FORUM EDITORIAL

Redox Proteomics: A Key Tool for New Insights into Protein Modification with Relevance to Disease

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Abstract

Oxidatively modified proteins are characterized by elevations in protein-resident carbonyls or 3-nitrotyrosine, measures of protein oxidation, or protein bound reactive alkenals such as 4-hydroxy-2-nonenal, a measure of lipid peroxidation. Oxidatively modified proteins nearly always have altered structure and function. Redox proteomics is that branch of proteomics used to identify oxidized proteins and determine the extent and location of oxidative modifications in the proteomes of interest. This technique nearly always employs mass spectrometry as the major platform to achieve the goals of identifying the target proteins. Once identified, oxidatively modified proteins can be placed in specific molecular pathways to provide insights into protein oxidation and human disease. Both original research and review articles are included in this Forum on Redox Proteomics. The topics related to redox proteomics range from basic chemistry of sulfur radical-induced redox modifications in proteins, to the thiol secretome and inflammatory network, to reversible thiol oxidation in proteomes, to the role of glutamine synthetase in peripheral and central environments on inflammation and insulin resistance, to bioanalytical aspects of tyrosine nitrated proteins, to protein oxidation in human smokers and models thereof, and to Alzheimer disease, including articles on the brain ubiquitinylome and the “triangle of death” composed of oxidatively modified proteins involved in energy metabolism, mammalian target of rapamycin activation, and the proteostasis network. This Forum on Redox Proteomics is both timely and a critically important resource to highlight one of the key tools needed to better understand protein structure and function in oxidative environments in health and disease. *Antioxid. Redox Signal.* 26, 277–279.

Keywords: redox proteomics, oxidative modifications

THE FIRST REPORT OF REDOX PROTEOMICS to identify oxidatively modified proteins was from our laboratory and dealt with oxidized hippocampal proteins in subjects with Alzheimer disease (AD) (5). Since then, numerous studies using redox proteomics have appeared, most often directed toward understanding how alteration of protein structure affects function in health and disease [compiled or reviewed in Refs. (3, 6)]. Advances in mass spectroscopy methods for use of redox proteomics in neurodegenerative disorders have been reviewed recently (2).

The most common oxidative post-translational modifications to proteins involve (a) carbonyl formation associated with free radical scission of the primary peptide chain, oxidation of side chains of selected amino acids, the products of

glycation reactions between the protein and a reducing sugar, or the product of a highly reactive alkenal product of lipid peroxidation (4, 7, 9); (b) covalent adducts resulting from Michael addition of 4-hydroxy-2-nonenal or other reactive alkenals bound to Cys, Lys, or His residues (7, 9); and (c) nitration of Tyr residues; and nitrosylation of Cys residues (8).

In addition to these major oxidative post-translational modifications of proteins, Cys residues are exquisitely sensitive to cellular redox state, and the thiol functionality often controls the activity of proteins. Consequently, Cys residues are prime targets of redox modification, not only by nitrosylation as already noted but also by the three oxidation states of the sulfur atom of the Cys thiol moiety that can change in response to numerous stimuli and changes in cellular redox

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state (8). Other oxidative modifications of proteins identified by redox proteomics have been less well studied [reviewed in Ref. (2)].

Given that many of these oxidative modifications of proteins often occur on hydrophobic amino acid residues that are normally buried within the three-dimensional structure of proteins, and that such modifications often introduce a dipole on these amino acids that then force the protein to dramatically alter its conformation to expose the polar end of the dipole to water, oxidative modifications can cause dramatic alterations to the structure of proteins with consequent loss of function (4). As there is a growing appreciation that oxidative stress underlies numerous disease processes in both peripheral and central systems (4, 5, 6, 9), a greater knowledge of which proteins are selectively oxidatively or nitrosatively modified in disease conditions is crucially important to better understand the molecular bases of diseases and to identify potential therapeutic targets that potentially can be disease modifying.

These goals are the subjects of redox proteomics and the topic of this *Antioxidants and Redox Signaling* Forum. Figure 1 provides an overview of the content of this ARS Forum on Redox Proteomics. In a review article, Christian Schöneich delves deeply into one-electron oxidation processes of the sulfur atom in methionine and cysteine residues on proteins. Normally, two-electron oxidation of the thioether functionality in Met is the case, but in an increasing number of cases, including in aging and age-related

disorders, one-electron oxidation occurs, sometimes in signaling processes but sometimes leading to oxidation processes in lipids with damaging consequences for the cell. Prof. Schöneich's review provides new insights into Met and Cys modifications on proteins.

Continuing the theme of Cys modification, Prof. Elena Hidalgo and colleagues review proteomics utilization for characterizing reversible thiol oxidations in proteomes and proteins. Prof. Hidalgo and colleagues review the notion that reversible thiol oxidations of Cys in response to elevated cellular peroxides can serve as to protect thiols from irreversible oxidation and to potentially serve as reporters for future toxicity. Proteomics methods to characterize reversible thiol oxidations and how these can be potential sensors are expertly described. Prof. Pietro Ghezzi and Dr. Phillip Chan review a hitherto underreported use of redox proteomics, namely, the analysis of secreted proteins and their possible functions. In particular, these authors discuss the redox state of protein cysteines in both cytosolic and plasma environments. Under inflammatory conditions, secreted proteins and their Cys redox states may function as cytokines. Methods for detection of secreted glutathionylation of Cys residues and potential problems in the study of the thiol secretome are elegantly addressed.

Prof. Rafael Radi and colleagues review redox proteomics studies of 3-nitrotyrosine (3-NT), a post-translational modification of selected Tyr residues and a formal protein oxidation after formation of peroxynitrite from reaction of the two free radicals, superoxide radical anion, and nitric oxide. This team of researchers review aspects of resulting pathophysiology, key features of Tyr nitration *in vitro* and *in vivo*, especially selectivity, site-specificity, and effects on protein structure and function, and challenges of 3-NT identification using redox proteomics methodologies. Researchers interested in nitrosative stress will find this article highly useful.

In their original research article, Prof. Alessandra Castegna and colleagues demonstrate that glutamine synthetase (GS), a known protein target for oxidative modification, is expressed in microglia. When oxidized or conditionally knocked down, GS enhances release of inflammatory mediators that cause disruption of the redox state and loss of viability of neurons. This study is the first to report a metabolic mechanism mediating microglia response to the proinflammatory stimulator, lipopolysaccharide, suggesting that normally GS modulates microglia inflammatory function. As GS has been identified by redox proteomics previously as a target for oxidation, the implications of this study support the notion of a new role for GS in pathogenic mechanisms associated with oxidative damage and a potential therapeutic target to modulate neuroinflammation.

Tobacco smoking has significant and harmful effects on smokers and those exposed to secondhand smoke. Prof. Isabella Dalle-Donne reviews redox proteomics studies of protein carbonylation in smokers and models thereof. Critical discussion of the relevance and limitations of models of cigarette smoke and their congruence to clinical presentation of smokers is provided. Prof. Dalle-Donne outlines how progress in redox proteomics methodologies permits enrichment, identification, and characterization of protein carbonyls and how these advances make redox proteomics a powerful tool for investigations of cigarette smoke-related diseases and their potential treatment strategies.

FIG. 1. Overview of this ARS Forum on Redox Proteomics. Review and original articles dealing with oxidative and nitrosative stress that is associated with and possibly causative of aging, neurodegenerative diseases, inflammatory diseases, and cardiovascular diseases are shown (*top*). Proteins modified by or associated with indices of oxidative and nitrosative stress, including indices of protein oxidation [protein carbonyls (PC); 3-nitrotyrosine (3-NT); methionine sulfoxide (MetOX); thiol oxidation (S-S); polyubiquitinylation (polyUb)]; and lipid peroxidation [covalent binding of 4-hydroxy-2-nonenal (HNE)] are identified by redox proteomics (*bottom*).

Defects in protein quality control mechanisms are proposed common features of neuropathology in persons with Down syndrome (DS) and AD. One process within the proteostasis network is the ubiquitin proteasome system (UPS), which requires polyubiquitinylation before degradation of aggregated or damaged proteins through the 26S proteasome. In this original research article, Prof. Marzia Perluigi and colleagues report results of proteomics analyses that identified brain proteins throughout the spectrum of DS with altered patterns of polyubiquitinylation as a function of age and, therefore, a function of AD neuropathology. Correlation of polyubiquitinylation with protein oxidation was found for the majority of identified proteins. This first report of altered polyubiquitinylation profiles in DS brain compared with those of healthy controls identified mainly proteins involved in glucose metabolism and protein quality control in DS. The authors posit that such changes may contribute to age-associated cognitive decline in this syndrome.

It is well known that (a) decreased glucose utilization; (b) increased activation of mammalian target of rapamycin, with downstream consequences of inhibited apoptosis and induction of insulin resistance; and (c) defects in the proteostasis network (UPS, autophagy, and unfolded protein response) are neuropathological features of AD brain (1, 4, 5, 9). Prof. D. Allan Butterfield and colleagues review redox proteomics studies of this “triangle of death” experienced by neurons in brain of subjects with this devastating dementing disorder (13). There is altered crosstalk among the components of this triangle of death in AD brain assessed by redox proteomics identification of oxidatively modified and, therefore, dysfunctional proteins. Aberrant oxidative stress plays a crucial role in fueling and integrating this triangle of processes that are associated with neuronal death and cognitive decline. Redox proteomics has revealed potential therapeutic targets to slow or arrest progression of AD, even from its earliest stages.

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Abbreviations Used

3-NT = 3-nitrotyrosine
AD = Alzheimer disease
DS = Down syndrome
GS = glutamine synthetase
UPS = ubiquitin proteasome system