S-Mercuration of ubiquitin carboxyl-terminal hydrolase L1 through Cys152 by methylmercury causes inhibition of its catalytic activity and reduction of monoubiquitin levels in SH-SY5Y cells

Takashi Toyama1,a, Yumi Abiko1, Yuko Katayama2, Toshiyuki Kaji3 and Yoshito Kumagai1,2

1Faculty of Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, Japan
2Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki, Japan
3Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, Japan

aPresent address: Laboratory of Molecular and Biochemical Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba-ku, Sendai, Miyagi, Japan

[Contributed by Toshiyuki Kaji] (Received October 14, 2015; Accepted October 15, 2015)

ABSTRACT — Methylmercury (MeHg) is an environmental electrophile that covalently modifies cellular proteins. In this study, we identified proteins that undergo S-mercuration by MeHg. By combining two-dimensional SDS-PAGE, atomic absorption spectrometry and ultra performance liquid chromatography mass spectrometry (UPLC/MS/MS), we revealed that ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is a target for S-mercuration in human neuroblastoma SH-SY5Y cells exposed to MeHg (1 μM, 9 hr). The modification site of UCH-L1 by MeHg was Cys152, as determined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. MeHg was shown to inhibit the catalytic activity of recombinant human UCH-L1 in a concentration-dependent manner. Knockdown of UCH-L1 indicated that this enzyme plays a critical role in regulating mono-ubiquitin (monoUb) levels in SH-SY5Y cells and exposure of SH-SY5Y cells to MeHg caused a reduction in the level of monoUb in these cells. These observations suggest that UCH-L1 readily undergoes S-mercuration by MeHg through Cys152 and this covalent modification inhibits UCH-L1, leading to the potential disruption of the maintenance of cellular monoUb levels.

Key words: Electrophile, Methylmercury, S-Mercuration, UCH-L1

INTRODUCTION

Methylmercury (MeHg) is a global organometal contaminant. Under certain conditions, MeHg-mediated toxicity involves the modification of cellular proteins through the S-mercuration reaction (Rabenstein and Saetre, 1977). Once MeHg invades cells, this environmental electrophile undergoes glutathione (GSH) conjugation, resulting in the excretion of a polar metabolite into the extracellular spaces through multidrug resistance-associated proteins (MRPs) (Toyama et al., 2007; Kumagai et al., 2013; Ballatori, 2002; Madejczyk et al., 2007). We reported previously that MeHg activates transcription factor Nrf2 through S-mercuration of its negative regulator Keap1 and up-regulates its downstream genes, such as glutamate-cysteine ligase (GCL), a late-limiting enzyme for GSH synthesis, GSH S-transferases and MRPs, resulting in detoxification of the organomercury compound (Toyama et al., 2007, 2011; Kumagai et al., 2013; Itoh et al., 1997; Vollrath et al., 2006). However, MeHg exposure at higher concentrations causes dysfunction of central nervous systems (Harada, 1978, 1995) and daily intake of MeHg through a predatory fish diet may represent a health risk (Grandjean et al., 2010). High levels of MeHg target proteins such as tubulin and a disintegrin and metalloproteinases (Imura et al., 1980; Wasteneys et al., 1988; Tamm et al., 2008). We also found that MeHg inactivates neuronal nitric oxide synthase, sorbitol dehydrogenase and arginase-I through S-mercuration of these proteins (Shinyaishi et al., 1998;...
Kanda et al., 2012, 2008). We therefore hypothesize that an approach to detect electrophilic modification of proteins by MeHg is required for understanding the toxicity or health effects of MeHg.

To determine post-transcriptional modifications of proteins, western blotting with antibodies against the modifier or radiolabeled compounds are used (Garcia et al., 1974; Zheng and Hammock, 1996). We have developed previously an assay to detect chemical modifications using biotin-PEAC₅-maleimide (BPM), which can label free thiols (Toyama et al., 2013; Abiko et al., 2015). However, global analysis for detection of S-mercurated proteins is challenging to perform with these assays. In this study, we have identified molecular targets that undergo S-mercuration by MeHg in SH-SY5Y cells using a combination of two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2D SDS-PAGE), atomic absorption spectrometry and ultra performance liquid chromatography mass spectrometry (UPLC/MS/MS).

MATERIALS AND METHODS

Materials
MeHg, avidin-HRP and avidin-agarose were obtained from Sigma-Aldrich (St. Louis, MO, USA). Escherichia coli BL21 cells and trypsin were purchased from Promega Co. (Madison, WI, USA). Anti-ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) and anti-GAPDH antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). BPM was obtained from Dojindo (Kumamoto, Japan).

Cells and cell culture
Human neuroblastoma SH-SY5Y cells were cultured in Dulbecco’s modified Eagle’s medium/Ham’s nutrient mixture F-12 with 10% fetal bovine serum, 2 mM L-alanyl-L-glutamine and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). Cultured cells were seeded at a density of 1 × 10⁵ cells/cm² on a culture plate and maintained at 37°C in a humidified incubator under an atmosphere of CO₂ (5%) and ambient air (95%). Before treatment, cells were cultured in serum-free medium overnight, and then were exposed to MeHg.

2D SDS-PAGE following thermal decomposition gold amalgamation atomic absorption spectrophotometry (AAS) analysis
Cells were dissolved in lysis buffer containing 9.8 M urea, 4% CHAPS, 2% IPG buffer and bromophenol blue. Protein concentrations of cell lysates were determined by the Bradford method. Cell lysates (50 μg of proteins) were applied to an Immobiline DryStrip pH 3-10, 7 cm (GE Healthcare, Buckinghamshire, UK) for 10 hr under silicon oil. Isoelectric focusing was performed for 1 min at 200 V, 90 min at 3500 V and 65 min at 3500 V using a Multiphor II (GE Healthcare). Proteins separated by isoelectric focusing were further separated by SDS-PAGE. The gel was stained with a CBB solution [0.1% Coomassie brilliant blue (w/v), 50% methanol (v/v), 7.5% acetic acid (v/v)] for 1 hr, and bleached for an additional 5 hr in bleaching solution [5% methanol (v/v) and 7.5% acetic acid (v/v)]. The gel was cut into pieces (3 × 3 × 1 mm) by a microtome blade and mercury concentrations in the obtained gels were analyzed by thermal decomposition gold amalgamation atomic absorption spectrophotometry (Mercury analyzer MA-3000; Nippon Instruments Corporation, Tokyo, Japan). Detection limit for Hg was 0.012 ng under the conditions used. The concentrations of Hg in the gels were expressed in Hg ng/segment.

Western blotting
Proteins separated by SDS-PAGE (Laemmli, 1970) were electro-transferred onto PVDF membranes (Bio-Rad Laboratories Inc., Hercules, CA, USA) at 2 mA/cm² for 1 hr, according to the method of Kyhse-Andersen (Kyhse-Andersen, 1984). After blocking with 5% skim milk, the membrane-bound proteins were incubated with primary antibodies. Secondary antibodies coupled to horse-radish peroxidase were used to detect primary antibodies on the membrane. Proteins were detected with an ECL system (Nacalai Tesque Inc., Kyoto, Japan) and exposed to X-ray film (Konica Minolta Health Care Co., Tokyo, Japan).

UPLC/MS/MS analysis
Identification of target proteins for MeHg was performed using a nanoAcquity UPLC system (Waters, Milford, MA, USA) coupled to a Synapt High Definition Mass Spectrometry (Synapt HDMS; Waters). The extracted proteins from the gel were digested with 5 μL of MS grade modified trypsin (100 ng) for 16 hr at 37°C. The eluted peptides (2 μL) were loaded onto a BEH130 nanoAcquity C₁₈ column (100 mm × 75 μm i.d., 1.7 μm) held at 35°C. Mobile phases A [0.1% formic acid] and B [acetonitrile containing 0.1% formic acid] at a flow rate of 0.3 μL/min were linearly mixed using a gradient system as follows: 3% B for 1 min; linear increase over 30 min to 40% B; linear increase over 2 min to 95% B; maintain at 95% B for 5 min before returning linearly to 3% B over 2 min. The total running time, including the conditioning of the column to the initial conditions, was 70 min. The eluted peptides were then transferred to the nano-Electrospray source of a quadrupole time-of-flight...
(Q-TOF) mass spectrometer (Synapt HDMS system, Waters) through a Teflon capillary union and a pre-cut PicoTip (Waters). The initial parameters of the Synapt HDMS were set as follows: capillary voltage 3.0 kV, sampling cone voltage 35 V and source temperature 100°C. Low (6 eV) or elevated (step from 15 to 30 eV) collision energy was used to generate either intact peptide precursor ions (low energy) or peptide product ions (elevated energy). The MS survey scan was m/z 200 to 2000. The data were acquired using an independent reference spray using the nanoLockSpray interference procedure in which Glu-1-fibrinopeptide B (m/z 785.8426), infused via the nanolockSpray ion source, was sampled every 10 sec as the external mass calibration.

Purification of recombinant human UCH-L1
RNA was extracted from the human neuroblastoma SH-SY5Y cells with Sepasol-RNA I super reagent (Nacalai Tesque Inc.) and complementary DNA (cDNA) synthesis was performed using Prime Script RT (Takara Bio, Shiga, Japan). Amplification of the UCH-L1 gene was performed by PCR with Prim Script (Takara Bio). cDNA corresponding to human UCH-L1 was cloned by the StrataClone Blunt PCR Cloning Kit (Stratagene, La Jolla, CA, USA). The cloned UCH-L1 cDNA was placed downstream of the phage T7 RNA polymerase promoter at the Xhol and BamHI sites of the pET15b vector (Novagen, Madison, WI, USA). The plasmid sequence was verified on an ABI 310 DNA sequencer (Applied Biosystems, Foster City, CA, USA). The resulting plasmids were transformed into E. coli BL21 (DE3) pLysS (Invitrogen Co., Carlsbad, CA, USA) and control siRNA (Qiagen, Valencia, CA, USA) was performed using the HiPerfect transfection reagent (Qiagen), according to the manufacturer’s protocol. Briefly, siRNA duplex (0.6 μg) and the HiPerFect reagent (12 μL) were incubated with OPTI-MEM (Invitrogen) for 5 min at room temperature to encourage the formation of complexes. The complex was added into 35-mm dishes, in which 1 × 10^5 cells were seeded. The band intensities were quantified by the ImageJ software, version 1.37. Statistical significance was assessed by the t-test. All p values are two tailed.
RESULTS AND DISCUSSION

Exposure of SH-SY5Y cells to 1 μM of MeHg for 9 hr led to the modification of a large number of cellular proteins, as detected by 2D SDS-PAGE/AAS (Fig. 1). Consistent with this, we indirectly detected S-modified proteins by the BPM labeling assay (Toyama et al., 2013). To identify S-merculated proteins in SH-SY5Y cells exposed to MeHg, we selected five MeHg bound proteins with isometric points of 5-6 and higher covalent modification of MeHg (> 0.06 ng) (Fig. 1), and then identified these proteins by UPLC/MS/MS analysis. As shown in Table 1 and Fig. 1, spots 1, 2, 3, 4 and 5 were 150 kDa oxygen regulated protein, 78 kDa glucose regulated protein, nucleolin isoform CRA c, UCH-L1, UCH-L1 and heat shock protein beta 1, respectively. The 150 kDa oxygen regulated protein and 78 kDa glucose regulated protein, which are known to be chaperone proteins at the endoplasmic reticulum, have four and two cysteine residues, respectively (Bando et al., 2000; Hendershot et al., 1994; Wisniewska et al., 2010; Ikeda et al., 1997). Nucleolin isoform CRA c and heat shock protein beta 1 have a cysteine residue in the structure (Srivastava et al., 1989; Sinsimer et al., 2008). Of interest, UCH-L1 contained the highest amount of MeHg (0.116 ng), indicating that this deubiquitinating enzyme mediating stabilization of monoUb (monoUb), is a key target of S-mercuration in SH-SH5Y cells.

To confirm that UCH-L1 is modified by MeHg, purified recombinant human UCH-L1 protein was incubated with MeHg for 30 min and the modification was detected by the BPM labeling assay. UCH-L1 was covalently modified by MeHg and thus its catalytic activity was markedly suppressed by MeHg in a concentration-dependent manner (Fig. 2A and Toyama et al., unpublished observation). Under these conditions, MALDI-TOF/MS revealed that the modification site was Cys152 in UCH-L1 (Fig. 2C and Table 2) among the six cysteine residues in the protein (Fig. 2B). While Cys90 and His161 are found to be active site residues for UCH activity (Larsen et al., 1996), Cys90 has been reported to not play an important role in the stabilization of monoUb (Osaka et al., 2003). Interestingly, endogenous electrophiles such as 4-hydroxyl-2-nonenal and 15-deoxy-Δ12,14-prostaglandin J2 covalently bind to Cys152, resulting in aggregation and/or disruption of UCH-L1 enzyme activity (Koharudin et al., 2010; Kabuta et al., 2008; Li et al., 2004). Consistent with this notion, we previously found that an endogenous dopamine derivative covalently modifies Cys152 in UCH-L1 and an atmospheric electrophile, 1,2-naphthoquinone, also modifies the Cys and inhibits the deu-

![Fig. 1. Proteomics analysis of the target proteins for MeHg.](image_url)

Table 1. S-Mercurated proteins identified by 2D SDS-PAGE-AAS following UPLC/MS/MS.

| Spot No. | Accession | Description                  | MW (Da) | pI (pH) | Coverage (%) |
|----------|-----------|------------------------------|---------|---------|-------------|
| 1        | A8C1Z0    | 150 kDa oxygen regulated protein | 111,266 | 4.97    | 11.71       |
| 2        | P11021    | 78 kDa glucose regulated protein | 72,288  | 4.87    | 52.91       |
| 3        | B3KM80    | Nucleolin isoform CRA c       | 58,519  | 4.36    | 32.84       |
| 4        | P09936    | Ubiquitin carboxyl terminal hydrolase isozyme L1 | 24,808  | 5.18    | 53.36       |
| 5        | P09936    | Ubiquitin carboxyl terminal hydrolase isozyme L1 | 24,808  | 5.18    | 59.64       |
| 5        | P04792    | Heat shock protein beta 1     | 22,768  | 5.96    | 57.07       |

“Coverage” corresponds to the percentage of identified amino acid sequence in the protein.
biquitination activity of this enzyme in SH-SY5Y cells (Contu et al., 2014; Toyama et al., 2014). Taken together, we suggest that residue Cys152 of UCH-L1 is a common target for not only endogenous but also exogenous electrophiles.

As shown in Fig. 3A, knockdown of UCH-L1 by siRNA significantly diminished the monoUb level. This result indicates that UCH-L1 predominantly stabilizes monoUb in SH-SY5Y cells. In addition to this, MeHg significantly inhibited UCH activity (Toyama et al., unpublished observation) and presumably decreased cellular monoUb levels in a concentration-dependent manner (Fig. 3B).

In the present study, we found that UCH-L1 undergoes S-mercuration by MeHg through Cys152, and such a reaction reduces the catalytic activity, i.e., mono-ubiquitin levels, of UCH-L1 in SH-SY5Y cells. Loss of UCH-L1 activity, which is an abundant protein in neurons, causes neurodegeneration and UCH-L1 is linked to Parkinson’s disease (Wilkinson et al., 1989; Saigoh et al., 1999; Kurihara et al., 2001; Leroy et al., 1998). Exposure of MeHg could be one of the risk factors associated with neurodegenerative disorders such as Parkinson’s disease and the disruption of neuronal cells (Petersen et al., 2008; Annau and Cuomo, 1988). Our study therefore indicates that UCH-L1 is a potential target for the development of therapeutic strategies for Parkinson’s disease.

### Table 2. Site of MeHg modification in UCH-L1.

| Peak No. | Position | Peptide sequence | Calculated MS | Observed MS |
|---------|----------|------------------|---------------|-------------|
| 1       | 214-221  | FSAVALCK         | 838.45        | 838.5       |
| 2       | 20-27    | LGVAGQWR         | 886.48        | 886.5       |
| 3       | 1-15     | MQLKMEINPEMLNK  | 1,815.9       | 1,815.7     |
| 4       | 179-195  | MPFPVNHGASSEDTLLK | 1,842.9     | 1,842.6     |
| 5       | 136-153  | NEAIQAADAVAEGQCR | 1,910.87     | 1,910.6     |
| 6       | 158-178  | NHHFILFVNDGHLYELDGR | 2,519.24 | 2,519.6 |
| P1      | 136-153  | NEAIQAADAVAEGQCR*R +MeHg | 2,127.6 | 2,127.4 |

*MeHg-modified Cys. “Position” corresponds to the portion of the amino acid sequence of human UCH-L1.
that inactivation of UCH-L1 by S-mercuration, resulting in a reduction of cellular mono-ubiquitin levels, might be, at least in part, involved in MeHg-mediated neurotoxicity.

ACKNOWLEDGMENTS

This work was supported by a Grant-in-Aid (#25220103 to Y.K.) for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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

REFERENCES

Abiko, Y., Luong, N.C. and Kumagai, Y. (2015): A Biotin-PEAC5-maleimide labeling assay to detect electrophiles. J. Toxicol. Sci., 40, 405-411.

Annau, Z. and Cuomo, V. (1988): Mechanisms of neurotoxicity and their relationship to behavioral changes. Toxicology, 49, 219-225.

Ballatori, N. (2002): Transport of toxic metals by molecular mimicry. Environ. Health Perspect., 110 Suppl 5, 689-694.

Bando, Y., Ogawa, S., Yamauchi, A., Kuwabara, K., Ozawa, K., Horii, O., Yanagi, H., Tamatani, M. and Tohyama, M. (2000): 150-kDa oxygen-regulated protein (ORP150) functions as a novel molecular chaperone in MDCK cells. Am. J. Physiol. Cell Physiol., 278, C1172-1182.

Contu, V.R., Kotake, Y., Toyama, T., Okuda, K., Miyara, M., Sakamoto, S., Samizo, S., Sanoh, S., Kumagai, Y. and Ohta, S. (2014): Endogenous neurotoxic dopamine derivative covalently binds to Parkinson’s disease-associated ubiquitin C-terminal hydrolase L1 and alters its structure and function. J. Neurochem., 130, 826-838.

Garcia, J.D., Yang, M.G., Wang, J.H. and Belo, P.S. (1974): Carbon-mercury bond cleavage in blood of rats fed methyl merccuric chloride. Proc. Soc. Exp. Biol. Med., 146, 66-70.

Grandjean, P., Satoh, H., Murata, K. and Eto, K. (2010): Adverse effects of methylmercury: environmental health research implications. Environ. Health Perspect., 118, 1137-1145.

Harada, M. (1978): Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology, 18, 285-288.

Harada, M. (1995): Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit. Rev. Toxicol., 25, 1-24.

Hendershot, L.M., Valentine, V.A., Lee, A.S., Morris, S.W. and Shapiro, D.N. (1994): Localization of the gene encoding human BiP/GRP78, the endoplasmic reticulum cognate of the HSP70 family, to chromosome 9q34. Genomics, 20, 281-284.

Ikeda, J., Kaneda, S., Kuwabara, K., Ogawa, S., Kobayashi, T., Matsumoto, M., Yura, T. and Yanagi, H. (1997): Cloning and expression of cDNA encoding the human 150 kDa oxygen-regulated protein, ORP150. Biochem. Biophys. Res. Commun., 230, 94-99.

Imura, N., Miura, K., Inokawa, M. and Nakada, S. (1980): Mechanism of methylmercury cytotoxicity: by biochemical and mor-
phological experiments using cultured cells. Toxicology, 17, 241-254.

Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Odake, T., Hayashi, N., Satoh, K., Hayataki, I., Yamamoto, M. and Nabeshima, Y. (1997): An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem. Biophys. Res. Commun., 236, 313-322.

Kabuta, T., Sessue, R., Mitsui, T., Kinugawa, A., Sakurai, M., Aoki, S., Uchida, K. and Wada, K. (2008): Aberrant molecular properties shared by familial Parkinson's disease-associated mutant UCH-L1 and carbonyl-modified UCH-L1. Hum. Mol. Genet., 17, 1482-1496.

Kanda, H., Sumi, D., Endo, A., Toyama, T., Chen, C.L., Kikushima, K., Itoh, K., Chiba, T., Takahashi, S., Wada, K. and Yasutake, A., Taguchi, K., Tong, I., Gajdusek, D., Shibata, M. and Kumagai, Y. (2008): Reduction of arginase I activity and manganese levels in the liver during exposure of rats to methylmercury: a possible mechanism. Arch. Toxicol., 82, 803-808.

Kanda, H., Toyama, T., Shinkai, Y., Yasutake, A., Uchida, K., Kikushima, K., and Kumagai, Y. (2012): S-Mercuration of rat sorbitol dehydrogenase by methylmercury causes its aggregation and the release of the zin c ion from the active site. Arch. Toxicol., 86, 1693-1702.

Koharudin, L.M., Liu, H., Di Maio, R., Kodali, R.B., Graham, S.H. and Gronenborn, A.M. (2010): Cyclopentenone prostaglandin-induced unfolding and aggregation of the Parkinson disease-associated UCH-L1. Proc. Natl. Acad. Sci. USA, 107, 6835-6840.

Kumagai, Y., Kanda, H., Shinkai, Y. and Toyama, T. (2013): The role of the Keap1/Nrf2 pathway in the cellular response to methylmercury. Oxid. Med. Cell. Longev., 2013, 848279.

Kurti, I., H. Di, K., Maio, R., Kodali, R.B., Graham, G.H. and Gronenborn, A.M. (2010): Cyclopentenone prostaglandin-induced unfolding and aggregation of the Parkinson disease-associated UCH-L1. Proc. Natl. Acad. Sci. USA, 107, 6835-6840.

Kythe-Andersen, J. (1984): Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polycrylamide to nitrocellulose. J. Biochem. Biophys. Methods, 10, 203-209.

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685.

Larsen, C.N., Price, J.S. and Wilkinson, K.D. (1996): Substrate binding and catalysis by ubiquitin C-terminal hydrolases: identification of two active site residues. Biochemistry, 35, 6735-6744.

Leroy, E., Boyer, R., Auburger, G., Leube, B., Ulm, G., Mezey, E., Harta, G., Brownstein, M.J., Jonnalagada, S., Chernova, T., Dehejia, A., Lavedan, C., Gasser, T., Steinbach, P.B., Wilkinson, K.D. and Polymeropoulos, M.H. (1998): The ubiquitin pathway in Parkinson's disease. Nature, 395, 451-452.

Li, Z., Melandri, F., Berdo, I., Jansen, M., Hunter, L., Wright, S., Valbrun, D. and Figueiredo-Pereira, M.E. (2004): Delta12-Prostaglandin J2 inhibits the ubiquitin hydrolase UCH-L1 and elicits ubiquitin-protein aggregation without proteasome inhibition. Biochem. Biophys. Res. Commun., 319, 1171-1180.

Madejczyk, M.S., Aremu, D.A., Simmons-Willis, T.A., Clarkson, T.W. and Ballatori, N. (2007): Accelerated urinary excretion of methylmercury following administration of its antidote N-acetylcysteine requires Mrp2/Abcc2, the apical multidrug resistance-associated protein. J. Pharmacol. Exp. Ther., 322, 378-384.

Osaka, H., Wang, Y.L., Takada, K., Takizawa, S., Sessue, R., Li, H., Sato, Y., Nishikawa, K., Sun, Y.J., Sakurai, M., Harada, T., Hara, Y., Kimura, I., Chiba, S., Namikawa, K., Kiyama, H., Noda, M., Aoki, S. and Wada, K. (2003): Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Hum. Mol. Genet., 12, 1945-1958.

Petersen, M.S., Halling, J., Bech, S., Wermuth, L., Weihe, P., Nielsen, F., Jorgensen, P.J., Budtz-Jorgensen, E. and Grandjean, P. (2008): Impact of dietary exposure to food contaminants on the risk of Parkinson’s disease. Neurotoxicology, 29, 584-590.

Rabenstein, D.L. and Saeure, R. (1977): Mercury-based electrochemical detector of liquid chromatography for the detection of glutathione and other sulfur-containing compounds. Anal. Chem., 49, 1036-1039.

Saigoh, K., Wang, Y.J., Suh, J.G., Yamanishi, T., Sakai, Y., Kiyosawa, H., Harada, T., Ichihara, N., Wukana, S., Kikuchi, T. and Wada, K. (1999): Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. Nature Genet., 23, 47-51.

Shinya, M., Kumagai, Y., Nakajima, H., Nagaofune, J., Homma-Takeda, S., Sagai, M. and Shimojo, N. (1998): Differential changes in rat brain nitric oxide synthase in vivo and in vitro by methylmercury. Brain Res., 798, 147-155.

Sinsimer, K.S., Gratacós, F.M., Knapsinska, A.M., Lu, J., Krause, C.D., Wierzbowski, A.V., Maher, L.R., Scrudato, S., Rivera, Y.M., Gupta, S., Turrin, D.K., De La Cruz, M.P., Pesta, S. and Brewer, G. (2008): Chaperone Hsp27, a novel subunit of AUF1 protein complexes, functions in AU-rich element-mediated mRNA decay. Mol. Cell. Biol., 28, 5223-5237.

Srivastava, M., Fleming, P.J., Pollard, H.B. and Burns, A.L. (1989): Cloning and sequencing of the human nucleolin cDNA. FEBS Lett., 250, 99-105.

Tamm, C., Duckworth, J.K., Hermanson, O. and Ceccatelli, S. (2008): Methylmercury inhibits differentiation of rat neural stem cells via Notch signalling. Neuroreport, 19, 339-343.

Toyama, T., Shinkai, Y., Kaji, T. and Kumagai, Y. (2013): Convenient method to assess chemical modification of protein thiols by electrophilic metals. J. Toxicol. Sci., 38, 477-484.

Toyama, T., Shinkai, Y., Yasutake, A., Uchida, K., Yamamoto, M. and Kumagai, Y. (2011): Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury. Environ. Health Perspect., 119, 1117-1122.

Toyama, T., Shinkai, Y., Yazawa, A., Kakeshashi, H., Kaji, T. and Kumagai, Y. (2014): Glutathione-mediated reversibility of covalent modification of ubiquitin carboxy-terminal hydrolase L1 by 1,2-naphthoquinone through Cys152, but not Lys4. Chem. Biol. Interact., 214, 41-48.

Toyama, T., Sumi, D., Shinkai, Y., Yasutake, A., Taguchi, K., Tong, K.I., Yamamoto, M. and Kumagai, Y. (2007): Cytotoxic protective role of Nrf2/Keap1 system in methylmercury toxicity. Biochem. Biophys. Res. Commun., 363, 645-650.

Vollrath, V., Wielandt, A.M., Iruetagoyena, M. and Chianale, J. (2006): Role of Nrf2 in the regulation of the Mrp2 (ABCC2) gene. Biochem. J., 395, 599-609.

Wasteneys, O.G., Cadrin, M., Reuhl, K.R. and Brown, D.L. (1988): The effects of methylmercury on the cytoskeleton of murine embryonic carcinoma cells. Cell Biol. Toxicol., 4, 81-60.

Wilkinson, K.D., Lee, K.M., Deshpande, S., Duerksen-Hughes, P., Wisniewska, M., Carlberg, T., Lehto, L., Johansson, I., Kotenyouva, T., Moche, M. and Schuler, H. (2010): Crystal structures of the ATPas domains of four human Hsp70 isoforms: HsP41/Hsp70-hom, HsP42/Hsp70-2, HsP46/Hsp70B', and HsP5/Hsp72. PLoS One, 5, e6825.

Zeng, J. and Hammond, B.D. (1996): Development of polyclonal antibodies for detection of protein modification by 1,2-naphthoquinone. Chem. Res. Toxicol., 9, 904-909.

Vol. 40 No. 6