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Review

Methionine oxidation and aging

Earl R. Stadtman[a,*], Holly Van Remmen[b], Arlan Richardson[b], Nancy B. Wehr[a], Rodney L. Levine[a]

[a]*Laboratory of Biochemistry, National Heart, Lung and Blood Institute, Bethesda, Maryland USA*
[b]*Department of Cellular and Structural Biology and the Barshop Center for Longevity Studies, University of Texas Health Science Center at San Antonio, and South Texas Veterans Health Care System, San Antonio, Texas, USA*

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Abstract

It is well established that many amino acid residues of proteins are susceptible to oxidation by various forms of reactive oxygen species (ROS), and that oxidatively modified proteins accumulate during aging, oxidative stress, and in a number of age-related diseases. Methionine residues and cysteine residues of proteins are particularly sensitive to oxidation by ROS. However, unlike oxidation of other amino acid residues, the oxidation of these sulfur amino acids is reversible. Oxidation of methionine residues leads to the formation of both R- and S-stereoisomers of methionine sulfoxide (MetO) and most cells contain stereospecific methionine sulfoxide reductases (Msr's) that catalyze the thioredoxin-dependent reduction of MetO residues back to methionine residues. We summarize here results of studies, by many workers, showing that the MetO content of proteins increases with age in a number of different aging models, including replicative senescence and erythrocyte aging, but not in mouse tissues during aging. The change in levels of MetO may reflect alterations in any one or more of many different mechanisms, including (i) an increase in the rate of ROS generation; (ii) a decrease in the antioxidant capacity; (iii) a decrease in proteolytic activities that preferentially degrade oxidized proteins; or (iv) a decrease in the ability to convert MetO residues back to Met residues, due either to a direct loss of Msr enzyme levels or indirectly to a loss in the availability of the reducing equivalents (thioredoxin, thioredoxin reductase, NADPH generation) involved. The importance of Msr activity is highlighted by the fact that aging is associated with a loss of Msr activities in a number of animal tissues, and mutations in mice leading to a decrease in the Msr levels lead to a decrease in the maximum life span, whereas overexpression of Msr leads to a dramatic increase in the maximum life span.
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Keywords: Methionine sulfoxide; Methionine; Methionine sulfoxide reductase; Aging

All amino acid residues of proteins are susceptible to oxidative modification by one or more forms of reactive oxygen species (ROS); see Refs. [1,2] for review. However, the only oxidative modifications that can be repaired are those involved in the oxidation of the sulfur-containing amino acids (cysteine and methionine). Methionine (Met) residues of proteins are the most susceptible to oxidation by almost all forms of ROS [1]. As illustrated in Fig. 1, the ROS-mediated oxidation of Met residues leads to a mixture of the S- and R-epimers of methionine sulfoxide (MetO) [3–7], hereafter referred to as MetO-S and MetO-R, respectively. However, most cells contain two different reductases that catalyze the thioredoxin [$Th(SH)_2$]-dependent reduction of MetO back to Met [4,8]. One of these, MsrA, is specific for reduction of the S-epimer back to Met and the other, MsrB, is specific for reduction of the R-epimer. Significantly, the oxidized form of thioredoxin (Th-S-S) produced in the reduction of MetO can be converted back to $Th(SH)_2$ by the enzyme thioredoxin reductase (Thr) in an NADPH-dependent reaction. Thus, the cyclic oxidation and reduction of a protein Met residue by an organic hydroperoxide (ROOH) can be described by the following reactions:

$$\text{Met} + \text{ROOH} \text{ -------> } \text{MetO} + \text{ROH}$$
$$\text{MetO} + \text{Th(SH)}_2 \text{ ------> } \text{Met} + \text{Th-S-S}$$
$$\text{Th-S-S} + \text{NADPH} + \text{H}^+ \text{ ------> } \text{Th(SH)}_2 + \text{NADP}^+ + \text{H}_2\text{O}$$
$$\text{Sum: } \text{NADPH} + \text{ROOH} + \text{H}^+ \text{ ------> } \text{NADP}^+ + \text{ROH} + \text{H}_2\text{O}$$

* Corresponding author. NIH, Bldg 50, Room 2140, Bethesda, MD 20892-8012. Tel.: +1 301 496 4096; fax: +1 301 496-0599.
E-mail address: EarlStadtman@nih.gov (E.R. Stadtman).

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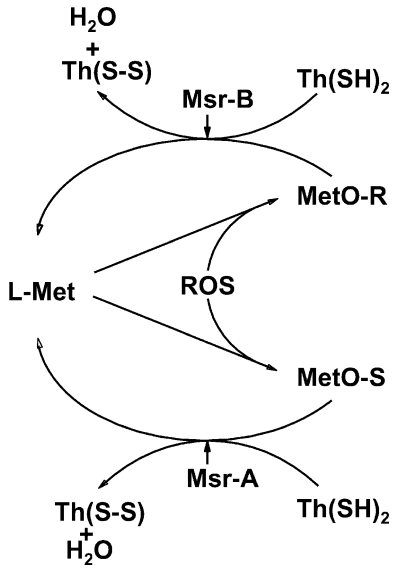

Fig. 1. Cyclic interconversion of Met and the R- and S-isomers of MeO. L-Met, L-isomer of Met; MetO-R and MetO-S, R- and S-isomers of MetO, respectively; MsrA and MsrB refer to the methionine sulfoxide reductases that are specific for reduction of the S- and R-isomers of MetO, respectively; $Th(SH)_2$ and Th(S-S) refer to the reduced and oxidized forms of thioredoxin, respectively.

It is evident from this series of reactions that the cyclic oxidation–reduction of Met residues of proteins provides a mechanism for the NADPH-dependent conversion of ROOH to an innoxious derivative, ROH. Moreover, if ROOH is replaced by another form of ROS, the cyclic oxidation and reduction of a Met residue will lead to the NADPH-dependent inactivation of that ROS. Based on this consideration, it was proposed that the cyclic oxidation–reduction of Met residues of proteins may represent an important antioxidant mechanism [9]. This proposition is supported by results of studies showing that mutant strains of yeast [10], bacteria [11–13], and mice [14] that lack the MsrA gene are more sensitive to oxidative stress (i.e., exposure to hyperoxia, H_2O_2, or radical generating systems such as paraquat) than are the wild-type strains. Moreover, overexpression of the MsrA gene in yeast [15], neuronal PC-12 cells [16], human T cells [15] and *Drosophila* [17] leads to increased resistance to oxidative stress. In the studies with yeast [10] it was shown that the levels of MetO in the protein fraction accounted for 32%, 74%, and 24% of the total Met content of the wild-type, MsrA knockout, and MsrA overexpressing strains, respectively. Similar variations were observed for levels of free Met and free MetO in cell extracts of these yeast strains. Although the results summarized above are consistent with the antioxidant hypothesis of Met oxidation/reduction, results of studies by Miller et al. [18] and Schöneich and Yang [19] have shown that the oxidation of Met residues by some oxygen free radicals and by transition metal-catalyzed reactions is dependent upon the presence of molecular oxygen and involve the generation of Met free radical intermediates. It remains to be determined whether the cyclic oxidation/reduction of Met residues by these mechanisms will scavenge all of the free radical intermediates.

1. Role of methionine oxidation in aging

The singular importance of methionine oxidation in aging is highlighted by the following findings: (i) The level of MsrA in various rat tissues declines with age [20,21] and in some age-related diseases, i.e., Alzheimer's disease [22], emphysema (especially in cigarette smokers) [1,23], bronchitis [24], and Parkinson's disease [25,26]. (ii) Mutations leading to a loss of MsrA activity in mice lead to a 40% decrease in the maximum life span [14]. (iii) Overexpression of msrA in flies leads to a nearly doubling of the life span [17]. (iv) There is a slight age-related increase in the

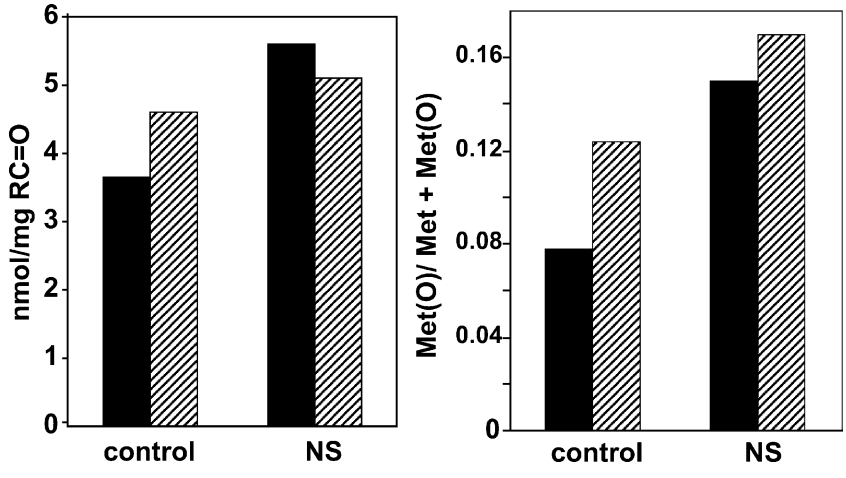

Fig. 2. Effect of nitrogen starvation on levels of oxidized proteins. Cells were grown on minimal medium (control), and under conditions of nitrogen starvation (NS); i.e., in minimal medium containing 10× the glucose level of the control. The levels of protein carbonyl derivatives and the fraction of Met residues that were converted to MetO (the MetO/Met+MetO ratio) were determined 24 h (black bars) and 48 h (hatched bars) after the cells went into stationary phase of development. (B.S. Berlett and E.R. Stadtman, unpublished observations).

Table 1

Age	Genotype	Liver			Heart			Kidney			Muscle			Brain Stem			Cortex			Hippocampus			Hypothalamus			Cerebellum		
		n	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.	*n*	Mean	S.D.
Young	Wt	7	4.72	0.77	5	5.26	1.14	7	4.97	0.33	5	5.86	1.27	4	9.81	1.43	4	7.70	1.64	4	6.90	4.26	4	6.90	4.26	4	4.30	0.67
Young	Ht	7	3.97	0.60	5	5.02	0.50	6	4.33	0.55	5	5.80	0.95	4	8.84	1.42	4	7.09	1.23	4	6.60	3.65	4	6.60	3.65	4	4.98	0.90
Young	Both	14	4.34	0.69	10	5.14	0.88	13	4.65	0.45	10	5.83	1.12	8	9.33	1.43	8	7.40	1.45	8	6.75	3.97	8	6.75	3.97	8	4.64	0.80
Middle	Wt	7	4.49	1.15	5	5.25	0.42	6	5.24	0.87	5	4.56	0.76	4	9.81	0.83	4	8.64	0.84	4	9.45	1.74	4	9.45	1.74	4	4.42	0.61
Middle	Ht	7	4.70	0.97	5	5.64	1.45	6	5.89	1.31	5	4.19	0.62	4	9.66	0.25	4	8.20	0.77	4	6.25	0.98	4	6.25	0.98	4	5.26	1.15
Middle	Both	14	4.59	1.06	10	5.44	1.07	12	5.57	1.11	10	4.37	0.69	8	9.73	0.61	8	8.42	0.81	8	7.85	1.41	8	7.85	1.41	8	4.84	0.92
Old	Wt	7	4.12	1.09	5	6.28	1.87	7	5.77	0.47	5	4.92	0.74	4	9.29	0.91	4	7.91	1.17	4	7.72	1.02	4	7.72	1.02	4	4.46	0.59
Old	Ht	7	4.51	0.79	5	6.04	0.62	7	5.87	0.90	5	4.45	0.79	4	9.40	0.68	4	8.51	1.06	4	9.00	2.50	4	9.00	2.50	4	4.55	0.79
Old	Both	14	4.32	0.95	10	6.16	1.39	14	5.82	0.72	10	4.68	0.77	8	9.34	0.81	8	8.21	1.11	8	8.36	1.91	8	8.36	1.91	8	4.50	0.70

The methionine sulfoxide content is given as the percentage of (methionine+methionine sulfoxide). S.D. stands for standard deviation and *n* gives the number of animals in each analysis. The genotypes are wt, wild-type, and ht, heterozygous knockout of *Sod2*. The colony of wild-type and *Sod2*+/- mice used for this study was generated by breeding heterozygous male *Sod2*+/- mice to female wildtype C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME). The *Sod2*+/- mice, designated Sod2tm1Cje, were originally produced in the CD1 strain of mice [44]. The mice used for this study were backcrossed to C57BL/6 for 13 generations. All procedures followed the guidelines approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and South Texas Veterans Health Care System, Audie L. Murphy Division. The mice were screened at 4 to 5 weeks of age for the Sod2 mutation by PCR analysis of DNA obtained from tail clips of these animals as previously described [44]. Mice were maintained under barrier conditions in a temperature-controlled environment and fed a commercial mouse chow (Teklad Diet LM485) ad libitum. The life span and age-related pathology of these mice have been reported [45]. Young (3- to 6-month-old), middle age (15- to 17-month-old), and old (2628 month-old) female wild-type and *Sod2*+/- mice were killed by CO_2 inhalation followed by cervical dislocation, and the various tissues were collected and frozen immediately in liquid nitrogen and stored at -80 or -85 °C until analyzed.

Two pieces of each tissue of liver, muscle, and heart tissue were cut using a clean razor blade from partially frozen tissue held with forceps in a plastic weighing dish. Samples were placed in tared 4-ml Wheaton vials (#224882, Millville, NJ) held on dry ice, then weighed. Brain (cut frozen) and kidney samples (partially frozen) were placed into 2-ml tared glass autosampler vials (Sun SRI #501-300, Duluth, GA). Teflon crimp caps (Sun SRI #200148) or lids (Kimble #73802, Vineland, NJ) were used for all digestion steps. One of the duplicate samples was treated with cyanogen bromide and the other was not. We added 200-çl 100 mM cyanogen bromide (Pierce, Rockford, IL) in 70% formic acid (Janssen Chimica, Geel, Belgium), incubated at 70 °C for 1 h, then removed reagent by drying in a vacuum centrifuge (Savant). Cyanogen bromide converts methionine to homoserine but does not affect methionine sulfoxide [46]. Both samples were then subjected to acid hydrolysis and amino acid analysis as described [47].

level of MetO in the α-crystallins [27] and in the intrinsic membrane fraction [28] of normal human lenses, and a dramatic age-related increase (two-thirds of the total Met) in the level of MetO in aged cataractous lenses [28,29]. (v) The MetO/Met ratio in acetic insoluble proteins from trabecular meshwork increases from 10 to 40 during aging, over the range of 10 to 80 years [30,31]. (vi) There is a three- to fourfold increase in the level of MetO in human skin collagen, over the range of 10 to 80 years [32]. (vii) The level of MetO from young mature and senescent human erythrocytes (separated by density gradient sedimentation) is about 50% and 60% higher, respectively, than in the young erythrocytes [33,34]. (viii) It is reported that there is a progressive age-related increase in the oxidation of Met residues in Fisher 344 rat brain calmodulin, over the range of 6–27 months, which is associated with a loss in ability of the protein to regulate plasma membrane ATP hydrolysis and ATP-dependent Ca^{2+} transport [35,36]. However, more recent studies demonstrate that oxidation of calmodulin need not occur in currently available strains of rats, raised under present husbandry conditions (C. Schöneich, personal communication). (ix) MsrA and MsrB (hCBS-1) gene expression is down-regulated during replicative senescence of WI-38 human fibroblasts [37]. (x) Evidence that enhanced oxidation of protein 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 [24]. Furthermore, as shown in Fig. 2, during *E. coli* senescence, induced by nitrogen starvation, there is a 1.5- to 2-fold increase in the levels of MetO and carbonyl content of proteins.

These various studies are consistent with a generalized age-dependent increase in MetO content of proteins. However, no systematic examination of tissues has yet been reported. This prompted us to determine the MetO content of tissues taken from young, middle-aged, and old mice. We determined methionine and methionine sulfoxide content in liver, heart, skeletal muscle, kidney, and five regions of the brain. No clear age-dependent changes in MetO content were detected (Table 1). Animals lacking mitochondrial SOD2 die in the neonatal period; heterozygotes survive but show evidence of chronic oxidative stress [38]. We therefore performed MetO analyses on the same tissues from the SOD2 heterozygotes and again found no age-dependent change in MetO content (Table 1). The levels in wild-type and SOD2 heterozygotes did not differ, so the results can be combined with an example shown in Fig. 3 which illustrates the variation among tissues. There are higher levels of MetO in the brain regions compared to other organs, with the exception of the cerebellum which has relatively high activity of MsrA [39]. These assays measure total tissue protein MetO following hydrolysis of the proteins. There is currently no method available to detect methionine sulfoxide following a "proteomics" separation, so that substantial changes in the MetO content of specific proteins cannot be detected. Attempts by several investigators to raise an antibody against MetO have thus far been unsuccessful. Similarly, no specific method of chemical derivatization of MetO has yet been developed.

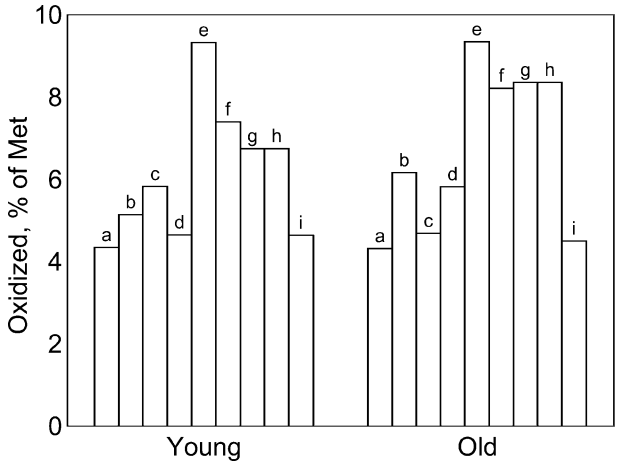

Fig. 3. Methionine sulfoxide content of mouse tissues. The data are for both genotypes, taken from Table 1. a, liver; b, heart; c, skeletal muscle; d, kidney; e, brainstem; f, cerebral cortex; g, hippocampus; h, hypothalamus; i, cerebellum.

It is evident from the results of studies summarized above that aging is associated with the accumulation of oxidized methionine residues of proteins in a number of aging models. However, the mechanisms responsible for these age-related changes may vary from one model to another. The accumulation of oxidized protein is a complex function of the balance between the rates of ROS generation on the one hand, and the ability to scavenge ROS (antioxidant capacity) on the other. Furthermore, oxidation of some Met residues of proteins to MetO can lead to an increase in hydrophobicity [9,40] and in susceptibility of the protein to proteolytic degradation by the 20S proteasome [9]. Therefore, the accumulation of MetO residues in protein could also reflect a loss in the proteolytic activity as has been observed in some studies of aging [41–43].

References

[1] W. Vogt, Oxidation of methionyl residues in proteins: tools, targets, and reversal, Free Radic. Biol. Med. 18 (1995) 93–105.
[2] N. Brot, H. Weissbach, Biochemistry and physiological role of methionine sulfoxide reductase in proteins, Arch. Biochem. Biophys. 223 (1983) 271–281.
[3] B.L. Miller, K. Kuczera, C. Schöneich, One-electron photooxidation of *N*-methionyl peptides. Mechanism of sulfoxide and azasulfonium diastereomer formation through reaction of sulfide radical cation complexes with oxygen or superoxide, J. Am. Chem. Soc. 120 (1998) 3345–3356.
[4] W.T. Lowther, H. Weissbach, F. Etienne, N. Brot, B.W. Matthews, The mirrored methionine sulfoxide reductases of *Neisseria gonorrhoeae* pilB, Nat. Struct. Biol. 9 (2002) 348–352.

[5] V.S. Sharov, D.A. Ferrington, T.C. Squier, C. Schöneich, Diastereoselective reduction of protein-bound methionine sulfoxide by methionine sulfoxide reductase, FEBS Lett. 455 (1999) 247–250.
[6] V.S. Sharov, C. Schöneich, Diastereoselective protein methionine oxidation by reactive oxygen species and diastereoselective repair by methionine sulfoxide reductase, Free Radic. Biol. Med. 29 (2000) 986–994.
[7] R. Grimaud, B. Ezraty, J.K. Mitchell, D. Lafitte, C. Briand, P.J. Derrick, F. Barras, Repair of oxidized proteins. Identification of a new methionine sulfoxide reductase, J. Biol. Chem. 276 (2001) 48915–48920.
[8] J. Moskovitz, V.K. Singh, J. Requena, B.J. Wilkinson, R.K. Jayaswal, E.R. Stadtman, Purification and characterization of methionine sulfoxide reductases from mouse and *Staphylococcus aureus* and their substrate stereospecificity, Biochem. Biophys. Res. Commun. 290 (2002) 62–65.
[9] R.L. Levine, L. Mosoni, B.S. Berlett, E.R. Stadtman, Methionine residues as endogenous antioxidants in proteins, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 15036–15040.
[10] J. Moskovitz, B.S. Berlett, J.M. Poston, E.R. Stadtman, The yeast peptide-methionine sulfoxide reductase functions as an antioxidant in vivo, Proc. Natl. Acad. Sci. U. S. A. 94 (1997 Sep. 2) 9585–9589.
[11] J. Moskovitz, M.A. Rahman, J. Strassman, S.O. Yancey, S.R. Kushner, N. Brot, H. Weissbach, *Escherichia coli* peptide methionine sulfoxide reductase gene: regulation of expression and role in protecting against oxidative damage, J. Bacteriol. 177 (1995) 502–507.
[12] T. Douglas, D.S. Daniel, B.K. Parida, C. Jagannath, S. Dhandayuthapani, Methionine sulfoxide reductase A (MsrA) deficiency affects the survival of *Mycobacterium smegmatis* within macrophages, J. Bacteriol. 186 (2004) 3590–3598.
[13] G. StJohn, N. Brot, J. Ruan, H. Erdjument-Bromage, P. Tempst, H. Weissbach, C. Nathan, Peptide methionine sulfoxide reductase from *Escherichia coli* and *Mycobacterium tuberculosis* protects bacteria against oxidative damage from reactive nitrogen intermediates, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 9901–9906.
[14] J. Moskovitz, S. Bar-Noy, W.M. Williams, J. Requena, B.S. Berlett, E.R. Stadtman, Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and life span in mammals, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12920–12925.
[15] J. Moskovitz, E. Flescher, B.S. Berlett, J. Azare, J.M. Poston, E.R. Stadtman, 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 (1998) 14071–14075.
[16] O. Yermolaieva, R. Xu, C. Schinstock, N. Brot, H. Weissbach, S.H. Heinemann, T. Hoshi, Methionine sulfoxide reductase A protects neuronal cells against brief hypoxia/reoxygenation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 1159–1164.
[17] H. Ruan, X.D. Tang, M.L. Chen, M.L. Joiner, G. Sun, N. Brot, H. Weissbach, S.H. Heinemann, L. Iverson, C.F. Wu, T. Hoshi, M.L. Chen, M.A. Joiner, S.H. Heinemann, High-quality life extension by the enzyme peptide methionine sulfoxide reductase, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2748–2753.
[18] B.L. Miller, T.D. Williams, C. Schöneich, Mechanism of sulfoxide formation through reaction of sulfur radical cation complexes with superoxide or hydroxide ion in oxygenated aqueous solution, J. Am. Chem. Soc. 118 (1996) 11014–11025.
[19] C. Schöneich, J. Yang, Oxidation of methionine peptides by Fenton systems: the importance of peptide sequence, neighboring groups, and EDTA, J. Chem. Soc., Perkin Trans. 2 (1996) 915–923.
[20] E.R. Stadtman, J. Moskovitz, B.S. Berlett, R.L. Levine, Cyclic oxidation and reduction of protein methionine residues is an important antioxidant mechanism, Mol. Cell. Biochem. 234–235 (2002) 3–9.
[21] I. Petropoulos, J. Mary, M. Perichon, B. Friguet, Rat peptide methionine sulfoxide reductase: cloning of the cDNA, and down-regulation of gene expression and enzyme activity during aging, Biochem. J. 355 (2001) 819–825.
[22] S.P. Gabbita, M.Y. Aksenov, M.A. Lovell, W.R. Markesbery, Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain, J. Neurochem. 73 (1999) 1660–1666.
[23] W.A. Pryor, M.M. Dooley, D.F. Church, Inactivation of human alpha-1-proteinase inhibitor by gas-phase cigarette smoke, Biochem. Biophys. Res. Commun. 122 (1984) 676–681.
[24] K. Maier, L. Leuschel, U. Costabel, Increased levels of oxidized methionine residues in bronchoalveolar lavage fluid proteins from patients with idiopathic pulmonary fibrosis, Am. Rev. Respir. Dis. 143 (1991) 271–274.
[25] S. Krishnan, E.Y. Chi, S.J. Wood, B.S. Kendrick, C. Li, W. Garzon-Rodriguez, J. Wypych, T.W. Randolph, L.O. Narhi, A.L. Biere, M. Citron, J.F. Carpenter, Oxidative dimer formation is the critical rate-limiting step for Parkinson's disease alpha-synuclein fibrillogenesis, Biochemistry 42 (2003) 829–837.
[26] M.J. Hokenson, V.N. Uversky, J. Goers, G. Yamin, L.A. Munishkina, A.L. Fink, Role of individual methionines in the fibrillation of methionine-oxidized alpha-synuclein, Biochemistry 43 (2004) 4621–4633.
[27] N. Fujii, Y. Ishibashi, K. Satoh, M. Fujino, K. Harada, Simultaneous racemization and isomerization at specific aspartic acid residues in alpha B-crystallin from the aged human lens, Biochim. Biophys. Acta 1204 (1994) 157–163.
[28] M.H. Garner, A. Spector, Selective oxidation of cysteine and methionine in normal and senile cataractous lenses, Proc. Natl. Acad. Sci. U. S. A. 77 (1980) 1274–1277.
[29] P.M. van Haard, H.J. Hoenders, J. Wollensak, J. Haverkamp, Pyrolysis mass spectra, sulfhydryl and tryptophan content of the embryonic nuclei from adult human normal and nuclear-cataractous lenses, Biochim. Biophys. Acta 631 (1980) 177–187.
[30] H.J. Horstmann, J.W. Rohen, K. Sames, Age-related changes in the composition of proteins in the trabecular meshwork of the human eye, Mech. Ageing Dev. 21 (1983) 121–136.
[31] H.J. Horstmann, K. Sames, J.W. Rohen, Methionine sulfoxide is found in the protein of the trabecular meshwork of the human eye, Eur. J. Cell Biol. 20 (1979) 122.
[32] M.C. Wells-Knecht, T.J. Lyons, D.R. McCance, S.R. Thorpe, J.W. Baynes, Age-dependent increase in *ortho*-tyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes, J. Clin. Invest. 100 (1997) 839–846.
[33] A. Brovelli, C. Seppi, A.M. Castellana, M.R. De Renzis, A. Blasina, C. Balduini, Oxidative lesion to membrane proteins in senescent erythrocytes, Biomed. Biochim. Acta 49 (1990) S218–S223.
[34] C. Seppi, M.A. Castellana, G. Minetti, G. Piccinini, C. Balduini, A. Brovelli, Evidence for membrane protein oxidation during in vivo aging of human erythrocytes, Mech. Ageing Dev. 57 (1991) 247–258.
[35] M.L. Michaelis, D.J. Bigelow, C. Schoneich, T.D. Williams, L. Ramonda, D. Yin, A.F. Huhmer, Y. Yao, J. Gao, T.C. Squier, Decreased plasma membrane calcium transport activity in aging brain, Life Sci. 59 (1996) 405–412.
[36] J. Gao, D. Yin, Y. Yao, T.D. Williams, T.C. Squier, Progressive decline in the ability of calmodulin isolated from aged brain to activate the plasma membrane Ca-ATPase, Biochemistry 37 (1998) 9536–9548.
[37] C.R. Picot, M. Perichon, J.C. Cintrat, B. Friguet, I. Petropoulos, The peptide methionine sulfoxide reductases, MsrA and MsrB (hCBS-1), are downregulated during replicative senescence of human WI-38 fibroblasts, FEBS Lett. 558 (2004) 74–78.
[38] M.D. Williams, H. Van Remmen, C.C. Conrad, T.T. Huang, C.J. Epstein, A. Richardson, Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice, J. Biol. Chem. 273 (1998) 28510–28515.
[39] J. Moskovitz, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, F. Jursky, H. Weissbach, N. Brot, Chromosomal localization of the mammalian

peptide-methionine sulfoxide reductase gene and its differential expression in various tissues, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 3205–3208.
[40] C.C. Chao, Y.S. Ma, E.R. Stadtman, Modification of protein surface hydrophobicity and methionine oxidation by oxidative systems, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 2969–2974.
[41] E.R. Stadtman, P.E. Starke-Reed, C.N. Oliver, J.M. Carney, R.A. Floyd, Protein Modification in Aging, in: I. Emerit, B. Chance (Eds.), Free Radicals and Aging, Birkhäuser Verlag, Basel, 1992, pp. 65–72.
[42] J.M. Carney, P.E. Starke-Reed, C.N. Oliver, R.W. Landrum, M.S. Cheng, J.F. Wu, R.A. Floyd, Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity, and loss in temporal and spatial memory by chronic administration of the spin-trapping compound *N-tert*-butyl-a-phenylnitrone, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 3633–3636.
[43] P.E. Starke-Reed, C.N. Oliver, Protein oxidation and proteolysis during aging and oxidative stress, Arch. Biochem. Biophys. 275 (1989) 559–567.
[44] Y. Li, T.T. Huang, E.J. Carlson, S. Melov, P.C. Ursell, J.L. Olson, L.J. Noble, M.P. Yoshimura, C. Berger, P.H. Chan, Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase, Nat. Genet. 11 (1995) 376–381.
[45] H. Van Remmen, Y. Ikeno, M. Hamilton, M. Pahlavani, N. Wolf, S.R. Thorpe, N.L. Alderson, J.W. Baynes, C.J. Epstein, T.T. Huang, J. Nelson, R. Strong, A. Richardson, Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging, Physiol. Genomics 16 (2003) 29–37.
[46] H. Fliss, H. Weissbach, N. Brot, Oxidation of methionine residues in proteins of activated human neutrophils, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 7160–7164.
[47] C. Taggart, D. Cervantes-Laurean, G. Kim, N.G. McElvaney, N. Wehr, J. Moss, R.L. Levine, Oxidation of either methionine 351 or methionine 358 in alpha 1-antitrypsin causes loss of anti-neutrophil elastase activity, J. Biol. Chem. 275 (2000) 27258–27265.