Proteomic Analysis of S-Nitrosoylated Proteins in Mesangial Cells*

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NO participates in numerous biological events in a variety of cell types including activated glomerular mesangial cells. Many of these events appear to be independent of the known effects of NO on soluble guanylyl cyclase. NO derived from all major isoforms of NO synthase can S-nitrosylate cysteine residues in target proteins, potentially altering their functional activities. Recent evidence suggests that S-nitrosylation is specific, is regulated, and may play an important regulatory role akin to phosphorylation. In the present study, the “biotin-switch” method of isolating S-nitrosylated proteins was coupled with two-dimensional PAGE protein separation followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and peptide mass fingerprinting to identify target proteins for S-nitrosylation in murine mesangial cells treated with NO donors or appropriate controls. This approach resolved 790 protein spots. We analyzed the most abundant spots and identified 34 known proteins. Of these, 31 are unique S-nitrosylated proteins not previously identified, including signaling proteins, receptors and membrane proteins, cytoskeletal or cell matrix proteins, and cytoplasmic proteins. Prominent among these were peroxisome proliferator activated receptor γ, uroguanylin, GTP-binding protein α, protein 14-3-3, NADPH-cytochrome P450 oxidoreductase, transcription factor IIα, melusin, mitosin, phospholipase A2-activating protein, and protein-tyrosine phosphatase. The in vivo induction of S-nitrosylation was assayed by treating mesangial cells with interleukin-1β followed by the biotin-switch and Western blot of selected targets. These results broaden our knowledge of potential signal transduction pathways and other cell functions mediated by NO S-nitrosylation. Molecular & Cellular Proteomics 2: 156–163, 2003.

NO is synthesized from l-arginine by the nitric-oxide synthase (NOS)1 isozymes. Neuronal NOS and endothelial NOS isozymes have restricted tissue distributions and are regulated in part by intracellular Ca2+ transients. Inducible nitric-oxide synthase (iNOS) is expressed in a number of cell types in mammals after induction by cytokines and/or lipopolysaccharide, and once expressed, it is active at resting levels of intracellular Ca2+ (1). In the kidney, NO plays prominent roles in the homeostatic regulation of glomerular, vascular, and tubular function as well as a variety of fundamental cellular functions, including DNA replication, transcription, energy metabolism, and apoptosis (1–3). Recent studies provide clear evidence for participation of iNOS-generated NO in the induction, progression, or protection of several types of experimental and human glomerulonephritis. In human glomerulonephritis, iNOS gene expression has been described in glomerular mesangial cells as well as in local and infiltrating macrophages (4). Glomerular mesangial cells play a central role in maintaining the structure and function of the glomerular capillary ultrafiltration apparatus and, under physiological or pathological conditions, regulate the amount and composition of the surrounding extracellular matrix. Despite their prominent role in glomerulonephritis and the evidence for NO-mediated injury, little is known about the protein targets of NO in these cells upon cytokine activation.

NO exerts its actions by chemical modification of targets, preferentially interacting with thiol groups, transition metals, and free radicals. S-Nitrosylation of cysteine residues in target proteins is a principal reaction of NO and of several NO-derived species. This redox-based post-translational modification has been implicated in the cGMP-independent control of a broad spectrum of cellular functions in a variety of cell types (5). A growing number of proteins has been found to undergo S-nitrosylation in vivo, including hemoglobin (6), creatine kinase (7), glyceraldehyde-3-phosphate dehydrogenase (8), the N-methyl-d-aspartate receptor (9) and ryanodine receptors (10), and several caspases (11), resulting in altered function. In addition, S-nitrosylation appears to be a mechanism for signal transduction in cells. For example S-nitrosylation of NF-κB p50 modifies its function in mediating changes in expression of target genes (12). Gow et al. (13) provided

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1 The abbreviations used are: NOS, nitric-oxide synthase; iNOS, inducible NOS; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MS, mass spectrometry; GSNO, S-nitrosothiolene; PPAR, peroxisome proliferator activated receptor; biotin-(2-pyridyldithio)propionamide; DM, dystrophia myotonica; 2D, two-dimensional; IL, interleukin; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; SHP-2, Src homology 2-containing phosphotyrosine phosphatase-2; TF, transcription factor; RFG, RET fused gene; ADAM, a disintegrin and metalloproteinase; MBL, mannose-binding lectin; MASP, MBL-associated serine protease.
evidence that Ca\(^{2+}\), growth factors, and developmental transitions regulate S-nitrosylation in diverse tissues. The identification of the full complement of S-nitrosylated proteins and the functional consequences of this modification is essential for understanding the mechanisms of action of NO and the signaling events that arise from its release.

Recently Jaffrey et al. (14) reported a proteomic approach, termed the “bistin-switch” method, to detect proteins that are S-nitrosylated in vitro and in vivo. The method involves the substitution of a bistin group at every Cys sulfur modified by nitrosylation. Biotinylated proteins are then purified by avidin-affinity chromatography or identified by immunoblotting. Using this method in mice, these authors identified 16 proteins S-nitrosylated by NO derived from neuronal NOS (14). In the present study, we identified 31 novel protein targets of S-nitrosylation in NO-treated and cytokine-activated murine mesangial cells using the bistin-switch method combined with two-dimensional (2D) gel electrophoresis and MALDI-TOF MS, and here we discuss their potential relevance to iNOS biology and mesangial cell function.

**EXPERIMENTAL PROCEDURES**

**Reagents**—S-Nitrosoglutathione (GSNO), S-nitroso-N-acetylpenicillamine, GSNO, and GSH were from Sigma. IL-1β was from R&D Systems. NeutrAvidin-agarose, biotin-HPDP, polyacrylamide 6000 desalting columns, and goat anti-biotin were from Pierce. Hybond-P membranes and ECL reagents were from Amersham Biosciences. Biospin-6 columns and the BCA protein concentration assay were from Bio-Rad. Antibody directed against p38 MAPK was from BIOMOL Research Laboratories (Plymouth Meeting, PA). Anti-NADPH-cytochrome P450 oxidoreductase antibody was from Accurate Chemical & Scientific Corp. (Westbury, NY). Anti-uroguanylin antibody was from Calbiochem.

**Cell Culture**—Mouse mesangial cells (ATCC CLR-1927) were cultured at 37°C in complete medium (Dulbecco’s modified Eagle’s medium supplemented with 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 5% fetal bovine serum). In some experiments, cells were treated with 10 ng/ml IL-1β to induce iNOS expression.

**Sample Preparation for Proteomic Analysis**—To prepare extracts of cytosolic and membrane-associated proteins, cells were harvested by scraping, resuspended in 20 volumes of HEN buffer (25 mM HEPES, pH 7.9, 1 mM EDTA, 0.1 mM neocuproine) containing 0.4% CHAPS, and allowed to swell for 15 min at 4°C. The cells were centrifuged at 2000 × g for 5 min at 4°C, and the pellet was washed in HEN buffer. The supernatant was recovered, and the particulate fraction was pelleted by centrifugation at 4°C. The resulting samples were used immediately or stored at −80°C until used.

**In Vitro S-Nitrosylation**—The procedure detailed by Jaffrey et al. (14) was followed in detail with a few modifications. The cytosolic and particulate fractions were used immediately or stored at −80°C until use. The treated proteins were incubated with 20 μM methyl nitrosothiols were then acetone-precipitated with 2 volumes of −20°C acetone, or biotin-HPDP was removed by desalting columns. After centrifugation, the pellet was resuspended in 0.1 ml HEN buffer containing 1% SDS/mg of protein in the initial protein sample. Two volumes of Neutralization buffer (20 mM HEPES, pH 7.7, 100 mM NaCl, 1 mM EDTA, and 0.5% Triton X-100) were added, and 15 μl of NeutrAvidin-agarose/mg of protein used in the initial protein sample were added. The biotinylated proteins were incubated with the resin for 1 h at room temperature. The resin was extensively washed in 10 volumes of Neutralization buffer containing 600 mM NaCl. Bound proteins were then eluted in a solution containing 20 mM HEPES, pH 7.7, 100 mM NaCl, 1 mM EDTA, and 100 mM 2-mercaptoethanol. The samples were then mixed in SDS sample buffer.

**Two-dimensional Gel Electrophoresis and Gel Image Analysis**—Immediately after affinity separation by the bistin-switch method, the purified proteins were suspended in a buffer containing 8 mM urea, 2 mM thiourea, 1% Triton X-100, 1% dithiothreitol, and 1% ampholytes pH 3–10 (15). An aliquot of 100 μg of protein isolated by the bistin-switch method was loaded onto an 11-cm isoelectric focusing strip, pH 4–7. Focusing was conducted on isoelectric focusing cells at 250 V for 20 min followed by a linear increase to 8000 V for 2 h. The focusing was terminated at 20,000 V-h. Strips were then equilibrated in 375 mM Tris buffer, pH 8.8, containing 6 M urea, 20% glycerol, and 2% SDS (16). Fresh dithiothreitol was added to the buffer at a concentration of 30 mg/ml. Fifteen minutes later, fresh buffer was added containing 40 mg/ml iodoacetamide. Strips were equilibrated for an additional 15 min and then loaded onto the second dimension using Criterion gradient gels (Bio-Rad) with an acrylamide gradient of 10–20%. Gels were then stained using SyproRuby fluorescent dye (17). The results of digital fluorescent image analysis of gel images from samples treated with NO donors were compared with their respective control samples using PDQuest software (Bio-Rad). Analysis included spot detection and comparison of protein patterns using internal protein standards as landmarks.

**Tryptic Digestion, MALDI-TOF MS, and Peptide Mass Fingerprinting**—For protein spot cutting and MS identification, gels were loaded with 200 μg of the proteins isolated by the bistin-switch method. Following software analysis, unique spots were excised from the gel using the ProteomeWorks robotic spot cutter (Bio-Rad). In-gel spots were robotically digested on a MultiPROBE II (Packard Instrument Co.) as follows. Gel spots were washed twice in 100 mM NH₄HCO₃ buffer followed by incubation at 37°C for 5 min, aspiration of the acetonitrile, and drying of the gels for 30 min. Rehydration of the gels using 20 μg/ml trypsin (Promega, Madison, WI) suspended in 25 mM NH₄HCO₃ buffer followed by soaking in 100% acetonitrile for 5 min, aspiration of the acetonitrile, and drying of the gels for 30 min. Rehydration of the gels using 20 μg/ml trypsin (Promega, Madison, WI) suspended in 25 mM NH₄HCO₃ buffer followed by incubation at 37°C for 14–20 h. The digested peptides were extracted twice using a solution of 50% acetonitrile and 5% trifluoroacetic acid for 40 min. Peptide extracts were desalted and concentrated using Zip-tips (Millipore, Bedford, MA) and robotically placed on MALDI chips using SymBiot I (Applied Biosystems, Foster City, CA). Mass spectral analyses were conducted on a MALDI-TOF Voyager DE PRO mass spectrometer (Applied Biosystems). Data searches were performed using the National Center for Biotechnology Information (NCBI) protein database with a minimum matching peptides setting of 4, a mass tolerance setting of 50–200 ppm, and a single triysin miss cut setting.

**Western Analysis**—Proteins isolated by the bistin-switch method were also resolved by SDS-PAGE, electropholeted to Hybond-P membranes, and immunoblotted with primary antibodies as indicated in the text and figure legends. Bound antibody was visualized by the ECL chemiluminescence detection system (Amersham Biosciences) using peroxidase-conjugated sheep anti-mouse or goat anti-rabbit IgG as appropriate.
RESULTS

An understanding of the functional consequences of S-nitrosylation requires knowledge of the identity of the proteins that are modified. To determine the proteomic profile of S-nitrosylated proteins in mesangial cells, we used the biotin-switch method combined with a proteomic approach. Cytoplasmic extracts were treated with NO donors or control chemicals GSH and GSSG, and the resulting S-nitrosylated proteins were purified using the biotin-switch method. The purified proteins (100 μg) were separated by 2D gel electrophoresis (isoelectric focusing plus SDS-PAGE), the gels were stained with SyproRuby, and digital fluorescent image analysis was performed. Two-dimensional patterns of the resulting gels were then matched by computer analysis. On separate gels loaded with 200 μg of the proteins isolated by the biotin-switch method, selected protein spots were digested with trypsin, and tryptic peptides were analyzed by MALDI-TOF MS. Database inquiries with the peptide masses then identified the specific proteins.

Under basal conditions in the absence of NO donors, relatively few peptide spots were evident on 2D gels of the biotin-switch protein isolates (Fig. 1A). Similarly only a few peptide spots were evident in the negative control samples first treated with GSH and then subjected to the biotin-switch assay (data not shown). In contrast 790 protein spots were evident in the samples treated with GSNO (Fig. 1B). The protein pattern of the 2D gel in Fig. 1B reveals that most of these proteins were detected in the acidic region of the pH gradient. A similar pattern and number of protein spots were identified in the GSSG-treated samples (data not shown) in keeping with the biochemistry of the biotin-switch method and published reports of S-glutathiolation of target cysteine residues in proteins that are also S-nitrosylated (18–21). To identify the S-nitrosylated proteins of interest in the NO donor-treated samples, additional 2D gels were produced with the same cellular extracts obtained by the biotin-switch method and stained as described under “Experimental Procedures.” Forty-four protein spots of interest were then excised from the gels, digested with trypsin, and subsequently analyzed by MALDI-TOF mass spectrometry. The resulting spectra were used to identify the tryptic peptide mass fingerprints (Fig. 1B and Table I). Of these 44 spots excised from the gels, 35 S-nitrosylated proteins, 34 of which were known proteins, were unambiguously identified (Table I). The proteins identified were members of specific functional families including receptors and membrane proteins, signaling proteins, cytoskeletal and cell matrix proteins, and cytoplasmic proteins (Table I). Three of these, glycerinaldehyde-3-phosphate dehydrogenase, caspase-6, and dimethylarginine dimethylaminohydrolase, have been previously reported to be S-nitrosylated in other systems (8, 22, 23).

To determine whether representative proteins identified as susceptible to S-nitrosylation by NO donors in vitro (Table I) are bona fide targets for S-nitrosylation in vivo, protein extracts were prepared from mesangial cells that had been treated for 24 h with or without IL-1β (10 ng/ml), a stimulus known to induce de novo iNOS expression and high output NO generation in these cells (24). Mesangial cells do not express any NOS and do not generate NO under basal conditions. These protein extracts were subjected to the biotin-switch assay, the S-nitrosylated proteins were purified on NeutrAvidin-agarose, and Western blots of the purified S-nitrosylated proteins were prepared. The blots were probed with antibodies specific for three selected proteins identified...
by mass fingerprinting in the in vitro nitrosylation experiments. As seen in Fig. 2, mesangial cells stimulated to produce NO by treatment with IL-1β exhibited evidence for S-nitrosylation of PPARγ, uroguanylin, and NADPH-cytochrome P450 oxidoreductase. These results indicate that these proteins, found to be S-nitrosylated by the exogenous addition of NO donors to mesangial cell extracts, are also S-nitrosylated endogenously by iNOS-derived NO within the intact cell.

**Table I**

*Analysis by MALDI-TOF MS of the tryptic peptide profiles of the protein spots followed by search in the NCBI database*

<table>
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<tr>
<th>SSP</th>
<th>NCBI ID</th>
<th>NCBI accession no.</th>
<th>Molecular mass</th>
<th>pl</th>
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<tr>
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<td>4</td>
<td>14-3-3 ε</td>
<td>5803225</td>
<td>29.2</td>
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<td>31.8</td>
<td>6.68</td>
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<td>Phospholipase A2-activating protein</td>
<td>110808M</td>
<td>35.8</td>
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<td>70.3</td>
<td>6.04</td>
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<tr>
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<td>Cytochrome P450 oxidoreductase</td>
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<tr>
<td>32</td>
<td>TFIIA-like factor</td>
<td>12963759</td>
<td>51.5</td>
<td>4.56</td>
</tr>
</tbody>
</table>

**III: cytoskeleton and cell matrix proteins**

| 8   | β-Tropomyosin | 346655 | 32.8 | 4.64 |
| 18  | Melusin | 7305191 | 38.8 | 4.79 |
| 19  | Annexin A5 | 13277612 | 35.7 | 4.83 |
| 33  | Otoconin-95 precursor | 4176764 | 52.4 | 4.76 |
| 34  | α1(XI) collagen | 12082365 | 50.1 | 4.46 |
| 35  | α1(XI) collagen | 12082365 | 50.1 | 4.46 |

**IV: cytoplasmic proteins**

| 10  | Glycerol-3-phosphate dehydrogenase | 10048296 | 33.8 | 5.33 |
| 13  | Dimethylarginine dimethylaminohydrolase 1, putative | 1284568 | 31.4 | 5.64 |
| 31  | Similar to α internexin neuronal intermediate filament protein | 17390900 | 55.4 | 5.35 |

**V: transcription factors**

| 22  | Peroxisome proliferator-activated receptor γ | 514307 | 54.5 | 6.23 |
| 23  | Peroxisome proliferator-activated receptor γ | 514307 | 54.5 | 6.23 |
| 24  | Zinc finger protein 297B | 23397003M | 52.6 | 5.4 |
| 32  | TFIIA-α/β-like factor | 12963759 | 51.5 | 4.56 |

*a Sample spot.*
Protein Targets of Nitric Oxide in Mesangial Cells

**Proteomic studies** represent a powerful complement to transcriptomic studies because they allow assessment of the expressed protein and potential post-translational modification. S-Nitrosylation is such a post-translational modification, and it has become increasingly recognized as a ubiquitous, specific, and reversible regulatory reaction potentially comparable to phosphorylation or acetylation (5, 13). Since S-nitrosylated proteins often serve as major effectors of NO-related bioactivity in various cell types and tissues, identification of the full complement of proteins that can be modified in this manner has been an emerging area of investigation.

In the course of glomerular injury, mesangial cells are exposed to endogenously produced NO and to NO generated from local phagocytes (4, 25). Thus NO and derived species could potentially exert important regulatory control on mesangial cell function via S-nitrosylation of proteins involved in a variety of cellular processes. To identify proteins uniquely sensitive to S-nitrosylation, we incubated mesangial cell lysates with the NO donor GSNO. GSNO is present in the membrane guanylate cyclase C and signals through cGMP as a second messenger (31). Mesangial cells are a primary target of natriuretic peptides, and these peptides are thought to play a role in regulating glomerular filtration rate.

Protein tyrosine phosphatase, non-receptor type 11, or SHP-2, a member of a small subfamily of cytosolic protein-tyrosine phosphatases, was also S-nitrosylated. SHP-2 is ubiquitously expressed and is involved in the cellular response to growth factors, hormones, cytokines, and cell adhesion molecules. Recent studies showed that bradykinin inhibits mesangial cell proliferation by a novel mechanism involving a direct protein-protein interaction between the bradykinin B2 receptor and SHP-2 (32). Defects in myotonic dystrophy (DM-kinase) result in myotonic dystrophy. NO synthase activity is required for the transcription factor MyoD to induce DM-kinase (33). Since this kinase is localized to gap junctions (34), S-nitrosylation of DM-kinase may play a role in cell-cell communication.

The release of NO seems to protect a significant number of proteins from ubiquitination and degradation mediated by ubiquitin ligase A3 (30). The mechanism of this NO-mediated protection is unclear, but it presumably requires S-nitrosylation (35). Our evidence of S-nitrosylation of ubiquitin ligase A3 could account for this protective action. Conversely, INOS itself is subject to ubiquitin-dependent degradation (36).

In this report, we show that a K⁺ channel-interacting protein KchIP4A (37) is S-nitrosylated. A recent report indicates the activation of K⁺ channels by NO (38). Three proteins involved in phospholipid metabolism and cell signaling, choline kinase, phospholipase A₂-activating protein, and otoconin-95, were also S-nitrosylated in mesangial cells. Early reports indicated that IL-1 partially inhibits the choline kinase activity in tumors (39), while it induces the synthesis of phospholipase A₂-activating protein (40). The role of otoconin-95 in the kidney has not yet been identified. The protein is a homologue of the phospholipase enzyme phospholipase A₂.
protein of the utricular and saccular otoconia, and is expressed by various non-sensory cell types (41).

Mitosin is a nuclear protein that associates with the mitotic apparatus, especially the kinetochore, during mitosis (42). Nitrosylation of mitosin could explain its signaling role in NO-mediated cell cycle progression. Proteins 14-3-3, an emerging family of proteins and protein domains that bind to serine/threonine-phosphorylated residues, were found to be S-nitrosylated. These proteins regulate key proteins involved in various physiological processes such as intracellular signaling, cell cycling, apoptosis, and transcriptional regulation (e.g., FKHRL1, DAF-16, p53, TAZ, TLX-2, and histone deacetylase) (43). These proteins also act as adaptor molecules, stimulating protein-protein interactions, and regulate the subcellular localization of proteins (43, 44). Annexin V belongs to a family of calcium-binding and phospholipid-binding proteins and participates in multiple biological processes such as the regulation of calcium concentration and certain endothelial functions (44). This protein was also identified as an S-nitrosylated protein in mesangial cells. Recently GSNO was found to inhibit the activity of the related protein annexin II tetramer (45).

Several proteins that regulate transcription were found to be S-nitrosylated. The human general transcription factor TFIIA is one of several proteins involved in specific transcription by RNA polymerase II, possibly by regulating the activity of the TATA-binding subunit of TFIIID (46). PPARγ is a member of the nuclear hormone receptor superfamily of ligand-dependent transcription factors. PPARs play an important role in transcription of cells including glucose and lipid homeostasis, cell cycle progression, differentiation, inflammation (47), and extracellular matrix remodeling. PPARγ is known to be expressed in mesangial cells (48) and to be up-regulated in the presence of glomerular injury, including diabetic glomerular disease (49). PPARγ has also been shown to trans-repress iNOS expression in murine macrophages (50, 51). It is intriguing to speculate about the existence of a feedback inhibition cycle in which NO derived from iNOS in activated mesangial cells and perhaps macrophages S-nitrosylates PPARγ, which, so modified, becomes competent to trans-repress iNOS gene expression. NO has been shown to inactivate zinc finger proteins (52), most likely by S-nitrosation of thiols in zinc-sulfur clusters leading to reversible disruption of zinc finger structures. Evidence of S-nitrosylation of the zinc finger protein 297B presented here could provide an additional molecular mechanism underlying the regulation of gene transcription. RFG (RET fused gene), a chimeric oncogene coactivator that results from a structural rearrangement between the ELE1 and the RET genes, was also found to be S-nitrosylated. Transducin β-like 1 protein, a ubiquitously expressed WD-40 repeat-containing protein, partners with nuclear receptor co-repressor and histone deacetylases to form the SMRT (silencing mediator of retinoid and thyroid hormone receptor) corepressor complex that directs diverse repressor pathways (53–55). Neither RFG nor transducin β-like 1 protein has been reported to be expressed in mesangial cells.

A number of cell matrix and cytoskeletal proteins were S-nitrosylated. These include the actin-binding protein β-tropomyosin, which is thought to stabilize actin filaments and influence aspects of F-actin, α-intemexin, a type IV intermediate filament protein, which may act as a scaffold for the formation of neuronal intermediate filaments during early development, drebrin, an F-actin-binding protein typically expressed in neuronal cells (56), and testase 3, a member of the ADAM (a disintegrin and metalloproteinase) family of metalloproteinases (57). ADAM-15 has been shown to participate in mesangial cell migration (58), but a similar role for testase 3 has not yet been demonstrated. Although members of the ADAM family have not been reported previously to be S-nitrosylated, matrix metalloproteinase-9 was recently shown to be activated by S-nitrosylation, resulting in the formation of a stable sulfenic or sulfonic acid with pathological activity in neurons (59). Melusin interacts with β-integrin and presumably plays a role in protein-protein interactions, and having a cysteine-rich domain, it is a strong biological candidate for S-nitrosylation by NO. The β-integrins play a role in mesangial cell adhesion, migration, survival, and proliferation (60). Collagen XI regulates the assembly of cartilaginous matrices by co-polymerizing with collagen II trimers. Collagen plays a critical role in thrombosis by enhancing platelet aggregation through its interaction with von Willebrand factor. S-Nitrosylation of collagen α1(XI), prothrombin (coagulation factor II), and annexin V may contribute to the ability of NO to inhibit platelet aggregation.

Mannose-binding lectin (MBL) is a serum protein of the innate immune system that circulates as a complex with a family of MBL-associated serine proteases (MASPs). Complexes of MBL-MASP-2 activate the complement system. MASP-3, which we show to be S-nitrosylated, down-regulates the C4 and C2 cleaving activity of MASP-2 (61). NADPH-cytochrome P450 oxidoreductase is an essential component of the microsomal P450 mixed function oxidase system, mediating electron transfer from NADPH to cytochrome P450s and several other acceptors. Thus, the ability of NO to S-nitrosylate this protein may impact a variety of signaling pathways.

Several factors may govern the specificity of S-nitrosylation for selected targets. Cysteine-rich proteins could be more susceptible to S-nitrosylation, and the spatial relationship of NO sources and targets within the cell has been shown to be important for the specificity of this post-translational modification (62). A Cys-based motif for S-nitrosylation has been proposed based on screenings of large databases: XHRKD-ECDE, where X can be any amino acid (63). The motif has been postulated to impart a degree of specificity for S-nitrosylation. However, other cysteine residues in each protein could also be candidates for nitrosylation based on tertiary structural conformation. The possibility also exists that S-nitrosyl-


