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Mini Review

Nitrosative protein tyrosine modifications: biochemistry and functional significance

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Nitrosative modifications regulate cellular signal transduction and pathogenesis of inflammatory responses and neurodegenerative diseases. Protein tyrosine nitration is a biomarker of oxidative stress and also influences protein structure and function. Recent advances in mass spectrometry have made it possible to identify modified proteins and specific modified amino acid residues. For analysis of nitrated peptides with low yields or only a subset of peptides, affinity 'tags' can be bait for 'fishing out' target analytes from complex mixtures. These tagged peptides are then extracted to a solid phase, followed by mass analysis. In this review, we focus on protein tyrosine modifications caused by nitrosative stresses and proteomic methods for selective enrichment and identification of nitrosative protein modifications. [BMB reports 2008; 41(3): 194-203]

INTRODUCTION

Nitration and nitrosylation of tyrosine and cysteine residues are mediated by reactive nitrogen species (RNS) produced during development, aging, and oxidative stress. Increases in RNS production result from excess or deregulated nitric oxide (·NO) reacting with reactive oxygen species (ROS). ·NO, as a signaling molecule, is generated during inflammation by neutrophils and phagocytes, and it reacts with superoxide ($\cdot O_2^-$) to generate RNS, including peroxynitrite ($ONOO^-$) and ·NO itself, which in turn reacts with tyrosyl radicals to add $-NO_2$ or -NO to tyrosine residues, forming 3-nitrotyrosine or 3-nitrosotyrosine residues, respectively (1, 2). These reactive species of nitrogen and oxygen modify biomolecules, including DNA, lipids, and proteins (3). Such modifications affect signal transduction pathways and cellular processes, including inflammatory disease pathogenesis, cancer, and age-related disorders. High levels of RNS and impaired antioxidant enzymatic systems are responsible for diverse chemical modifications of target proteins (4).

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Elevated levels of protein tyrosine nitration are indicated in the pathogenesis of various age-related neurodegenerative diseases. Indeed, protein tyrosine nitration is a biomarker of oxidative stress and the molecular signal of $ONOO^-$ formation during development, oxidative stress, and aging. However, the lack of easily available means to identify modified proteins and residues has been a roadblock in understanding the potential mechanistic contributions of such modifications to acute and chronic inflammation.

Mechanisms of protein tyrosine nitration

The nitration of tyrosine residues to form 3-nitrotyrosine (3-NT) can be a marker of $ONOO^-$ formation and permanent loss of protein function. Tyrosine nitration, a covalent modification, adds a nitro group ($-NO_2$) to one ortho carbon of tyrosine's phenolic ring. An alternative nitrosative modification on tyrosine residues is the reaction of a tyrosyl radical with ·NO to form 3-nitrosotyrosine, which is in turn oxidized by a two-electron oxidation step to form 3-NT. Formation of 3-nitrosotyrosine is an alternative pathway to form the 3-NT observed in transition metal-containing proteins, such as prostaglandin endoperoxide synthase (PHS) and cytochrome c (5-7). Since nitration of tyrosine residues is prevented *in vivo* by the action of strong physiological reductants, such as glutathione, the formation of 3-nitrosotyrosine followed by 3-NT circumvent the reducing environment *in vivo* (7). Interestingly, 3-nitrosotyrosine in angiotensin and bovine serum albumin molecules generated by tetranitromethane (TNM) treatment is stable enough to be identified by ESI-MS/MS following enzymatic digestion of TNM-treated proteins (8).

Protein tyrosine nitration dramatically changes protein structure and function due to a shift in the pKa of the tyrosine hydroxyl group from 10.1 to 7.2 (9). This introduces a net negative charge to the nitrated tyrosine at physiological pH, thus altering structural properties and catalytic activity of the protein in the local chemical environment. Protein tyrosine nitration, which covalently modifies the phenolic ring, may also affect tyrosine phosphorylation. There has been increasing interest in the effects of tyrosine nitration on changes in protein structure in diverse pathogenesis (10).

Among the three isoforms of nitric oxide synthase, inducible

nitric oxide synthase (iNOS) plays the major role in producing nitric oxide (·NO) that mediates inflammatory responses. During pathological conditions, the expression of iNOS is strongly up-regulated at the transcriptional level (11). ·NO produced from L-arginine by iNOS contributes to the nitrosative modifications on tyrosine residues. Under pathological conditions with elevated ·NO or superoxide (·O_2^-), the powerful oxidizing and nitrating $ONOO^-$ is generated through a rapid, diffusion-controlled reaction of ·NO and ·O_2^- (1, 12). Peroxynitrite is an extremely short-lived chemical species ($<$ 10 ms *in vivo*), which can damage cells or tissues by reacting with several chemicals, including metal centers and CO_2, via a one- or two-electron oxidation. While $ONOO^-$ does not react directly with tyrosine residues, it can yield potent oxidizing and nitrating species, including hydroxyl radical (·OH) and nitrogen dioxide (·NO_2) as a result of homolytic cleavage of the protonated form of $ONOO^-$, ONOOH (Fig. 1 ①) (13). Since the pKa of $ONOO^-$ is 6.8, about 20 % of $ONOO^-$ becomes protonated to peroxynitrous acid (ONOOH), which is more unstable than $ONOO^-$ at physiological pH (14). The resulting radicals remove hydrogen from the hydroxyl group of tyrosine residues, promoting tyrosyl radical formation. The tyrosyl radical then adds nitrogen dioxide to form 3-NT.

The proton-mediated decomposition of $ONOO^-$ is a relatively slow process, so $ONOO^-$ has a higher chance of reacting with strong Lewis acids, including CO_2 and metal ions, in heme proteins (15). The reaction of $ONOO^-$ with CO_2 generates an intermediate adduct, nitroso-peroxocarboxylate ($ONOOCO_2^-$), which spontaneously decomposes to carbonate (·CO_3^-) and nitrogen dioxide (·NO_2) radicals (16). Therefore, CO_2 might contribute to the activity of ONOO– in nitrating tyrosine residues at physiological pH (Fig. 1 ②) (17).

$ONOO^-$ is not the sole source of 3-NT formation *in vivo*. Nitrogen dioxide, nitrous acid, nitryl chloride, and certain peroxidases (18) derived from inflammatory cells can mediate the nitration of tyrosine to form 3-NT. For example, nitrite (NO_2^-) is a primary autoxidation product of ·NO (19). Nitrite is then further oxidized to form nitrogen dioxide, which is a nitrating species, by the action of peroxidases, such as myeloperoxidase and eosinophil peroxidase, which are heme proteins abundantly expressed in activated leukocytes. The resulting nitrogen dioxide nitrates tyrosine residues in the presence of H_2O_2 (Fig. 1 ③) (20).

Factors that influence direct site- and protein-specific nitration

Protein tyrosine nitration exhibits a certain degree of selectivity, and not all tyrosine residues are nitrated. To be biochemically relevant, the targeted nitration of certain tyrosine residues must be correlated with the structure-function relationship of nitrated proteins. The nitration of protein tyrosine residues could dramatically change protein structure and consequently alter their function (1, 21, 22). Tyrosine nitration sites are localized within specific functional domains of nitrated proteins (23). For example, the nitrated sites (356Y and 366Y) of sphingosine-1-phosphate lysase 1 (SIP lysase 1) are located in the catalytic domain of the protein, and nitration of leukocyte immunoglobulin-like receptor subfamily A member 4 (LIRA4) occurs in the antigen-interacting domain (23). In addition, nitration of Rho-GTPase-activating protein 5 (RHOGAP5) at 550Y, which is located between two domains, FF and Rho-GAP, might affect signal transduction pathways mediated by Rho-GTPase (23). In general, tyrosine residues are specifically targeted for nitration *in vivo*.

The factors that control site- and protein-specificity in tyrosine nitration have recently been reviewed (24). First is the proximity to the site of nitrating agent generation. Second is the abundance of the protein and the number of tyrosine residues within a single protein. Third is the amino acid sequence or a specific nitrating environment.

Tyrosine nitration occurs at inflammatory sites within specific cell types (25, 26), suggesting that the proximity to the sites of nitrating agent formation is critical to inducing site- and protein-specific tyrosine nitration. Intra- or extra-cellular distribution of nitrated proteins confirmed by electron microscopy also provides information on the sub-cellular localization where nitration occurs. For instance, nitrated proteins were detected in mitochondria during disease and also in Lewy bodies in dystrophic neuritis (27). Thus, nitrating agents generated from dysfunctional mitochondria nitrate the tyrosine residues in proximal proteins.

The peroxynitrite-mediated nitration of Mn superoxide dismutase (Mn-SOD) is an additional example of intramolecular proximity regulating site-specific nitration. During inflammatory

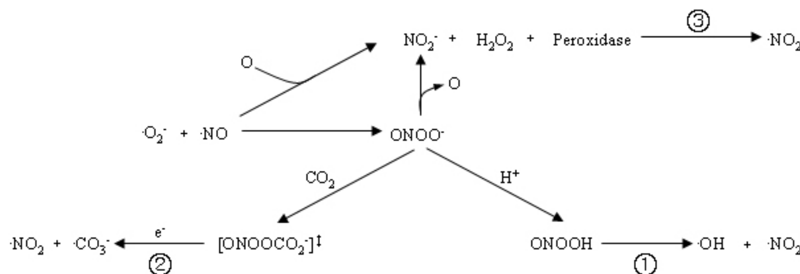

Fig. 1. Pathways of $ONOO^-$-mediated protein nitrating agent, ·NO_2, generation. $ONOO^-$ is generated by the diffusion-controlled reaction of ·NO and ·O_2^- radicals. The generated $ONOO^-$ can lead to oxidation with Lewis acids, CO_2, and physiological protons resulting in ·NO_2. Under inflammatory conditions, heme peroxidases are activated and catalyze the oxidation of nitrite (NO_2^-) in the presence of H_2O_2 to form ·NO_2.

responses, $ONOO^-$ reacts with the metal center of Mn-SOD to generate reactive free radicals. The production of such nitrating agents, which is close to Tyr34 in the active site of the enzyme, facilitates site-specific nitration (28, 29).

Protein tyrosine nitration usually occurs near acidic residues in loop regions (24, 30) in areas free of steric hindrances. Also, the presence of amino acids that compete for nitrating agents proximal to tyrosine residues, including tryptophan (Trp), cysteine (Cys), and methionine (Met), may prevent tyrosine nitration by removing the nitrating agents (31). The location of tyrosine residues in favorable environments for nitration within the secondary and tertiary protein structure may also influence site-specific nitration. Tyrosine residues are frequently located within carboxylic acid-rich environments (24), and the neighboring negative charge on the carboxyl group forms hydrogen bonds with the adjacent hydroxyl group of tyrosine within 2 - 4 Å. These electrostatic forces may affect the local concentration of nitrating agents proximal to tyrosine residues for directing site-specific nitration.

In addition, the presence of transition metal centers and binding sites for heme peroxidase increase peroxynitrite-mediated nitration (20, 24, 31). Interactions between positively charged myeloperoxidase and negatively charged clusters of a target protein can also promote protein nitration (20).

Affinity tags for enrichment of target analytes

The complexity of biological samples and diversity of information to be gleaned are both vast, probably due to post-translational modifications (PTMs) and large dynamic ranges in protein expression levels, ranging from 10^4 in yeast to $10^9 \sim 10^{12}$ in plasma (32). Today, instrumental and technological advances, including bioinformatics tools and mass spectrometry, make it possible to analyze crude proteome mixtures and thousands of proteins simultaneously. Nonetheless, the analysis of low abundant peptides or subsets of peptides remains a challenging task with regard to sensitivity and accuracy. Therefore, much of the work in this field uses affinity 'tag,' which can bait target analytes from complexes. Only a subset of proteolytic peptide fragments of a specific protein or protein complex are selectively labeled with tags, which can specifically interact with other biological and chemical moieties on solid supports such as polymeric beads and inorganic (nano) particles (Fig. 2). Some tags can covalently react with chemical counterparts on a solid support and can later be released on demand by experimental procedures. These tagged peptides are extracted to the solid phase, followed by mass analysis. This affinity tagging strategy not only isolates a desired analyte from the remainder of the sample, but also allows enrichment (i.e. concentration) of a desired analyte for subsequent mass analysis, thus rendering higher sensitivity and signal/noise values.

Three different enrichment methods are widely used, depending on tagging and isolation schemes. First, one can harness interactions between biomolecules, such as protein-protein interactions (ex. antibody-antigen interactions) and protein-ligand interactions (ex. lectin-carbohydrate interactions). Second, immobilized metal-ion affinity chromatography (IMAC) (33) utilizes metal chelating interactions between positively charged metal ions bound to solid supports via iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA) and chelating groups on tagged analytes. The interaction between metal ions on solid supports and chelators on analytes is not intrinsically covalent, but is strong enough to maintain the interaction through the isolation steps. The tagged analytes can be easily released from the solid support by salt, pH adjustment, or competing chelators. A similar method is to use intrinsically non-covalent chemical bonding. Metal oxides and boronic acids have been used for phosphorylated peptides and glycoproteins, respectively. Another method to enrich tagged analytes uses chemical modifications, resulting in covalent bond formation between the analyte and the tag. For instance, β-elimi-

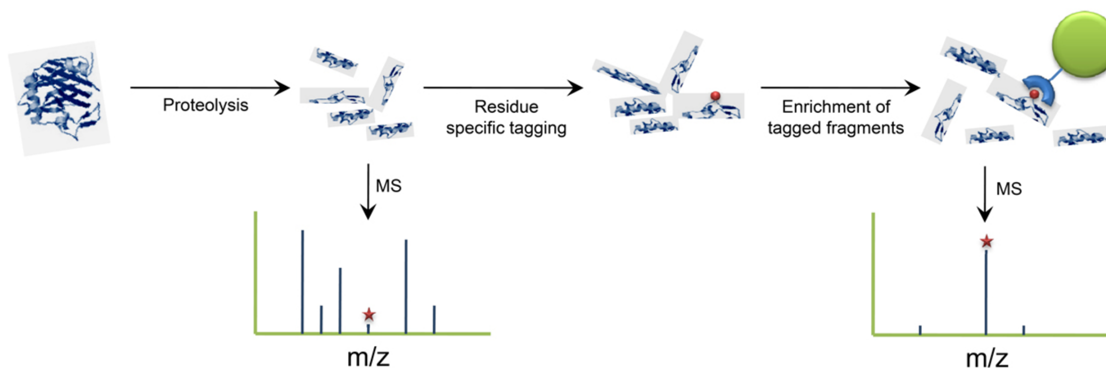

Fig. 2. Specific tagging and enrichment strategies for mass analysis of a subset of peptides in a proteolytic mixture. A subset of proteolytic fragments are selectively labeled with tags that can specifically interact with biological or chemical moieties. Solid supports, such as polymeric beads and inorganic (nano) particles, are coated with those moieties, and hence selectively isolate the tagged peptides. The enriched peptides undergo subsequent mass analysis, rendering higher sensitivity and signal-to-noise values.

nation/Michael addition for phosphate groups and disulfide formation for cysteine residues are used for proteolytic complex analysis and PTM site identification.

Enrichment methods of proteins and peptides for mass analysis (34)

Enrichment approaches using tags can be categorized into two groups: residue-specific and PTM-specific strategies. Residue-specific methods employ the chemical reactivities of certain amino acid side chain functional groups to enrich peptides. In this context, the thiol group of the cysteine side chain has a unique chemoselectivity that is used to label peptide fragments containing cysteine. Thiols can react efficiently and selectively with α-iodoacetate/iodoacetamide under physiological conditions. In addition, thiols can effectively be modified to disulfides, exchange with other thiol containing molecules, and then be released to free thiol by reducing agents such as dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP). A number of cysteine-specific tagging strategies have been introduced for enrichment and isolation, based on the chemical reactivities of thiol (35-38). For instance, Liu *et al*. described a quantitative cysteinyl-peptide enrichment technology (QCET) where cysteine-containing peptides were captured by a thiol-specific covalent resin, released by DTT, and analyzed by mass spectrometry (39). Brittain *et al*. reported an intriguing method for enrichment and mass analysis of peptide subsets using 'fluorous' affinity tags (40). As a tagging step, a tryptic digest of bovine serum albumin was treated with perfluorooctylpropyl iodoacetamide to selectively perfluorinate cysteine-containing peptides. The fluorous peptide subset was readily separated from other mixture components by a fluorous-functionalized silica gel because of strong fluorine-fluorine interactions (41). Similarly, enrichment strategies can target the chemical reactivities of additional amino acid side chains (42, 43). The combined fractional diagonal chromatography (COFRADIC) approach is based on the concept of diagonal chromatography, in which the collected fraction of the 'primary' run is subjected to chemical or enzymatic modifications of certain residues, thereby changing the polarity of the peptides (44, 45). In the 'secondary' run, the modified peptides can be identified and collected because of the change in retention times of the chromatographic separations due to the changes in polarity, while the unmodified peptides in the fraction come off at identical retention times.

PTMs are chemical alterations of proteins that may explain extremely diverse protein activities in living organisms. Because PTMs are accompanied with mass changes, for example an 80 Da increment for phosphorylation, the characterization of PTMs on a certain protein or on a subset of proteins is possible using mass spectrometry with proteolytic digestions, if necessary. This approach is challenging because the characterization of PTMs using mass analysis is severely hampered by the complexity of post-translationally modified samples and is susceptible to the incoherence of the components' physicochemical properties. As discussed above, the introduction of affinity tags and subsequent enrichment of post-translationally modified peptides is a plausible solution for PTM characterization in complicated samples, including immunoprecipitation of phosphorylated components with specific antibodies, particularly phosphotyrosine (46). IMAC can also enrich phosphorylated fractions, by taking advantage of the affinity of chelated Fe(III) or Ga(III) for phosphate groups (47). Recently, metal oxides have emerged as alternative materials for phosphate group enrichment since the capability of IMAC to prevent non-specific adsorption remains an issue despite extensive washing procedures (48). Affinity tags can be introduced to phosphoserine or phosphothreonine groups using chemical conversions. Oda *et al*. successfully biotinylated and enriched phosphorylated residues by generating a double bond through β-elimination of phosphate groups under basic conditions, followed by Michael addition of a dithiol to the resulting double bond and successive treatment with a biotinylation reagent (49). In the case of glycosylated proteins, lectins, and carbohydrate binding proteins are widely used as affinity tag for solid phase extraction (50). Glycoproteins can also be captured on solid supports presenting hydrazides, which form an essentially covalent bond with aldehydes, and analyzed by MS (51). Cis-diol groups of carbohydrates are specifically oxidized by periodate-yielding aldehydes, which are subsequently coupled with hydrazide groups on solid supports, leading to the extraction of glycoproteins on the solid support.

As a non-specific enrichment method, Yang and colleagues reported selective absorption and separation of desired peptides, enrichment, and isolation of low abundant peptides based on nano-zeolite materials (52, 53). In addition, they developed a particle-free enrichment method in which $CaCO_3$ nanoparticles were derivatized with poly(methyl methacrylate), and then the $CaCO_3$ core was destroyed for MALDI analysis after peptide extraction (54).

Enrichment methods of nitrated peptides for mass analysis

There are no effective enrichment methods for nitrated peptides, probably because of the poor chemical reactivity of nitric oxide groups. However, nitric oxide groups can be converted to amine groups, which are chemically reactive and hence can be used as a chemical handle to employ tagging groups. In this regard, Crowley *et al*. described an analytical method for nitrotyrosine, in which nitric oxide was reduced to an amino group by dithionite (55). The resulting aminotyrosine was then treated with heptafluorobutyric anhydride, resulting in a fluoracyl derivative, which has excellent gas chromatographic and electron-capturing properties. The subsequent GC/MS analysis showed a detection limit as low as 400 amol of 3-aminotyrosine. Nikov *et al*. reported a method for the detection and characterization of low abundance nitrotyrosine-containing peptides existing in a complex mixture (56). As a model, nitrated human serum albumin was treated with DTT,

and the resulting free thiols were blocked with iodoacetamide. Reduction of nitrotyrosine with sodium dithionite resulted in aminotyrosine, then cleavable biotin tags were selectively anchored to the aminotyrosines, and the protein sample was proteolyzed. The biotinylated peptides were extracted and enriched on the solid support, which presented streptavidin. Next, DTT treatment released the nitrated peptides from the solid support and the eluted peptides were analyzed by MALDI-TOF mass spectrometry. Zhang *et al*. introduced an improved enrichment strategy by hooking thiol groups, which have better chemical reactivity than nitric oxide and amine, to nitrotyrosine (57). They then reduced nitrotyrosine to aminotyrosine, which was derivatized with N-succinimidyl S-acetylthioacetate (SATA). Treatment with hydroxylamine revealed free thiol groups by removing an acetyl group from thioacetate, and the free thiol groups were specifically incorporated into thiopropyl sepharose beads, essentially affording enrichment of nitrotyrosine-containing peptides. The enriched peptides were released and analyzed by LC-MS/MS, showing 6～10 times more nitrotyrosine-containing peptides identified than

Table 1. Selected nitrated proteins in various diseases

Protein	Biological functions	Diseases	References
	Cellular Compartment		
	Extracellular space		
Fibrinogen	Signal transduction	Coagulation disorder	(71)
Plasminogen	Plasmin activity	Diabetes, atherosclerosis	(70)
Surfactant protein A	Lipid transporter activity	Inflammation	(79)
	Nucleus		
p130 adhesion protein	Protein binding	Stroke, neurodegenerative disorders	(80)
Cu, Zn-SOD	Negative regulation of apoptosis	Neurodegenerative disorders	(74)
p53 tumor suppressor	Transcription factor activity	Cancer	(72)
Histones	DNA binding	Cancer	(73)
Profilin	Regulation of transcription from RNA polymerase II promoter	Platelet dysfunction	(81)
	Cytoplasm		
Insulin receptor substrate-I	Transmembrane receptor protein kinase docking	Diabetes	(82)
Glutamine synthetase	Glutamate-ammonia ligase activity	Sepsis, liver disease	(83)
iNOS	Nitric-oxide synthase activity	Inflammation	(75)
Histone deacetylase II	Chromatin modification	Inflammation	(84)
Profilin	Regulation of transcription from RNA polymerase II promoter	Platelet dysfunction	(81)
Tau protein	Exocytosis	Alzheimer's disease	(85)
Alpha synuclein	Central nervous system development	Parkinson's disease	(86)
Alpha-enolase	Glycolysis	Alzheimer's disease	(86)
Triosephosphate isomerase	Glycolysis	Alzheimer's disease	(86)
	Mitochondria		
Carnitine palmitoyltransferase I	Acyltransferase activity	Septic myocardial dysfunction	(87)
Creatine kinase	Creatine kinase activity	Myocardial infarction	(88)
GAPDH	Glycolysis	Cardiovascular and neurological diseases	(89)
Mn-SOD	Superoxide dismutase activity	Neurodegenerative disorders	(76)
Nicotinamide nucleotide transhydrogenase	Electron transport	Inflammation, shock, ischemia	(90)
Cytochrome c	Aerobic respiration	Inflammation, shock, ischemia	(91)
ATP synthase complex 5	Hydrogen ion transporting ATP synthase activity	Inflammation, shock, ischemia	(92)
	Plasma membrane		
SERCA2A	Cation transport	Myocardial infarction	(93)
Amiloride-sensitive Na+ channel	Ion channel activity	Inflammation	(7)
	Cytoskeleton		
Desmin	Cytoskeleton organization and biogenesis	Chronic heart failure	(94)
Tubulin	Protein polymerization	Shock, ischemia	(77)
Neurofilament L	Structural constituent of cytoskeleton	Amyotrophic lateral sclerosis	(95)
Actin	Muscle thin filament assembly	Endothelial dysfunction	(78)
	Endoplasmic reticulum		
Prostacyclin synthase	Fatty acid biosynthetic process	Endothelial dysfunction, diabetes	(96)

global analysis of unenriched samples.

Mass spectrometry of nitrated proteins

Protein tyrosine nitration is typically a low-yield process and requires sensitive analytical methods for characterization. Nevertheless, the measurement of protein tyrosine nitration is usually performed by immunoblot analysis using an anti-3-nitrotyrosine antibody (58, 59) in two dimensional PAGE separation and Western blot analysis, followed by mass spectrometric identification of immunopositive protein spots (60). Until recently, however, the lack of specific enrichment and sensitive identification methods for nitrated residues has prevented the analysis of such low-level protein modifications.

Recently, tandem mass spectrometry has been applied to the identification of nitrosative modification of tyrosine residues (61, 62). These approaches made it possible to identify several potentially important modification sites (60, 63, 64). For the mass spectrometric analysis of nitrated target proteins, proteins of interest were digested into peptides by site-specific proteases, such as trypsin, GluC, and chymotrypsin, similar to general proteomic techniques, followed by mass spectrometry. In the case of MS analysis of tyrosine-nitrated peptides, a characteristic mass increase of 45 Da of the nitrated peptides was observed by both electrospray ionization (ESI) (63) and matrix-assisted laser desorption ionization (MALDI) methods (61). During MALDI analysis of the nitrated peptides, two additional modified peaks with mass shifts of +13 Da and +29 Da were observed (65-68). These ions are generated during the ionization process with UV-laser irradiation and correspond to the loss of one or two oxygen atoms from the nitro group that are added to the tyrosine residues (65-68). The characteristic loss of oxygen atoms was not observed in ESI-MS. A mass increase of 29 Da observed in ESI-MS was due to the addition of a nitrosyl group (-NO, +29 Da) to the target tyrosine residue, unlike the photodecomposition of nitrate groups on tyrosine residues (8).

Protein tyrosine nitration under pathophysiological states

Aberrant protein tyrosine nitration contributes to inflammatory diseases and age-related cardiac and neurodegenerative diseases (69). Since several efficient enrichment and mass spectrometric methods are now available, a number of studies have successfully characterized protein nitration sites in various diseases (Table 1).

Human plasma proteins, such as plasminogen (70) and fibrogen (71) are nitrated in diabetes and coagulation disorders, respectively. Among the nitrated proteins found in cell nuclei, p53 (72), histone (73), and Cu, Zn-SOD (74) regulate apoptosis and tumor growth. Many of the cytosolic proteins with nitrated tyrosine residues are also implicated in diseases such as diabetes, inflammation, and neurodegenerative diseases. Interestingly, increased iNOS expression and concomitant nitrotyrosine formation correlate with many inflammatory responses (75). The pathologies summarized in Table 1 are also associated with mitochondrial dysfunction, resulting in significant nitration of mitochondrial proteins. A well-documented example is the *in vivo* nitration and inactivation of MnSOD (76), which serves as a mitochondrial antioxidant enzyme, under inflammatory disease conditions and in neurodegenerative diseases. Many cytoskeleton proteins are also modified by nitrosative stress. Nitration of α-tubulin significantly alters microtubule formation and structure during ischemic conditions (77). Actin contains many tyrosine residues critical to actin polymerization, and nitration of actin induces stabilization of actin nucleus and filament formation, resulting in the contractile dysfunction of cardiomyocytes (78).

Taken together, these results suggest that the extent of the nitrosative modification, nitration, correlates with various pathogenic states in humans, although such modification may also play important roles in metabolic homeostasis, the endothelium, and immune system activation under normal physiological conditions.

Conclusions

Protein tyrosine nitration is an important posttranslational modification, occurring in a variety of diseases. The extent of protein tyrosine nitration, regulated by the presence of a strong reducing system (e.g. glutathione), can be a biomarker of oxidative stress in the pathogenesis of acute and chronic inflammatory responses. We outlined recent studies to identify nitrosatively-modified proteins on a whole proteome scale. We focused on the enrichment methods for low abundant peptides and peptides with specific modifications. Proteomic-based methods have successfully been used to characterize the biological consequences of such modifications. However, only a limited number of affected target proteins have been identified; therefore, more sensitive and effective analytical methods, such as specific enrichment and mass spectrometry, must be developed to precisely characterize the effects of nitration in human disease.

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