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**

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Byung Cheon Lee, Dung Tien Le, and Vadim N. Gladyshev

From the Redox Biology Center and Department of Biochemistry, University of Nebraska, Lincoln, Nebraska 68588-0664

Methionine is an essential amino acid in mammals at the junction of methylation, protein synthesis, and sulfur pathways. However, this amino acid is highly susceptible to oxidation, resulting in a mixture of methionine-S-sulfoxide and methionine-R-sulfoxide. Whether methionine is quantitatively regenerated from these compounds is unknown. Here we report that SK-Hep1 hepatocytes grew on methionine-S-sulfoxide and consumed this compound by import and methionine-S-sulfoxide reductase (MsrA)-dependent reduction, but methionine-R-sulfoxide reductases were not involved in this process, and methionine-R-sulfoxide could not be used by the cells. However, SK-Hep1 cells expressing a yeast free methionine-R-sulfoxide reductase proliferated in the presence of either sulfoxide, reduced them, and showed increased resistance to oxidative stress. Only methionine-R-sulfoxide was detected in the plasma of wild type mice, but both sulfoxides were in the plasma of MsrA knock-out mice. These results show that mammals can support methionine metabolism by reduction of methionine-S-sulfoxide, that this process is dependent on MsrA, that mammals are capable of reducing free Met sulfoxides (Met-S-sulfoxide (Met-SO) and Met-R-sulfoxide (Met-RO)) (6, 7).

To repair Met-SO and Met-RO residues in proteins, cells have evolved two families of enzymes known as Met sulfoxide reductases: MsrA that is specific for Met-SO and MsrB that specifically reduces Met-RO (8). The active sites of proteins in both Msr families are better adapted for binding Met sulfoxide residues than free Met sulfoxides (6). Although both MsrA and MsrB are capable of reducing free Met sulfoxides (9), a contribution of these enzymes to the reduction of these amino acids is not known (10).

Mammals have one MsrA and three MsrB isoforms, with selenoprotein MsrB1 localized to cytosol and nucleus, MsrB2 to mitochondria, and MsrB3 to the endoplasmic reticulum (9). A single MsrA is partitioned into various cellular compartments by alternative first exon splicing and folding-dependent cytosolic retention of the protein containing a mitochondrial targeting signal (11–14). MsrA and MsrB are thought to both repair oxidatively damaged Met residues and serve as antioxidant proteins, thus supporting the role of Met residues in scavenging ROS (15). Consistent with these ideas, deletion of MsrA reduces life span in yeast and mice (16, 17), whereas their overexpression can increase life span in fruit flies and yeast cells (17–19).

In comparison with the repair of oxidized Met residues, reduction of free Met sulfoxides in mammals received little attention. On the other hand, several cellular fractions with proteins distinct from MsrA and MsrB were found to reduce free Met sulfoxides in bacteria (20). Furthermore, by analyzing bacterial cells deficient in MsrA and MsrB, two enzymes were identified that acted exclusively on free Met sulfoxides. A molybdoprotein-containing biotin sulfoxide reductase Bisc (21) was found to reduce free Met-SO, whereas a GAF domain-containing protein rFMr was specific for free Met-RO (22).

In this work, we characterized the ability of mammals to reduce free Met sulfoxides. Whereas Met-SO was reduced by MsrA, mammalian cells were deficient in the reduction of free reactive oxygen species (ROS)** generated during oxidative stress and normal cellular metabolism. ROS can oxidize both free Met and Met residues in proteins, resulting in a diastereomeric mixture of Met sulfoxides: Met-S-sulfoxide (Met-SO) and Met-R-sulfoxide (Met-RO) (6, 7).

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1 To whom correspondence should be addressed: N151 Beadle Center, University of Nebraska, Lincoln, NE 68588. Tel.: 402-472-4948; Fax: 402-472-7842; E-mail: vgladyshev1@unl.edu.

2 The abbreviations used are: ROS, reactive oxygen species; ATF3, activating transcription factor 3; CBS, cystathionine β-synthase; rFMr, free methionine-R-sulfoxide reductase; OPA, p-phthalaldehyde; Met-SO, methionine-S-sulfoxide; Met-RO, methionine-R-sulfoxide; MsrA, methionine sulfoxide reductase A; MsrB, methionine sulfoxide reductase B; HPLC, high pressure liquid chromatography; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium.
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Met-RO. However, cells expressing a yeast fRMsr homolog could efficiently utilize Met-RO. Thus, this study both revealed an inherent deficiency of mammalian cells in an important antioxidant repair process and identified a protein that can compensate for this deficiency.

EXPERIMENTAL PROCEDURES

Preparation of Free Met-SO and Met-RO—Free Met-SO and Met-RO were prepared from mixed L-Met-R,S-sulfoxide (Sigma) according to the method of Lavine (23). To obtain diastereomers of higher purity, we repeated the separation process twice for each sulfoxide. Purity of Met-SO and Met-RO was assessed by an HPLC procedure using o-phthalaldehyde (OPA) (Sigma)-derivatized amino acids (7) and found to exceed 98%.

Cell Culture—SK-Hep1 (ATCC: HTB-52™) and fRMsr-transfected SK-Hep1 cells were cultured in DMEM or Met-free DMEM (Invitrogen) supplemented with 0.1 mM Met, 0.1 mM Met-RO, or 0.1 mM Met-RSO. The medium also contained 10% dialyzed fetal bovine serum and an antibiotics-antimycotic mixture (Invitrogen) of 100 units/ml penicillin G sodium, 100 µg/ml streptomycin sulfate, and 0.25 µg/ml amphotericin B. In initial experiments, we examined media containing different amounts of Met and found that 0.1 mM Met was optimal and avoided Met deficiency. Thus, 0.1 mM Met sulfoxides were used in further experiments. Cell culture experiments, with the exception of a proliferation assay, were carried out in 6-well plates, and the cells were maintained at 37 °C in a 5% CO₂ atmosphere.

Growth of SK-Hep1 cells was analyzed in modified DMEM containing Met, Met-SO, Met-RO, or Met-RSO (0.1 mM of each amino acid) or with no addition of these compounds. In addition, the media were supplemented or not with 100 nM sodium selenite. The cells were analyzed at 0, 24, 48, and 72 h. Another experiment involving SK-Hep1 cells was done with cells grown in serum-free modified DMEM containing insulin (5 µg/ml) and transferrin (10 µg/ml), Met or Met-RSO (0.1 mM of each amino acid) and 100 nM sodium selenite (or with no addition of this compound), and separately in 10% dialyzed fetal bovine serum-containing DMEM containing 0.1 mM Met with 100 nM sodium selenite (or not). The cells were analyzed at 0, 24, 48, and 72 h. Cell growth assays were carried out as described below.

Proliferation Assay—Cell growth was quantified using colorimetric MTS assay (Promega). The cells in regular DMEM
were plated in 96-well plates at 5 × 10³ cells/well, washed with PBS, and specialized DMEM were added that contained Met, Met-RO, Met-RO, or Met-RSO 24 h after plating. To assay for cell proliferation, a tetrazolium compound (inner salt; MTS) and an electron coupling reagent (phenazine methosulfate) were mixed according to the manufacturer’s protocol, and then 20 µl of the mixture were added to 100 µl of phenol red-excluded medium that further replaced cell culture medium. After 90 min of incubation at 37 °C in the atmosphere of 5% CO₂, cell proliferation was measured at indicated time periods from 0 to 96 h at 450 nm in a plate reader. Direct counting of viable cells using 0.2% trypan blue was done in 6-well plates.

An HPLC Analysis of Media Samples—The cells were plated in 6-well plates at 7.5 × 10⁴ cells/well in 1.5 ml of regular DMEM. After 24 h, the cells were washed with PBS, and the medium was changed to modified DMEM containing Met, Met-RO, Met-RSO, or Met-RSO (0.1 m of each amino acid). 90 µl of medium from each well was collected at 0, 24, 48, 72, and 96 h and mixed with 10 µl of 100% trichloroacetic acid. After incubation at 4 °C for 10 min and centrifugation at 13,000 rpm for 15 min, the supernatant was diluted 10 times with distilled water and prepared for OPA derivatization. OPA derivatization of amino acids and HPLC analysis were performed as described (7) with minor modifications. The derivatization reagent was freshly prepared as a stock solution (40 mg of o-phthalaldehyde, 1 ml of methanol, 50 µl of 2-mercaptoethanol, 5 ml of 0.1 M Na₂B₄O₇, pH 9.5) at room temperature in a capped amber vial. Sample solutions (2–5 µl) were mixed with the OPA derivatization reagent to a 100-µl final volume. Following a 2-min reaction at room temperature, the mixture was fractionated on a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm). Changes in Met level in the fRMsr activity assay were similarly quantified. Detection was by fluorescence of Met derivatives using a Waters 474 scanning fluorescence detector with excitation at 330 nm and emission at 445 nm.

Knockdown of MsrA in SK-Hep1 Cells by Small Interfering RNA—Double-stranded small interfering RNAs (Dharmacon) for targeting human MsrA mRNA were used to knockdown this gene in SK-Hep1 cells. The cells were transfected with small interfering RNA by using DharmaFECT™ 1 Transfection Reagent (Dharmacon) according to the manufacturer’s protocol. Proliferation of these cells was assayed 60 h after transfection in comparison with control transfected cells.

Transfection of Yeast fRMsr Gene into SK-Hep1 Cells—A His₆ tag sequence was cloned at the C terminus of yeast fRMsr, the sequence was inserted into a mammalian expression vector, pCI-neo (Promega), and the resulting construct was verified by DNA sequencing. SK-Hep1 cells were transfected or cotransfected with the expression vector coding for fRMsr and pEGFP-N1 (Clontech) vector or with an empty pCI-neo vector and pEGFP-N1 vector using FuGENE 6 transfection reagent according to the manufacturer’s suggestion. To establish a stable cell line, the cells were selected in the presence of 800 µg/ml G418 sulfate (Promega) and further maintained in the presence of 400 µg/ml G418 sulfate. Expression of recombinant fRMsr was verified by Western blotting using anti-His tag antibodies, and Msr activities were assayed by an HPLC method using OPA derivatization.

Cloning and Expression of Yeast fRMsr—Characterization of S. cerevisiae fRMsr (YKL069W) is described in a separate paper. Briefly, a yeast fRMsr cDNA, which encodes a protein homologous to fRMsr from Escherichia coli, was amplified from Saccharomyces cerevisiae genomic DNA and subcloned into pET21b vector (Novagen) using primers 5’-AAACATATG-ATGGGCTCATACAACGGGTTC-3’ (sense) and 5’-AAAG-CGGCCCGACACATGATTTATATATTTAGCAAG-3’ (antisense). After transformation into BL21 (DE3) E. coli, the subcloned sequence was verified by DNA sequencing. Cells with the plasmid in 500 ml of LB medium containing 100 µg/ml ampicillin were grown until A₆₀₀ reached 0.6–0.8, followed by the addition of isopropyl β-D-thiogalactopyranoside to 0.3 mM. Protein expression was at 30 °C for 4 h, followed by harvesting cells by centrifugation at 4,000 rpm for 5 min. The cells were washed with PBS and stored at −70 °C until use.

To purify the protein, cell pellet was dissolved in resuspension buffer (Tris-HCl, pH 7.5, 15 mM imidazole, 300 mM NaCl), and phenylmethylsulfonyl fluoride was added to a final concentration of 0.5 mM. After sonication, supernatant was collected by centrifugation at 8,000 rpm for 30 min. The supernatant was loaded onto a cobalt Talon resin (Clontech) pre-equilibrated with resuspension buffer. Following washing with the same buffer, the protein was eluted with elution buffer (Tris-HCl, pH 7.5, 300 mM imidazole, 300 mM NaCl). Fractions containing yeast fRMsr were pooled together and dialyzed overnight against PBS in a dialysis cassette (Pierce).

Activity Assays of fRMsr—Reaction mixture (40 µl) included 50 mM dithiotheritol, 1 mM substrate (Met-RO or Met-SO), and purified enzyme or cell lysate prepared by treatment with CelLytic™ M cell lysis reagent (Sigma). 20 µl of the reaction mixture was mixed with 2 µl of trichloroacetic acid and subjected to OPA derivatization as described above and then injected onto the column to measure endogenous Met level in the samples. An additional 20 µl from the same original reaction mixture were incubated at 37 °C for 30 min and then subjected to OPA derivatization by the same procedure using trichloroacetic acid. After derivatization of the sample (2–5 µl) by adding OPA solution to 100 µl as a final volume, 50 µl of the mixture were subjected to an HPLC separation as described above.

Analysis of SK-Hep1 and fRMsr-transfected SK-Hep1 Cells—SK-Hep1 and fRMsr-transfected SK-Hep1 cells were plated in 6-well plates at 7.5 × 10⁴ cells/well in regular DMEM. The medium was changed to Met-free DMEM supplemented with either Met or individual Met sulfoxides at 24 h after plating. The cells were then collected every 24 h until 96 h. Protein concentration was measured by the Bradford assay, and the samples were probed by standard immunoblot assays with polyclonal MsrA (kindly provided by Bertrand Friguet), cystathionine β synthase (CBS) (kindly provided by Ruma Banerjee), and activating transcription factor 3 (ATF3) (Santa Cruz Biotechnology) antibodies.

3 D. T. Le, B. C. Lee, Y. Zhang, D. E. Fomenko, S. M. Marino, E. Hacioglu, G. H. Kwak, A. Koc, H. Y. Kim, and V. N. Gladyshev, submitted for publication.
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Microscopy—A Nikon TE-300 microscope was used to prepare images of SK-Hep1 cells grown on different Met and Met sulfoxide media. Microscopy analyses were carried out in the University of Nebraska-Lincoln Microscopy Core Facility.

75Se Metabolic Labeling—SK-Hep1 cells were plated in 6-well plates at 7.5 × 104 cells/well in 1.5 ml of regular DMEM. After 24 h, the cells were washed with PBS, and the medium was changed to modified DMEM containing Met, Met-SO, Met-RO, or Met-RSO (0.1 mM of each amino acid). 0.01 mCi of freshly neutralized [75Se]selenonic acid (specific activity, 1,000 Ci/mmol; University of Missouri Research Reactor) was added in each well at 48 h after changing the medium, and the cells were incubated at 37°C for 24 h in 5% CO2 atmosphere, followed by harvesting the cells. The cell extract (30 µg of total protein) was electrophoresed on 10% Bis-Tris gels and transferred onto polyvinylidene difluoride membranes (Invitrogen). The 75Se radioactivity pattern was visualized by using a PhosphorImager (GE Healthcare).

Resistance of fmMr-expressing and Control SK-Hep1 Cells to Oxidative Stress—SK-Hep1 cells stably transfected with either an empty pcDNA vector and fmMr construct were plated in 96-well plates at 3.0 × 104 cells/well. The medium was changed to a serum-free medium, and H2O2 was added to each well at indicated concentrations (0–1000 µM). Cell viability was measured by using colorimetric MTS assay.

Mice—MsrA knock-out mice (16) were kindly provided by Drs. Rodney Levine and Geumsoo Kim (National Institutes of Health). These mice and control C57BL6 mice were used for blood sampling. In addition, C57BL6 mice, which were subjected to selenium deficiency or a control diet containing 0.4 ppm Se in the form of sodium selenite for 8 months, were used.

HPLC Analysis of Met-RO and Met-SO Level in Mouse Plasma—Mouse blood was centrifuged at 13,000 rpm for 15 min. The supernatant was prepared for OPA derivatization without dilution following trichloroacetic acid precipitation and analyzed at isocratic flow rate of 2 min/ml at 89:11 (v/v) of 20 mM sodium acetate, pH 5.8 (solvent A) and methanol (solvent B) as described in the supplemental materials.

RESULTS

SK-Hep1 Cells Grow in the Presence of Met and Met-SO, but Not in the Presence of Met-RO—To examine the capacity of mammalian cells to provide Met for cellular metabolism by Met sulfoxide reduction, human hepatoma SK-Hep1 cells were grown in Met-free DMEM supplemented with 0.1 mM Met, 0.1 mM Met-SO, 0.1 mM Met-RO, or 0.1 mM mixed Met-RO/Met-SO (Met-RSO) (Fig. 2A). SK-Hep1 hepatocytes grew best in the presence of Met and could also proliferate in Met-SO and Met-RSO media, although at a reduced rate. In contrast, Met-RO did not support the growth of SK-Hep1 cells. Morphology of cells maintained on Met-SO and Met-RO media was also different (see below). After 96 h on the Met-RO medium, SK-Hep1 hepatocytes were long and narrow, similar in shape to Met-restricted cells, whereas the cells grown on Met-SO resembled those maintained in the presence of Met. These data suggested that SK-Hep1 cells have a system for import and reduction of Met-SO, which provides them with Met, whereas these cells are unable to utilize free Met-RO.

To determine whether Met, Met-RO, and Met-SO were consumed by SK-Hep1 hepatocytes, levels of these compounds were determined in each growth medium at 0, 24, 48, 72, and 96 h. For this purpose, we developed an HPLC procedure that utilized OPA derivatization of free amino acids following trichloroacetic acid precipitation of medium components (Fig. 2B and supplemental Fig. S1). We found this method to be superior to the currently available procedures for quantitative analysis of free Met sulfoxides and Met. It was recently reported (25) that many Met sulfoxide derivatives, such as widely used dabsyl chloride-reacted forms, are subject to nonenzymatic Met sulfoxide reduction at high temperatures, but the corresponding derivatization procedures themselves require heating. In contrast, the OPA derivatization process is completed at room temperature after 2–3 min, thus avoiding a heating step and associated nonenzymatic Met sulfoxide reduction.

With this procedure, we found that relative amounts of Met in the Met-supplemented medium decreased to ~60% of the initial level at 96 h. In contrast, Met-RO levels were not changed in Met-RO and Met-RSO media at any time points. Met-SO was decreased to 88% in the Met-SO-supplemented medium and to 77% in the Met-RSO medium (Fig. 2B). These data show that SK-Hep1 cells could import and consume both Met-SO and Met from the medium to support cell proliferation. On the other hand, consistent with the inability of SK-Hep1 cells to grow on Met-RO, this compound was not utilized. Moreover, when cells were grown on Met-RSO, only Met-SO was consumed.

SK-Hep1 Cell Extracts Are Active in the Reduction of Met-SO but Do Not Reduce Met-RO—Because both MsrA and MsrB were reported to support low level reduction of free Met

FIGURE 2. Growth and consumption of free Met sulfoxides by SK-Hep1 cells. A, growth of SK-Hep1 hepatocytes on Met and Met sulfoxide media. The cells were grown in Met-free DMEM supplemented with 0.1 mM Met (diamonds), 0.1 mM Met-RO (triangles), 0.1 mM Met-SO (circles), or 0.1 mM Met-RSO (squares). Cell growth was quantified in a cell proliferation assay at 0, 24, 48, 72, and 96 h. The error bars represent the standard deviations from five independent experiments. B, consumption of Met in Met medium (diamonds), Met-SO in Met-RSO medium (squares), Met-RO in Met-RSO medium (triangles), Met-SO in Met-SO medium (circles), and Met-RO in Met-RO medium (stars) at 0, 24, 48, 72, and 96 h. The initial concentration of each amino acid was 0.1 mM. The error bars represent the standard deviations from three independent experiments.
TABLE 1
Specific activities of SK-Hep1 cells towards free Met sulfoxides
The table shows the values ± standard deviations from three independent experiments.

| Specific activity for each Met-O | Met-RO | Met-SO |
|----------------------------------|--------|--------|
| yfRMser*                        | 30115 ± 2652 | ND³ |
| SK-Hep1                          | ND³    | 38.6 ± 3.6 |
| yfRMser transfected SK-Hep1      | 46.4 ± 5.0  | 42.7 ± 5.9 |

* yfRMser is yeast-free methionine-β-sulfoxide reductase.
³ A signal corresponding to specific activity less than 5 pmol/min/mg is shown as ND (not detectable).

sulfoxides in in vitro assays (9), it was of interest to examine the contribution of these enzymes to Met sulfoxide reduction under physiological conditions in mammalian cells. We measured specific activities of SK-Hep1 cell extracts for the reduction of free Met-RO and Met-SO. No free Met-RO reductase activity was detected (it was within background), whereas the activity for free Met-SO reduction was ≈38.6 pmol/min/mg of protein (Table 1).

Role of MsrA in the Reduction of Free Met-SO—To examine the role of MsrA in providing cells with Met by reduction of free Met-SO, we knocked down its expression by small interfering RNA. Decreased MsrA expression was verified by Western blot assays (Fig. 3A). We found that MsrA-deficient cells grew neither in Met-SO nor Met-RO medium, whereas Met still supported their growth. Thus, MsrA is responsible for the reduction of Met-SO acquired from the media by SK-Hep1 cells. These data also indicate that none of the three mammalian Mrsb isozymes contributes significantly to the enzymatic reduction of free Met sulfoxides and to providing Met to support cell growth.

A Yeast Enzyme Specific for Free Met-RO—A recent study identified a bacterial enzyme specific for free Met-RO (22). This GAF domain–containing protein was designated as fRMsr. We analyzed sequence databases for homologs of this protein and found that mammals and other animals lack fRMsr. However, homologs of this protein were detected in many lower eukaryotes (supplemental Fig. S2).³ We cloned fRMsr homolog from S. cerevisiae and expressed it in E. coli as a His-tagged protein. The recombinant protein was soluble and had the expected molecular weight as determined by SDS-PAGE and mass spectrometry, whereas the native yeast protein functioned as fRMsr in S. cerevisiae cells.³ The recombinant yeast fRMsr protein exhibited high activity toward free Met-RO (≈33 nmol/min/mg of protein), whereas it was inactive with Met-SO as well as with dabsyl-Met-RO and dabsyl-Met-SO.

Yeast fRMsr-expressing SK-Hep1 Cells Grow on Met-RO—Yeast fRMsr was stably expressed in SK-Hep1 cells in the form of His-tagged fRMsr (lane 1) and control SK-Hep1 cells (lane 2) with anti-His antibodies. B, yeast fRMsr-transfected SK-Hep1 cells were grown in media containing 0.1 mM Met (diamonds), 0.1 mM Met-RO (circles), or 0.1 mM Met-SO (squares) for 96 h. Cell growth was measured by an MTS cell proliferation assay at 0, 24, 48, 72, and 96 h. Cell growth was measured by an MTS cell proliferation assay at 0, 24, 48, 72, and 96 h. C, resistance of fRMsr-expressing SK-Hep1 cells to hydrogen peroxide treatment. Control (diamonds) and fRMsr-expressing SH-Hep1 (squares) cells were treated with the indicated concentrations of hydrogen peroxide, and their viability was assayed at 24 h. The error bars show the standard deviations from three independent experiments.
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FIGURE 5. Morphology of cells grown on sulfoxide media. A, an image of SK-Hep1 cells grown in the Met-SO medium for 96 h. B, an image of SK-Hep1 cells grown in Met-RO medium for 96 h. C, an image of SK-Hep1 cells stably expressing yeast fRMsr grown in Met-RO medium for 96 h.

DISCUSSION

Prior to this study, there was little understanding of the pathways for reduction of free Met-SO and Met-RO in mammalian cells. Although both Met residues and free Met are known to be susceptible to oxidation by ROS, and the reduction of Met sulfoxide residues is carried out by four enzymes in mammals,
whether free Met sulfoxides are reduced or wasted was not known. Surprisingly, analysis of human SK-Hep1 hepatocytes grown on media containing Met, Met-RSO or individual Met sulfoxide diastereomers showed a significant difference in cell proliferation and Met sulfoxide consumption between Met-SO and Met-RO (Fig. 2). Met-SO, but not Met-RO, supported the growth of SK-Hep1 cells and was consumed from the medium. In addition, extracts of SK-Hep1 cells showed high free Met-SO reductase activity, whereas free Met-RO activity was not detected (Table 1). Certainly, MsrA was an obvious candidate for the role in free Met-SO reduction, and further experiments confirmed this idea because MsrA-deficient SK-Hep1 cells

![Figure 6](image-url)
were no longer able to utilize free Met-SO and could not grow on either Met sulfoxide. These findings, as well as experiments with selenium deficiency and supplementation in SK-HeP1 hepatocytes, also demonstrated that none of the three mammalian MsrBs had a role in providing these cells with Met by enzymatic free Met sulfoxide reduction.

We were able to extend these observations from cell culture to an animal model. Plasma samples from wild type mice had undetectable Met-SO, whereas the bulk of Met sulfoxide occurred in the form Met-RO (9 μM). Moreover, MsrA knock-out mice had both diastereomers at similar levels, indicating a specific elevation in Met-SO. Thus, not only hepatocytes, but also mice were deficient in Met-RO reduction and accumulated this compound in plasma. In addition, low levels of Met-SO were clearly caused by reduction of this compound by MsrA. We also found that subjecting wild type mice to selenium deficiency slightly elevated both diastereomers, likely because of increased oxidative stress caused by systemic selenium-protein deficiency. This finding again excluded the role of MsrB1 in the reduction of Met-RO in mice.

A recent report by Lowther and co-workers (22) identified an enzyme, fRMsr, that catalyzes the reduction of free Met-RO in E. coli. However, our sequence analyses revealed that mammals lack this protein. Nevertheless, fRMsr homologs were detected in a variety of prokaryotes as well as in many single-celled eukaryotes. We characterized an fRMsr homolog from S. cerevisiae, which showed robust free Met-RO reductase activity. Furthermore, SK-HeP1 cells expressing this protein grew equally well on Met and Met-RO media (Fig. 4B). Thus, fRMsr could fully compensate for inability of SK-HeP1 cells to reduce free Met-RO and could provide these cells with Met in quantities sufficient for cell growth. In addition, we observed increased resistance of fRMsr-expressing cells to hydrogen peroxide treatment, suggesting that reduction of free Met sulfoxides provides cells with antioxidant defense as previously proposed (15).

In view of such an important function, it is puzzling why mammals lack fRMsr. Perhaps, environment at the time when animals evolved could have played a role. However, once the enzyme is lost, the ability for Met-RO reduction may be difficult to replace, and mammalian cells are now faced with this defect. In this regard, the finding that a yeast reductase fRMsr can fully compensate for the deficiency in Met-RO reduction could provide avenues for correcting this shortcoming in mammals and other animals.

Met is an essential amino acid in mammals, which is exclusively derived from food sources and linked to many metabolic processes, including protein synthesis, methylation, sulfur metabolism, and ROS scavenging. In particular, Met metabolism has an important role in the synthesis of antioxidant compounds, such as cysteine, taurine, and GSH, through the transsulfuration pathway in liver, kidney, and small intestine. Thus, free Met plays a role in antioxidant defense not only by scavenging ROS (through reversible oxidation) but also as precursor of other antioxidant compounds.

In conclusion, this work revealed an ability of mammalian cells to utilize free Met sulfoxides; however, of the two stereoisomers, only Met-SO is reduced in sufficient quantities, and this occurs in a MsrA-dependent manner. In contrast, mammals are deficient in the reduction of free Met-RO because of a loss of fRMrs in animals during evolution. Importantly, we found that this potential Achilles’ heel of the mammalian antioxidant repair system could be addressed by expression of a yeast fRMsr, suggesting strategies for improved protection of mammals from oxidative stress and disease and for influencing the aging process.

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FIGURE 7. Analysis of Met-SO and Met-RO in mouse plasma. Concentrations of Met-SO and Met-RO were determined in plasma of wild type, heterozygous MsrA knock-out (+/−), homozygous MsrA knock-out (−/−), and selenium-deficient (SD) mice. The error bars represent the standard deviations from four independent experiments.
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