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Forum Mini Review

Oxidation of Methionine Residues of Proteins: Biological Consequences

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ABSTRACT

Most reactive oxygen species (ROS) can oxidize methionine (Met) residues of proteins to methionine sulfoxide (MetO). However, unlike the ROS-dependent oxidation of other amino acid residues of proteins (except cysteine residues), the oxidation of Met residues is readily reversed by the action of methionine sulfoxide reductase (Msr) that catalyzes the thioredoxin-dependent reduction of MetO residues of proteins back to Met. We summarize here results of studies showing that the cyclic interconversion of Met and MetO residues of proteins is involved in several different biological processes: (a) It is the basis of an important antioxidant mechanism for the scavenging of ROS. (b) It is likely involved in the regulation of enzyme activities. (c) It is involved in cell signaling. (d) It can target proteins for proteolytic degradation. Furthermore, a loss in the ability to catalyze the reduction of protein MetO to Met residues leads to a decrease in the maximum life span, whereas overexpression of this activity leads to an increase in the life span of animals. In addition, a decrease in Msr activities in brain tissues is associated with the development of Alzheimer's disease. *Antioxid. Redox Signal.* 5, 577–582.

INTRODUCTION

THE NONENZYMIC OXIDATION of surface-exposed methionine (Met) residues of proteins by reactive oxygen species (ROS) generates mixtures of the *R*- and *S*-isomers of methionine sulfoxide (MetO) (32). If unrepaired, these oxidations can lead to changes in hydrophobicity (4, 15, 37), alterations in protein conformation (2, 33, 37, 38), and loss of biological activity of the oxidized proteins (3, 37). However, cells contain two kinds of methionine sulfoxide reductases (Msr) that catalyze the reduction of MetO residues of proteins back to Met residues. One of these, MsrA, is specific for reduction of the *S*-isomer of MetO (12, 21–23), and the other, MsrB, is specific for reduction of the *R*-isomer of MetO (12, 13, 23, 24). Based on the consideration that the cyclic interconversion of Met and MetO residues of proteins leads to the consumption of multiple forms of ROS, it was proposed that this interconversion may play an important antioxidant role in the scavenging of ROS (15). Also, in view of the fact that interconversion of proteins between phosphorylated and unphosphorylated forms is involved in the regulation of enzyme activities and cell signaling, it is generally believed that cyclic interconversion of Met and MetO residues of proteins may play an important role in enzyme regulation and signal transduction (10, 21, 37). We summarize here results of studies in various laboratories showing that deficiencies in the ability to catalyze the Met/MetO interconversion makes organisms more sensitive to oxidative stress and can have a profound effect on their life span, and may also contribute to the development of some diseases; conversely, increasing the capacity to reduce MetO back to Met increases life span.

POSSIBLE ROLE OF MET OXIDATION IN CELLULAR REGULATION

Because the oxidation of Met residues of some proteins to MetO derivatives leads to a significant loss of their biological

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activities (3, 37), it is generally believed that the reversible oxidation of protein Met residues is involved in the regulation of some enzyme and peptide hormone activities. This is a reasonable hypothesis because the cyclic interconversion of proteins between unmodified and covalently modified forms is basic to the regulation of many enzyme activities and cell signaling processes. As illustrated in Fig. 1, the cyclic phosphorylation and dephosphorylation of tyrosine, serine, or threonine hydroxyl groups of proteins and the cyclic adenylylation and deadenylylation of tyrosine hydroxyl groups are implicated in the regulation of some enzyme activities. By analogy, the cyclic interconversion of particular Met residues between unmodified and oxidized forms could serve an important regulatory role. Indeed, the oxidation of Met residues of the bacterial glutamine synthetase can mimic the effects of obtained by adenylylation of the enzyme (Fig. 2).

If the cyclic interconversion of Met and MetO residues of proteins is involved in the regulation of enzyme/hormone function, then cells should possess Met oxidases and MetO reductases that catalyze oxidation and reduction of particular Met residues in a specific protein. The random oxidation of surface-exposed Met residues of proteins by multiple forms of ROS would lack the specificity needed to serve an important regulatory function. Until now, there is no evidence for the presence of oxidases and reductases that possess such specificity. Flavin monooxygenases that can catalyze the NADPH-dependent oxidation of a Met residue in model peptides have been described (6, 11, 27), but their physiological roles, if any, remain to be established. If they are involved in cellular regulation, then their specific substrates must be identified. In any case, the capacity of Msr to reduce MetO residues to Met may have an important role in the repair of some oxidatively damaged proteins, and also in protection of cells against oxidative stress.

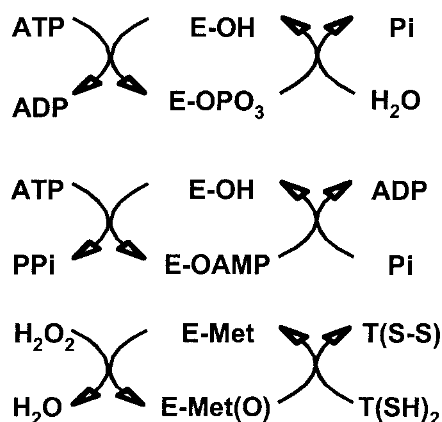


FIG. 1. Cyclic interconversion of enzymes between unmodified and covalently modified forms. (**Upper figure**) Phosphorylation and dephosphorylation of enzyme serine, threonine, or tyrosine hydroxyl groups (E-OH). (**Middle figure**) Adenylylation and deadenylylation of enzyme tyrosine hydroxyl groups (E-OH). (**Lower figure**) Oxidation–reduction of enzyme Met (E-Met) and enzyme MetO [E-Met(O)] residues by hydrogen peroxide (H_2O_2) and thioredoxin [$T(SH)_2$], respectively.

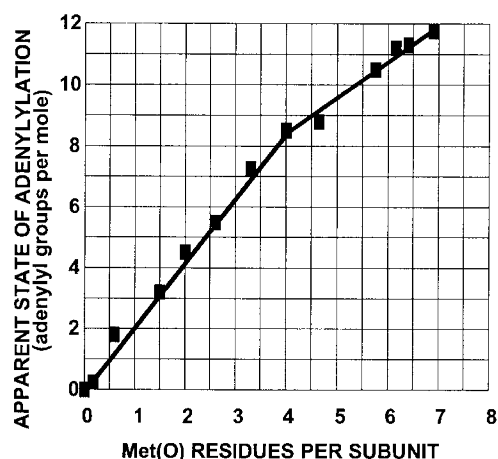


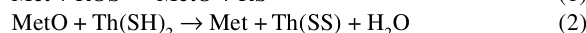
FIG. 2. Oxidation of Met residues of the unadenylylated form of *E. coli* glutamine synthetase converts it to a form that mimics affects of adenylylation of the enzyme. Data are from reference 2.

There is growing evidence that the cyclic interconversion of Met and MetO residues of proteins may have an important role the regulation of cell signaling processes (for review, see 10).

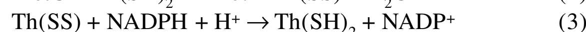
ANTIOXIDANT ROLE OF THE MET–METO INTERCONVERSION

Any one of many different kinds of ROS can oxidize surface-exposed Met residues of proteins to their MetO derivatives (reaction 1). But, in contrast to ROS-mediated oxidation of other amino acid residues (except cysteine residues), the oxidation of Met residues can be reversed by the action of Msr that catalyze the thioredoxin [$Th(SH)_2$]-dependent conversion of MetO back to Met (reaction 2); moreover, the oxidized form of thioredoxin [Th(SS)] formed in this reaction is readily reduced back to $Th(SH)_2$ by the NADPH-driven reaction catalyzed by thioredoxin reductase (ThR) (reaction 3). It follows that the coupling of these three reactions is described by the overall reaction 4 and, therefore, provides a mechanism for the NADP-dependent conversion of the ROS to an inactive derivative (RS).

$$\text{Met} + \text{ROS} \rightarrow \text{MetO} + \text{RS} \quad (1)$$



$$\text{MetO} + \text{Th(SH)}_2 \rightarrow \text{Met} + \text{Th(SS)} + \text{H}_2\text{O} \quad (2)$$



$$\text{Th(SS)} + \text{NADPH} + \text{H}^+ \rightarrow \text{Th(SH)}_2 + \text{NADP}^+ \quad (3)$$

$$\text{NADPH} + \text{H}^+ + \text{ROS} \rightarrow \text{NADP}^+ + \text{RS} + \text{H}_2\text{O} \quad (4)$$

This antioxidant mechanism would apply for reactions in which ROS is a nonradical species, such as hydrogen peroxide, peroxynitrite, hypochlorous acid, ozone, lipid peroxides, cholesterolesterperoxide, and singlet oxygen, which are among the most common forms of ROS organisms are exposed to. However, oxidation of Met to MetO is also mediated by various oxygen free radicals ($^{\bullet}OH$, $O_2^{\bullet-}$) and by transition metal-catalyzed reactions that are dependent upon the presence of molecular oxygen. These processes have been studied in great

detail by Schoeneich *et al.* (19, 30, 31), who have established that they involve the generation of Met free radical intermediates. It remains to be determined if the cyclic interconversion of Met and MetO can scavenge all of these free radical intermediates.

A role of Met/MetO interconversion in the scavenging of some ROS species is supported by the results of studies showing that overproduction of MsrA in yeast (22) and in *Drosophila* (28) increased their resistance to oxidative stress induced by toxic levels of paraquat or hydrogen peroxide, whereas strains of bacteria (20, 35) and yeast (22) lacking the MsrA gene are more susceptible to paraquat and hydrogen peroxide toxicity. In the studies with yeast, it was shown also that the levels of MetO in the protein fraction accounted for 32, 74, and 24% of the total Met content of the wild-type, Msr knockout, and MsrA-overexpressing strains, respectively. Comparable variations were observed for the levels of free Met and free MetO in the cell extracts of these yeast strains.

Evidence that MsrA may also serve an antioxidant function in mammals was derived from studies showing that deletion of the MsrA gene in mice leads to a decrease in their maximum life span when exposed to 100% oxygen atmosphere (23). In this study, we also learned that the MsrA knockout mouse strain contained substantial amounts of another previously unrecognized form of Msr, which was purified from extracts of the MsrA knockout mouse and, like the MsrB from other sources (9, 12, 25), was shown to specifically catalyze $Th(SH)_2$-dependent reduction of the *R*-isomer of MetO. Thus, organisms contain two different Msr—one specific for reduction of the *S*-isomer and one specific for the *R*-isomer. This is understandable in view of the fact that the ROS-mediated oxidation of Met residues of proteins leads to a racemic mixture of both *S*- and *R*-MetO isomers, and consequently provides a mechanism for the regeneration of Met from both isomeric forms of MetO (as illustrated in Fig. 3). Based on computational analyses of genome data bases, it was deduced that the mammalian MsrB contains a selenocysteine moiety at the catalytic site (12, 24). This was confirmed by studies showing that radioactive selenium is incorporated into the mouse MsrB (1) and that substitution of the selenocysteine moiety of this MsrB with cysteine leads to a dramatic decrease in its activity (24). In the meantime, MsrB proteins exhibiting *R*-MetO specificity have been shown to be present in many other organisms, but in contrast to the mammalian enzyme these do not contain a selenocysteine moiety (12). Curiously, the MsrA and MsrB activities of *Neisseria meningitidis* and several other bacteria are fused together in a single protein (17, 25). Significantly, it has been established that *Drosophila* contains two forms of MsrB, one of which requires zinc for its activity (34).

A very important antioxidant function of Met oxidation is suggested by the observation that lipid peroxides and cholesterolester peroxides generated in low-density lipoprotein (LDL) or high-density lipoprotein (HDL) can be reduced to their corresponding hydroxyl derivatives by reaction with surface-exposed Met residues of these lipoproteins (29) (Fig. 4). HDL is distinctly more effective than LDL in mediating these reductions. HDL can also reduce cholesterolester peroxides transferred from LDL by the cholesterylester transfer protein (5). Significantly, the hydroxyl derivative is selectively removed from the circulation by the liver (7), and thus these reactions

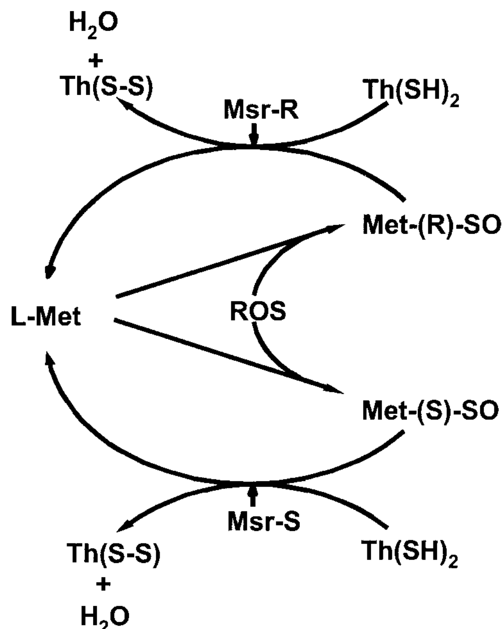


FIG. 3. Cyclic interconversion of Met and the *R*- and *S*-isomers of MetO. L-Met, L-isomer of Met; Met-(R)-SO and Met-(S)-SO, *R*- and *S*-isomers of MetO, respectively; Msr-R, the MsrB specific for reduction of the *R*-isomer of MetO; Msr-S, the MsrA specific for the *S*-isomer of MetO; $Th(SH)_2$ and Th(SS), reduced and oxidized forms of thioredoxin, respectively.

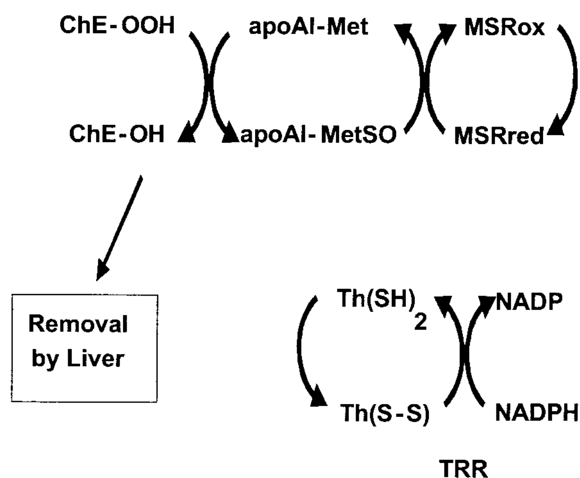


FIG. 4. Apolipoprotein A1-mediated reduction and removal of cholesterolester peroxides. Cholesterolester peroxides (ChE-OOH) are converted to their hydroxyl derivatives (ChE-OH) by reaction with Met residues of apoprotein-1 Met residues (apoA1-Met) to form the sulfoxide derivative (apoA1-MetSO). This can be converted back to the Met derivative by its interaction with the reduced form (MSRred) of Msr, leading to the oxidized form of Msr (MSRox), which can be reduced back to MSRred by thioredoxin [$Th(SH)_2$], which is regenerated by reaction with NADPH. The importance of this mechanism follows from the fact that after its conversion to the hydroxyl derivative, cholesterolester is readily transferred to the liver.

may have an important role in preventing the development of atherosclerotic lesions. However, if the HDL-mediated destruction of peroxides plays a catalytic role in the prevention of atherosclerosis, then the MetO residues of oxidized HDL should be reduced back to Met as is illustrated in Fig. 4. Whereas MetO residues of HDL have been shown to be reduced to Met by the *E. coli* Msr preparations (33), whether such reduction occurs *in vivo* remains to be established.

MSR AND AGING

The possibility that Msr may play a role in aging is supported by the demonstration that deletion of the MsrA gene in mice leads to a 40% decrease in the maximal life span of mice grown under normal conditions (23), and that overexpression of the MsrA gene in *Drosophila* leads to an almost doubling of the maximal life span (28). Moskovitz *et al.* (for review, see 36) demonstrated that there is progressive decrease in the level of MsrA in brain and kidney tissues of rats with increasing age, over a range of 5–25 months, but little or no age-related change of the enzyme in liver (Fig. 5). In a more detailed study, it was found that there was a progressive age-related decline in the level of MsrA gene expression (*i.e.,* in the MsrA-RNA level) in rat brain, kidney, and liver, but this was not reflected in substantial decreases in the levels of MsrA protein or MsrA enzyme activity in these tissues until the animals reached 26 months of age (26). Nevertheless, results of other studies (4) demonstrated that there is an age-related increase in the surface hydrophobicity of rat liver proteins (Fig. 6), and that ROS-mediated oxidation of proteins in cell-free extracts of rat liver leads to parallel increases in both protein hydrophobicity and MetO content (Fig. 6, inset).

A role of Met oxidation in aging is supported also by the observation that there is an age-dependent increase in *ortho*-tyrosine and MetO in human skin collagen (39). Moreover, in this study, it was shown also that these derivatives along with N^{ϵ}-(carboxymethyl)lysine and pentosidine are formed when collagen is exposed to glycoxidation *in vitro,* suggesting that glycoxidation may contribute to the observed age-related increase in MetO.

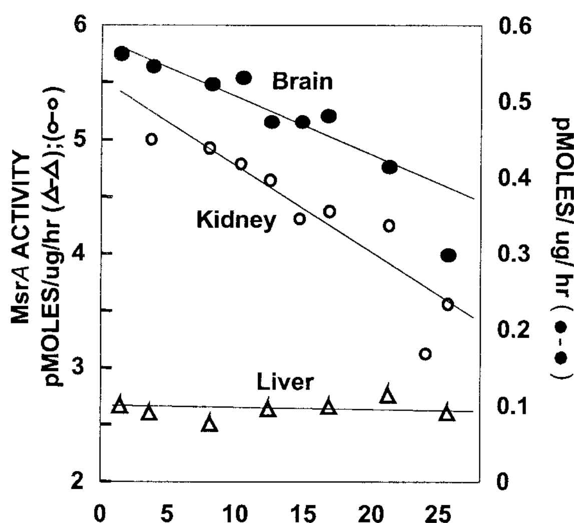


FIG. 5. Age-related changes in MsrA activity in rat tissues. ●, brain; ○, kidney; △, liver. Data are from reference 36.

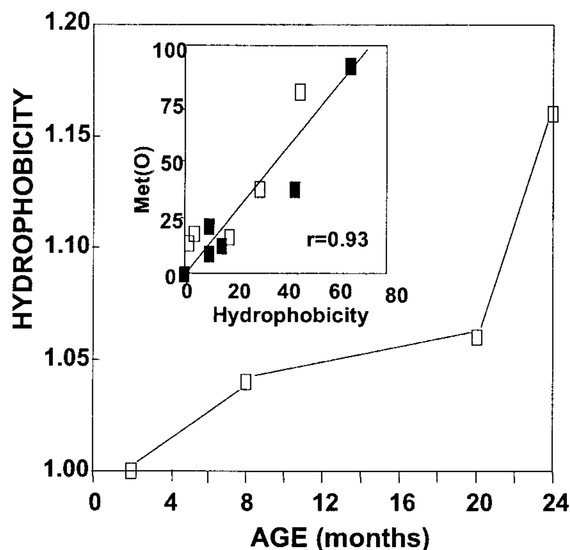


FIG. 6. Age-related changes in surface hydrophobicity and Met oxidation of proteins in rat liver. Hydrophobicity was measured by the increase in fluorescence associated with the binding of 1-anilinonaphthalene-8-sulfonic acid (ANSA) to the proteins. (**Inset**) Relationship between hydrophobicity and level of MetO residues in liver proteins after treatment with 2,2′-azobis(2-aminopropane)dihydrochloride; □, 2-month-old rats; ■, 24-month-old rats. See reference 4 for details.

INTERRELATIONSHIP BETWEEN MET OXIDATION, PROTEIN FOLDING, ENZYME ACTIVITY, AND PROTEOLYTIC SUSCEPTIBILITY

Results of *in vitro* studies have shown that surface-exposed Met residues of proteins are major targets for oxidation by almost all ROS (37). However, the consequences of these oxidations are variable. Whereas oxidation of only one or a few Met residues of some proteins leads to loss of biological function, the oxidation of many different Met residues in other proteins may have little or no effect on their biological function (16). Contrary to the expectation that oxidation of Met residues to MetO would lead to an increase in protein hydrophilicity, Met oxidation is sometimes associated with an increase in surface hydrophobicity. This has been interpreted to indicate that oxidation leads to conformational changes that expose buried hydrophobic amino acid residues (37). In this regard, a detailed study of *E. coli* glutamine synthetase showed that oxidation of up to seven of the 10 surface-exposed Met residues had no detectable effect on the hydrophobicity of the protein, whereas further oxidation of just two of the remaining exposed Met residues led to a dramatic increase in surface hydrophobicity and coincidentally to an increase in the susceptibility of the protein to proteolytic degradation by the 20S proteasome (15) (Fig. 7). This suggests that, in addition

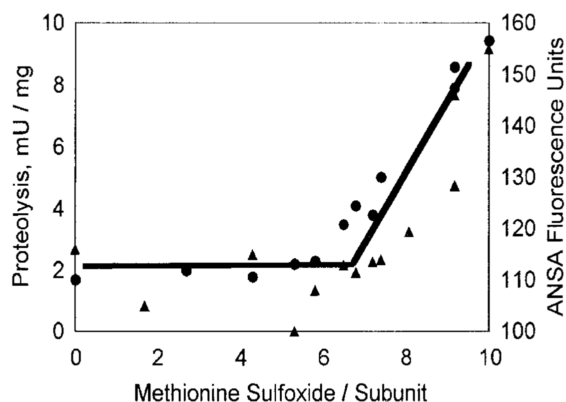


FIG. 7. Relationship between Met oxidation, surface hydrophobicity, and susceptibility to proteolytic degradation. ▲, proteolysis by 20S proteasome; ●, hydrophobicity as measured by increased fluorescence associated with binding of 1-anilinonaphthalene-8-sulfonic acid (ANSA). Data are from Levine *et al.* (16).

to its role in the cellular regulation of enzyme and antioxidant activities, the oxidation of Met residues may have a role in the targeting of some enzymes for proteolytic degradation.

POSSIBLE IMPLICATION OF MET OXIDATION IN DISEASE

The oxidation of Met residues of proteins is associated with the development of several diseases, including respiratory distress syndrome, emphysema, reperfusion injury, and α-crystalline degeneration (for reviews, see 16, 37). A potential role in Alzheimer's disease is implied by the demonstration that the level of MsrA in various regions of the brain of Alzheimer's disease patients is significantly lower than in brains of normal individuals, and this is reflected by increased levels of MetO in these regions (8). Furthermore, those regions of the brain containing elevated levels of MetO contain higher than normal levels of protein carbonyls, which is a widely accepted measure of oxidative stress-mediated oxidative damage (14). Collectively, these observations support the view that a decrease in the level of MsrA activity leads to a loss of antioxidant activity and consequently to an increase in sensitivity to ROS-mediated cellular damage. Evidence that enhanced oxidation of protein Met residues by neutrophil-generated ROS contributes to the development of chronic and acute bronchitis is supported by the observation that these abnormalities are associated with substantial increases in the neutrophil content and in the MetO/Met ratio of bronchoalveolar lavage fluid of individuals suffering from these disorders (18).

ABBREVIATIONS

HDL, high-density lipoprotein; LDL, low-density lipoprotein; Met, methionine; MetO, methionine sulfoxide; Msr, methionine sulfoxide reductase(s); MsrA, the reductase specific for reduction of the *S*-form of methionine sulfoxide residues of proteins; MsrB, the reductase specific for reduction of the *R*-form of methionine sulfoxide residues of proteins; ROS, reactive oxygen species; ThR, thioredoxin reductase; $Th(SH)_2$, thioredoxin; Th(SS), oxidized form of thioredoxin.

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