Free Radical Biology and Medicine ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

The cysteine proteome

Young-Mi Go, Joshua D. Chandler, Dean P. Jones*

Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Emory University, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form
17 March 2015
Accepted 22 March 2015

Keywords:
Cysteine proteome
Redox proteome
Redox signaling
Functional network
Thiol
Free radicals

ABSTRACT

The cysteine (Cys) proteome is a major component of the adaptive interface between the genome and the exposome. The thiol moiety of Cys undergoes a range of biologic modifications enabling biological switching of structure and reactivity. These biological modifications include sulfenylation and disulfide formation, formation of higher oxidation states, S-nitrosylation, persulfidation, metalation, and other modifications. Extensive knowledge about these systems and their compartmentalization now provides a foundation to develop advanced integrative models of Cys proteome regulation. In particular, detailed understanding of redox signaling pathways and sensing networks is becoming available to allow the discrimination of network structures. This research focuses attention on the need for atlases of Cys modifications to develop systems biology models. Such atlases will be especially useful for integrative studies linking the Cys proteome to imaging and other omics platforms, providing a basis for improved redox-based therapeutics. Thus, a framework is emerging to place the Cys proteome as a complement to the quantitative proteome in the omics continuum connecting the genome to the exposome.

Contents

* Corresponding author. Fax: +404 712 2974.
E-mail address: dpjones@emory.edu (D.P. Jones).

http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.022

Introduction

The sulfur atom of cysteine (Cys) provides a considerable range of chemical reactivity and structural flexibility in the proteome. The presence of conserved Cys in motifs of proteins found in essentially all life forms indicates that these chemical characteristics were harnessed in early evolution to support enzyme catalysis, transcriptional regulation, protein folding, and three-dimensional structure. A remarkable range of biologic functions is supported by Cys because sulfur is stable in multiple coordinate covalent bonds with the major atoms of living organisms (C, H, O, N, P), forms stable coordinate complexes with transition metal ions (Zn, Fe, Cu), and is stable at a range of oxidation states (–SH, –SS–, $–SO_2^{-1}$, $–SO_3^{-1}$). Additionally, Cys thiols differentially undergo reversible ionization to the negatively charged thiolate form over the physiologic range of pH to flexibly optimize the functions of specific peptidyl Cys.

The human genome encodes about 214,000 Cys. Cys is widely distributed among proteins, with most expressing at least one and many having multiple coordinated Cys and Cys-rich domains. The reactivity and diverse functions of Cys are mirrored by a spectrum of susceptibilities and dysfunctions of their respective proteins, resulting in central roles of the Cys proteome in development, signal transduction, biologic defenses, aging, and disease. Considerable knowledge has accumulated for the symptoms and etiology of major human diseases, such as cardiovascular disease, Alzheimer disease, lung disease, metabolic syndrome, eye disease, and diseases of other organ systems. These often share related oxidative phenotypes and mechanisms, and progress in redox systems biology is beginning to provide an understanding of the integration of the underlying redox systems.

Early photosynthetic organisms transformed the earth by fixing the carbon atom of CO_2 into hydrocarbons and releasing bound atomic oxygen as gaseous molecular O_2. This changed the atmosphere from $<1\%$ to about 21% O_2 and had a profound effect on life forms by creating a diversity of external environments with different oxidative conditions. Contemporary redox proteomic systems have evolved as a result of the selective pressures of an oxygen environment and aerobic respiration, with extant genomes having selectively advantageous redox proteomic structures that protect the genome and ensure its replication despite environmental, organismal, and developmental variation in oxidative and reductive challenges. Although oxidative pressure influences multiple critical loci of the redox proteome, such as methionine (Met) and selenocysteine (Sec), the Cys proteome is central within this spectrum of biological plasticity and is the focus of this review.

The Cys proteome (Fig. 1) serves as a central adaptive interface between the functional genome and the external environment of an organism [1]. The external environment is a critical determinant of individual survival; in human health, this is now discussed in terms of an individual's "exposome" [2]. The exposome is defined as the cumulative measure of environmental influences and associated biological responses throughout the life span, including exposures from the environment, diet, behavior, and endogenous processes [3]. In addition to utility in contemporary environmental health research [3,4], this provides a useful way to think about the evolution of complex multicellular organisms as the content of O_2 increased in the atmosphere. Specifically, the redox sensing and signaling mechanisms supporting responses to challenge also provided sensing and signaling systems for temporal and spatial execution of differentiation and development in multicellular organisms.

In the present review of the Cys proteome, we start with a summary of lessons learned from study of the redox proteome, that portion of reversible and irreversible covalent modifications that link redox metabolism to biologic structure and function [1]. We follow this with a more specific consideration of the chemistry of sulfur and the spectrum of oxidative reactions that defines Cys proteomic functions. In a third section, we address the redox regulation systems, especially focusing on major reducing systems that balance the oxidative reactions and support a rapidly evolving understanding of the spatiotemporal nature of redox regulation. In this, we provide specific examples illustrating the organizational structure of stable, kinetically controlled functional redox networks and summarize other modifications such as S-nitrosylation and persulfidation. Finally, we discuss the Cys proteome within the context of integrated omics approaches, highlighting the ongoing need for development of quantitative redox biology models.

Lessons from the redox proteome

Central dogma

Cys is incorporated into protein as the thiol (RSH) form, with apparently no exception. Thiols are oxidized to sulfenic acids (RSO^-) intermediate to formation of disulfides (RSSR) and higher oxidation states (e.g., RSO_2^-). Thiols and disulfides also undergo exchange reactions in which the thiol reacts to form a new disulfide and liberates a different thiol ($RSH + R_1SSR_1 \leftrightarrow RSSR_1 + R_1SH$) [5]. Disulfide is a common posttranslational modification for three-dimensional structure, as a component of multistep vectorial processing and transport, as a switching mechanism in regulation or signaling, and as a consequence of oxidative stress. Research with redox Western- and mass spectrometry-based redox proteomics methods shows that partial oxidation is common for Cys residues throughout the proteome of mammalian systems [6–10]. This contradicts earlier interpretations that Cys thiol oxidation represents only an artifact of extraction [11] and supports the hypothesis that significant speciation of different peptidyl Cys in basal oxidation, organization, and function occurs within the Cys proteome.

Discussions of procedures and pitfalls in measurements of thiols and thiol/disulfide have been reviewed [12–14]. Measurements involving extraction and chemical modification reflect the efficiency of alkylation relative to oxidation in trapping the thiol form (see Hansen and Winther [13] for detailed discussion). Importantly, processing must be rapid under various conditions to minimize both oxidative *and* reductive artifacts. Artifacts are more likely in experimental studies with added oxidants, metal ions, or electrophiles, especially if these are not removed before processing biologic materials for thiol analysis. Extraction at 0 °C slows reaction rates about 10-fold relative to 37 °C, and higher O_2 in air-equilibrated solution increases rates about 5-fold that of tissues in vivo in mammals. Thus, in the absence of added oxidants and metals, oxidation of Cys during assay occurs at relatively slow rates, similar to the ongoing autoxidation rates in vivo. High concentrations of thiol-reactive chemicals can be readily achieved

Please cite this article as: Go, Y-M; et al. The cysteine proteome. *Free Radic. Biol. Med.* (2015), http://dx.doi.org/10.1016/j.freeradbiomed.2015.03.022