Dimethyl sulfoxide elevates hydrogen peroxide-mediated cell death in *Saccharomyces cerevisiae* by inhibiting the antioxidant function of methionine sulfoxide reductase A

*Geun-Hee Kwak, Seung Hee Choi & Hwa-Young Kim**

Department of Biochemistry and Molecular Biology, Yeungnam University College of Medicine, Daegu 705-717, Korea

Dimethyl sulfoxide (DMSO) can be reduced to dimethyl sulfide by MsrA, which stereospecifically catalyzes the reduction of methionine-*S*-sulfoxide to methionine. Our previous study showed that DMSO can competitively inhibit methionine sulfoxide reduction ability of yeast and mammalian MsrA in both *in vitro* and *in vivo*, and also act as a non-competitive inhibitor for mammalian MsrB2, specific for the reduction of methionine-*R*-sulfoxide, with lower inhibition effects. The present study investigated the effects of DMSO on the physiological antioxidant functions of methionine sulfoxide reductases. DMSO elevated hydrogen peroxide-mediated *Saccharomyces cerevisiae* cell death, whereas it protected human SK-Hep1 cells against oxidative stress. DMSO reduced the protein-carbonyl content in yeast cells in normal conditions, but markedly increased protein-carbonyl accumulation under oxidative stress. Using Msr deletion mutant yeast cells, we demonstrated the DMSO's selective inhibition of the antioxidant function of MsrA in *S. cerevisiae*, resulting in an increase in oxidative stress-induced cytotoxicity. [BMB reports 2010; 43(9): 622-628]

INTRODUCTION

Dimethyl sulfoxide (DMSO) is used as a cryoprotectant for the preservation of cells and as a solvent for chemical compounds. Due to its anti-inflammatory properties, DMSO is also therapeutically used in the treatment of amyloidosis, interstitial cystitis, and rheumatic, gastrointestinal, and dermatologic disorders (1). In addition, DMSO has reactive oxygen species (ROS) scavenger properties. It reduces arsenite- or hydrogen peroxide (H_2O_2)-induced ROS intracellular production in human hamster hybrid and mouse embryo cells (2, 3) and is capable of trapping nitric oxide free radicals in human umbilical

vein endothelial cells (4).

Oxidation of free and protein-bound methionine residues can occur readily by ROS. This oxidation generates a diastereomeric mixture of methionine-*S*-sulfoxide (Met-*S*-O) and methionine-*R*-sulfoxide (Met-*R*-O), but can be reversed by methionine sulfoxide reductases (Msrs) (5, 6). Three distinct families of Msrs have evolved for the stereospecific reduction of Met-O (7-10). MsrA reduces free and protein-bound Met-*S*-O (11-13). MsrB reduces protein-based Met-*R*-O primarily due to the enzyme's lower activity toward free Met-*R*-O (13, 14). fRMsr, a new type of Msr, only reduces the free form of Met-*R*-O (10, 15). MsrA and MsrB are widely present in all three kingdoms of life (16, 17), whereas the occurrence of fRMsr is limited to unicellular organisms, including *Saccharomyces cerevisiae* (15).

MsrA and MsrB are essential enzymes that repair oxidatively damaged proteins and also function as antioxidants to protect cells against oxidative stress (5, 18). Deletion or knockdown of MsrA and MsrB genes in organisms ranging from bacteria to mammals results in increased sensitivity to oxidative stress (17, 19-23). Conversely, overexpression of these genes protects against oxidative stress-induced cell death (24-27). In addition, fRMsr also has an antioxidant function in yeast (15).

In yeast and animals, DMSO can be reduced to dimethyl sulfide by MsrA, but cannot be reduced by MsrB or fRMsr (10-12, 28). We previously showed that DMSO competitively inhibits the Met-O reduction ability of MsrA and non-competitively inhibits mammalian MsrB2 enzyme with lower inhibition effects compared to MsrA (12). Furthermore, we demonstrated that DMSO inhibits *in vivo* Met-*S*-O reduction in *S. cerevisiae* and human SK-Hep1 cells. Thus, it is possible that in these cells, DMSO could directly affect the physiological functions of Msr enzymes including protection of cells from oxidative stress.

In the present study, DMSO increased oxidative stress-induced cell death in *S. cerevisiae* cells, whereas it protected SK-Hep1 cells against oxidative stress-induced cell death. Furthermore, DMSO selectively inhibited the antioxidant function of MsrA in yeast cells, resulting in higher sensitivity to oxidative stress.

RESULTS

DMSO increases oxidative stress-induced cell death in *S. cerevisiae* cells

The first experiment investigated the influence of DMSO on yeast cell growth under oxidative stress conditions. Wild-type *S. cerevisiae* cells were grown in non-appended medium or medium containing 0.5% DMSO with or without 1.5 mM H_2O_2. In the absence of H_2O_2, growth of cells treated with DMSO was similar to that of untreated cells (Fig. 1A). As expected, cell death increased in the presence of H_2O_2. Surprisingly, the treatment of DMSO further increased the H_2O_2-mediated cell death. Similar results were observed in cells treated with 1.2 mM H_2O_2 and 0.5% DMSO (data not shown). Fig. 1B shows that DMSO increased oxidative stress-induced cell death of yeast in a concentration-dependent manner.

DMSO protects human SK-Hep1 cells against oxidative stress-induced cell death

The next experiment tested whether DMSO could affect oxidative stress-induced cell death in mammalian cells. Human SK-Hep1 cells were cultured in non-appended medium or medium containing 0.5 or 1.0% DMSO in the presence or absence of 500 μM H_2O_2. Neither concentration of DMSO affected SK-Hep1 cell growth in the absence of H_2O_2 (Supplementary Fig. S1). However, and in contrast to the observations with *S. cerevisiae*, DMSO protected against oxidative stress-induced cell death caused by H_2O_2 in a concentration-dependent manner. The DMSO's protective effect was also observed in 700 μM H_2O_2 treatment (data not shown).

Effects of DMSO on yeast MsrA, MsrB, and fRMsr activities

Our previous study showed that DMSO competitively inhibits yeast and mouse MsrAs and non-competitively inhibits mouse MsrB2; however, the DMSO inhibition effect on MsrB2 was much lower when compared with that on MsrAs. DMSO did not inhibit human MsrB3 (12). Presently, as shown above, DMSO was capable of increasing the oxidative stress-induced cell death in yeast, despite the compound's ROS scavenging activity. The previous and present observations led to the hy-
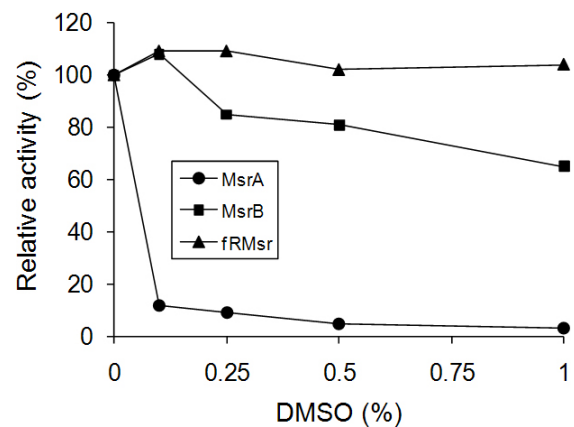
Fig. 2. Effect of DMSO on Met-O reduction by yeast Msr enzymes. Specific activities (nmol/min/mg) corresponding to 100% relative activity are as follows: 187 for MsrA; 26 for MsrB; 45 for fRMsr.
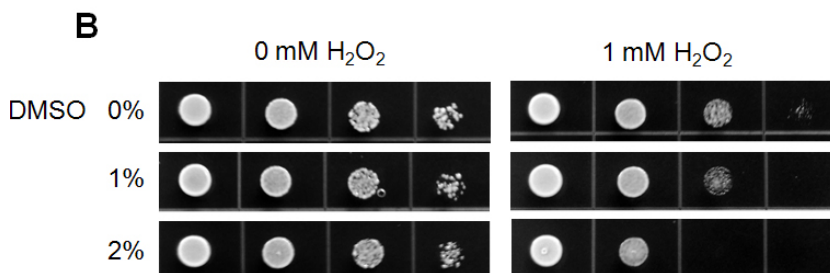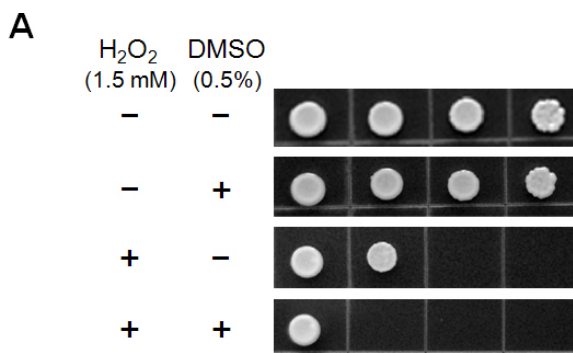
Fig. 1. DMSO increases oxidative stress-induced cytotoxicity in *S. cerevisiae*. (A) Wild-type yeast cells were incubated on non-appended YNBD medium or medium with 1.5 mM H_2O_2 and/or 0.5% DMSO. Photographs were taken after 3 days incubation at 30°C. (B) Wild-type yeast cells were spotted on YNBD medium untreated (left) or treated with 1 mM H_2O_2 (right) containing 0, 1, or 2% DMSO and incubated for 2 days at 30°C.

pothesis that the increased oxidative stress-induced yeast cell death by DMSO was due to its direct prevention of Msr antioxidant functions. To test this idea, *in vitro* enzyme assays were conducted to assess whether DMSO could inhibit yeast MsrB and fRMsr activities, such as MsrA (Fig. 2). Purified Msrs (1 μg) was used in the reaction mixture along with concentrations of DMSO ranging from 0-1%. As expected, MsrA was severely inhibited by 0.1% DMSO, with only 12% relative activity being detected. At this DMSO concentration, however, yeast MsrB and fRMsr enzymes were not inhibited. At higher concentrations, DMSO inhibited MsrB activity with a lower inhibition effect, consistent with mouse MsrB2 (12), whereas it did not inhibit fRMsr enzyme activity at all. At a DMSO concentration of 1%, the relative activities of yeast MsrA, MsrB, and fRMsr were 3%, 65%, and 104%, respectively.

DMSO selectively inhibits antioxidant function of MsrA in yeast

Next, growth assays were conducted under the oxidative stress condition using an *S. cerevisiae* mutant lacking all three known Msrs (triple *msrA/msrB/fRMsr* deletion mutant) to investigate whether DMSO could directly affect the antioxidant functions of Msrs. Also, yeast cells containing each Msr gene only (i.e. double *msrB/fRmsr*, *msrA/fRmsr*, and *msrA/msrB* deletion *mutants*) were used. The spotted cells were incubated in the medium with or without 0.5 mM H_2O_2 and/or 0.5% DMSO and cell growth was monitored. As shown in Fig. 3, DMSO conferred a slightly positive effect on the growth of all yeast mutant strains in the absence of H_2O_2 (upper panels). Interestingly, in contrast to its inhibitory effect on the wild-type cell

growth under oxidative stress, DMSO did not affect cell growth of the triple Msr deletion mutant in the presence of H_2O_2 (lower panels). These data suggested a direct inhibitory effect of DMSO on Msr antioxidant functions under oxidative stress tested but not on other antioxidant enzymes. Furthermore, under oxidative stress DMSO inhibited growth of the double *msrB/fRmsr* deletion mutant, which contains the MsrA gene only; however, it did not affect growth of other double Msr deletion mutants. These data were consistent with the idea that DMSO increased the oxidative stress-induced cell death through a selective inhibition of MsrA antioxidant function in yeast. These results corroborated the enzymatic data where MsrA was potently inhibited by DMSO. The DMSO's selective inhibition of MsrA antioxidant function under oxidative stress was also observed using single Msr deletion mutants (Supplementary Fig. S2).

DMSO increases protein-carbonyl content under oxidative stress in yeast

Protein-carbonyl content is generally used as a marker of protein oxidation and oxidative stress. It has been reported that accumulation of protein-carbonyl is elevated in yeast and mice that are deficient in MsrA, suggesting the involvement of Met oxidation in the formation and accumulation of protein-carbonyl (29-31). If DMSO inhibits the antioxidant function of MsrA that protects against oxidative stress, protein oxidation levels in yeast should be increased by DMSO under oxidative stress. Appropriately, an experiment tested whether DMSO could facilitate protein-carbonyl accumulation under oxidative stress. Wild-type *S. cerevisiae* cells were aerobically grown
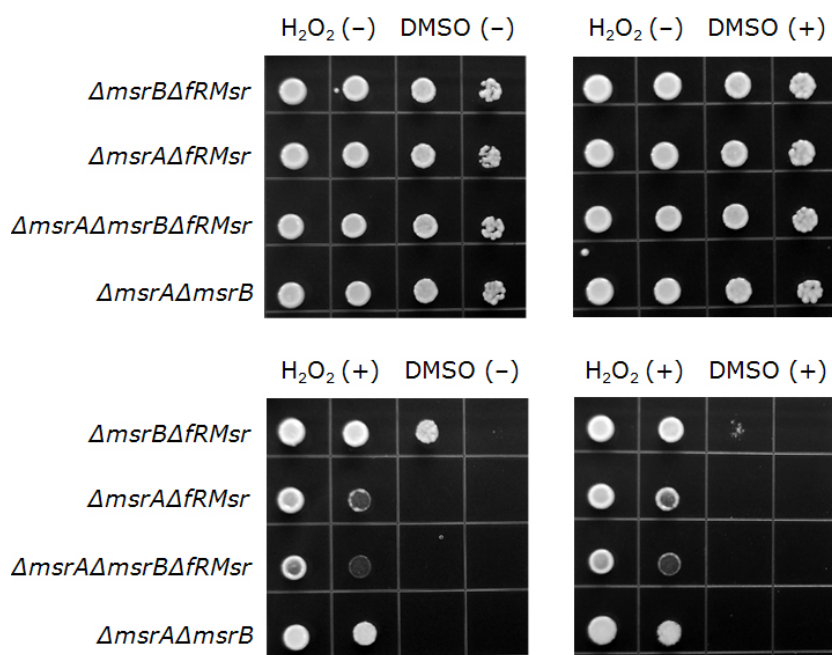

Fig. 3. DMSO selectively inhibits the antioxidant function of MsrA. *S. cerevisiae* double or triple Msr deletion mutants as indicated in the left of the figure were analyzed for cell growth on YNBD medium untreated (upper panels) or treated (lower panels) with 0.5 mM H_2O_2 in the absence or presence of 0.5% DMSO. The plates were incubated for 3 days at 30°C.

overnight in the liquid medium lacking H_2O_2 and medium with 2 mM H_2O_2 and/or 1% DMSO. Cell growth was further inhibited by DMSO in the medium with H_2O_2, similar to the observation in the spotting assay shown in Fig. 1. Cell extracts
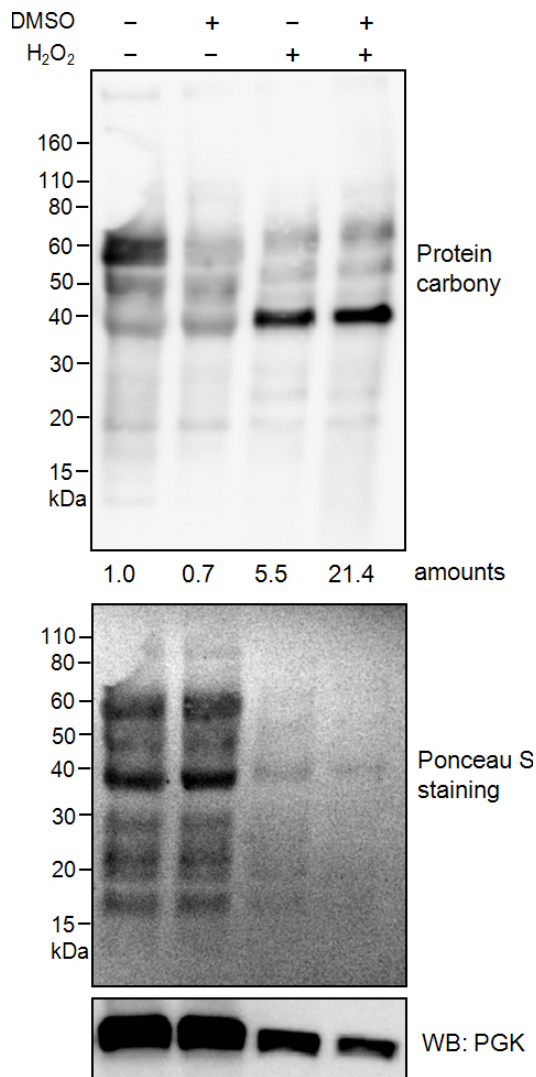

Fig. 4. DMSO elevates protein-carbonyl content in yeast under oxidative stress. Wild-type *S. cerevisiae* cells were aerobically grown in non-appended YNBD medium or medium with 2 mM H_2O_2 and/or 1% DMSO for 12 h at 30°C. After growth, optical density at 600 nm was measured as follows: 12.2 for untreated control cells; 13.1 for cells treated with DMSO only; 5.4 for cells treated with H_2O_2 only; and 4.4 for cells treated with H_2O_2 and DMSO. Protein extracts were subjected to Western blot analysis using anti-dinitrophenol antibodies for protein-carbonyl detection. Relative protein-carbonyl content was determined by normalizing the Western blot signals to the protein amounts loaded. Ponceau S stained blot and Western blot for 3-phosphoglycerate kinase (PGK) represent the protein amounts loaded.

were used to measure the protein-carbonyl content using Western blotting. As shown in Fig. 4, the presence of H_2O_2 was associated with the accumulation of 5-fold more protein-carbonyl than the H_2O_2-free medium. DMSO reduced the protein-carbonyl content by 30% in cells not treated with H_2O_2. In contrast, DMSO markedly increased the protein-carbonyl content of cells when co-treated with H_2O_2 (4-fold more protein-carbonyl accumulation relative to H_2O_2 treatment only). Together, these data are consistent with the suggestion that, even though DMSO has an ROS scavenger property, it causes an increase in protein oxidation levels under oxidative stress conditions through a selective inhibition of MsrA antioxidant function that is important for protecting against oxidative stress.

DISCUSSION

There have been a great number of reports describing the antioxidant function of MsrA. This enzyme has been demonstrated to play a crucial role in cell viability ranging from bacteria to humans by providing resistance against oxidative stress. In yeast, among the three Msrs, MsrA appears to have the strongest antioxidant effect (Fig. 3) (15, 17, 23). MsrA is specific for the reduction of free and protein-bound Met-*S*-O, and can also reduce other compounds such as DMSO, sulindac, and sulforaphane (11, 12, 18, 32). Our previous study showed that DMSO can competitively inhibit MsrA for the reduction of Met-*S*-O as it serves as a substrate for this enzyme (12). Inhibition of *in vivo* Met-*S*-O reduction by DMSO was also demonstrated in *S. cerevisiae* and SK-Hep1 cells.

The present study examined the effects of DMSO on the physiological antioxidant functions of Msrs. DMSO is known to have an ROS scavenger effect. Interestingly, it was demonstrated that DMSO could elevate oxidative stress-induced cell death of *S. cerevisiae*, whereas it could protect that of SK-Hep1. Using various Msr deletion mutant yeast cells, the present study further revealed that the increased oxidative stress-induced cell death in yeast by DMSO was due to its selective inhibition of the antioxidant function of MsrA.

It has been shown that DMSO also inhibits yeast MsrB activity, but the inhibition effect is much lower, compared with its inhibition ability for MsrA. DMSO appeared to have no effect on the antioxidant function of MsrB in cells treated with a 0.5% concentration, whose concentration gave rise to a clear inhibition effect on MsrA antioxidant function. This can be explained by the much lower inhibitory property of DMSO on MsrB enzyme; i.e. 0.5% DMSO would have not been sufficient for inhibiting enzymatic activity and the antioxidant function of MsrB.

This study has shown that DMSO acts in two completely different ways against oxidative stress-induced cytotoxicity between yeast and mammalian cells. In sharp contrast to its cytotoxic effect in yeast under oxidative stress, DMSO showed a protective effect on oxidative stress-induced cytotoxicity in

SK-Hep1 cells, although it has been demonstrated that DMSO can inhibit *in vivo* Met-*S*-O reduction in these cells by competitively inhibiting MsrA activity. It has been reported that DMSO can eliminate intracellular ROS induced by arsenite and H_2O_2 in mammalian cells, leading to the protective effect on ROS-induced cytotoxicity (2, 3). Also, DMSO was observed to reduce the protein-carbonyl content in yeast cells in the absence of H_2O_2 treatment, suggesting its ROS scavenging activity even in normal culture conditions. This ROS scavenging activity may give rise to a slightly positive effect on the growth of yeast cells without H_2O_2 treatment (Fig. 3).

These completely different effects of DMSO on oxidative stress-induced cytotoxicity between yeast and mammalian cells may be explained by a dominant result of DMSO actions as an ROS scavenger and as an inhibitor for MsrA antioxidant function. The antioxidant function of MsrA that contributes to protection against oxidative stress-induced cell death seems more critical in *S. cerevisiae* than in SK-Hep1. In yeast cells, the inhibition effect outcome of DMSO on MsrA antioxidant function may be much greater under oxidative stress and, consequently, may compromise the protective effect by its ROS scavenging activity (opposite in SK-Hep1 cells), thus resulting in a phenotype of an increased oxidative stress-induced cytotoxicity. Notably, the *in vitro* inhibition activity of DMSO is greater for yeast MsrA than for the mammalian version (12).

In conclusion, this study shows that DMSO increases oxidative stress-induced cell death in *S. cerevisiae* via the selective inhibition of MsrA antioxidant function. DMSO can present either protective or harmful effects against oxidative stress according to its functions as an ROS scavenger or as an inhibitor of MsrA.

MATERIALS AND METHODS

S. cerevisiae strains
Wild-type *S. cerevisiae* (BY4741) and the Msr deletion mutant strains (15) used are summarized in Supplementary Table S1.

Cell growth assays
For the *S. cerevisiae* cell growth assay, wild-type or Msr deletion cells were grown aerobically at 30°C in yeast nitrogen base minimal medium supplemented with 2% glucose (YNBD). The overnight broth cultures were each adjusted to an optical density of 2.5, 0.25, 0.025, and 0.0025 at 600 nm via serial dilution. Each diluted sample (5 μl) was spotted onto YNBD agar medium with or without H_2O_2 and/or DMSO. The spotted plates were incubated at 30°C and the cell growth was monitored.

For the SK-Hep1 cell growth assay, cells were seeded in wells of 24-well plates at a density of 7×10^3 cells/well and cultured for 24 h in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum at 37°C in a 5% CO_2 incubator. Cells were then maintained in the medium with or without H_2O_2 and/or DMSO. Cell growth was ana-

lyzed at 0, 24, and 48 h using an established colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-based viability assay.

Preparation of purified Msr enzymes
S. cerevisiae MsrA and fRMsr genes, based on a pET21 vector (12, 15), were overexpressed in *Escherichia coli* BL21 (DE3) and the proteins were purified by Talon-metal affinity chromatography (Clontech), as described previously (12, 15). For *S. cerevisiae* MsrB, a coding region of the MsrB gene was PCR-amplified using a genomic DNA and cloned into *Nde*I/*Xho*I sites of pET28a. The resulting construct, designated pET28-yMsrB, coded for the full-length yeast MsrB with an N-terminal His-tag. The yeast MsrB protein was purified from *E. coli* BL21 (DE3) cells by Talon-metal affinity chromatography. The eluted MsrA, MsrB and fRMsr proteins were dialyzed against 50 mM sodium phosphate (pH 7.5) and 50 mM NaCl, and the purity of proteins was checked by SDS-PAGE. Protein concentrations were determined by the Bradford method using a Bio-Rad protein assay reagent and bovine serum albumin as a standard.

DMSO inhibition of Msr activity
MsrA and MsrB activities were assayed in a dithiothreitol (DTT)-dependent reaction mixture in the presence of different concentrations of DMSO. The reaction mixture (100 μl) contained 50 mM sodium phosphate (pH 7.5), 50 mM NaCl, 20 mM DTT, 200 μM dabsyl-Met-O (dabsyl-Met-*S*-O for MsrA or dabsyl-Met-*R*-O for MsrB), 0-1% (v/v) DMSO, and 1 μg purified enzyme. The reaction was carried out at 37°C for 30 min and the reaction product, dabsyl-Met, was analyzed by HPLC. fRMsr activity was assayed using thioredoxin (Trx) as a reducing agent. The reaction mixture (200 μl) contained 50 mM sodium phosphate (pH 7.5), 50 mM NaCl, 0.2 mM NADPH, 0.1 mM EDTA, 10 μg rat Trx2 (33), and 5.8 μg human Trx reductase 1 (33), 1 mM free Met-*R*-O, 0-1% (v/v) DMSO, and 1 μg purified fRMsr. The reactions were carried out at 25°C for 10 min, and a decrease in absorbance of NADPH at 340 nm was monitored. A control for normalization purposes was the reaction mixture without enzyme. Enzyme activity was calculated using the molar extinction coefficient of NADPH (6220 $M^{-1} cm^{-1}$).

Measurement of protein-carbonyl content
Wild-type *S. cerevisiae* cells were grown aerobically at 30°C for 12 h in YNBD liquid medium with or without 2 mM H_2O_2 and/or 1% DMSO. Cells were harvested by centrifugation, resuspended in phosphate buffered saline containing 1 mM phenylmethylsulfonyl fluoride and 2% β-mercaptoethanol, and lysed using BeadBeater® glass beads (BioSpec Products). The lysates were centrifuged at 15,000 rpm for 20 min and the supernatant was used for protein-carbonyl measurement. Protein-carbonyl content was measured using OxyBlot protein oxidation detection kit (Chemicon International) according to the manufacture's protocol. The Western blot signals were quanti-

fied by densitometric analysis using ImageJ software, followed by normalizing to amounts of protein loaded. Relative amount of protein carbonyl was described.

REFERENCES

1. Santos, N. C., Figueira-Coelho, J., Martins-Silva, J. and Saldanha, C. (2003) Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects. *Biochem. Pharmacol.* **65**, 1035-1041.

2. Liu, S. X., Athar, M., Lippai, I., Waldren, C. and Hei, T. K. (2001) Induction of oxyradicals by arsenic: implication for mechanism of genotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1643-1648.

3. Perez-Pasten, R., Martinez-Galero, E., Garduno-Siciliano, L., Lara, I. C. and Cevallos, G. C. (2006) Effects of dimethylsulphoxide on mice arsenite-induced dysmorphogenesis in embryo culture and cytotoxicity in embryo cells. *Toxicol. Lett.* **161**, 167-173.

4. Sugimoto, K., Fujii, S., Takemasa, T. and Yamashita, K. (2000) Detection of intracellular nitric oxide using a combination of aldehyde fixatives with 4,5-diaminofluorescein diacetate. *Histochem. Cell Biol.* **113**, 341-347.

5. Kim, H. Y. and Gladyshev, V. N. (2007) Methionine sulfoxide reductases: selenoprotein forms and roles in antioxidant protein repair in mammals. *Biochem. J.* **407**, 321-329.

6. Weissbach, H., Resnick, L. and Brot, N. (2005) Methionine sulfoxide reductases: history and cellular role in protecting against oxidative damage. *Biochim. Biophys. Acta* **1703**, 203-212.

7. Brot, N., Weissbach, L., Werth, J. and Weissbach, H. (1981) Enzymatic reduction of protein-bound methionine sulfoxide. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2155-2158.

8. Sharov, V. S., Ferrington, D. A., Squier, T. C. and Schoneich, C. (1999) Diastereoselective reduction of protein-bound methionine sulfoxide by methionine sulfoxide reductase. *FEBS Lett.* **455**, 247-250.

9. Grimaud, R., Ezraty, B., Mitchell, J. K., Lafitte, D., Briand, C., Derrick, P. J. and Barras, F. (2001) Repair of oxidized proteins. Identification of a new methionine sulfoxide reductase. *J. Biol. Chem.* **276**, 48915-48920.

10. Lin, Z., Johnson, L. C., Weissbach, H., Brot, N., Lively, M. O. and Lowther, W. T. (2007) Free methionine-(R)-sulfoxide reductase from *Escherichia coli* reveals a new GAF domain function. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 9597-9602.

11. Moskovitz, J., Weissbach, H. and Brot, N. (1996) Cloning the expression of a mammalian gene involved in the reduction of methionine sulfoxide residues in proteins. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2095-2099.

12. Kwak, G. H., Choi, S. H., Kim, J. R. and Kim, H. Y. (2009) Inhibition of methionine sulfoxide reduction by dimethyl sulfoxide. *BMB Rep.* **42**, 580-585.

13. Lee, B. C., Le, D. T. and Gladyshev, V. N. (2008) Mammals reduce methionine-S-sulfoxide with MsrA and are unable to reduce methionine-R-sulfoxide, and this function can be restored with a yeast reductase. *J. Biol. Chem.* **283**, 28361-28369.

14. Kim, H. Y. and Gladyshev, V. N. (2004) Methionine sulfoxide reduction in mammals: Characterization of methionine-R-sulfoxide reductases. *Mol. Biol. Cell* **15**, 1055-1064.

15. Le, D. T., Lee, B. C., Marino, S. M., Zhang, Y., Fomenko, D. E., Kaya, A., Hacioglu, E., Kwak, G. H., Koc, A., Kim, H. Y. and Gladyshev, V. N. (2009) Functional analysis of free methionine-R-sulfoxide reductase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **284**, 4354-4364.

16. Delaye, L., Becerra, A., Orgel, L. and Lazcano, A. (2007) Molecular evolution of peptide methionine sulfoxide reductases (MsrA and MsrB): on the early development of a mechanism that protects against oxidative damage. *J. Mol. Evol.* **64**, 15-32.

17. Kryukov, G. V., Kumar, R. A., Koc, A., Sun, Z. and Gladyshev, V. N. (2002) Selenoprotein R is a zinc-containing stereo-specific methionine sulfoxide reductase. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4245-4250.

18. Lee, B. C., Dikiy, A., Kim, H. Y. and Gladyshev, V. N. (2009) Functions and evolution of selenoprotein methionine sulfoxide reductases. *Biochim. Biophys. Acta* **1790**, 1471-1477.

19. Alamuri, P. and Maier, R. J. (2004) Methionine sulphoxide reductase is an important antioxidant enzyme in the gastric pathogen *Helicobacter pylori*. *Mol. Microbiol.* **53**, 1397-1406.

20. Kantorow, M., Hawse, J. R., Cowell, T. L., Benhamed, S., Pizarro, G. O., Reddy, V. N. and Hejtmancik, J. F. (2004) Methionine sulfoxide reductase A is important for lens cell viability and resistance to oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 9654-9659.

21. Salmon, A. B., Perez, V. I., Bokov, A., Jernigan, A., Kim, G., Zhao, H., Levine, R. L. and Richardson, A. (2009) Lack of methionine sulfoxide reductase A in mice increases sensitivity to oxidative stress but does not diminish life span. *FASEB J.* **23**, 3601-3608.

22. Marchetti, M. A., Pizarro, G. O., Sagher, D., Deamicis, C., Brot, N., Hejtmancik, J. F., Weissbach, H. and Kantorow, M. (2005) Methionine sulfoxide reductases B1, B2, and B3 are present in the human lens and confer oxidative stress resistance to lens cells. *Invest. Ophthalmol. Vis. Sci.* **46**, 2107-2112.

23. Koc, A., Gasch, A. P., Rutherford, J. C., Kim, H. Y. and Gladyshev, V. N. (2004) Methionine sulfoxide reductase regulation of yeast lifespan reveals reactive oxygen species-dependent and -independent components of aging. *Proc. Natl. Acad. Sci. U.S.A.* **101**, 7999-8004.

24. Moskovitz, J., Flescher, E., Berlett, B. S., Azare, J., Poston, J. M. and Stadtman, E. R. (1998) Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyces cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 14071-14075.

25. Picot, C. R., Petropoulos, I., Perichon, M., Moreau, M., Nizard, C. and Friguet, B. (2005) Overexpression of MsrA

protects WI-38 SV40 human fibroblasts against H_2O_2-mediated oxidative stress. *Free Radic. Biol. Med.* **39**, 1332-1341.

26. Cabreiro, F., Picot, C. R., Perichon, M., Castel, J., Friguet, B. and Petropoulos, I. (2008) Overexpression of mitochondrial methionine sulfoxide reductase B2 protects leukemia cells from oxidative stress-induced cell death and protein damage. *J. Biol. Chem.* **283**, 16673-16681.

27. Kwak, G. H., Kim, J. R. and Kim, H. Y. (2009) Expression, subcellular localization, and antioxidant role of mammalian methionine sulfoxide reductases in *Saccharomyces cerevisiae*. *BMB Rep.* **42**, 113-118.

28. Hansen, J. (1999) Inactivation of MXR1 abolishes formation of dimethyl sulfide from dimethyl sulfoxide in *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* **65**, 3915-3919.

29. Oien, D. and Moskovitz, J. (2007) Protein-carbonyl accumulation in the non-replicative senescence of the methionine sulfoxide reductase A (msrA) knockout yeast strain.

Amino Acids **32**, 603-606.

30. Moskovitz, J., Bar-Noy, S., Williams, W. M., Requena, J., Berlett, B. S. and Stadtman, E. R. (2001) Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12920-12925.

31. Moskovitz, J. and Oien, D. B. (2010) Protein carbonyl and the methionine sulfoxide reductase system. *Antioxid. Redox Signal.* **12**, 405-415.

32. Etienne, F., Resnick, L., Sagher, D., Brot, N. and Weissbach, H. (2003) Reduction of Sulindac to its active metabolite, sulindac sulfide: assay and role of the methionine sulfoxide reductase system. *Biochem. Biophys. Res. Commun.* **312**, 1005-1010.

33. Kim, H. Y. and Kim, J. R. (2008) Thioredoxin as a reducing agent for mammalian methionine sulfoxide reductases B lacking resolving cysteine. *Biochem. Biophys. Res. Commun.* **371**, 490-494.