ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 4, 2007

DOI: 10.1089/ars.2006.1473

News & Views

Does *S*-Methyl Methanethiosulfonate Trap the Thiol–Disulfide State of Proteins?

ANNA–RIIKKA KARALA AND LLOYD W. RUDDOCK

ABSTRACT

***S*-Methyl methanethiosulfonate (MMTS) is a reagent used to trap the natural thiol–disulfide state of the proteins. The efficiency of trapping mixed disulfides *in vivo* has been found to be higher for MMTS than for the more commonly used *N*-ethylmaleimide. MMTS has also been used for studying protein *S*-nitrosylation and the role of catalytic and structural cysteines on enzyme activities. However, the treatment of a protein with MMTS can potentially generate additional protein disulfide bonds. These results indicate that *in vitro* MMTS is able to form both intramolecular and intermolecular protein disulfide bonds in addition to dithiomethane adducts.** *Antioxid. Redox Signal.* 9, 527–531.

PROTEIN DISULFIDE ISOMERASES

Secreted and outer membrane proteins are translated, folded, and transported through the endoplasmic reticulum (ER) with the assistance of many ER resident protein catalysts and chaperones (3, 5, 23, 24). Disulfide bond formation is an important step in the folding of many of these proteins and this requires assistance from members of the protein disulfide isomerase (PDI) family (9). PDI catalyzes all of the steps in native disulfide bond formation, including oxidation, reduction, and isomerization (7, 9). During these thiol–disulfide exchange reactions, transient mixed disulfides are formed between PDI and substrates. These mixed disulfides are broken by nucleophilic attack by free cysteines in PDI, the folding protein or external factors such as glutathione, resulting in oxidation or reduction of the proteins. In addition to PDI, there are now 17 human PDI-like enzymes in the ER (6). Many of these ER-located proteins have been shown to have activity in thiol–disulfide exchange assays. However, the function of these PDI family members in the ER, the different substrate specificities of family members, as well as their interactions with other ER-resident protein folding catalysts, is far from clear.

STUDYING OF THE NATURAL THIOL–DISULFIDE STATE OF THE PROTEINS

Methods to study the thiol–disulfide state of proteins, including the transient formation of intermolecular disulfides, are important in identifying folding pathways, substrates of thiol–disulfide reductases, and novel redox regulated proteins. As a first step, these methods often involve the blocking of all free thiols with thiol-reactive reagents to prevent thiol–disulfide exchange during sample preparation. There are many compounds available to block free thiols. The most commonly used are iodoacetamide (IAM) and *N*-ethylmaleimide (NEM), which block thiol groups irreversibly (13). *S*-Methyl methanethiosulfonate (MMTS) is also used to trap thiol groups, but it reacts with thiols reversibly. The efficiency of trapping mixed disulfides has been found to be higher for MMTS than for NEM (18, Ellgaard and Molinari personal communications). MMTS has also been used for studying protein *S*-nitrosylation (8, 11) and to study the role of catalytic and structural cysteines on enzyme activities (14, 15, 19). For all of these uses there is the assumption that MMTS traps the natural thiol–disulfide state of the proteins.

Biocenter Oulu and Department of Biochemistry, University of Oulu, Oulu, Finland.

THIOL MODIFICATION BY MMTS

The reaction of MMTS with thiol groups results in their modification to dithiomethane (–S–S–CH_3). Dithiomethane can be reduced by agents such as DTT or β-mercaptoethanol, but it is also a potential target for nucleophilic attack by free thiol groups of proteins. Hence, during the treatment of a protein with MMTS, protein cysteine residues can potentially react with dithiomethane, resulting in protein disulfide bond formation (Fig. 1); the resulting protein disulfide may then undergo isomerization reactions, but it cannot react with MMTS. Such a reaction could potentially give false results when MMTS is used to determine the free thiol content of a protein and could potentially generate artificial disulfides when used as a trapping agent. The potential for forming protein disulfide bonds depends on kinetic partitioning between the reaction of the second cysteine residue with the dithiomethane moiety or with another MMTS molecule. This will depend on the location, accessibility, and reactivity of the second cysteine residue and the concentration of MMTS. For example, if MMTS is in large excess, the formation of a second dithiomethane adduct (Fig. 1, reaction 1) is favored. In contrast, the formation of a protein disulphide bond (Fig. 1, reaction 2) is favored when MMTS concentrations are low and/or where the second cysteine is juxtaposed to the dithiomethane adduct formed from the first reaction.

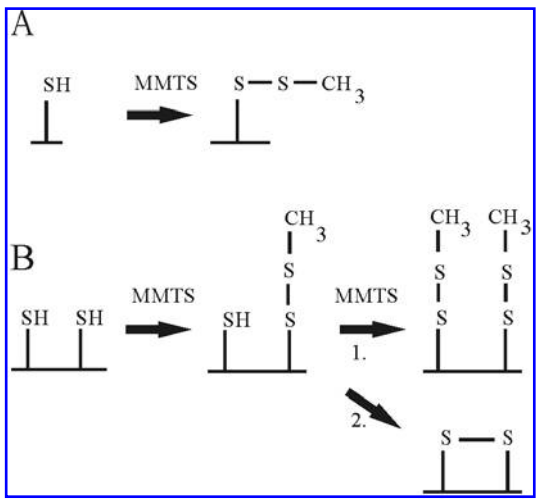

FIG. 1. Schematic representation of the reaction of MMTS with protein thiols. (**A**) When a protein contains a single thiol group, MMTS reacts with this to form dithiomethane adduct. (**B**) When a protein contains multiple thiol groups after the initial formation of the first dithiomethane adduct, a partitioning occurs between the formation of a second dithiomethane adduct via nucleophilic attack by the second cysteine on another molecule of MMTS (reaction 1) and the formation of a protein disulfide bond (reaction 2) via the nucleophilic attack by the second cysteine on the dithiomethane adduct formed from the first reaction. Reaction 1 is favored at high concentrations of MMTS. Reaction 2 is favored at low concentrations of MMTS and/or where the second cysteine is juxtaposed to the dithiomethane adduct formed from the first reaction.

REDUCED PDIA IS OXIDIZED BY MMTS

To test the possibility of protein disulfide bond formation by MMTS, isolated reduced PDI**a** and a 10 amino acid peptide (NRCSQGSCWN), both of which contain two cysteine residues that are known to be able to form an intramolecular disulphide bond (20), were treated with MMTS and then iodoacetamide (IAM), and analyzed by mass spectrometry. When the peptide (100 μ*M*) was treated with excess MMTS (1 m*M*), conditions that should favour the formation of a second dithiomethane adduct, the MS analysis revealed that both cysteines reacted with MMTS and were in dithiomethane form. However, when the MMTS concentration was reduced to approximately the equivalent concentration of the peptide, conditions that should favor protein disulfide bond formation, the MS spectrum showed three distinct peptide masses. These corresponded to the oxidized peptide (35.8%), one cysteine in dithiomethane form (22.5%), and two cysteines in dithiomethane form (41.7%).

A similar experiment was performed treating reduced PDI**a** with MMTS and then IAM and the subsequent mass of PDI**a** measured by ESI-mass spectrometry. PDI**a** contains two active site cysteines and after MMTS treatment of reduced PDI**a** sample, these cysteines were in an intramolecular disulfide and no dithiomethane adducts of PDI**a** were seen. In the control sample, which was only alkylated with iodoacetamide, 79% of the PDI**a** was in the reduced form, that is, both of the active site cysteines were alkylated (+57 Da). The oxidation of the active site cysteines of PDI**a** is very efficient, and during the removal of DTT some oxidation occurred and hence 21% of the control PDI**a** was in the oxidized form. When the experiment was repeated with 0.5 m*M* of DTT being present throughout, the control sample of PDI**a** was fully reduced, while the addition of 1 m*M* MMTS still resulted in the exclusive formation of a disulfide between active site cysteines of PDI**a** (*i.e.*, no dithiomethane adducts of PDI**a** were seen).

MMTS TREATMENT OF PROTEIN MIXTURE RESULTS IN THE FORMATION OF PROTEIN MIXED DISULFIDES

To test whether MMTS treatment can result in the generation of mixed disulfide bonds between proteins, reduced members of the PDI family were incubated with reduced PDI-substrate proteins. The PDI family members chosen were those most commonly studied *in vivo* and *in vitro*, PDI and ERp57, both of which have two catalytic domains containing a C*XX*C motif (6). The PDI-substrates chosen were RNase A and BPTI, which have four and three disulfides in their native state, respectively, whose oxidative folding has been widely studied, and that are routinely used in activity assays for the PDI family (4, 17, 21, 25). Specifically, reduced PDI or ERp57 (20 μ*M*) were mixed together with reduced BPTI or RNase A (100 μ*M*) and subsequently treated with 50 m*M* MMTS or 50 m*M* NEM as a control. Since at this concentration the MMTS is in large excess, the complete formation of dithiomethane adducts should be favored.

Nonreducing SDS-PAGE revealed that MMTS treatment of a mixture of reduced proteins resulted in the appearance of additional protein bands, while NEM treatment did not (Fig. 2A). When the MMTS samples were run on reducing SDS-PAGE these additional bands disappeared (Fig. 2B), indicating that they were mixed disulfides. Based on the molecular weights of the additional bands, the mixed disulfides were formed between the PDI family members PDI or ERp57, and the substrate proteins BPTI or RNase A. In addition, a ladder of bands indicated the formation of mixed disulfide dimers, trimers, tetramers, etc. of BPTI. The protein mixed disulfides, especially those between PDI or ERp57 and BPTI or RNase, were still efficiently formed even when the MMTS concentration was raised to 1*M* (data not shown).

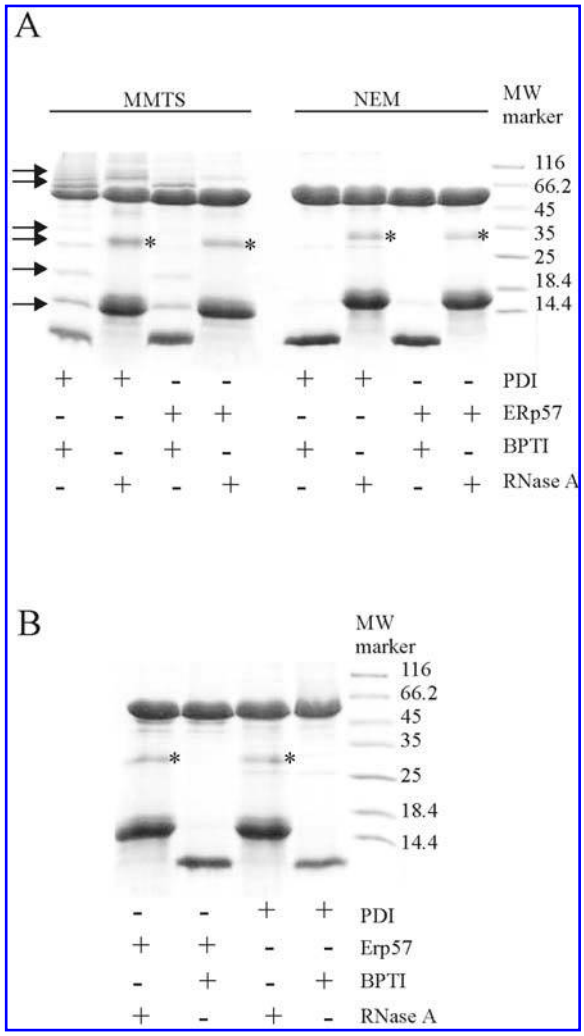

FIG. 2. Trapping the reduced state of proteins. (**A**) Reduced PDI + BPTI, PDI + RNase A, ERp57 + BPTI, and ERp57 + RNase A were incubated with 50 m*M* MMTS or NEM at pH 7.2 for 3 min. Nonreducing sample buffer was added to the samples before they were analyzed by SDS-PAGE. MMTS treatment of reduced proteins resulted in the formation of additional protein bands (*lanes 1–4*, marked with *arrows*), but NEM treatment did not (*lanes 6–9*). (**B**) Samples from Fig. 2A *lanes 1–4* to which β-mercaptoethanol was added. In the reducing SDS-PAGE, the additional bands seen under nonreducing SDS-PAGE were not present. This indicates that the additional bands upon MMTS treatment were due to the formation of intermolecular disulfide bonds. *Asterisk* (*) indicate contaminant in RNase A sample.

CONCLUSIONS

The efficient trapping of the thiol–disulfide state of proteins is a requisite for studying folding pathways, including the formation of transient mixed disulfides during catalyzed disulfide bond formation and during ER-associated degradation (ERAD). *In vitro*, acid quenching is widely used since it very efficiently quenches the thiol–disulfide exchange reaction and is readily reversible; however, it results in protein denaturation and aggregation events. To avoid this, chemical thiol-blocking agents such as IAM and NEM are widely used. *In vitro* both reagents are usually added in very large excess as the intermolecular reaction of free thiol groups with the blocking agent is in competition with thiol–disulphide exchange reactions, which even at physiological pH values may happen on the subsecond timescale. Thiol-blocking reagents need to be highly specific for thiol groups, with minimal side reactions and highly reactive. Both IAM and NEM are usually considered to meet these requirements. However, IAM is often contaminated with iodine, which can result in thiol oxidation to the disulfide or higher oxidation states such as the sulfinic acid (10) and can also derivatize methionine (12), while NEM can react with some other groups in proteins (22). IAM and NEM react with thiols irreversibly. *In vivo* the situation is more complex than *in vitro* with the requirement for the blocking reagent to enter the appropriate subcellular compartment and the presence of a highly complex mixture of proteins at concentrations in excess of 100 mg/ml. Furthermore, the mixed disulfides formed between protein folding catalysts and their substrates are often extremely short-lived and may be present at very low concentrations. Hence, any system used to trap these intermediates need to be extremely efficient and specific. While both NEM and IAM have been widely used *in vivo*, the efficiency of trapping is generally thought to be low. Recently MMTS was reported to result in more efficient trapping of the ERp57-tapasin complex *in vivo* (18), with similar apparent increased trapping efficiency being seen for other systems in the ER (Molinari and Ellgaard, personal communications). MMTS traps the cysteine–disulfide state in proteins by modifying their thiol groups to dithiomethane (–S–S–CH_3). MMTS should not be able to react with other amino groups (*e.g.*, methionine), and we have seen no evidence from our mass spectrometric analysis of the reactions of MMTS with peptides or proteins with other residues (*i.e.*, MMTS is highly specific for cysteine residues). This reaction of MMTS with cysteine residues is reversible by reducing agents, allowing regeneration of the free thiol groups; however, dithiomethane is also a potential target for nucleophilic attack by free thiol groups of proteins. Hence, during the treatment of a protein with MMTS, protein cysteine residues can potentially react with dithiomethane, resulting in protein disulfide bond formation.

By using a combination of mass spectrometry and nonreducing SDS-PAGE, we were able to show that *in vitro* MMTS treatment of both peptides and proteins resulted in the formation of intramolecular and intermolecular protein disulfide bonds. The potential for forming protein disulfide bonds depends on the kinetic partitioning between the reaction of the second cysteine residue with the dithiomethane moiety formed by the first reaction or with another MMTS molecule. This will depend on the relative location, accessibility, and reactivity of the second cysteine residue and the concentration of MMTS. Hence for the unstructured 10 amino acid peptide, dithiomethane formation on both cysteine groups was very efficient and no intramolecular disulfide formation was detected as long as the MMTS was in excess. Only when MMTS concentrations were low did an intramolecular protein disulfide bond form. In contrast, for PDI**a** the reaction of the second cysteine with the dithiomethane moiety formed from the first reaction was far faster than the reaction of the second cysteine with another molecule of MMTS and hence an intramolecular protein disulfide bond was efficiently formed, and no dithiomethane adducts were seen, even with MMTS in large excess. In addition to the formation of intramolecular protein disulfide bonds, the addition of MMTS to a mixture of reduced proteins resulted in the formation of intermolecular protein disulfides. These results imply that methods using MMTS as thiol–disulfide trapping reagent may give false results. The possibility of MMTS acting as an oxidant, generating protein intra- or intermolecular disulfide bonds will be protein dependent, the dependency arising due to the position, accessibility, and reactivity of the second cysteine residue to react. However, the formation of intramolecular disulfide bonds in PDI**a** and the formation of intermolecular disulfide bonds between PDI or ERp57 and BPTI or RNase, or between BPTI monomers to form small oligomers suggest this effect may be commonplace. Furthermore, the partitioning reaction will depend on the concentration of MMTS. Hence, for the unstructured 10 amino acid peptide, disulfide bond formation only occurred when the MMTS concentration was lowered to near that of the free thiol concentration. In contrast, the formation of intermolecular protein disulfides between PDI and BPTI or RNase A occurred even at 1 *M* MMTS (*i.e.*, 50,000-fold molar excess). The concentration of MMTS usually used to trap the thiol–disulfide state *in vivo* is around 20 m*M*. Based on intracellular protein concentration and the frequency of cysteine encoding codons (16), an estimate of 20 m*M* can be made of the concentration of proteinaceous cysteine groups *in vivo*, to which must be added up to 10 m*M* for the cysteine groups found in peptides such as glutathione. Hence the concentration of MMTS commonly used *in vivo* is not in excess over the thiol content of the cell, a fact likely to exacerbate the effects of artificial protein disulfide bond formation by MMTS.

In addition to its use in trapping the thiol–disulfide state of proteins *in vivo*, MMTS is widely used to study the role of catalytic and structural cysteines on enzyme activities (14, 15, 19) and *S*-nitrosylation (8, 11). For all of these uses there is the assumption that MMTS traps the natural thiol–disulfide state of the proteins, an assumption that must now be re-evaluated.

ACKNOWLEDGMENTS

This work was supported by the Department of Biochemistry, University of Oulu and Biocenter Oulu, Oulu, Finland.

ABBREVIATIONS

BPTI, bovine pancreatic trypsin inhibitor; DTT, dithiothreitol; ESIMS, electrospray ionization mass spectrometry; IAM, iodoacetamide; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight; MMTS, *S*-methyl methanethiosulfonate; NEM, *N*-ethylmaleimide; PAGE, polyacrylamide gel electrophoresis; PDI, protein disulfide isomerase; SDS, sodium dodecyl sulfate; RNase A, ribonuclease A.

APPENDIX

Notes

1. Generation of expression vectors

N-terminally His-tagged mature PDI**a**, PDI, and ERp57 expression vectors were generated for previous study (1). Mature BPTI (bovine pancreatic trypsin inhibitor) with an additional initiating methionine was constructed from synthetic oligonucleotides with optimal codon usage for *Escherichia coli* expression and subcloned as an NdeI/BamHI fragment into pET23 (Novagen, Madison, WI).

2. Protein expression and purification

BPTI, PDI**a,** PDI, and ERp57 were expressed in *E. coli* strain BL21(DE3) pLysS grown in LB medium at 37°C and induced at an A_{600} of 0.3 for 3 h with 1 m*M* isopropyl-β-D-thiogalactoside. Lysis of bacteria was performed by freeze-thawing the samples twice. PDI**a,** PDI, and ERp57 were purified by immobilized metal affinity chromatography and anion exchange chromatography as described for ERp18 (2), except for PDI**a** the anion exchange column was run in 20 m*M* Tris buffer, pH 8.6, instead of 20 m*M* phosphate buffer, pH 7.2.

While PDI**a**, PDI, and ERp57 were expressed solubly in *E. coli*, BPTI formed insoluble inclusion bodies. To purify BPTI, the insoluble cellular material was collected by centrifugation (8,000 rpm for 20 min). The cell pellet was then washed twice with wash buffer (50 m*M* Tris, 10 m*M* EDTA, 0.5% Triton-X100, pH 8.0) and twice with double distilled water. The cell pellet was then solubilized in 50 m*M* Tris-buffer, pH 8.75, containing 5 *M* guanidium chloride and 20 m*M* DTT and incubated at room temperature for 45 min. The sample was filtered through a 0.45 μ*M* filter before being applied to a SOURCE 5 RPC ST 4.6/150 column (Amersham Biosciences, Uppsala, Sweden) from which the proteins were eluted with a linear gradient from buffer A (2% ACN, 0.1% TFA) to 60% of buffer B (90% ACN, 0.1% TFA) over 20 column volumes. Analysis of the fractions that absorbed at 280 nm was performed by SDS-PAGE with Coomassie staining. Fractions that apparently contained pure BPTI were combined, lyophilized, and then resuspended in 10 m*M* HCl, pH 2. The concentrations of proteins were determined spectrophotometrically using a calculated absorption at 280 nm (PDI 45040 M^{-1} cm^{-1}, M_r = 56386; ERp57 45200 M^{-1} cm^{-1}, M_r = 55276; RNase A 9440 M^{-1} cm^{-1}, M_r = 13690; BPTI 5680 M^{-1} cm^{-1}, M_r = 6648; PDI**a** 19720 M^{-1} cm^{-1}, M_r = 14342). All proteins were stored in aliquots at −20°C.

For trapping experiments, all of the proteins were reduced with DTT at room temperature. PDI and ERp57 (50 μ*M*) were reduced with DTT (5 m*M*) in 20 m*M* phosphate buffer pH 7.2 for 30 min. RNase A (Sigma, St. Louis, MO) (1.7 m*M*) was reduced with DTT (700 m*M*) in 200 m*M* phosphate buffer containing 6 *M* guanidium chloride and 1 m*M* EDTA for 2 h. DTT and guanidium chloride were removed from RNase A by gel filtration (25 ml of Sephadex G-25 medium from Amersham Biosciences, XK16 column from Pharmacia Biotech, Uppsala, Sweden) into 10 m*M* HCl, pH 2.0.

3. Analysis of NRCSQGSCWN–peptide and reduced PDIa after MMTS treatment

NRCSQGSCWN–peptide (100 μ*M*) was treated with MMTS (1 m*M*) in 20 m*M* phosphate buffer pH 7.2 at room temperature for 10 min. The excess of MMTS was removed with PepClean C-18 spin column (Pierce, Rockford, IL) according to the product protocol and the purified samples were dried in a speed vacuum. Following the alkylation of the peptide with iodoacetamide (1.1 *M* solution in base buffer) and purification with PepClean C-18 spin column, the peptide masses were measured by mass spectrometry (A VOYAGER DE-STR MALDI-TOF, Applied Biosystems, Foster City, CA).

PDI**a** (400 μ*M*) was reduced with DTT (5 m*M*) in 20 m*M* phosphate buffer at room temperature for 30 min. One part of the sample was purified from DTT by gel filtration into 20 m*M* phosphate buffer pH 7.2 (NAP-10 column, Pharmacia Biotech). The other part of the reduced PDI**a** was left with DTT. Gel-filtered reduced PDI**a** was concentrated using a Millipore ultrafree-0.5 centrifugal filter device (5-kDa nominal molecular weight limit membrane filter) and was split into two parts. One part of the reduced PDI**a** (40 μ*M*) sample and PDI**a** (40 μ*M*) in 500 μ*M* DTT was alkylated with iodoacetamide (1.1 *M* solution in 20 m*M* phosphate buffer, pH 7.2) and purified with PepClean C-18 spin column. The other part of reduced PDI**a** (40 μ*M*) and PDI**a** (40 μ*M*) in 500 μ*M* DTT were incubated with MMTS (1 m*M*) in 20 m*M* phosphate buffer, pH 7.2, at room temperature for 10 min. MMTS was removed with PepClean C-18 spin column and the purified samples were dried in a speed vacuum. Purified proteins were further alkylated with iodoacetamide (1.1 *M* solution in 20 m*M* phosphate buffer, pH 7.2) and purified with PepClean C-18 spin column. The masses of PDI**a** proteins were measured by electrospray ionization mass spectrometry (ESIMS) (Micromass, Manchester, UK).

4. Analysis of the effects of MMTS treatment on mixtures of reduced proteins

Reduced PDI + BPTI, PDI + RNase A, ERp57 + BPTI, and ERp57 + RNase A were treated with MMTS and NEM. The protein concentration of PDI and ERp57 was 20 μ*M* and BPTI and RNase A 100 μ*M*. Proteins were mixed together and then incubated in 50 m*M* MMTS or NEM in 50 m*M* phosphate buffer, pH 7.2, for 3 min. Nonreducing sample buffer was added to the samples before they were separated by SDS-PAGE (15% acrylamide).

REFERENCES

1. Alanen HI, Salo KE, Pekkala M, Siekkinen HM, Pirneskoski A, and Ruddock LW. Defining the domain boundaries of the human protein disulfide isomerases. *Antioxid Redox Signal* 5: 367–374, 2003.
2. Alanen HI, Williamson RA, Howard MJ, Lappi AK, Jantti HP, Rautio SM, Kellokumpu S, and Ruddock LW. Functional characterization of ERp18, a new endoplasmic reticulum-located thioredoxin superfamily member. *J Biol Chem* 278: 28912–28920, 2003.
3. Bukau B, Weissman J, and Horwich A. Molecular chaperones and protein quality control. *Cell* 125: 443–451, 2006.
4. Creighton TE, Zapun A, and Darby NJ. Mechanisms and catalysts of disulfide bond formation in proteins. *Trends Biotechnol* 13: 18–23, 1995.
5. Ellgaard L and Helenius A. Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4: 181–191, 2003.
6. Ellgaard L and Ruddock LW. The human protein disulphide isomerase family: substrate interactions and functional properties. *EMBO Rep* 6: 28–32, 2005.
7. Ferrari DM and Söling HD. The protein disulphide-isomerase family: unraveling a string of folds. *Biochem J* 339: 1–10, 1999.
8. Foster MW and Stamler JS. New insights into protein *S*-nitrosylation. Mitochondria as a model system. *J Biol Chem* 279: 25891–25897, 2004.
9. Freedman RB, Klappa P, and Ruddock LW. Catalytic and binding domains in the mechanism and specificity of protein disulphide isomerases: a theme with variations. *EMBO Rep* 3: 136–140, 2002.
10. Guzzetta A. 2000–2006. Mass Spectrometry and Biotechnology Resource [Online]. IonSource, LLC. http://www.ionsource.com/Card/cmc/mono0004.htm. 2006, Aug. 15.
11. Jaffrey SR, Erdjument–Bromage H, Ferris CD, Tempst P, and Snyder SH. Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. *Nat Cell Biol* 3: 193–197, 2001.
12. Lapko VN, Smith DL, and Smith JB. Identification of an artifact in the mass spectrometry of proteins derivatized with iodoacetamide. *J Mass Spec* 35: 572–575, 2000
13. Leichert LI and Jakob U. Global methods to monitor the thiol-disulfide state of proteins *in vivo. Antioxid Redox Signal* 8: 763–772, 2006.
14. Lowther WT, Brot N, Weissbach H, Honek JF, and Matthews BW. Thiol-disulfide exchange is involved in the catalytic mechanism of peptide methionine sulfoxide reductase. *Proc Natl Acad Sci USA* 97: 6463–6468, 2000.
15. Millian NS and Garrow TA. Human betaine-homocysteine methyltransferase is a zinc metalloenzyme. *Arch Biochem Biophys* 356: 93–98, 1998.
16. Miseta A and Csutora P. Relationship between the occurrence of cysteine in proteins and the complexity of organisms. *Mol Biol Evol* 17: 1232–1239, 2000.
17. Narayan M, Welker E, Wedemeyer WJ, and Scheraga HA. Oxidative folding of proteins. *Acc Chem Res* 33: 805–812, 2000.
18. Peaper DR, Wearsch PA, and Cresswell P. Tapasin and ERp57 form a stable disulfide-linked dimer within the MHC class I peptide-loading complex. *EMBO J* 24: 3613–3623, 2005.
19. Rodriguez–Romero A, Hernandez–Santoyo A, del Pozo Yauner L, Kornhauser A, and Fernandez–Velasco DA. Structure and inactivation of triosephosphate isomerase from *Entamoeba histolytica. J Mol Biol* 322: 669–675, 2002.
20. Ruddock LW, Hirst TR, and Freedman RB. pH-dependence of the dithiol-oxidizing activity of DsbA (a periplasmic protein thiol:disulphide oxidoreductase) and protein disulphide-isomerase: studies with a novel simple peptide substrate. *Biochem J* 315**:** 1001–1005, 1996.
21. Ruoppolo M, Orrù S, Talamo F, Ljung J, Pirneskoski A, Kivirikko KI, Marino G, and Koivunen P. Mutations in domain *a*' of protein disulfide isomerase affect the folding pathway of bovine pancreatic ribonuclease A. *Protein Science* 12: 939–952, 2003.
22. Smyth DG, Blumenfeld OO, and Konigsberg W. Reactions of *N*-ethylmaleimide with peptides and amino acids. *Biochem J* 91: 589–595, 1964.
23. Trombetta ES and Parodi AJ. Quality control and protein folding in the secretory pathway. *Annu Rev Cell Dev Biol* 19: 649–676, 2003.
24. van Anken E and Braakman I. Versatility of the endoplasmic reticulum protein folding factory. *Crit Rev Biochem Mol Biol* 40: 191–228, 2005.
25. Weissman JS and Kim PS. Efficient catalysis of disulfide bond rearrangements by protein disulfide-isomerase. *Nature* 365: 185–188, 1993.

Address reprint requests to:
Lloyd W. Ruddock
University of Oulu
Department of Biochemistry
P.O. Box 3000
90014 University of Oulu
Oulu, Finland

E-mail: lloyd.ruddock@oulu.fi

Date of first submission to ARS Central, September 28, 2006; date of final revised submission, October 20, 2006; date of acceptance, October 22, 2006.

This article has been cited by:

1. Jay R. Laver , Samantha McLean , Lesley A.H. Bowman , Laura J. Harrison , Robert C. Read , Robert K. Poole . Nitrosothiols in Bacterial Pathogens and Pathogenesis. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Hai Po Helena Liang, Teresa M. Brophy, Philip J. Hogg. 2011. Redox properties of the tissue factor Cys 186 –Cys 209 disulfide bond. *Biochemical Journal* **437**:3, 455-460. [CrossRef]
3. Marika Lindahl , Alejandro Mata-Cabana , Thomas Kieselbach . 2011. The Disulfide Proteome and Other Reactive Cysteine Proteomes: Analysis and Functional Significance. *Antioxidants & Redox Signaling* **14**:12, 2581-2642. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
4. M. Luciana Lanteri, Lorenzo Lamattina, Ana M. Laxalt. 2011. Mechanisms of xylanase-induced nitric oxide and phosphatidic acid production in tomato cells. *Planta* . [CrossRef]
5. Van Dat Nguyen, Mirva J. Saaranen, Anna-Riikka Karala, Anna-Kaisa Lappi, Lei Wang, Irina B. Raykhel, Heli I. Alanen, Kirsi E.H. Salo, Chih-chen Wang, Lloyd W. Ruddock. 2011. Two Endoplasmic Reticulum PDI Peroxidases Increase the Efficiency of the Use of Peroxide during Disulfide Bond Formation. *Journal of Molecular Biology* **406**:3, 503-515. [CrossRef]
6. Maike Thamsen, Ursula Jakob. 2011. The redoxomeProteomic analysis of cellular redox networks. *Current Opinion in Chemical Biology* **15**:1, 113-119. [CrossRef]
7. Anna-Riikka Karala, Anna-Kaisa Lappi, Lloyd W. Ruddock. 2010. Modulation of an Active-Site Cysteine pKa Allows PDI to Act as a Catalyst of both Disulfide Bond Formation and Isomerization. *Journal of Molecular Biology* **396**:4, 883-892. [CrossRef]
8. Feras Hatahet , Lloyd W. Ruddock . 2009. Protein Disulfide Isomerase: A Critical Evaluation of Its Function in Disulfide Bond Formation. *Antioxidants & Redox Signaling* **11**:11, 2807-2850. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
9. Rosa E. Hansen, Jakob R. Winther. 2009. An introduction to methods for analyzing thiols and disulfides: Reactions, reagents, and practical considerations. *Analytical Biochemistry* **394**:2, 147-158. [CrossRef]
10. Wenyun Lu, Bryson D. Bennett, Joshua D. Rabinowitz. 2008. Analytical strategies for LC–MS-based targeted metabolomics#. *Journal of Chromatography B* **871**:2, 236-242. [CrossRef]
11. Nicolas Brandes, Andrea Rinck, Lars Ingo Leichert, Ursula Jakob. 2007. Nitrosative stress treatment of E. coli targets distinct set of thiol-containing proteins. *Molecular Microbiology* **66**:4, 901-914. [CrossRef]