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Review

The physiological role of reversible methionine oxidation ☆

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ABSTRACT

Sulfur-containing amino acids such as cysteine and methionine are particularly vulnerable to oxidation. Oxidation of cysteine and methionine in their free amino acid form renders them unavailable for metabolic processes while their oxidation in the protein-bound state is a common post-translational modification in all organisms and usually alters the function of the protein. In the majority of cases, oxidation causes inactivation of proteins. Yet, an increasing number of examples have been described where reversible cysteine oxidation is part of a sophisticated mechanism to control protein function based on the redox state of the protein. While for methionine the dogma is still that its oxidation inhibits protein function, reversible methionine oxidation is now being recognized as a powerful means of triggering protein activity. This mode of regulation involves oxidation of methionine to methionine sulfoxide leading to activated protein function, and inactivation is accomplished by reduction of methionine sulfoxide back to methionine catalyzed by methionine sulfoxide reductases. Given the similarity to thiol-based redox-regulation of protein function, methionine oxidation is now established as a novel mode of redox-regulation of protein function. This article is part of a Special Issue entitled: Thiol-Based Redox Processes.

1. Introduction

Reactive oxygen species (ROS) and reactive chlorine species (RCS) are a major source of cysteine (Cys) and methionine (Met) oxidation. Both reactive species are the inevitable consequence of aerobic life style. ROS are produced as by-product of respiration, i.e., when oxygen is reduced to water. Upon incomplete reduction of oxygen, superoxide and hydrogen peroxide (H_2O_2) are generated, the latter of which can further react with metal ions to form hydroxyl radicals ($HO^{\bullet}$). Further, ROS are major constituents of the innate immune response. Upon bacterial infection, neutrophils produce H_2O_2 and chloride ions, and the enzyme myeloperoxidase catalyzes the generation of hypochlorous acid (HOCl) [1,2]. Thus, H_2O_2 and HOCl are two potent effector molecules of the immune system responsible for killing invading microorganisms. HOCl is also produced by mucosal barrier epithelia to control bacterial colonization [3]. Its strong bactericidal activity is derived from its high reactivity with macromolecules including DNA, proteins, and lipids. As a consequence, amines may be chlorinated/oxidized generating RCS such as chloramines [4,5], chromosomal mutations and lipid peroxidation may occur [6,7], and proteins may be inactivated or aggregated [8–10] leading to loss of cellular energy [11]. Accumulation of oxidative damage causes a condition called oxidative stress, which is hazardous to all kinds of organisms and may cause killing or apoptosis. Thus, ROS and RCS are ubiquitous sources of oxidation reactions and oxidative stress.

Cys and Met residues are extremely vulnerable to oxidation. While Cys oxidation and its role in redox regulation and cell signaling are well-established and have been extensively studied, Met oxidation and its role in cells are the topics of this review. Groundbreaking work by Weissbach, Brot, Schöneich, Levine, Moskovitz, Stadtman, Squier, Gladyshev and their co-workers unraveled Met oxidation as damage to proteins and the function of methionine sulfoxide reductases (Msrs) in repairing such damage as well as the underlying mechanisms (for reviews see for example the special issue on "Methionine Oxidation and Methionine Sulfoxide Reductases" in Biochim Biophys Acta 2005, volume 1703). Met oxidation and accordingly Msrs play an important role during oxidative stress; they are thus associated with the aging process and several pathophysiological conditions such as neurodegenerative diseases and cancer. In this review we will give an overview about the role of ROS and RCS in Met oxidation to methionine sulfoxide (Met-SO), the function of Msrs in damage repair, and detection methods for reduced and oxidized Met residues. We will shed light on the implications of Met oxidation on microorganisms and higher eukaryotes, focusing on oxidative stress and related effects. Further, we will eventually discuss the consequences of Met oxidation for individual proteins, namely inactivation of proteins as well as activation of protein function. Especially the role of Met oxidation to reversibly trigger the activation of proteins will expand the focus of current reviews in order to establish Met oxidation as a novel mode of redox-regulation of proteins.

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2. Mechanisms of methionine oxidation

2.1. Chemical reactions causing methionine oxidation

All nascent polypeptides start with Met as first amino acid, which can be modified, for example by acetylation or formylation, or become processed, e.g. by cleavage from a precursor. On average, about 1.5% of amino acids in a protein are Met residues. In *Escherichia coli*, the Met content roughly ranges from 1 to 3% (most abundant protein: elongation factor TU: 2.8%; oxidative stress related proteins: KatE (1.3%), HypT (2%), MsrA (3.3%); according to EcoCyc). Similar values were reported for yeast and animal proteins [12,13]. Thus, the Met content is less than the theoretically possible content of 5% considering 20 proteinogenic amino acids. It should be noted that Met-rich proteins (MRP) constitute an exception; they are naturally occurring proteins with Met contents of 20% and higher and are used as model proteins to study Met oxidation [14].

Met is a strongly hydrophobic amino acid; thus, most Met residues are found in the hydrophobic core of proteins and Met residues in membrane proteins are often found to interact with the lipid bilayer. While such Met residues are fairly protected from oxidation, surface exposed Met residues are susceptible to oxidation [15,16]. Oxidation of Met generates Met-SO (Fig. 1), which is more hydrophilic than Met and this alteration may affect the protein structure. The sulfur in Met is present as thioether (R-S-R′) and has a low oxidation potential. Thus, Met is readily oxidized by a large number of oxidizing species. Although Met-SO is a fairly stable product, sulfur can be further oxidized to sulfone (Met-SO_2) by strong oxidants, even though this occurs to a low extent [4]. Met-SO_2 constitutes an irreversible reaction product and cannot be enzymatically reduced back to Met by Msrs (see Section 3). It is important to note that oxidation of Met yields two stereoisomers of the sulfoxide group, the *S*-(D-) and *R*-(L-) forms (Fig. 1), which will be discussed in detail in Section 3.

To provide the full picture of Met oxidation, it should be noted that oxidation of Met can occur via two distinct mechanisms that are dependent on the oxidant. Oxidants such as HOCl, H_2O_2, and singlet oxygen directly oxidize Met to Met-SO via a formal oxygen transfer by a two-electron oxidation. Radicals such as $HO^{\bullet}$ and metal ions such as Fe^{III} and Cu^{II}, in contrast, oxidize Met in a one-electron oxidation. One-electron oxidation yields sulfide radical cations (i.e., sulfur–nitrogen (S∴N)-three-electron-bonded radical cations), which are highly unstable and convert into intermediates, thus, causing posttranslational protein modifications [17,18].

2.2. Kinetics of Met oxidation

As mentioned above, reactive species with a high enough oxidation potential to yield Met-SO include H_2O_2, $HO^{\bullet}$, HOCl, and chloramines. These species differ in their reactivity with sulfur in Met leading to vastly different reaction constants for the formation of Met-SO (Table 1).

Table 1
Second-order rate constants determined for the reactions of various ROS and RCS with Met and Cys residues.

ROS/RCS	k_2 [M^{-1} s^{-1}] for Met	k_2 [M^{-1} s^{-1}] for Cys
H_2O_2	6.0×10^{-3} [4]	2×10^{1} [183] 10^{7}[a]
Chloramines		
Taurine chloramine	3.9×10^{1} [19]	2.1×10^{2} [19]
Glycine chloramine	2.0×10^{2} [19]	3.5×10^{2} [19]
N-acetyl-lysine chloramines	5.2×10^{1} [19]	4.8×10^{2} [19]
HOCl	3.8×10^{7} [20]	3.0×10^{7} [20]
$HO^{\bullet}$	8.3×10^{9} [182]	3.4×10^{10} [182]

[a] While ordinary Cys residues react slowly with H_2O_2, redox-sensing thiols are quickly and efficiently oxidized (e.g., OxyR [184], peroxiredoxins [185]).

While H_2O_2 oxidizes Met to Met-SO with a rather slow rate constant of 6×10^{-3} M^{-1} s^{-1} [4], the other oxidants are several orders of magnitude faster in oxidizing Met. Monochloramines such as taurine chloramine, glycine chloramine, and N-acetyl-lysine chloramine show second order rate constants of up to 2×10^{2} M^{-1} s^{-1} [19]. The most reactive species are $HO^{\bullet}$ and HOCl that oxidize Met to Met-SO with second order rate constants of ~8×10^{9} M^{-1} s^{-1} and 3.8×10^{7} M^{-1} s^{-1}, respectively (at pH 7 in water; [4,20]).

These rates are similar to the rate constants observed for the reaction of HOCl with Cys (3×10^{7} M^{-1} s^{-1}, [20]). Thus, Met are besides Cys residues highly vulnerable targets for oxidation (Table 1). Due to its high reactivity, HOCl preferentially reacts with Met and Cys in proteins. Pattison and Davies showed that at low molar ratios of HOCl to protein (up to 4:1), HOCl is almost exclusively consumed by Met and Cys residues in model proteins. Only at higher ratios, other amino acids, disulfide bonds, or backbone amides are attacked by HOCl, consistent with their reactivity with HOCl being orders of magnitude slower [20]. This is similar when analyzing the reactivity of HOCl with a mixture of amino acids. Here, HOCl is almost exclusively consumed by Met, Cys, and α-amino groups with the latter being a preferred target at high HOCl concentrations [20].

3. Methionine sulfoxide reductases — enzymes that reduce Met-SO

Given that Met oxidation occurs unavoidably under aerobic conditions, it is not unexpected that cells have developed sophisticated mechanisms and enzymes to counteract Met oxidation. In contrast to Met sulfone, Met-SO is a reversible post-translational modification. The enzymes catalyzing the reduction of Met-SO back to Met are methionine sulfoxide reductases (Msr). The first Msr was described in *E. coli* in 1981 [21] and later termed MsrA. MsrA turned out to be specific for the *S*-epimer of Met-SO. It took twenty more years until MsrB, the Msr specific for the *R*-epimer, was identified and characterized in *E. coli* [22]. Until now, additional Msrs in numerous organisms have been

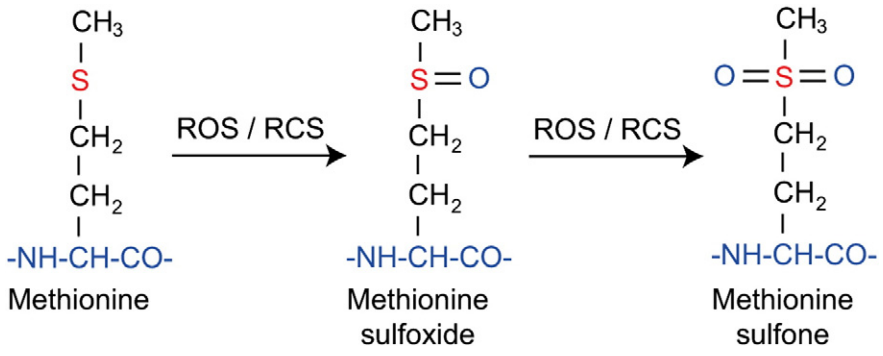

Fig. 1. Met can be reversibly oxidized to Met-SO by ROS/RCS. High concentrations of these oxidants can further oxidize Met-SO to the irreversible Met sulfone.