Biochimica et Biophysica Acta 1844 (2014) 2315–2330

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Chemical-proteomic strategies to investigate cysteine posttranslational modifications

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ARTICLE INFO

Article history:
Received 30 May 2014
Received in revised form 8 September 2014
Accepted 29 September 2014
Available online 5 October 2014

Keywords:
Oxidation
Nitrosation
Palmitoylation
Prenylation
Glutathionylation
Lipid-derived electrophile

ABSTRACT

The unique combination of nucleophilicity and redox-sensitivity that is characteristic of cysteine residues results in a variety of posttranslational modifications (PTMs), including oxidation, nitrosation, glutathionylation, prenylation, palmitoylation and Michael adducts with lipid-derived electrophiles (LDEs). These PTMs regulate the activity of diverse protein families by modulating the reactivity of cysteine nucleophiles within active sites of enzymes, and governing protein localization between soluble and membrane-bound forms. Many of these modifications are highly labile, sensitive to small changes in the environment, and dynamic, rendering it difficult to detect these modified species within a complex proteome. Several chemical-proteomic platforms have evolved to study these modifications and enable a better understanding of the diversity of proteins that are regulated by cysteine PTMs. These platforms include: (1) chemical probes to selectively tag PTM-modified cysteines; (2) differential labeling platforms that selectively reveal and tag PTM-modified cysteines; (3) lipid, isoprene and LDE derivatives containing bioorthogonal handles; and (4) cysteine-reactivity profiling to identify PTM-induced decreases in cysteine nucleophilicity. Here, we will provide an overview of these existing chemical-proteomic strategies and their effectiveness at identifying PTM-modified cysteine residues within native biological systems.

1. Introduction

Cysteine residues play critical roles within protein scaffolds as sites of nucleophilic and redox catalysis, allosteric regulation, metal binding and structural stabilization [1]. These diverse functions of cysteine are facilitated by the unique nucleophilic and redox properties of the cysteine thiol group. Due to the low dissociation energy of the S-H bond and the large atomic radius of sulfur, the cysteine thiol has a pK_a value of ~8.0 [2]. This side-chain pK_a is easily perturbed by the local protein environment, with pK_a values as low as 3.5 reported for members of the glutaredoxin family [3,4]. These altered pK_a values serve to enhance the nucleophilicity and redox sensitivity of thiols. This increased reactivity thereby promotes a multitude of electrophilic and oxidative posttranslational modifications (PTMs) (Fig. 1).

Cysteine PTMs can be categorized into spontaneous and enzyme-catalyzed modifications. Spontaneous cysteine PTMs are generally driven by the encounter of reactive cysteines with endogenous oxidants and reactive electrophiles, such as reactive oxygen/nitrogen species (ROS/RNS) and lipid-derived electrophiles (LDEs). Oxidative modifications of cysteine result in the formation of sulfenic/sulfinic/sulfonic acids [5], S-nitrosothiols [6], intra- and inter-chain disulfides [7], persulfides [8] and mixed disulfides with glutathione and cysteine [9]. LDEs such as 4-hydroxy-2-nonenal (HNE) and 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), commonly form Michael adducts with nucleophilic cysteines [10]. Enzyme-catalyzed modifications of cysteine include prenylation [11] and palmitoylation [12], whereby select enzymes mediate the transfer of the lipid or isoprenoid moiety from an activated donor to the thiol side chain of cysteine. It is important to note that some modifications, e.g. disulfide formation, can be both spontaneous and enzyme catalyzed. In addition to the common eukaryotic PTMs discussed above, many other rare modifications of cysteine, such as methylation and phosphorylation, have been discovered in both eukaryotic and prokaryotic organisms [13]. All of these cysteine PTMs have been shown to modulate protein activity as well as localization, and dysregulation of these PTMs are critical in driving a variety of proliferative and degenerative diseases [14–16].

Methods to enrich, identify and quantify cysteine PTMs are of paramount importance to our understanding of the scope and physiological role of these modifications. Chemical-proteomic approaches that investigate these modifications within the context of a native proteome have been developed over several decades [17]. These methods range from early radioisotope-labeling experiments to more recent advances in chemical-probe design and mass spectrometry (MS), which enable detailed interrogation of these modifications. The recent advent of bioorthogonal reactions that allow for selective tagging of functional groups with minimal perturbations to biomolecules [18] have further accelerated studies into cysteine PTMs. Here we will discuss the technologies currently available to investigate the common cysteine PTMs.

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http://dx.doi.org/10.1016/j.bbapap.2014.09.024
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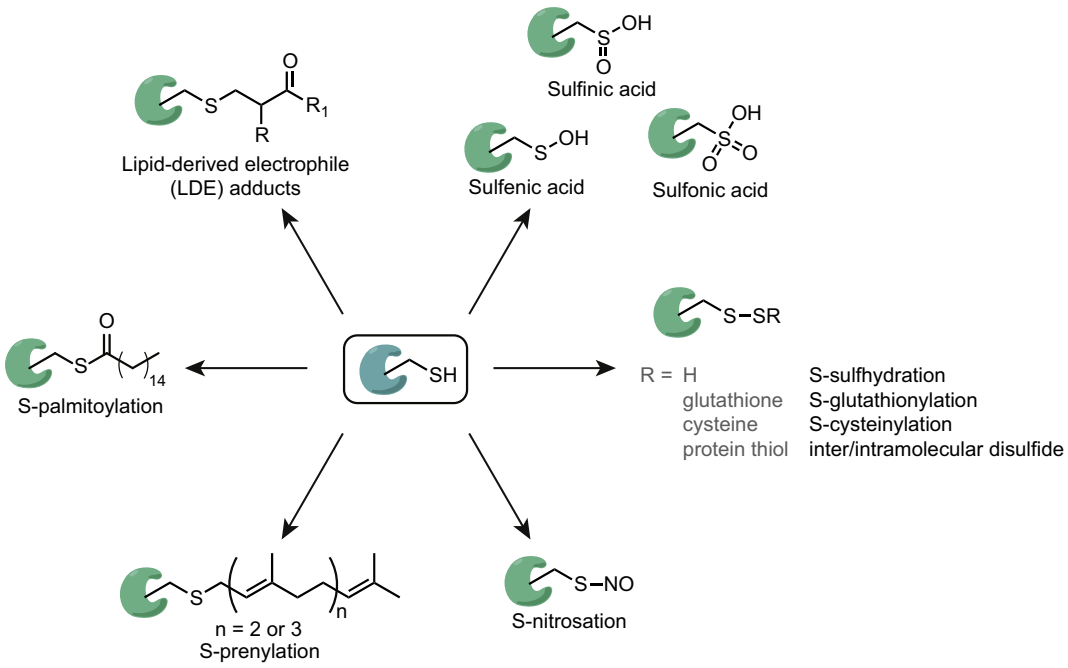

Fig. 1. Common posttranslational modifications of cysteines.

Advantages and limitations of each method and biological insight that has been gained through utilization of these methods will be highlighted where relevant.

2. Cysteine oxidation

Cysteine residues are highly susceptible to oxidation, resulting in the formation of a variety of cysteine oxoforms [19]. Of these, the most common is intra- and inter-molecular disulfide formation, which is known to facilitate protein folding and structural stability. In addition to disulfide bonds, other oxoforms of cysteine include sulfenic, sulfinic and sulfonic acids (Fig. 2A) [5]. Sulfenic acids are generated by the reaction of a cysteine thiol with biological oxidants such as hydrogen peroxide, hypochlorous acid and hydroxyl radicals, as well as the hydrolysis of S-nitrosothiols [19]. Further oxidation of sulfenic acids result in sulfinic and sulfonic acids. Sulfinic acids are stable to most cellular reductants, and are reduced only in the presence of a newly discovered ATP-dependent sulfiredoxin enzyme [20]. Sulfonic acids are the most highly oxidized thiol species and are considered to be irreversible. The reversibility of cysteine sulfenic acids has resulted in the evolution of this PTM into a key mode of regulation in biology, with functions analogous to phosphorylation, acetylation and ubiquitination [21]. Proteins from diverse functional classes have been identified to be sulfenylated and sulfinylated, and regulate key signaling pathways in (patho)physiology [5]. Examples include the protein-tyrosine phosphatases (PTPs), whereby sulfenylation of the active-site cysteine nucleophile is a well-characterized mechanism for inactivation of these enzymes [22]. The formation of the sulfenic acid is subsequently followed by either disulfide formation with an adjacent cysteine, or sulfonamide formation with a backbone amide-nitrogen [23,24]. Similarly, cysteine proteases such as the deubiquitinating enzymes (DUBs) that cleave ubiquitin from target proteins, are known to be sulfenylated at the active-site cysteine nucleophile. In particular, sulfenylation of both USP1, a DUB involved in DNA damage response [25], and A20, a known tumor suppressor [26], are known to inhibit deubiquitinase activity. These and other studies into the function of cysteine oxoforms have been accelerated by the availability of methods to identify these modifications. Although S-glutathionylation and S-nitrosation are described in separate sections of this review, it is important to note that many of the methods outlined in the cysteine oxidation section can also be used to detect these other oxidative cysteine PTMs.

2.1. Two-dimensional gel electrophoresis (2DE)

The oxidation of cysteine residues into the various cysteine oxoforms serves to quench the nucleophilicity of the cysteine thiol. Existing two-dimensional gel electrophoresis (2DE) methods rely on well-characterized cysteine-reactive agents such as iodoacetamide (IAM) or N-ethylmaleimide (NEM) to cap all reduced cysteines in the proteome, leaving behind oxidized cysteines that are unreactive to the capping agent. These oxidized cysteine residues are then reduced with well-known reducing agents such as dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP), and capped with IAM and NEM derivatives containing a radiolabel [27] or fluorophore [28,29] for detection. The resulting proteome samples are subject to 2DE and imaged by autoradiography or fluorescence to visualize oxidized proteins [30]. DTT and TCEP treatment results in reduction of numerous cysteine oxoforms [31], including sulfenic and sulfinic acids, nitrosothiols, and disulfides, thereby allowing for the facile visualization of multiple oxoforms of cysteine within a biological sample. However, this method relies on the initial cysteine capping step proceeding to completion; reduced efficiency in this step often results in false positives. Furthermore, comprehensive visualization of all oxidized proteins are limited by the resolution of 2DE, which results in co-elution of proteins with similar physicochemical properties, as well as the limit of detection afforded by the imaging methods, which hinders detection of low abundance oxidized proteins.

To enable direct comparison of the degree of oxidation in two samples, differential labelling with orthogonal fluorophores in a method known as redox differential in-gel electrophoresis (redox-DIGE) was developed [32,33]. This method allows for facile visualization of differences in oxidized protein content across samples, but is limited by the inability to inform on the fraction of protein oxidized, since information on total protein levels is lacking. Differential labeling of reduced and oxidized cysteines with orthogonal fluorescent dyes helps to circumvent this problem by providing the fraction of oxidized protein [34]. However, this method loses the advantage of multiplexing, since each sample needs to be analyzed independently. A variety of methods exist for