ELSEVIER

Cardiovascular Research 75 (2007) 220–228

Cardiovascular Research

www.elsevier.com/locate/cardiores

Review

Signalling by NO-induced protein S-nitrosylation and S-glutathionylation: Convergences and divergences

Antonio Martínez-Ruiz [a,b,*], Santiago Lamas [a,*]

[a] Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas and Instituto "Reina Sofía" de Investigaciones Nefrológicas, Ramiro de Maeztu, 9, Madrid E-28040, Spain

[b] Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Melchor Fernández Almagro, 3, Madrid E-28029, Spain

Received 16 January 2007; received in revised form 15 March 2007; accepted 19 March 2007

Available online 24 March 2007

Time for primary review 15 days

Abstract

The role of nitric oxide in several signalling routes has been clearly established. In recent years increasing attention has been paid to its ability to produce covalent protein post-translational modifications in conjunction with other reactive oxygen and nitrogen species. Among these, the modification of cysteine residues has been shown to be of particular importance due to the functional relevance of many of them. In this review, we focus on the modification of the cysteine thiol by incorporation of a NO moiety (S-nitrosylation) or of a glutathione moiety (S-glutathionylation). Both modifications are produced by different reactions induced by nitric oxide-related species. We discuss the differences and similarities of both modifications, and their relationships, in regard to the biochemical mechanisms that produce them, including the enzymatic activities that may catalyze some of them and their subcellular compartmentalization. Even when biochemical knowledge is one step ahead of the demonstration of their pathophysiological relevance, we also describe the potential role of both modifications in several processes in which both post-translational modifications are involved.

© 2007 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Nitric oxide; Glutathione; Oxidative stress; Post-translational modifications

1. Nitric oxide and glutathione as conveyors of signals

As research on signal transduction has evolved, many pathways based on the successive involvement of defined proteins have been described. These are related one to another in a more or less clearly defined order that can be attributed to specific patterns of interaction among the components of the pathway. In many of these pathways, small molecules are involved as messengers, interacting with relatively specific receptors, and hence they can be included as defined elements of the pathway. Also, protein post-translational modifications such as phosphorylation are a common mechanism for many pathways, but the specificity relies on the enzymes participating in the pathway.

Nitric oxide (NO), is considered a "classical" second messenger in signalling pathways, as it interacts by means of a thoroughly described reversible coordination bonding with its specific target, soluble guanylate cyclase (sGC). This interaction changes the conformation of sGC and activates it to produce cyclic GMP (cGMP), another small molecule that gives way to the activation of specific kinases. Indeed, other roles of NO rely on a similar kind of reversible coordination bonding that can change or modulate protein activities; one of the best characterized is the competition with O_2 for cytochrome c oxidase, leading to the inhibition of mitochondrial respiration.

Nitric oxide is a free radical, which makes it very unstable compared to many other chemical species (although it is relatively stable compared to other free radicals). This implies not only that it is rapidly decomposed but that, as it is able to

* Corresponding authors. Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu, 9, E-28040 Madrid, Spain. Tel.: +34 91 8373112x4302; fax: +34 91 5360432.

E-mail addresses: amartinez@cnic.es (A. Martínez-Ruiz), slamas@cib.csic.es (S. Lamas).

0008-6363/$ - see front matter © 2007 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.cardiores.2007.03.016

react with other species, it produces a variety of radical and non radical compounds, collectively named "reactive nitrogen species" (RNS). RNS can modify lipids and DNA, as well as different protein residues, producing post-translational modifications that can alter protein functions. Among these post-translational modifications, S-nitrosylation (also called S-nitrosation; for a discussion of the terminology see [1,2]) has been thoroughly studied in the last decade. It is believed to be easily formed and destroyed in cellular environments, and it is involved in several cellular processes, thus being considered as a feasible signalling mechanism. When compared to other signalling mechanisms like phosphorylation, it presents unique features, the main one being the fact that its formation and degradation depend on chemical reactions without the aid of enzymatic catalysis. Thus, some concepts like specificity are based on different biochemical bases, and hence it has been proposed that it may represent a new paradigm in signal transduction [1].

Many features of S-nitrosylation, conceived as a signal transduction mechanism are shared by oxidative modifications induced by the so-called "reactive oxygen species" (ROS), and by RNS itself. The change of paradigm has been discussed for both sets of modifications [3], although each modification has special features. In this regard, S-nitrosylation shows some parallelism with sulfenic acid formation, as it is easily reversible [4]. Sulfinic and sulfonic acid formation are more stable modifications, that have been considered as irreversible in cell systems until the recent description of reversion enzymatic mechanisms for sulfinic acid [5,6]. Protein disulfide bond formation is a key redox modification that has been studied at length; it is largely dependent not only on the redox environment but also on protein structure. S-thiolation, the formation of a mixed disulfide between a protein thiol and a low molecular mass thiol, is also a reversible process. As glutathione is the most abundant intracellular low molecular mass thiol and a key regulator of the redox state, S-glutathionylation, the formation of a mixed disulfide with glutathione, can be considered as the most important thiolation, as it has been also shown to be the most abundant [7–9]. In this review, we will explore the similarities, differences and commonalities between S-nitrosylation and S-glutathionylation, as both modifications share many mechanistic and functional features that make them solid candidates as general mechanisms for signal transduction within the new paradigm described.

2. Biochemical mechanisms involved in the formation of the modifications

There are several chemical mechanisms that can lead to the formation of both S-nitrosylation and S-glutathionylation in protein cysteine thiols. A number of *in vitro* studies, using low molecular mass thiols or different proteins, provide detailed information, which may help to extend studies to the cell environment. However, the key issue in cellular systems is the need to understand not only the mechanisms, but its potential occurrence in specific cellular compartments. We now describe some of these mechanisms and their relationship.

Formation of both modifications does not occur directly by addition of the species nitric oxide (·NO) or glutathione (GSH) to the cysteine thiol (P–S–H), as a redox reaction is needed by loosing one electron per sulphur atom (one in the case of nitrosylation and two in the case of glutathionylation), and every electron matters in chemistry. A direct reaction is only possible if a thyil radical is formed in the cysteine thiol (P–S·) that would easily react with nitric oxide, a radical itself,

$$\mathrm{P{-}S\cdot + \cdot NO \rightarrow P{-}S{-}NO}$$

or with the thyil radical of glutathione,

$$\mathrm{P{-}S\cdot + GS\cdot \rightarrow P{-}S{-}SG}$$

Probably, this is restricted to certain special protein environments, as it has been described for calbindin in the presence of copper [10].

Oxidized species are the most important candidates for modifying a reduced thiol. N_2O_3 is the higher oxidation state (one-electron oxidation) for nitrogen oxides, formed by the reaction of NO with O_2 or with peroxynitrite, or from acidified nitrite, and has been considered the quintessential S-nitrosylating species [11,12].

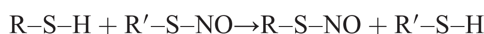
$$\mathrm{N_2O_3 + RSH \rightarrow RSNO + H^+ + NO_2^-}$$

Oxidized glutathione (GSSG) would be the equivalent for glutathione,

$$\mathrm{P{-}S{-}H + GS{-}SG \rightarrow P{-}S{-}SG + GSH}$$

and an increase in GSSG levels has been postulated as the main mechanism for S-glutathionylation. This mechanism provided a link with the concept that the GSH/GSSG pair is the central parameter when considering the redox state of the cell, and explained the higher S-glutathionylation levels observed after oxidative bursts [13–17]. However, evidences have accumulated that argue against significant involvement of GSSG in many cell situations, based mainly on the fact that GSSG levels do not increase and the GSH/GSSG levels are maintained [7,18–20].

Another mechanism that can theoretically share some common features is the transfer of the modification between two different thiols. In the case of S-nitrosylation, it is transnitrosation



$$\mathrm{R{-}S{-}H + R'{-}S{-}NO \rightarrow R{-}S{-}NO + R'{-}S{-}H}$$

and is a fast reaction that is likely to occur quite easily in cells [21,22]. However, the disulfide exchange between glutathione and another thiol (including S–glutathionylation by GSSG), what could be called "transglutathionylation",

$$\mathrm{R{-}S{-}H + R'{-}S{-}S{-}G \rightarrow R{-}S{-}S{-}G + R'{-}S{-}H}$$

is a slow reaction that is thermodynamically unfavoured based on typical redox potentials for cysteine residues [23]. This

implies that the formation of protein thiols by transnitrosation is likely to occur promptly, but can be easily reverted by other thiols, a circumstance that may explain in part the high lability of protein S-nitrosylation. In contrast, S-glutathionylation by GSSG is less likely to be a predominant mechanism if not helped by enzymes such as those described later, but the modification may become more stable in the presence of other thiols like glutathione itself (Fig. 1).

2.1. Modification by activation of protein cysteine thiols

An alternative mechanism for protein S-glutathionylation is the formation of "activated" forms of the cysteine residue thiol, which could "prime" the mixed disulfide formation. To express it "in redox terms", the electron loss in the thiol would occur in the cysteine thiols before the incorporation of GSH takes place. A good example is the formation of sulfenic acid, a two-electron oxidation that may result from the reaction with several oxidants, e.g. H_2O_2, and that can react with GSH to form the mixed disulfide [4]:

$$\mathrm{PSH + H_2O_2 \rightarrow PSOH + H_2O}$$

$$\mathrm{PSOH + GSH \rightarrow PS{-}SG + H_2O}$$

This mechanism has been described for protein tyrosine phosphatase 1B (PTP1B), whose active cysteine is reversibly inactivated when the sulfenic acid is formed, linking oxidant production with the phosphorylation status [24,25], and can be S-glutathionylated by GSH, preventing it from being overoxidized [26,27]. However, the structural data obtained reveal the formation of a sulfenilamide that could be the intermediate for S-glutathionylation [28,29].

In the case of S-nitrosylation primed by sulfenic acid, there has been a report of its formation at the active cysteine of PTP1B with H_2O_2 treatment [30]. This probably needs further confirmation, as the evidences for S-nitrosylation are based just on one method, the biotin switch. Hence, because there are many possible artifacts in the S-nitrosylation assays, the use of different complementary methods is recommended [1,31,32].

Fig. 1. Scheme depicting several of the chemical pathways leading to the formation of protein S-nitrosylation and S-glutathionylation. Horizontal dotted lines separate one-electron oxidative states. Dashed lines represent oxidations by oxidative species not depicted, including molecular oxygen (O_2). For the sake of simplicity, lines representing reaction of P-SH with GSSG or GSNO to yield P-SS-G have been omitted. GSH: reduced glutathione; GSNO; S-nitrosoglutathione; GSSG: oxidized glutathione.

2.2. S-nitrosylation as an intermediate for S-glutathionylation

The nitrosothiol itself represents another activated form of the protein cysteine thiol. It can react with GSH leading to S-glutathionylation.

$$\mathrm{PS{-}N{=}O + GSH \rightarrow PS{-}SG + HNO}$$

which is chemically equivalent to the S-glutathionylation produced by S-nitrosoglutathione (GSNO):

$$\mathrm{PSH + GSNO \rightarrow PS{-}SG + HNO}$$

Isolated analysis of each mechanism is very difficult, especially if we add the fast transnitrosation reaction between both types of nitrosothiols:

$$\mathrm{PSNO + GSH \leftrightarrows PSH + GSNO}$$

Thus, the role of S-nitrosothiols as primers for S-glutathionylation should be considered as a whole, with special emphasis given to GSNO as a reagent that can induce not only S-nitrosylation but also S-glutathionylation in proteins.

In experiments with yeast alcohol dehydrogenase, J.W. Park showed in 1998 that the enzyme was inactivated by GSNO, accompanied by the reduction of protein free thiols, and that it was reversible by 2-mercaptoethanol [33]. Based on additional experiments on reactivity of GSNO with Cys to give mixed disulfides, Park postulated that although there could be a fast transnitrosation reaction, the inactivation could be based on the glutathionylation of the protein thiol. Since then, S-glutathionylation has been described for many purified proteins treated with S-nitrosoglutathione (GSNO), or NO or other RNS plus GSH [34]. We have compiled and updated these reports, as well as proteins identified in cell studies, in Table 1.

In some proteins the "competition" between S-glutathionylation and S-nitrosylation has also been compared, showing that there are big differences among them regarding their tendencies to suffer each modification [35–39]. Once it is clear that both modifications are possible if GSNO is produced in the cell, the relative selectivity of each protein for both modifications will be determinant to discriminate which of the cellular proteins will be more easily or more stably modified by one or other modification. The functional relevance of the modifications can also become significantly different depending on the modification. For example, in transcription factors that bear a cysteine residue in the DNA binding domain that needs to be in the reduced state, both modifications impair this functionality, as it is the case for cJun in AP-1 or p50 in NF-kB [40–44]. Hence, the

Table 1
Reports since 1996 of proteins S-glutathionylated when treated with nitric oxide, GSNO or other RNS, either on purified proteins or in cells

Protein	Stimulus	References
p50	GSNO	[105]
Cathepsin K	GSNO	[107]
Aldose reductase	GSNO	[108,109]
Hemoglobin	GSNO	[105]
Creatine kinase	GSNO	[36,105]
Alcohol dehydrogenase	GSNO	[105]
Glycerol phosphate dehydrogenase	GSNO	[105]
Glutaredoxin	GSNO	[105]
Thioredoxin	GSNO	[105]
Carbonic anhydrase	GSNO	[35,105]
Glycogen phosphorylase b	GSNO	[35,105]
Glutathione reductase	GSNO	[110]
Ryanodine receptor	GSNO	[39,46,111]
Papain	GSNO	[38,112]
CFTR	GSNO	[113]
GAPDH	GSNO, decomposed GSNO	[38,105,114,115]
Cu,Zn-SOD	GSNO, decomposed GSNO	[105,115]
Actin	GSNO, cell treatments	[48]
c-Jun	GSNO, NO/GSH	[42,105]
Caspase-3	GSNO, SNAP+GSH	[102,104,105]
H-Ras	GSNO, $ONOO^-$	[35,37,89]
Microsomal GST 1	GSNO, $ONOO^-$/GSH	[116]
Neurogranin/RC3	GSNO, decomposed GSNO	[117,118]
Neuromodulin/GAP-43	Decomposed GSNO	[118]
Calbindin	Decomposed GSNO	[115]
BSA	Decomposed GSNO	[115]
Ca^{2+}-ATPase (SERCA)	$ONOO^-$	[83,85]
MMP-1,-8,-9	$ONOO^-$/GSH	[119]
Tyrosine hydroxylase	$ONOO^-$, NO_2	[120]
PTEN	CysSNO*	[121]
Hsp70	SNAP in cells, eNOS activation	[48]
Vimentin	eNOS activation	[48]
Adenine nucleotide transporter	iNOS transgenesis	[48]
ATP synthase α-subunit	iNOS transgenesis	[48]

*S-nitrosocysteine (CysSNO) treatment of cells; S-glutathionylation not directly shown, although compatible with the results.

differences between the two modifications will gain importance in terms of stability or potential reversibility. Another case is constituted by proteins in which the different cysteines are selective for different modifications, and these affect protein function in distinct manners. An example of this is the ryanodine receptor channel (RyR1), where it was established that there were different reactive thiols with selectivity for S-nitrosylation [45] or S-glutathionylation [39,46], each leading to specific functional consequences.

The work of several laboratories has led to the conclusion that treatment with nitrosothiols induces protein S-glutathionylation in cells. In NIH-3T3 cells and rat hepatocytes, both S-glutathionylation and S-cysteinylation were detected when millimolar concentrations of S-nitrosocysteine were used, although the S-nitrosylation estimated was much higher [47]. More recently, a very appealing study describes several evidences for NO-induced protein S-glutathionylation by nitrosothiol treatment of cells, eNOS activation in aortas and iNOS transgenetic expression in mice hearts; indeed, they identify two or three glutathionylation targets in each system, thus providing additional evidence about the potential in vivo ocurrence of the reaction [48].

3. Enzymatic mechanisms for the formation and breakdown of the modifications

Although S-nitrosylation and S-glutathionylation in cells can be explained by biochemical mechanisms, some enzymatic systems have been described that can accelerate some steps of their turnover. Regarding S-nitrosylation, and apart from the fact that certain protein targets have been described to be specifically assisted for their modification (reviewed in [49]), the main enzymatic process described so far is the GSNO reductase activity. It is exerted by the glutathione-dependent formaldehyde dehydrogenase (also called alcohol dehydrogenase class III) [50,51] and would presumably have a function of controlling the levels of intracellular GSNO and, indirectly, of protein S-nitrosothiols. This perspective adds even more lack of stability to the nitrosothiols formed in the cell, and it has been corroborated in several studies dealing with the nitrosothiol equilibrium.

Disulfide exchange is a slow chemical reaction that is accelerated in the intracellular environment by several enzyme families that mainly help to maintain the reduced state of intracellular thiols, both of proteins and of low molecular mass compounds like glutathione, by transferring the oxidizing equivalents to an acceptor cosubstrate. Thioredoxin (Trx) and glutaredoxin (reviewed in [17,52,53]) have been involved in the role of catalyzing breakage of protein mixed disulfides like S-glutathionylation. More recently sulfiredoxin, the enzyme that reverts protein sulfinic acid, has been added to this role [54]. A role in the formation could also be possible, as the reactions catalyzed by these enzymes are generally reversible, but then the cosubstrate should be predominantly in the oxidized form. This implies that GSSG would be the predominant form of the pair, in the case of glutaredoxin. In addition, glutathione S-transferase P has also been involved in S-glutathionylation formation [55].

4. Compartmentalization

Another important issue when considering the feasibility of both modifications in cells is subcellular compartmentalization. This is specially important due to the "multimolecular" and "submolecular" specificity derived from the chemical mechanisms described [1,3]. Furthermore, the short lifespan of some of the reactive species needs to be taken into consideration. This implies that the probability of the reaction between certain reactive species and certain proteins will be higher if the protein is in the same subcellular localization where the reactive species is produced.

In the case of S-nitrosylation, this has been studied mainly in terms of the NOS enzymes as they are the source for NO and

thus for other RNS. Since its molecular characterization [56] knowledge on the regulation of eNOS expression and activation has accumulated in a vast proportion. Undoubtedly the most important mode of regulation of eNOS in endothelial cells is related to post-translational modifications based on its dual acylation and interactions with a significant number of proteins which determine its topological fate. There are several examples of interactions of target proteins with NOS isoforms (see [57,58] for review), especially eNOS and nNOS, which are very tightly regulated in their subcellular localization, although iNOS subcellular localization has been also described to be regulated by acylation mechanisms [59]. A recent example of this target-NOS association is the interaction of the ryanodine receptor with eNOS [60]. In addition, eNOS has been shown to undergo S-nitrosylation both in the purified protein [61] and in a receptor-coupled mode in endothelial and COS-7 cells [62,63]. Functional consequences of this modification include the localized inhibition of eNOS activity by the NOS product itself, as S-nitrosylation was showed to disrupt the zinc tetrathiolate cluster and hence the active dimeric conformation of eNOS [61,62]. The same functional relevance of feedback inhibition by NOS products has been attributed to the S-nitrosylation of Hsp90, an activator of eNOS [32]. Of interest, studies in cells by Erwin et al. addressed the relevant issue of subcellular targeting, as loss of myristoylation lead to hypernitrosylation and to lack of regulation by VEGF and related changes in eNOS subcellular localization [63]. The link between acylation and other post-translational modifications of eNOS such as phosphorylation and S-nitrosylation is important due to the dynamic regulation of eNOS activity by its subcellular localization. Recent data by Iwakiri et al. show that S-nitrosylation is preferentially localized in the regions where transfected eNOS is localized, and can alter Golgi trafficking function by altering proteins that are sensitive to S-nitrosylation [64]. Of note, mitochondrial production of ROS could also be necessary for S-nitrosylation, as deduced from a subcellular localization study performed with a NONOate NO donor [65], while this was not the case when nitrosothiols were used [66] (methodological differences were also important in these two studies, see [67] for review).

The subcellular localization of glutathione has been debated at the same time as analytical advances have been developed and showed more clear differences in glutathione distribution. Early experiments detected an important presence of glutathione in the nucleus [68,69] while a more recent study has highlighted the presence of glutathione in perinuclear locations including mitochondria, showing also an uneven distribution in the cytosol [70]. Some efforts have been done to localize S-glutathionylated proteins, showing nuclear, perinuclear and discrete membrane localization [70]. Studies on other redox pairs like cysteine/cystine or thioredoxin have shown that they are also unevenly distributed and that each pair may have distinct equilibria depending on the localization (reviewed in [71]). Thus, further work will be needed to address this issue.

5. Potential role of S-glutathionylation in pathophysiological processes. Similarities and contrasts with S-nitrosylation

One of the major problems with non enzymatic-mediated post-translational modifications is establishing functional consequences in cells, tissues and organisms. To this end, and since our last review on this topic [34] significant efforts have been devoted to identify S-glutathionylation in vivo. Among these, the Cotgreave lab has put forward a technique to identify subcellular compartments of glutathione and mixed disulfides [70] as well as different proteomic approaches to identify S-glutathionylated proteins [72,73]. Inkeeping, several groups also applied redox proteomics to the identification of thiolated proteins [74,75] (see also [76–78] for review). However, although these approaches have facilitated the discovery of many proteins susceptible of becoming thiolated, the major leap forward leading to the establishment of causal relationships between S-glutathionylation (or S-nitrosylation) and human disease has yet to occur.

Currently, a significant number of clinical disorders or pathophysiological entities have been related to redox post-translational modifications. These comprise cardiovascular disease, diabetes (see non-comprehensive lists in [79,80]) and neural protein conformational disorders [81] among others. We will now comment some of the best characterized examples of proteins modified by S-glutathionylation in relationship to NO production and their pathophysiological correlates.

5.1. *Sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA)*

In an effort to understand NO-mediated cGMP-independent vasodilatation, the laboratory of Richard Cohen identified that NO may stimulate calcium uptake into intracellular stores, thereby decreasing intracellular Ca^{2+} concentration and facilitating the uncoupling of the contractile machinery and vasorelaxation. SERCA is a key protein regulating the intracellular storage of Ca^{2+}, as it enhances refilling of Ca^{2+} sarcoplasmic and endoplasmic reticula. NO may regulate its function through both cGMP-dependent and independent mechanisms [82]. A key finding was the observation that this protein may result modified by several post-translational mechanisms in the presence of NO-related species [83,84]. Even more poignant was the discovery that peroxynitrite is able to activate SERCA by promoting S-thiolation in the context of arterial relaxation [85], an effect recapitulated by eNOS agonists. SERCA seems to have a specific target, Cys 674, whose modification results in the absence of NO-mediated increased activity. Furthermore this S-thiolation happens to occur in vivo and a decrease in its levels due to irreversible oxidation was found in atherosclerotic arteries. Overall, these data represent significant evidence suggesting the potential pathophysiological role of S-thiolated SERCA in vascular disease.

5.2. p21^{Ras}

Landmark work by Lander et al. showed that this oncogenic protein may become S-nitrosylated and that this may correlate with functional activation by converting it to its biologically active GTP-bound state [86]. Seminal work by the laboratory of Sharon Campbell has contributed to explain structure-functional relationships of Ras S-nitrosylation in Cys 118, and hence to gain mechanistic insight into the process of NO-mediated Ras guanine nucleotide dissociation in the presence of O_2 (see [87] for review). As it is the case with several other proteins, Ras may also become S-glutathionylated in the same cysteine and this was studied several years ago [35,37]. More recently, it has been suggested that this modification may mediate signalling by angiotensin II in vascular smooth muscle cells [88]. Peroxynitrite, albeit at moderately high concentrations, is able to thiolate Ras in endothelial cells, and this has been correlated with the activation of its downstream signaling effectors [89]. Of interest, the cysteine modification (not precisely determined) in myocites is potentially reversible by thioredoxin [90]. In terms of pathophysiological findings, one of the most interesting observations situates ROS-induced S-thiolation of Ras as a mediator of cardiac myocyte hypertrophy in a strain-stimulated model [91].

5.3. Hemoglobin

Pioneering work by Stamler and cols. showed that hemoglobin (Hb) is a target for S-nytrosylation and that this modification may play a role in blood flow regulation [92]. This concept has evolved into many detailed studies of this interaction and roles for S-nitrosohemoglobin [93]. The binding of glutathione to the Cys-93 of Hb beta chain has been known for more than 20 years, and is associated with inhibition of Hb S polymeryzation, increased oxygen affinity and reduced alkaline Bohr effect [94,95]. This made it an interesting candidate to combat sickle cell anemia. However, neither the modification of Hb by glutathione nor by NO [96] have translated into effective treatments for sickle cell disease. In contrast, glutathionyl Hb has been widely documented in disease states such as uremia or diabetes (see [97] for review), and it is currently believed to be a consequence of the oxidative stress underlying these diseases. However, this may convert it in a very useful marker of oxidative stress in these conditions.

5.4. Caspase-3

More than a decade ago the antiapoptotic effect of NO was related to its potential inhibition of caspase-3 activation through cGMP-dependent and independent mechanisms [98]. In several elegant studies Mannick et al. documented that the Fas ligand was under the control of the NO pathway and that S-nitrosylation-denitrosylation of caspase-3 were mechanisms by which this ligand was controlling the general process of apoptosis [99,100]. This same laboratory showed that S-nitrosylation of caspases could be linked to subcellular localization and that other caspases, such as caspase-9 could become S-nitrosylated [101]. Procaspase-3 undergoes S-nitrosylation in basal conditions in immune-response lineage cells on the catalytic cysteine, Cys163 [100]. Of interest, recent studies by Mitchell and Marletta described that the enzyme thioredoxin is involved in the process of S-nitrosylation of caspase-3 [102], and what is even more provocative, that S-nitrosylation of the critical cysteine residue within the catalytic site could be assisted by a nitrosopeptide that mimics the substrate configuration, thus providing an elegant example for a specific transnitrosation mechanism [103]. It should be noted that all these studies relied only on the indirect “biotin switch” technique.

S-glutathionylation of caspase-3 by GSNO or SNAP plus GSH has been described [102,104,105], and very recent observations claim that caspase-3 experiences S-glutathionylation on several thiol residues and that this process is intracelullarly regulated by the thiol transferase glutaredoxin by a mechanism that was relevant for the regulation of endothelial cell death promoted by TNF-α [106]. Clearly, caspase-3 constitutes an important model to study both post-translational modifications at the cellular level, specially given its transcendence for the regulation of cell death, even when a definitive evidence establishing whole organism level implications still needs to be provided.

6. Conclusions and perspectives

It is clear that there is a chemical relationship among different cysteine post-translational modifications produced by both ROS and RNS and especially between S-nitrosylation and S-glutathionylation. This is supported by different experimental approaches that have shown a potential functional relevance in signal transduction, especially those arising from NO production. Also there is accumulating evidences of their (patho) physiological relevance in the cardiovascular system.

Much work is still needed to more precisely define the balance between the different modifications, the detailed mechanisms underlying the relationships, and their functional implications in physiological settings. Some years ago, we concluded that we were facing mainly an analytical problem [34]. Recent years have seen the development of a bunch of useful new methodologies, for example in proteomics or subcellular localization studies, that have moved the frontier of knowledge a bit further, but significant improvements in the analytical methodologies are still needed in order to gain deeper insight into the physiological relevance of these modifications.

Beyond this, one concept still holds: if several modifications are possible and are in certain ways homologous, they may be found when they are searched for. However, insight into the relative quantitative relevance of each modification is the crucial issue still to be chiselled out of the mist.

Acknowledgements

Research in our laboratory is supported by grants from the *Plan Nacional de I+D+I, Ministerio de Educación y Ciencia* (SAF 2003-01039 and SAF 2006-02410) and *Ministerio de Sanidad y Consumo* (CP03/0025), all in Spain.

References

[1] Martínez-Ruiz A, Lamas S. S-nitrosylation: a potential new paradigm in signal transduction. Cardiovasc Res 2004;62:43–52.

[2] Forman HJ, Fukuto JM, Torres M. Redox signaling: thiol chemistry defines which reactive oxygen and nitrogen species can act as second messengers. Am J Physiol Cell Physiol 2004;287:C246–56.

[3] Nathan C. Specificity of a third kind: reactive oxygen and nitrogen intermediates in cell signaling. J Clin Invest 2003;111:769–78.

[4] Poole LB, Karplus PA, Claiborne A. Protein sulfenic acids in redox signaling. Annu Rev Pharmacol Toxicol 2004;44:325–47.

[5] Woo HA, Chae HZ, Hwang SC, Yang K-S, Kang SW, Kim K, et al. Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. Science 2003;300:653–6.

[6] Biteau B, Labarre J, Toledano MB. ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. Nature 2003;425: 980–4.

[7] Chai YC, Hendrich S, Thomas JA. Protein S-thiolation in hepatocytes stimulated by t-butyl hydroperoxide, menadione, and neutrophils. Arch Biochem Biophys 1994;310:264–72.

[8] Ravichandran V, Seres T, Moriguchi T, Thomas JA, Johnston Jr RB. S-thiolation of glyceraldehyde-3-phosphate dehydrogenase induced by the phagocytosis-associated respiratory burst in blood monocytes. J Biol Chem 1994;269:25010–5.

[9] Schuppe-Koistinen I, Gerdes R, Moldeus P, Cotgreave IA. Studies on the reversibility of protein S-thiolation in human endothelial cells. Arch Biochem Biophys 1994;315:226–34.

[10] Tao L, English AM. Mechanism of S-nitrosation of recombinant human brain calbindin D28K. Biochemistry 2003;42:3326–34.

[11] Wink DA, Darbyshire JF, Nims RW, Saavedra JE, Ford PC. Reactions of the bioregulatory agent nitric oxide in oxygenated aqueous media: determination of the kinetics for oxidation and nitrosation by intermediates generated in the NO/O2 reaction. Chem Res Toxicol 1993;6:23–7.

[12] Hogg N. The biochemistry and physiology of S-nitrosothiols. Annu Rev Pharmacol Toxicol 2002;42:585–600.

[13] Bellomo G, Mirabelli F, DiMonte D, Richelmi P, Thor H, Orrenius C, et al. Formation and reduction of glutathione-protein mixed disulfides during oxidative stress. A study with isolated hepatocytes and menadione (2-methyl-1,4-naphthoquinone). Biochem Pharmacol 1987;36:1313–20.

[14] Loeb GA, Skelton DC, Forman HJ. Dependence of mixed disulfide formation in alveolar macrophages upon production of oxidized glutathione: effect of selenium depletion. Biochem Pharmacol 1989;38: 3119–21.

[15] Schuppe I, Moldeus P, Cotgreave IA. Protein-specific S-thiolation in human endothelial cells during oxidative stress. Biochem Pharmacol 1992;44:1757–64.

[16] Thomas JA, Poland B, Honzatko R. Protein sulfhydryls and their role in the antioxidant function of protein S-thiolation. Arch Biochem Biophys 1995;319:1–9.

[17] Cotgreave IA, Gerdes RG. Recent trends in glutathione biochemistry-glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? Biochem Biophys Res Commun 1998;242: 1–9.

[18] Chai YC, Ashraf SS, Rokutan K, Johnston Jr RB, Thomas JA. S-thiolation of individual human neutrophil proteins including actin by stimulation of the respiratory burst: evidence against a role for glutathione disulfide. Arch Biochem Biophys 1994;310:273–81.

[19] Rossi R, Milzani A, Dalle-Donne I, Giannerini F, Giustarini D, Lusini L, et al. Different metabolizing ability of thiol reactants in human and rat blood. Biochemical and pharmacological implications. J Biol Chem 2001;276:7004–10.

[20] Dalle-Donne I, Giustarini D, Colombo R, Milzani A, Rossi R. S-glutathionylation in human platelets by a thiol-disulfide exchange-independent mechanism. Free Radic Biol Med 2005;38:1501–10.

[21] Rossi R, Lusini L, Giannerini F, Giustarini D, Lungarella G, Simplicio PD. A method to study kinetics of transnitrosation with nitrosoglutathione: reactions with hemoglobin and other thiols. Anal Biochem 1997;254:215–20.

[22] Liu Z, Rudd MA, Freedman JE, Loscalzo J. S-transnitrosation reactions are involved in the metabolic fate and biological actions of nitric oxide. J Pharmacol Exp Ther 1998;284:526–34.

[23] Gilbert HF. Thiol/disulfide exchange equilibria and disulfide bond stability. Methods Enzymol 1995;251:8–28.

[24] Lee S-R, Kwon K-S, Kim S-R, Rhee SG. Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. J Biol Chem 1998;273:15366–72.

[25] Denu JM, Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. Biochemistry 1998;37:5633–42.

[26] Barrett WC, DeGnore JP, Keng YF, Zhang ZY, Yim MB, Chock PB. Roles of superoxide radical anion in signal transduction mediated by reversible regulation of protein-tyrosine phosphatase 1B. J Biol Chem 1999;274:34543–6.

[27] Barrett WC, DeGnore JP, Konig S, Fales HM, Keng YF, Zhang ZY, et al. Regulation of PTP1B via glutathionylation of the active site cysteine 215. Biochemistry 1999;38:6699–705.

[28] Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, et al. Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature 2003;423:769–73.

[29] van Montfort RL, Congreve M, Tisi D, Carr R, Jhoti H. Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B. Nature 2003;423:773–7.

[30] Barrett DM, Black SM, Todor H, Schmidt-Ullrich RK, Dawson KS, Mikkelsen RB. Inhibition of protein-tyrosine phosphatases by mild oxidative stresses is dependent on S-nitrosylation. J Biol Chem 2005;280:14453–61.

[31] Lancaster Jr JR, Gaston B. NO and nitrosothiols: spatial confinement and free diffusion. Am J Physiol Lung Cell Mol Physiol 2004;287:L465–6.

[32] Martínez-Ruiz A, Villanueva L, de Orduña CG, López-Ferrer D, Higueras MÁ, Tarín C, et al. S-nitrosylation of Hsp90 promotes the inhibition of its ATPase and endothelial nitric oxide synthase regulatory activities. Proc Natl Acad Sci U S A 2005;102:8525–30.

[33] Park J-W. Reaction of S-nitrosoglutathione with sulfhydryl groups in protein. Biochem Biophys Res Commun 1988;152:916–20.

[34] Klatt P, Lamas S. Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur J Biochem 2000;267:4928–44.

[35] Ji Y, Akerboom TP, Sies H, Thomas JA. S-nitrosylation and S-glutathiolation of protein sulfhydryls by S-nitroso glutathione. Arch Biochem Biophys 1999;362:67–78.

[36] Konorev EA, Kalyanaraman B, Hogg N. Modification of creatine kinase by S-nitrosothiols: S-nitrosation vs. S-thiolation. Free Radic Biol Med 2000;28:1671–8.

[37] Mallis RJ, Buss JE, Thomas JA. Oxidative modification of H-ras: S-thiolation and S-nitrosylation of reactive cysteines. Biochem J 2001;355:145–53.

[38] Giustarini D, Milzani A, Aldini G, Carini M, Rossi R, Dalle-Donne I. S-nitrosation versus S-glutathionylation of protein sulfhydryl groups by S-nitrosoglutathione. Antioxid Redox Signal 2005;7:930–9.

[39] Aracena-Parks P, Goonasekera SA, Gilman C, Dirksen RT, Hidalgo C, Hamilton SL. Identification of cysteines involved in S-nitrosylation, S-glutathionylation, and oxidation to disulfides in RyR1. J Biol Chem 2006;281:40354–68.

[40] DelaTorre A, Schroeder RA, Bartlett ST, Kuo PC. Differential effects of nitric oxide-mediated S-nitrosylation on p50 and c-jun DNA binding. Surgery 1998;124:137–41.
[41] Klatt P, Pineda Molina E, García de Lacoba M, Padilla CA, Martínez-Galesteo E, Bárcena JA, et al. Redox regulation of c-Jun DNA binding by reversible S-glutathiolation. FASEB J 1999;13:1481–90.
[42] Klatt P, Pineda Molina E, Lamas S. Nitric oxide inhibits c-Jun DNA binding by specifically targeted S-glutathionylation. J Biol Chem 1999;274:15857–64.
[43] Pineda-Molina E, Klatt P, Vázquez J, Marina A, García de Lacoba M, Pérez-Sala D, et al. Glutathionylation of the p50 subunit of NF-kappaB: a mechanism for redox-induced inhibition of DNA binding. Biochemistry 2001;40:14134–42.
[44] Marshall HE, Stamler JS. Inhibition of NF-kappa B by S-nitrosylation. Biochemistry 2001;40:1688–93.
[45] Sun J, Xin C, Eu JP, Stamler JS, Meissner G. Cysteine-3635 is responsible for skeletal muscle ryanodine receptor modulation by NO. Proc Natl Acad Sci U S A 2001;98:11158–62.
[46] Aracena P, Sanchez G, Donoso P, Hamilton SL, Hidalgo C. S-glutathionylation decreases Mg^{2+} inhibition and S-nitrosylation enhances Ca^{2+} activation of RyR1 channels. J Biol Chem 2003;278:42927–35.
[47] Mallis RJ, Thomas JA. Effect of S-nitrosothiols on cellular glutathione and reactive protein sulfhydryls. Arch Biochem Biophys 2000;383:60–9.
[48] West MB, Hill BG, Xuan YT, Bhatnagar A. Protein glutathiolation by nitric oxide: an intracellular mechanism regulating redox protein modification. FASEB J 2006;20:1715–7.
[49] Hess DT, Matsumoto A, Kim S-O, Marshall HE, Stamler JS. Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 2005;6:150–66.
[50] Jensen DE, Belka GK, Du Bois GC. S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. Biochem J 1998;331:659–68.
[51] Liu L, Hausladen A, Zeng M, Que L, Heitman J, Stamler JS. A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. Nature 2001;410:490–4.
[52] Shelton MD, Chock PB, Mieyal JJ. Glutaredoxin: role in reversible protein S-glutathionylation and regulation of redox signal transduction and protein translocation. Antioxid Redox Signal 2005;7:348–66.
[53] Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochem Pharmacol 2006;71:551–64.
[54] Findlay VJ, Townsend DM, Morris TE, Fraser JP, He L, Tew KD. A novel role for human sulfiredoxin in the reversal of glutathionylation. Cancer Res 2006;66:6800–6.
[55] Tew KD. Redox in redux: emergent roles for glutathione S-transferase P (GSTP) in regulation of cell signaling and S-glutathionylation. Biochem Pharmacol 2007;73:1257–69.
[56] Lamas S, Marsden PA, Li GK, Tempst P, Michel T. Endothelial nitric oxide synthase: molecular cloning and characterization of a distinct constitutive enzyme isoform. Proc Natl Acad Sci U S A 1992;89:6348–52.
[57] Dudzinski DM, Igarashi J, Greif D, Michel TM. The regulation and pharmacology of endothelial nitric oxide synthases. Annu Rev Pharmacol Toxicol 2006;46:235–76.
[58] Gratton J-P, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. Circ Res 2004;94:1408–17.
[59] Navarro-Lérida I, Corvi MM, Alvarez-Barrientos A, Gavilanes F, Berthiaume LG, Rodríguez-Crespo I. Palmitoylation of inducible nitric-oxide synthase at Cys-3 is required for proper intracellular traffic and nitric oxide synthesis. J Biol Chem 2004;279:55682–9.
[60] Martínez-Moreno M, Alvarez-Barrientos A, Roncal F, Albar JP, Gavilanes F, Lamas S, et al. Direct interaction between the reductase domain of endothelial nitric oxide synthase and the ryanodine receptor. FEBS Lett 2005;579:3159–63.
[61] Ravi K, Brennan LA, Levic S, Ross PA, Black SM. S-nitrosylation of endothelial nitric oxide synthase is associated with monomerization and decreased enzyme activity. Proc Natl Acad Sci U S A 2004;101:2619–24.
[62] Erwin PA, Lin AJ, Golan DE, Michel T. Receptor-regulated dynamic S-nitrosylation of endothelial nitric oxide synthase in vascular endothelial cells. J Biol Chem 2005;280:19888–94.
[63] Erwin PA, Mitchell DA, Sartoretto J, Marletta MA, Michel T. Subcellular targeting and differential S-nitrosylation of endothelial nitric oxide synthase. J Biol Chem 2006;281:151–7.
[64] Iwakiri Y, Satoh A, Chatterjee S, Toomre DK, Chalouni CM, Fulton D, et al. Nitric oxide synthase generates nitric oxide locally to regulate compartmentalized protein S-nitrosylation and protein trafficking. Proc Natl Acad Sci U S A 2006;103:19777–82.
[65] Yang Y, Loscalzo J. S-nitrosoprotein formation and localization in endothelial cells. Proc Natl Acad Sci U S A 2005;102:117–22.
[66] Ckless K, Reynaert NL, Taatjes DJ, Lounsbury KM, van der Vliet A, Janssen-Heininger Y. In situ detection and visualization of S-nitrosylated proteins following chemical derivatization: identification of Ran GTPase as a target for S-nitrosylation. Nitric Oxide 2004;11:216–27.
[67] Martínez-Ruiz A, Lamas S. Nitrosylation of thiols in vascular homeostasis and disease. Curr Atheroscl Rep 2005;7:213–8.
[68] Hjelle OP, Chaudhry FA, Ottersen OP. Antisera to glutathione: characterization and immunocytochemical application to the rat cerebellum. Eur J Neurosci 1994;6:793–804.
[69] Voehringer DW, McConkey DJ, McDonnell TJ, Brisbay S, Meyn RE. Bcl-2 expression causes redistribution of glutathione to the nucleus. Proc Natl Acad Sci U S A 1998;95:2956–60.
[70] Söderdahl T, Enoksson M, Lundberg M, Holmgren A, Ottersen OP, Orrenius S, et al. Visualization of the compartmentalization of glutathione and protein-glutathione mixed disulfides in cultured cells. FASEB J 2003;17:124–6.
[71] Hansen JM, Go YM, Jones DP. Nuclear and mitochondrial compartmentation of oxidative stress and redox signaling. Annu Rev Pharmacol Toxicol 2006;46:215–34.
[72] Lind C, Gerdes R, Hamnell Y, Schuppe-Koistinen I, von Löwenhielm HB, Holmgren A, et al. Identification of S-glutathionylated cellular proteins during oxidative stress and constitutive metabolism by affinity purification and proteomic analysis. Arch Biochem Biophys 2002;406:229–40.
[73] Hamnell-Pamment Y, Lind C, Palmberg C, Bergman T, Cotgreave IA. Determination of site-specificity of S-glutathionylated cellular proteins. Biochem Biophys Res Commun 2005;332:362–9.
[74] Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmona M, et al. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. Proc Natl Acad Sci U S A 2002;99: 3505–10.
[75] Eaton P, Byers HL, Leeds N, Ward MA, Shattock MJ. Detection, quantitation, purification, and identification of cardiac proteins S-thiolated during ischemia and reperfusion. J Biol Chem 2002;277:9806–11.
[76] Bonetto V, Ghezzi P. Thiol-disulfide oxidoreduction of protein cysteines: old methods revisited for proteomics. In: Dalle-Donne I, Scaloni A, Butterfield DA, editors. Redox Proteomics. Wiley-Interscience; 2006. p. 101–22.
[77] Hampton MB, Baty JW, Winterbourn CC. Use of a proteomic technique to identify oxidant-sensitive thiol proteins in cultured cells. In: Dalle-Donne I, Scaloni A, Butterfield DA, editors. Redox Proteomics. Wiley-Interscience; 2006. p. 253–65.
[78] Eaton P. Protein thiol oxidation in health and disease: techniques for measuring disulfides and related modifications in complex protein mixtures. Free Radic Biol Med 2006;40:1889–99.
[79] Adachi T, Schoneich C, Cohen RA. S-glutathiolation in redox-sensitive signaling. Drug Discov Today Dis Mech 2005;2:39–46.
[80] Giustarini D, Rossi R, Milzani A, Colombo R, Dalle-Donne I. S-glutathionylation: from redox regulation of protein functions to human diseases. J Cell Mol Med 2004;8:201–12.

[81] Dinoto L, Deture MA, Purich DL. Structural insights into Alzheimer filament assembly pathways based on site-directed mutagenesis and S-glutathionylation of three-repeat neuronal Tau protein. Microsc Res Tech 2005;67:156–63.
[82] Cohen RA, Weisbrod RM, Gericke M, Yaghoubi M, Bierl C, Bolotina VM. Mechanism of nitric oxide-induced vasodilatation: refilling of intracellular stores by sarcoplasmic reticulum Ca^{2+} ATPase and inhibition of store-operated Ca^{2+} influx. Circ Res 1999;84:210–9.
[83] Viner RI, Williams TD, Schöneich C. Peroxynitrite modification of protein thiols: oxidation, nitrosylation, and S-glutathiolation of functionally important cysteine residue(s) in the sarcoplasmic reticulum Ca-ATPase. Biochemistry 1999;38:12408–15.
[84] Viner RI, Williams TD, Schöneich C. Nitric oxide-dependent modification of the sarcoplasmic reticulum Ca-ATPase: localization of cysteine target sites. Free Radic Biol Med 2000;29:489–96.
[85] Adachi T, Weisbrod RM, Pimentel DR, Ying J, Sharov VS, Schöneich C, et al. S-glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. Nat Med 2004;10:1200–7.
[86] Lander HM, Hajjar DP, Hempstead BL, Mirza UA, Chait BT, Campbell S, et al. A molecular redox switch on p21ras. Structrural basis for the nitric oxide-p21ras interaction. J Biol Chem 1997;272: 4323–6.
[87] Heo J, Campbell SL. Nitric oxide and cell signaling: redox regulation of Ras superfamiliy GTPases. In: Lamas S, Cadenas E, editors. Nitric Oxide, Cell Signaling and Gene Expression. CRC Press — Taylor and Francis; 2006. p. 263–89.
[88] Adachi T, Pimentel DR, Heibeck T, Hou X, Lee YJ, Jiang B, et al. S-glutathiolation of Ras mediates redox-sensitive signaling by Angiotensin ii in vascular smooth muscle cells. J Biol Chem 2004;279: 29857–62.
[89] Clavreul N, Adachi T, Pimental DR, Ido Y, Schoneich C, Cohen RA. S-glutathiolation by peroxynitrite of p21ras at cysteine-118 mediates its direct activation and downstream signaling in endothelial cells. FASEB J 2006;20:518–20.
[90] Kuster GM, Pimentel DR, Adachi T, Ido Y, Brenner DA, Cohen RA, et al. alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras. Circulation 2005;111:1192–8.
[91] Pimentel DR, Adachi T, Ido Y, Heibeck T, Jiang B, Lee Y, et al. Strain-stimulated hypertrophy in cardiac myocytes is mediated by reactive oxygen species-dependent Ras S-glutathiolation. J Mol Cell Cardiol 2006;41:613–22.
[92] Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. Nature 1996;380:221–6.
[93] Singel DJ, Stamler JS. Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohemoglobin. Annu Rev Physiol 2005;67:99–145.
[94] Garel MC, Domenget C, Caburi-Martin J, Prehu C, Galacteros F, Beuzard Y. Covalent binding of glutathione to hemoglobin. I. Inhibition of hemoglobin S polymerization. J Biol Chem 1986;261: 14704–9.
[95] Craescu CT, Poyart C, Schaeffer C, Garel MC, Kister J, Beuzard Y. Covalent binding of glutathione to hemoglobin. II. Functional consequences and structural changes reflected in NMR spectra. J Biol Chem 1986;261:14710–6.
[96] Head CA, Brugnara C, Martinez-Ruiz R, Kacmarek RM, Bridges KR, Kuter D, et al. Low concentrations of nitric oxide increase oxygen affinity of sickle erythrocytes in vitro and in vivo. J Clin Invest 1997;100:1193–8.
[97] Niwa T. Proteome analysis of oxidative stress: glutathionyl hemoglobin in diabetic and uremic patients. In: Dalle-Donne I, Scaloni A, Butterfield DA, editors. Redox Proteomics. Wiley-Interscience; 2006. p. 651–67.
[98] Kim Y-M, Talanian RV, Billiar TR. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. J Biol Chem 1997;272:31138–48.
[99] Mannick JB, Miao XQ, Stamler JS. Nitric oxide inhibits Fas-induced apoptosis. J Biol Chem 1997;272:24125–8.
[100] Mannick JB, Hausladen A, Liu L, Hess DT, Zeng M, Miao QX, et al. Fas-induced caspase denitrosylation. Science 1999;284:651–4.
[101] Mannick JB, Schonhoff C, Papeta N, Ghafourifar P, Szibor M, Fang K, et al. S-nitrosylation of mitochondrial caspases. J Cell Biol 2001;154: 1111–6.
[102] Mitchell DA, Marletta MA. Thioredoxin catalyzes the S-nitrosation of the caspase-3 active site cysteine. Nat Chem Biol 2005;1:154–8.
[103] Mitchell DA, Morton SU, Marletta MA. Design and characterization of an active site selective caspase-3 transnitrosating agent. ACS Chem Biol 2006;1:659–65.
[104] Zech B, Wilm M, van Eldik R, Brüne B. Mass spectrometric analysis of nitric oxide-modified caspase-3. J Biol Chem 1999;274:20931–6.
[105] Klatt P, Pineda Molina E, Pérez-Sala D, Lamas S. Novel application of S-nitrosoglutathione-Sepharose to identify proteins that are potential targets for S-nitrosoglutathione-induced mixed-disulphide formation. Biochem J 2000;349:567–78.
[106] Pan S, Berk BC. Glutathiolation regulates tumor necrosis factor-{alpha}-induced caspase-3 cleavage and apoptosis: key role for glutaredoxin in the death pathway. Circ Res 2007;100:213–9.
[107] Percival MD, Ouellet M, Campagnolo C, Claveau D, Li C. Inhibition of cathepsin K by nitric oxide donors: evidence for the formation of mixed disulfides and a sulfenic acid. Biochemistry 1999;38: 13574–83.
[108] Chandra A, Srivastava S, Petrash JM, Bhatnagar A, Srivastava SK. Modification of aldose reductase by S-nitrosoglutathione. Biochemistry 1997;36:15801–9.
[109] Srivastava SK, Ramana KV, Chandra D, Srivastava S, Bhatnagar A. Regulation of aldose reductase and the polyol pathway activity by nitric oxide. Chem Biol Interact 2003;143–144:333–40.
[110] Becker K, Savvides SN, Keese M, Schirmer RH, Karplus PA. Enzyme inactivation through sulfhydryl oxidation by physiologic NO-carriers. Nat Struct Biol 1998;5:267–71.
[111] Aracena P, Tang W, Hamilton SL, Hidalgo C. Effects of S-glutathionylation and S-nitrosylation on calmodulin binding to triads and FKBP12 binding to type 1 calcium release channels. Antioxid Redox Signal 2005;7:870–81.
[112] Xian M, Chen X, Liu Z, Wang K, Wang PG. Inhibition of Papain by S-Nitrosothiols. Formation of mixed disulfides. J Biol Chem 2000;275:20467–73.
[113] Wang W, Oliva C, Li G, Holmgren A, Lillig CH, Kirk KL. Reversible silencing of CFTR chloride channels by glutathionylation. J Gen Physiol 2005;125:127–41.
[114] Mohr S, Hallak H, de Boitte A, Lapetina EG, Brüne B. Nitric oxide-induced S-glutathionylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase. J Biol Chem 1999;274:9427–30.
[115] Tao L, English AM. Protein S-glutathiolation triggered by decomposed S-nitrosoglutathione. Biochemistry 2004;43:4028–38.
[116] Imaizumi N, Miyagi S, Aniya Y. Reactive nitrogen species derived activation of rat liver microsomal glutathione S-transferase. Life Sci 2006;78:2998–3006.
[117] Huang KP, Huang FL, Li J, Schuck P, McPhie P. Calcium-sensitive interaction between calmodulin and modified forms of rat brain neurogranin/RC3. Biochemistry 2000;39:7291–9.
[118] Li J, Huang FL, Huang K-P. Glutathiolation of proteins by glutathione disulfide S-oxide derived from S-nitrosoglutathione. Modifications of rat brain neurogranin/RC3 and neuromodulin/GAP-43. J Biol Chem 2001;276:3098–105.
[119] Okamoto T, Akaike T, Sawa T, Miyamoto Y, van der Vliet A, Maeda H. Activation of matrix metalloproteinases by peroxynitrite-induced protein S-glutathiolation via disulfide S-oxide formation. J Biol Chem 2001;276:29596–602.
[120] Sadidi M, Geddes TJ, Kuhn DM. S-thiolation of tyrosine hydroxylase by reactive nitrogen species in the presence of cysteine or glutathione. Antioxid Redox Signal 2005;7:863–9.
[121] Yu CX, Li S, Whorton AR. Redox regulation of PTEN by S-nitrosothiols. Mol Pharmacol 2005;68:847–54.