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REVIEW

Progress of Analytical Methods for Protein Cysteine Post-translational Modifications

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Abstract: Cysteine thiols have high reaction activity as sites of nucleophilic, redox catalysis, metal binding and allosteric regulation, and play significant roles in protein structures and functions. Due to the reactivity of the thiol group, cysteine residues are very prone to post-translational modifications (PTMs) such as oxidation, lipidation, and so on, which can regulate/damage protein functions, and are associated with many diseases. Thus, it is very important to qualitatively and quantitatively analyze PTMs in the cysteine residues for further understanding its biological functions. This review mainly focuses on the development of mass spectrometric and high-throughput proteomic approaches for investigating some common cysteine post-translational modifications.

Key Words: Cysteine; Post-translational modifications; Mass spectrometry; Proteomics; Review

1 Introduction

Since 1980s, with the advent of numerous soft ionization techniques, notably matrix-assisted laser desorption/ionization (MALDI)[1] and electrospray ionization (ESI)[2] methods, biological mass spectrometry (MS) technique has been rapidly developed. It serves as a high-throughput, high-sensitivity and high-resolution analytical method for the studies of life science, including protein's post-translational modifications (PTMs), and plays a critical role in the progress of modern biological techniques. MS analysis can provide not only species and sites information for the modified proteins, but also quantitative information about the modification and their relative change, which further deepens our understanding of PTMs. Cysteine (Cys, C) is one of the least abundant amino acid residues (approximately 1%–2%)[3] with high reactive activity as nucleophilicity and redox sensitivity. Cysteine residues often play key roles in active sites for redox catalysis, binding metals and allosteric regulation, which further participate in various biological events including cell recognition, signal transduction, and so on[4]. Due to their high sensitivity to minute changes in the surrounding environment, a multitude of non-enzymatic or enzyme-catalyzed modifications can arise on the cysteine residues to rapidly and dynamically regulate proteins' structures and activities, even with causing impairment of proteins' function, which is closely linked to many human diseases. Therefore, studying cysteine modifications shows great significance. However, the dynamic range of protein abundance is wide in complex biological samples, while proteins/peptides containing modified cysteine residues are mostly low-abundant and their signals are usually suppressed in MS analysis, thus specific enrichment is often required for effective detection. In addition, a variety of cysteine modifications with different chemical natures are often unstable during sample preparation and MS analysis, thus it is necessary to develop new and appropriate MS methods. Different ion fragmentation techniques, such as collision induced dissociation (CID), high energy collision dissociation (HCD), electron capture dissociation (ECD) and electron transfer dissociation (ETD), have their own characteristics and provide distinctive information about fragment ions in the study of PTMs. Herein, this review focuses on the research progresses of some common cysteine modifications analyzed by MS and

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proteomic methods (As shown in Fig.1), including redox-dependent modification (such as nitrosylation, cysteine oxidation and glutathionylation), lipid-derived electrophiles (LDEs) adducts (e.g. 4-hydroxy-2-nonenal, HNE) and lipidation (such as palmitoylation and prenylation).

2 Redox-dependent PTMs

Oxidation of cysteine residues is an important mechanism for cellular signal transduction. It is inevitable to produce highly reactive species during aerobic metabolism, such as "reactive nitrogen species" (RNS) and "reactive oxygen species" (ROS), which play important roles in cellular signal transduction pathways and homeostasis. On the other hand, excessive production of ROS and RNS can lead to oxidative stress and even impair cellular antioxidant defenses, which are closely associated with human diseases such as cardiovascular diseases and cancers.

2.1 S-Nitrosylation

Owing to its high reactivity, nitric oxide (NO) can bind to cysteine residues of target proteins to form S-nitrosothiols (S-NO), termed protein S-nitrosylation, which is a reversible and redox-based covalent modification. The S-nitrosylation functions in diverse biological systems and is associated with human diseases[5].

However, detection of protein S-nitrosylation is a challenging task because of its low abundance and the labile nature of the S-NO bond. When analyzed by MALDI-MS[6], S-NO bonds are more prone to fragmentation than peptide backbones and give rise to NO loss. Under relative mild conditions such as ESI-MS, peptides containing NO groups could be detected sometimes[7]. Hao *et al*[8] directly detected S-nitrosylated proteins and their modification sites by MS using the neutral loss of NO group. Wang *et al*[9] found that suitable MS parameters (e.g. cone voltage and collision energy) and buffers were necessary to preserve the labile S-NO bonds. To avoid the neutral loss of NO, Beuve *et al*[10] converted SNO-Cys to biotin-Cys using *N*-[6-(biotinamido) hexyl]-3'-(2'-pyridyldithio) propionamide (Biotin-HPDP) and then analyzed using different MS/MS fragmentation modes (CID and HCD). ETD induces fragmentation of large, multiply-charged cations such as proteins and peptides by transferring electrons to them, which can cause cleavage of peptide backbone into *c*- and *z*-ions while leaving labile PTMs intact. So it has been more and more widely used to investigate a various of PTMs including S-nitrosylation[11]. However, these approaches are limited to analyze simple samples such as recombinant proteins.

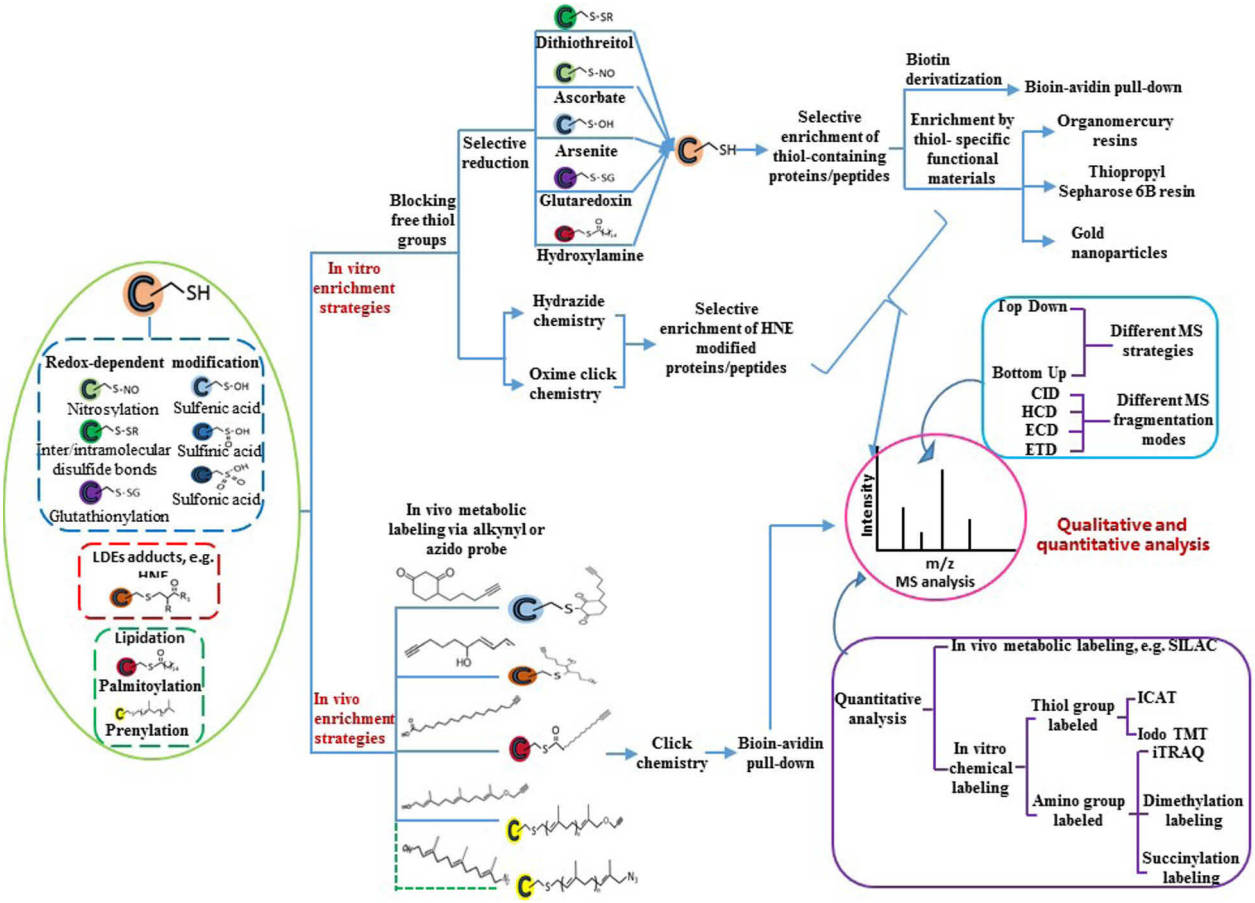

Fig.1 General strategies for mass spectrometric (MS) analysis of different cysteine post-translational modifications (PTMs) discussed in this review