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Review

Oxidative stress-mediated N-terminal protein modifications and MS-based approaches for N-terminal proteomics

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ABSTRACT

The N-termini of peptides and proteins can be subjected to highly diverse modifications, including acetylation, myristoylation, pyroglutamylation, and epimerization. These modifications affect protein stability, localization, and activity as well as alter the chemical properties of the N-terminus. Oxidative stress is known to induce the direct oxidation of amino acid side chains and peptide backbones in proteins. Alternatively, polyunsaturated fatty acids can be oxidized to lipid hydroperoxides, which further decompose to form highly reactive aldehydes such as 4-oxo-2(*E*)-nonenal (ONE) and 4-hydroxy-2(*E*)-nonenal (HNE). ONE and HNE modify various amino acid residues and induce protein cross-linking. However, there have been few studies on oxidative stress-mediated N-terminal modifications and the resulting functional changes. Our recent studies have reported several novel N-terminal modifications that result in the formation of α-ketoamide, transamination, cyclization, and epimerization. These novel N-terminal modifications are the focus of this review. We also outline recent advances in approaches for N-terminal analysis, which have been developed over the last several decades.

Contents

Abbreviations: AA, L-ascorbic acid; $A\beta_{1-11}$, amyloid β 1–11; Ala, alanine; Ang, angiotensin; Ang C, N-terminal cyclized-Ang II; Ang P, pyruvamide-Ang II; APA, aminopeptidase A; Arg, arginine; Asp, aspartic acid; AT_1, Ang II type 1; BSA, bovine serum albumin; COFRADIC, combined fractional diagonal chromatography; Cys, cysteine; DICAS, dimethyl isotope-coded affinity selection; ESI, electrospray ionization; Glc, D-glucose; Gln, glutamine; Glu, glutamic acid; Gly, glycine; Hb, hemoglobin; His, histidine; HNE, 4-hydroxy-2(*E*)-nonenal; HSA, human serum albumin; LC, liquid chromatography; Lys, lysine; MALDI, matrix-assisted laser desorption ionization; Met, methionine; $[M+H]^+$, protonated molecule; MS, mass spectrometry; MS/MS, tandem mass spectrometry; ONE, 4-oxo-2(*E*)-nonenal; pGlu, pyroglutamic acid; PITC, phenylisothiocyanate; PLP, pyridoxal 5′-phosphate; PM, pyridoxamine; Pro, proline; PSD, postsource decay; PTH, phenylthiohydantoin; PTM, post-translational modification; ROS, reactive oxygen species; SCX, strong cation exchange; Ser, serine; TAILS, terminal amine isotope labeling of substances; TMPP, trimethoxyphenyl phosphonium; TNBS, 2,4,6-trinitrobenzenesulfonic acid; TOF, time-of-flight; Val, valine.

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1. Introduction

Oxidative stress is caused by alterations in balance between the formation of reactive oxygen species (ROS), their removal by antioxidant systems, and damage repair by enzymes such as methionine (Met) sulfoxide reductase and disulfide reductase [1]. Increased ROS production is associated with a number of inflammatory and age-related degenerative diseases [2,3]. Highly reactive and unstable ROS can directly react with proteins, resulting in the oxidation of amino acid side chain Met and cysteine (Cys) [4]. The oxidation of lysine (Lys), arginine (Arg), and proline (Pro) residues in the presence of metal ions and hydrogen peroxide causes the formation of semialdehyde amino acids, and is known as direct protein carbonylation [5]. Polyunsaturated fatty acids in membrane phospholipids are the primary targets of ROS. The highly reactive aldehydes produced by ROS-mediated lipid peroxidation can be just as damaging as the initial ROS. 4-Oxo-2(*E*)-nonenal (ONE) and 4-hydroxy-2(*E*)-nonenal (HNE) have been identified as the most abundant, toxic, and reactive lipid-derived aldehydes [6,7], and they are involved in protein dysfunction and altered gene regulation through the modification of amino acid residues and protein cross-linking [7]. Upon reaction with proteins, ONE and HNE form Michael addition products with the nucleophilic amino acids, Cys, histidine (His), and Lys [7]. ONE can also modify Arg [8] and Lys [9] through the formation of a Schiff base intermediate to produce a substituted imidazole and a stable 4-ketoamide, respectively. In addition, unusual modifications by ONE or HNE can be derived from specific amino acid sequences and conformations of peptides or proteins. For example, ONE recognizes the specific amino acid motifs of His^{75}, alanine (Ala)[76], and Lys^{77} in bovine histone H4 and forms a cyclic structure [10]. HNE produces dehydrated Michael addition products upon reaction with His^{6} in angiotensin (Ang) II because of the specific conformation of Ang II [11].

The amino-terminal (N-terminal) region of peptides and proteins is an important area for co-translational and post-translational modifications (PTMs). In addition to altering the chemical properties of the N-terminus, these modifications influence the stability, localization, regulation and activity of peptides and proteins [12]. N-Terminal acetylation (Fig. 1) is an abundant PTM in eukaryotes. The enzyme complex of acetyltransferase catalyzes the transfer of acetyl groups to the N-termini of proteins during translation. N-Terminal acetylation predominantly occurs at the N-terminal Met residue. Alternatively, the N-terminal Met can be cleaved off by aminopeptidases followed by acetylation of the second residue when the second amino acid is a less bulky serine (Ser), Ala, or threonine [13]. Early studies demonstrated that N-terminal acetylation would protect proteins from premature degradation [14,15]. In contrast, it was recently proposed that N-terminal acetylated Met residue might act as a degradation signal [16,17]. Protein N-terminal myristoylation (Fig. 1) results in the irreversible addition of a myristic acid (C14:0) to the N-terminal glycine (Gly) residue [12]. N-Myristoyltransferase is responsible for transferring myristate from myristoyl-CoA to the N-terminus of the protein. Several studies have indicated that post-translational N-myristoylation occurs upon caspase-mediated cleavage during apoptosis [18,19]. The formation of N-terminal pyroglutamic acid (pGlu) (Fig. 1) is catalyzed by either glutaminyl cyclase or isoglutaminyl cyclase, which convert N-terminal glutamic acid (Glu) or glutamine (Gln) into pGlu, respectively. N-Terminal pGlu modulates the biological activities of peptide hormones, neuropeptides and chemokines [20]. It has also been shown to protect proteins from degradation by aminopeptidases [21]. In the case of amyloid β peptides, N-terminal pGlu enhances their hydrophobicity and stability, leading to accelerated aggregation [22]. Therefore, the reduction of N-terminal pGlu through inhibition of glutaminyl cyclase is considered a new therapeutic strategy for the treatment of Alzheimer's disease [23]. Epimerization of amino acids is also regarded as a PTM that can cause conformational changes and alter the biological activity of proteins [24]. D-Amino acids arising from epimerization have been detected in human and animal tissues [24,25] and are associated with certain diseases such as cataracts [25] and Alzheimer's disease [26], although epimerization occurs only on aspartic acid (Asp), Ser, and Pro. N-Terminal epimerization

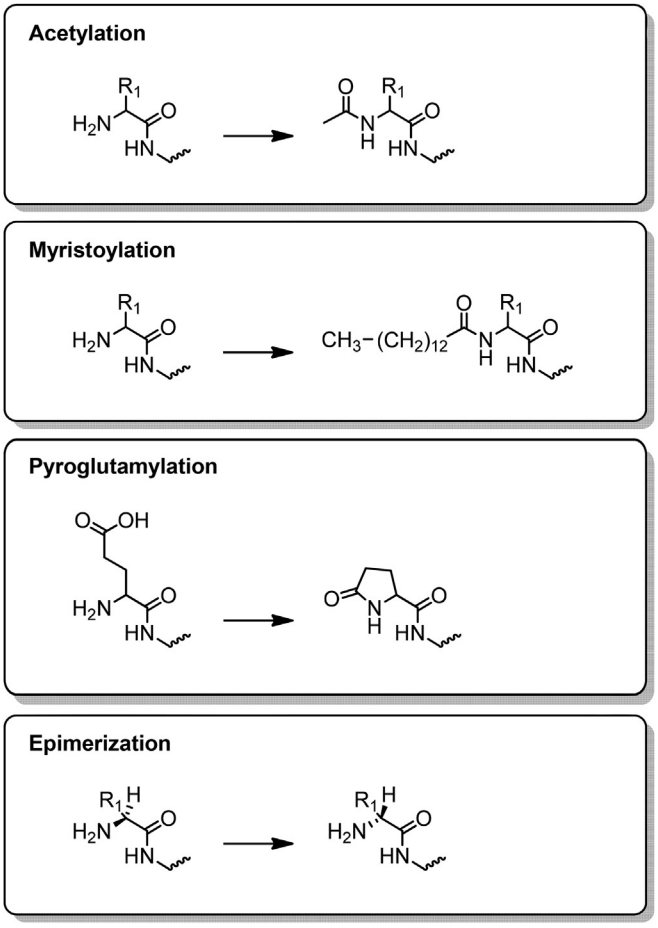

Fig. 1. Examples of N-terminal modifications: acetylation, myristoylation, pyroglutamylation, and epimerization.