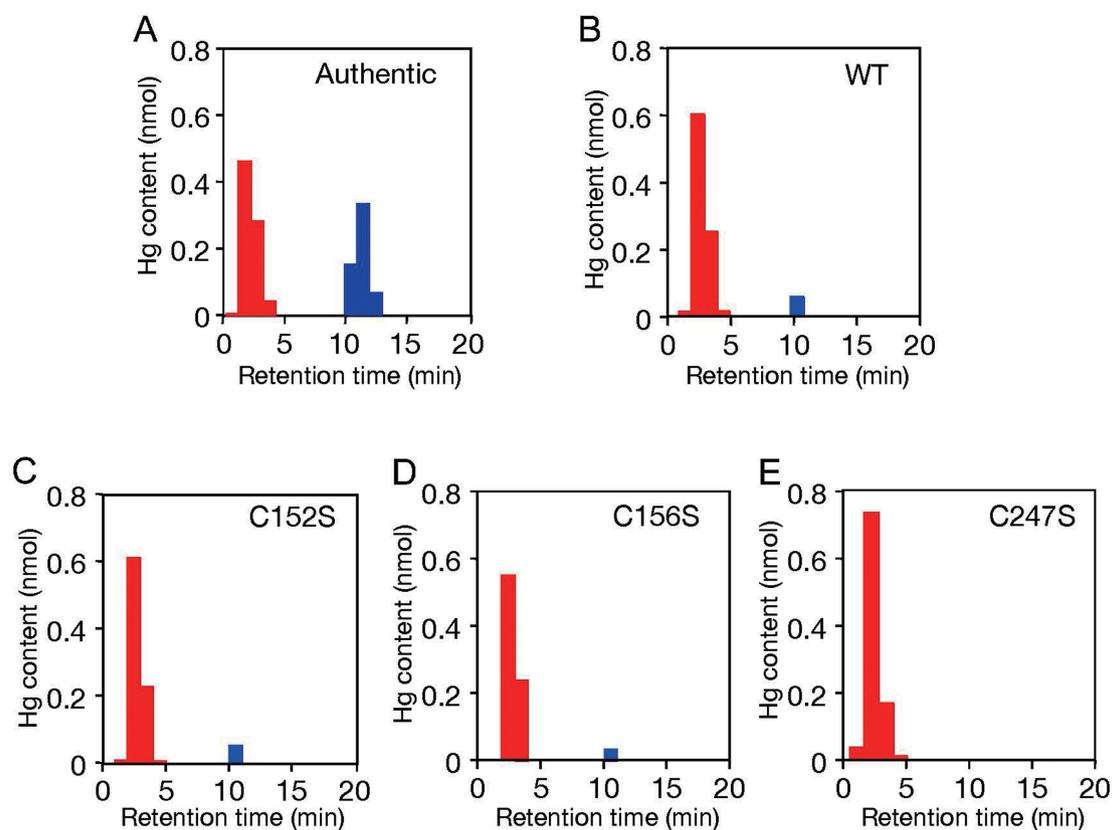


Fig. 2. Formation of (MeHg)₂S during incubation of hGAPDH with its cysteine-to-serine mutants. Red and blue bars indicate methylmercury (MeHg) and bismethylmercury sulfide [(MeHg)₂S], respectively. (A) Authentic MeHg and (MeHg)₂S. MeHg was incubated with WT hGAPDH (B) and Cys mutants: C152S (C), C156S (D), and C247S (E). MeHg and (MeHg)₂S were analyzed by HPLC/AAS after removing the protein, and representative data are shown.

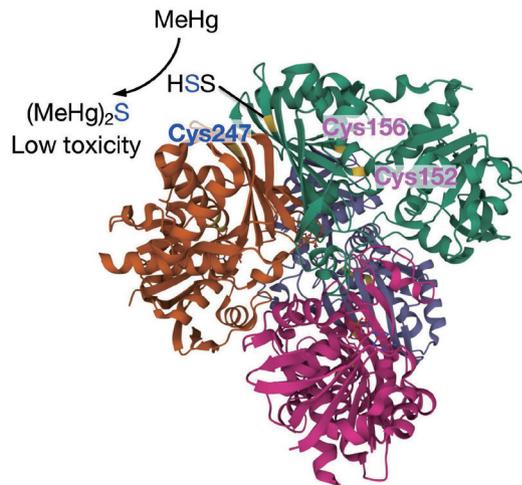


Fig. 3. Schematic formation of $(\text{MeHg})_2\text{S}$ from MeHg by a sulfane sulfur on hGAPDH. The three-dimensional structure of hGAPDH was visualized using Mol* and the structure from the RCSB protein data bank (PDB ID: 1U8F). Cys residues are highlighted by yellow. Methylmercury (MeHg) reacts with per/polysulfide (-SSH) on Cys247 to form the weakly toxic sulfur adduct, bismethylmercury sulfide $[(\text{MeHg})_2\text{S}]$.

DH C247S mutant (Fig. 2E). These results suggest that Cys247 of GAPDH mainly stores sulfane sulfur to inactivate the electrophilic organometal and form $(\text{MeHg})_2\text{S}$. Inspection of the structure of hGAPDH (Fig. 3) revealed that Cys247 is more surface exposed than the other two cysteine residues, indicating that MeHg may more readily access the sulfur on Cys247 when compared with that of Cys152 or Cys156. Jarosz *et al.* (2015) demonstrated that Cys247 readily underwent *S*-sulphydration when compared with that of Cys156 and that Cys152 was an unfavorable site for *S*-sulphydration. The preference of *S*-sulphydration on GAPDH may also explain the selectivity of $(\text{MeHg})_2\text{S}$ formation from MeHg in this study. Although Cys247 plays no active role in the dehydrogenase activity of GAPDH and *S*-sulphydration at Cys247 does not inactivate the enzyme (Jarosz *et al.*, 2015), post-translational modifications at Cys247 are reported to be associated with other functions of GAPDH (Jia *et al.*, 2012; Mustafa Rizvi *et al.*, 2021). For example, *S*-nitrosylation of Cys247 disrupts a protective function against degradation of phosphorylated L13a following ubiquitination, resulting in the loss of interferon- γ -activated inhibitor of translation complex activity (Jia *et al.*, 2012). Recent findings indicate that H_2O_2 -induced *S*-glutathionylation at Cys247 enhances nuclear translocation of GAPDH, which can trans-glutathionylate proteins such as sirtuin-1 in the

nucleus leading to apoptosis (Mustafa Rizvi *et al.*, 2021). We showed previously that proteins with reactive thiols (e.g., protein tyrosine phosphatase 1B) are readily *S*-sulphydrated, yielding the protein persulfides that play a role in protection against further oxidation of the reactive Cys residues (Doka *et al.*, 2020). Therefore, we postulate that the sulfane sulfur atom at Cys247 possibly acts as a sacrificial sulfur to block oxidation, as shown in Fig. 3. In the present study, it was found that the sulfane sulfur can trap MeHg, forming its sulfur adduct with lower toxicity. Overall, Cys247 may contribute to decreasing the reactivity of xenobiotic electrophiles such as MeHg through sulfur adduct formation (Abiko *et al.*, 2015; Nishida *et al.*, 2012; Kumagai and Abiko, 2017) in addition to the established post-translational modifications based on *S*-nitrosylation, *S*-glutathionylation, and *S*-sulphydration involved in the multiple functions of GAPDH.

In earlier work, we found that GSTP1 has per/polysulfides involved in $(\text{MeHg})_2\text{S}$ formation (Abiko *et al.*, 2015). The present study has revealed that *S*-sulphydrated GAPDH also reacts with MeHg to form $(\text{MeHg})_2\text{S}$. Because $(\text{MeHg})_2\text{S}$ is a low toxic metabolite of MeHg due to the loss of its electrophilicity, we hypothesize that a variety of protein-bound per/polysulfides may sacrifice the sulfane sulfur to reduce the level of *S*-mercuration and therefore protect cells from MeHg toxicity.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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