

Methionine and Antioxidant Potential

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Abstract

Methionine (Met) is a nutritionally essential amino acid and has been widely demonstrated to improve cellular oxidative balance and mediate oxidative stress. Met targets reactive oxygen species (ROS) directly by being oxidized to Met sulfoxide (MetO) [1]. Met can be metabolized to cysteine (Cys) through transsulfuration pathway, which is further metabolized to glutathione (GSH), taurine, and hydrogen sulfide (H₂S). All these metabolites exhibit antioxidant functions in various models (reviewed at [2]). More recently, Met also has been demonstrated to enhance cellular oxidative tolerance via pentose phosphate pathway (PPP) [3], which contributes to the balance of cellular reducing power and accelerates the reduction reaction of MetO and GSH oxidative product GSSH back to Met and GSH.

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Met Oxidation (Met sulfoxide)

Met, as a free amino acid or bound to a protein, is readily metabolized and interacts with various ROS, such as hydrogen peroxide (H₂O₂), hydroxyl radicals, hypochlorite, chloramines, and peroxyxynitrite [4]. These oxidants oxidize Met residues into a mixture of the R- and S-isomers of MetO [5]. Metabolome analysis reveals that dietary excess Met increases accumulation of Met oxidation products, such as MetO and Ac-Met sulfoxide (a MetO metabolite) [6]. Previous report suggests that Met oxidation mainly protects critical residues at the active site against oxidative modification of proteins [1]. For example, glutamine synthetase contains 16 Met residues, 8 surface exposed Met residues are oxidized with little effect on catalytic activity of the enzyme after exposure to H₂O₂, while the other intact residues are generally buried within the core of the protein and guard the entrance to the active site [7]. However, the recent experimental models suggested that Met oxidation involves in activation or inactivation of protein function and is now established as a novel mode of redox-regulation of protein function similar with thiol-based redox-regulation of protein function [8]. For example, calcium/calmodulin (Ca²⁺/CaM)-dependent protein kinase II is a prototypical methionine redox sensor and oxidation of paired regulatory domain Met residues enhances its activity by pro-oxidant conditions [1, 9], while oxidative stress-induced Met residues oxidation leads to the accumulation of chemically and functionally altered alpha-synuclein with reducing its affinity for biological membranes and impairing degradation the by 20S proteasome [10].

Met oxidation can be reversed by NADPH-dependent MetO reductases (Msr), which have been identified in all organisms from bacteria to mammals [11]. Currently, three classes of Msr are discovered:

MsrA and MsrB which are stereo-dependent when binding to the Met sulfoxide in the oxidized protein; and fRMsr which mainly reduces selectively free L-Met-R-O [12]. It is now well established that the chemical mechanism of the reductase step passes through formation of a sulfenic acid intermediate [12]. The Msr system provides a more efficient mechanism to scavenge ROS as the reduced Met residues in proteins allows them to react again with ROS. Msr family has been widely shown to repair proteins with oxidative damages and protect cells against oxidative damage [13]. Upregulation or activation of Msr lowers ROS generation and increase oxidative stress resistance in different models [14], suggesting a potential approach to treat oxidative injury related diseases. Conversely, knocking out or inhibition of Msr enhances cell susceptibility to oxidative stress [15]. Thus, the cyclic interconversion of Met and MetO residues of proteins may serve as a key pathway against oxidative stress [16].

Met Metabolism/Cys-GSH

Met is mainly metabolized in liver as some Met metabolic enzymes are liver-specific, such as Met adenosyltransferase (MAT I, II, and III) [17]. Firstly, MAT catalyzes Met into S-adenosylmethionine (SAM), which is a methyl donor for DNA and protein modification [18]. DNA methyltransferases (DNMTs) contributes to catalyzing the methylation reactions and transfers a methyl group from SAM to a variety of acceptors (DNA and histones) to form S-adenosylhomocysteine (SAH) [19]. SAH can be further metabolized into homocysteine by S-adenosylhomocysteinase (Ahcy). Ahcy is a bidirectional enzyme and also catalyzes homocysteine and adenosine to form SAH. Homocysteine regenerates Met via MTR or involves in Cys metabolism (reviewed at [2]). Cystathionine β-synthase (CBS) and cystathionine γ-

lyase (CSE) contribute to Cys generation from homocysteine [20]. Cys is further used for GSH synthesis in two successive enzymatic ATP-dependent reactions catalyzed by glutamate cysteine ligase (GCL) and GSH synthase (GS) [21, 22].

Met can be metabolized to cysteine (Cys) through transsulfuration pathway and Cys mainly contributes to generation of GSH, taurine, and H₂S. GSH, the most abundant cellular thiol antioxidant, can directly scavenge ROS and be oxidized into glutathione disulfide (GSSG) or serve as a cofactor for the enzyme glutathione peroxidase (GPx) in metabolizing H₂O₂ and lipid peroxides [2, 21]. Thioredoxin reductase-1 (TrxR1) and glutathione reductase (GR) are two major intracellular reducing power, which reduces GSSG back to GSH using nicotinamide adenine dinucleotide phosphate (NADPH). Mice genetically engineered to lack both [TrxR1](#) and GR cannot reduce oxidized GSSG and are more susceptible to oxidative stress, while dietary methionine provides the cytosolic disulfide-reducing power and methionine-fueled transsulfuration supplies the necessary Cys precursor for GSH synthesis in TR/GR-null livers [23], suggesting that hepatocytes can adequately sustain cytosolic redox homeostasis pathways after adequate methionine uptake. Taurine and H₂S also exhibit antioxidant function and has been reviewed at [2].

Pentose Phosphate Pathway (PPP)

PPP branches from glycolysis of glucose metabolism and plays an important role in the cellular redox homeostasis via providing NADPH, which powers reductive biosynthesis and contributes to detoxification of intracellular ROS [3, 24]. The PPP is the most direct route to produce NADPH from glucose and inhibition of PPP results in the reduction of cellular NADPH/NADP⁺ [25]. The PPP subdivides into two branches: the

oxidative branch and non-oxidative branch. The oxidative branch yields two NADPH per metabolized glucose-6-phosphate. Firstly, glucose-6-phosphate dehydrogenase (G6PD) catalyzes glucose and glucose-6-phosphate dehydrogenation to form NADPH and 6-phosphogluconolactone, which is subsequently hydrolyzed by phosphogluconolactonase (6PGL) into 6-phosphogluconate. G6PD-deficient patients, who are unable to regenerate enough NADPH for maintenance of GSH pool, exhibit high susceptibility to oxidative stress under a stressful situation [26]. 6-phosphogluconate dehydrogenase (6PGDH) further catalyzes the oxidative decarboxylation of 6-phosphogluconate to yield NADPH and ribulose-5-phosphate (Ru5P), which is then enters the non-oxidative branch and can be converted either to ribose 5-phosphate by ribose 5-phosphate isomerase (RPI) or to xylulose 5-phosphate by ribulose 5-phosphate epimerase (RPE) [27]. The oxidative branch of PPP mainly maintains cellular redox homeostasis, while the non-oxidative branch involves in nucleic acid metabolism. The PPP can be in response to different physiological conditions and then maintain metabolic demands of cells. For example, the PPP favors to accelerate the metabolism of oxidative branch under oxidative stress and then generate more NADPH to replenish the antioxidant system against the excess ROS generation [28].

Met has been demonstrated to target on PPP at various metabolic points. Firstly, Met has been demonstrated to regulate PPP via increasing NADPH production and 6PGDH activity [29]. In the thiol oxidizing agent diamide challenged cells, Met supplementation from 0 mg/L to 200 mg/L markedly enhances cell resistance to oxidative stress, while the protective effect is abolished by deletion of 6PGDH [29], the rate limiting enzyme of oxidative PPP, suggesting that Met improves oxidative PPP metabolism and the PPP serves as a potential mechanism of Met against

oxidative stress.

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