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Redox modification of cell signaling in the cardiovascular system

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Abstract

Oxidative stress is presumed to be involved in the pathogenesis of many diseases, including cardiovascular disease. However, oxidants are also generated in healthy cells, and increasing evidence suggests that they can work as signaling molecules. Intracellular reduction-oxidation (redox) status is tightly regulated by oxidant and antioxidant systems. Imbalance between them causes oxidative or reductive stress which triggers cellular damage or aberrant signaling, leading to dysregulation. In this review, we will briefly summarize the aspects of ROS generation and neutralization mechanisms in the cardiovascular system. ROS can regulate cell signaling through oxidation and reduction of specific amino acids within proteins. Structural changes during post-translational modification allow modification of protein activity which can result in alteration of cellular function. We will focus on the molecular basis of redox protein modification and how this regulatory mechanism affects signal transduction in the cardiovascular system. Finally, we will discuss some techniques applied to monitoring redox status and identifying redox-sensitive proteins in the heart.

Keywords

Oxidative stress; reactive oxygen species; posttranslational oxidative modification; thiol; heart

1. Introduction

Oxidative stress, defined as the excessive accumulation of reactive oxygen species (ROS), plays an important role in the pathogenesis of heart disease, including cardiac hypertrophy, ischemia/reperfusion injury and heart failure (Figure 1). Increasing lines of evidence suggest

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that ROS also serve as second messengers, thereby mediating both physiological and pathological responses [1, 2]. Downregulation of ROS below certain levels disrupts normal cellular functions, causing adverse effects. For example, the salutary effect of ischemic preconditioning (IPC) against ischemia/reperfusion injury is blocked by treatment with antioxidants [3–5]. In this case, protein oxidation may mediate the adaptive response of ischemic preconditioning. Recent evidence suggests that accumulation of electron donors and consequent increases in the glutathione/glutathione disulfide (GSH/GSSG) ratio, termed reductive stress, can cause cellular malfunctions and cardiovascular diseases. A mouse model overexpressing a chaperone mutant had an elevated GSH/GSSG level, which was accompanied by abnormal protein aggregation and dilated cardiomyopathy [6].

In general, a redox reaction is signified by a change in the oxidative state of an atom; reduction is defined by the gain of an electron whereas oxidation is defined by the loss of an electron. Post-translational oxidative modification (PTOM) of proteins can take place in multiple forms, thereby imposing substantial influences on their structure and function. In order to improve understanding of cardiac physiology and pathology, it has become increasingly imperative to elucidate how ROS regulate intracellular signaling mechanisms. We will discuss how PTOM affects the function of signaling molecules in the heart to regulate growth and death of cardiomyocytes.

2. Generation of ROS within cells

Intracellular redox status is controlled by the balance between oxidants and antioxidants (Figure 2). Various forms of oxidants are generated when electrons are transferred to molecular oxygen: An electron is transferred to molecular oxygen (O_2) to generate superoxide ($O_2^{\cdot-}$); transfer of a second electron, either spontaneously or mediated by superoxide dismutase (SOD), generates hydrogen peroxide (H_2O_2); and transfer of a third electron from ferrous (Fe^{2+}) generates hydroxyl radicals ($OH^{\cdot}$) through a Fenton reaction [7, 8]. Superoxide ($O_2^{\cdot-}$) also reacts with nitrogen monoxide (NO) to produce peroxynitrite ($ONOO^-$) in NO-producing cells, such as endothelial cells. Among these species, H_2O_2 is the most stable and can travel inside the cell and even across the cellular membranes, acting as a second messenger.

The cell produces electrons as byproducts of ATP synthesis at the electron transport chain (ETC), the most important energy-producing machinery, in mitochondria even under unstressed conditions. Importantly, the extent of electron leakage is increased dramatically when the components of the electron transport chain are downregulated or damaged in response to stress such as ischemia/reperfusion and pressure overload. Increased production of ROS further damages mitochondrial proteins, which creates a vicious cycle of increased oxidative stress, known as ROS-induced ROS damage. In particular, the ETC complex I and complex III are the major sources of oxidative stress during heart failure [9].

Cardiomyocytes also have an ability to increase ROS production quite rapidly in response to hypertrophic agonists, which suggests that they have an additional mechanism actively producing oxidants in a regulated manner. In fact, aside from spontaneous and passive generation of ROS in mitochondria, cells have several enzymes that can actively produce ROS. Perhaps the most important example is NADPH oxidases (Noxs), transmembrane proteins that actively produce $O_2^{\cdot-}$ and H_2O_2 [10]. Cardiomyocytes primarily express Nox2 and Nox4. Induction of ROS by Nox2 triggers cardiomyocyte death in response to angiotensin II (Ang II) and cardiac remodeling after myocardial infarction [11–13]. Nox4, which is mainly localized in mitochondria or the endoplasmic reticulum in cardiomyocytes, mediates mitochondrial dysfunction and apoptosis [14]. Expression of Nox4, stimulated by

cardiac stress including cardiac hypertrophy, heart failure and aging, is the major source of ROS production in the heart during pressure overload [15, 16].

Another ROS-producing enzyme is NO synthase (NOS). NOS functions to produce NO, which is involved in the production of an important second messenger molecule called cyclic guanosine monophosphate (cGMP). During NO production, the leakage and side reactions generate superoxide in the presence of NADPH [17]. This process is facilitated in the presence of ROS and a paucity of tetrahydrobiopterin (BH4) in endothelial cells [18]. Other molecules, such as cytochrome p450 and xanthine oxidase (XO), are structurally similar to NOS and also produce ROS inside the cell under pathological conditions such as volume overload [19, 20].

3. Neutralization of ROS within cells

Cellular redox status is precisely controlled by antioxidants as well. Superoxide (O_2^-) is very rapidly dismutated to H_2O_2 by SOD and, thus, highly restricted to certain cellular compartments. Three isoforms of SOD have been found and the major form, called SOD2 (or MnSOD), located in the mitochondrial matrix, can prevent O_2^- accumulation and subsequent mitochondrial dysfunction and DNA damage in the heart. [21–23]. H_2O_2 can penetrate the membrane and rapidly reach its targets, but it is neutralized by catalase localized in peroxisomes, that catalyzes decomposition of H_2O_2 to oxygen and water. In addition, peroxiredoxins are ubiquitously expressed in mammalian cells and also reduce H_2O_2 , where their peroxidatic thiol reacts with the oxidant to form a sulfenic acid. The sulfenic acid rapidly forms a disulfide with glutathione in the case of the glutathione-dependent peroxiredoxins, before being recycled back to the reduced state by glutaredoxin or ascorbic acid. Thioredoxin-dependent peroxiredoxin sulfenic acids rapidly react with proximal thiols to form a homo intermolecular disulfide, before being recycled back to the reduced state by thioredoxin [24–26]. Small molecules, including ascorbic acid (Vitamin C), retinol (Vitamin A), tocopherol (Vitamin E) and lipoic acid, also prevent cells from ROS-induced damage, and their activities are regenerated by other groups of small molecules, such as ubiquinol, glutathione reductase and thioredoxin reductase.

Glutathione (GSH) is a tripeptide whose active cysteine serves to protect cellular proteins from ROS. One of its mechanisms involves the reduction of disulfide bonds to cysteines within cellular proteins, where GSH acts as an electron donor and is in turn oxidized to glutathione disulfide (GSSG), which is then regenerated by glutathione reductase [27, 28]. Another mechanism occurs when GSH covalently binds to cellular proteins and forms mixed disulfide bonds (glutathiolation) in a reversible manner, preventing the thiol groups within proteins from undergoing further oxidation during extreme conditions. When the cellular environment returns to normal, the reduction of the GSH-protein complex is mediated by glutaredoxin instead of glutathione reductase [29–33].

Another powerful buffering system, thioredoxin1 (Trx1), ubiquitously present in mammalian cells, serves as an important antioxidant in cardiac myocytes by acting as a major disulfide reductase [34]. The two cysteines in the Trx1 common disulfide active motif (-Cys-Gly-Pro-Cys-) are able to catalyze the reduction of disulfide bonds within redox-sensitive proteins, which results in the oxidation of Trx1 itself [35]. The regeneration of Trx1 occurs through Trx reductase using electrons from NADPH [36, 37]. Trx1 is usually considered to be an antioxidant protein, which balances the intracellular redox state of cells by regenerating active peroxiredoxin. Interestingly, Trx1 also works as a signaling intermediate that senses the imbalance of the redox status and transduces signals to other effectors, including transcription factors and kinases. To elaborate, Trx1 interacts with AP-1, NF- κ B and p53 in order to enhance or reduce their transcriptional activity [38–42]. In the

heart, reduced Trx1, Trx2 and/or glutaredoxin1 (Grx1) bind to and negatively regulate apoptosis signal-regulating kinase 1 (ASK1), which inhibits ASK-1 from inducing apoptosis [43–45]. Trx1 interacts with gamma-actin to prevent stress fiber formation, thereby inhibiting apoptosis [46]. Trx1 regulates Ras to prevent α -adrenergic receptor-stimulated hypertrophy and apoptosis in adult rat ventricular myocytes [47]. Class II histone deacetylases (HDACs) are oxidized by the formation of an intramolecular disulfide bond during stimulation of hypertrophy. The intramolecular disulfide bond disrupts the binding of zinc to HDACs and induces nuclear export of HDACs. Trx1 can reduce HDACs in order to inhibit their nuclear export, which in turn inhibits cardiac hypertrophy [48]. Trx2 is expressed primarily in mitochondria, and gene disruption of Trx2 causes embryonic lethality with massive apoptotic cell death; embryonic fibroblasts are not viable in culture [49]. Although Trx2 is believed to play an essential role in regulating mitochondrial oxidative stress, the specific function of Trx2 in cardiovascular cell types remains to be elucidated.

4. Structural modification of signaling molecules in the cardiovascular system

Based on chemical characteristics, only a few kinds of amino acids can undergo oxidative modification: these include cysteine, methionine and tyrosine. Tyrosine can be modulated by NO. Cysteine, histidine and lysine can also undergo carbonylation reactions, and additionally, some amino acids can be modulated by products of lipid peroxidation.

4-1 Cysteine

4-1-1 Single cysteine oxidation—Among these amino acids, the thiol group within cysteine is the most reactive and is the most easily modified by oxidants (Figure 3A). Different species among ROS induce varied modifications of thiol within cysteine. For example, H_2O_2 and byproducts of lipid peroxidation directly react with thiol and form sulfenic acid. Sulfenic acid is unstable and rapidly reacts with another thiol group to form a disulfide bond through intra- or intermolecular interactions. Thiol-based reductants, including Trx and GSH, can reduce disulfide bonds via the thiol disulfide exchange reaction. The sulfur atom in sulfenic acid can also react with amide nitrogen within a given protein to form a cyclic sulfonamide. This structure was first discovered in protein tyrosine phosphatase 1B (PTP1B), which is oxidized by H_2O_2 and forms reversible sulfenic acid [50, 51]. Formation of the cyclic sulfonamide inhibits PTP1B catalytic activity. This process can be reversed by GSH, which indicates that this new sulfonamide structure can prevent further oxidation of the thiol group [52]. Sulfenic acid undergoes further oxidation to form sulfinic acid or sulfonic acid, which is thought to be irreversible. Increasing evidence suggests that sulfinic acid modification also modulates protein function. For example, oxidation of the zinc thiolate ligand to sulfinic acid activates matrix metalloprotease activity by modulating metal-binding properties [53], whereas sulfinic acid formation inhibits copper-zinc superoxide dismutase (SOD1) activity and induces cytotoxicity [54]. Sulfinylation of peroxiredoxin is reduced by ATP-dependent sulfinic acid reductase, called sulfiredoxin [55]. The crystal structures of peroxiredoxin and sulfiredoxin imply that conformational arrangement and local sulfinic acid reduction are mediated by sulfiredoxin [56]. All of these results suggest that sulfinic acid modification can modulate protein function through a reversible switch on-off action. Sulfinic acid is further oxidized to sulfonic acid, the highest oxidized sulfur species. Sulfonic acid modification is irreversible and generally inhibits the activities of enzymes in which the reduced thiol group is required for catalysis.

4-1-2 S-glutathiolation—S-glutathiolation is one of the most common S-thiolation reactions inside the cell. Usually proteins first undergo oxidation to form sulfenic acid before reacting with a reduced thiol such as GSH, resulting in a mixed disulfide that

prevents further oxidation to sulfinic or sulfonic acid. Alternatively, an oxidized form of glutathione such as GSSG (glutathione disulfide) or GSNO (S-nitrosoglutathione) may react with a reduced protein thiol to yield S-glutathiolation. S-thiolation provides a variety of products that can exert many different biological functions by rearranging structures and interacting with co-factors [57].

4-1-3 Disulfide bond—Some thiol active proteins can also form a disulfide structure through inter- or intramolecular interactions. Structural rearrangement caused by disulfide bond formation may alter the function of these proteins resulting in oxidant-induced signaling [58–60]. For example, H₂O₂ induces intermolecular disulfide bond formation of the catalytic subunit of cAMP-dependent protein kinase A (PKA) at Cys199 and Cys343. This disulfide bond formation inhibits kinase activity by inducing dephosphorylation of threonine 197 [61]. In another example, ROS-induced intermolecular disulfide bond formation of cGMP-dependent protein kinase (PKG1 α) at Cys42 enhances kinase activity and mediates oxidant-induced vasorelaxation [62]. In addition to intermolecular disulfide formation, two proximal cysteines within a given protein can form an intramolecular disulfide bond, thereby altering protein function. Intramolecular disulfide bond formation between Cys667/Cys669 in HDAC4 induces its nuclear export in response to hypertrophic stimuli [48].

4-1-4 S-nitrosylation—S-nitrosylation or S-nitrosation is the adduction of NO to a thiol group and can occur within proteins. This modification is reversible with the protein denitrosylation process, mediated by the GSH or Trx system [63, 64]. Brain-derived neurotrophic factor (BDNF) triggers NO synthesis and S-nitrosylation of HDAC2, which induces the release of HDAC2 from chromatin and promotes neurotrophin-dependent gene activation [65]. Since class I HDACs negatively regulate anti-hypertrophic genes, an intervention that induces S-nitrosylation of HDAC2 may inhibit cardiac hypertrophy. Interprotein disulfide formation between the two regulatory RI α subunits of protein kinase A (PKA) is induced by transnitrosylation, which in turn stimulates PKA to induce vasorelaxation independently of β -adrenergic signaling [66]. Increases in NO lead to elevation of the second messenger cGMP, which primarily signals via activation of PKG. In contrast, many proteins may be targets for and regulated by S-nitrosylation, potentially providing a greater scope of regulation by NO [67–69]. S-nitrosylation of mitochondrial proteins is stimulated by ischemic preconditioning, thereby playing a protective role in the heart [70]. In this case, mitochondrial proteins subjected to S-nitrosylation are protected from oxidation, resulting in the prevention of mitochondrial dysfunction as well as cell death.

4-2 Methionine

Methionine is subjected to oxidation and forms methionine sulfoxide, which is readily reversed by methionine sulfoxide reductase (MsrA), which catalyzes the thioredoxin-dependent reduction back to Methionine [71] (Figure 3B). Methionine oxidation can serve as a posttranslational regulatory modification, but it can also play a protective, sacrificial role that reduces oxidants and prevents irreversible oxidation of other amino acids [72]. Lack of Msr in mice and lower organisms increases oxidative stress and decreases life span [73–75], and is associated with accumulation of protein carbonylation which correlates with the aging process and neurodegeneration [76–78]. Methionine 281/282 oxidation of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), induced by angiotensin II, stimulates CaMKII independently of Ca²⁺/calmodulin. CaMKII oxidation, myocardial apoptosis and cardiac dysfunction are all enhanced in MsrA-deficient mice after myocardial infarction, suggesting that methionine oxidation mediates CaMKII activation and apoptosis during cardiac remodeling [12].

4-3 Tyrosine

Dityrosine is the predominant oxidation form of tyrosine when cells are exposed to oxygen free radicals, which include nitrogen dioxide, peroxynitrite and lipid hydroperoxides [79–81] (Figure 3C). Protein tyrosine nitration is modified in the 3-position of the phenolic ring of tyrosine by the addition of a nitro group (NO₂), usually mediated by peroxynitrite [82]. Biological denitration processes have been found in several organs [83], indicating that the reversible nitration process of tyrosine may be involved in the signaling transduction system. However, whether an enzymatic “denitrase” complex exists is still unknown.

4-4 Tryptophan

Tryptophan has been shown to be a specific target of nitration through exposure to nitrating reagents, such as peroxynitrite and peroxidase [84] (Figure 3D). Compared to tyrosine, the modification of tryptophan residues in proteins may occur at a more limited extent as tryptophan residues are less abundant and are often buried inside proteins. A novel structural modification, involving the addition of nitro and hydroxy groups to tryptophan, has been detected in the mitochondrial protein succinyl-CoA:3-oxoacid CoA transferase (SCOT) in the rat heart [85]. Modified SCOT accumulated progressively with age, which was associated with an elevation of its activity [86, 87]. However, whether or not tryptophan nitration is reversible remains unclear.

4-5 Carbonylation

Protein carbonylation is defined as the adduction of a carbonyl group to the side chain of a given amino acid, which includes cysteine, lysine, arginine, proline and threonine residues (Figure 3E). Carbonyl groups, including ketones and aldehydes, are usually generated by a lipid peroxidation process [88]. Protein carbonylation induces protein accumulation or enhances degradation in many oxidative stress-associated disease conditions [89–91]. Thus it is thought to be an irreversible process. However, carbonylation-driven degradation of annexin A1 by endothelin1 is responsible for maintaining cell growth and survival signaling in pulmonary artery smooth muscle cells, indicating that protein carbonylation events may have important functional effects [92, 93].

5. Specificity of protein modification in redox signaling

Oxidants transduce signals and regulate biological activities through redox modifications of signaling molecules that couple with alterations in their functions. However, since ROS-neutralizing defense mechanisms also exist in cells, the cellular environment is maintained in reduced conditions. Thus, posttranslational oxidative modification of proteins is tightly controlled both spatially and temporally.

The first level of specificity is determined by the concentration and localization of ROS. For example, a low dose of H₂O₂ induces cardiac myocyte hypertrophy while a high concentration induces myocyte apoptosis [94], indicating that ROS may regulate different signaling mechanisms in a dose-dependent manner. Superoxide, which is typically generated within an organelle compartment or outside the cell, cannot cross membranes due to its charge and thus functions only locally. However, H₂O₂ can diffuse everywhere inside a cell and across membranes, and it may affect signaling molecules in areas farther away from where it was generated. Nevertheless, the cellular response mediated by H₂O₂ is probably carried out by a local response via close contact between a protein and H₂O₂. Peroxiredoxin can be transiently phosphorylated and inactivated in the presence of growth factors, which allow local accumulation of H₂O₂ around the membrane, resulting in the oxidation of protein tyrosine phosphatase 1B (PTP1B) without ROS spreading to remote areas [95].

In theory, protein cysteines can undergo oxidation, but under physiological conditions, most do not. Most thiols have a pKa above 8.5, meaning that, at physiological pH, they principally exist in the reduced thiol (-SH) state which is relatively unreactive with oxidants; hence, they do not become posttranslationally modified. Redox active thiols tend to be in proximity to deprotonating basic amino acids, such as lysine, arginine or histidine. Such low pKa thiols exist as thiolate anions (S^-) at cellular pH, making them very reactive with oxidants. Thus, not all cysteines are equal in terms of their oxidant reactivity, and this provides a basis for selective oxidant signaling, as only certain proteins have the correct structure for these events to occur. Kinase substrates tend to have conserved sequences around their site of phosphorylation; similar consensus motifs have been reported in redox sensor proteins [96–99], but analyses of primary sequences have not been especially useful for predicting redox-active protein thiols. Perhaps analyzing the tertiary structure of a protein may eventually allow identification of the redox-sensitive motifs using bioinformatic approaches [100, 101].

6. Detection and identification of redox-sensitive molecules

Many methods have been developed to identify redox-sensitive proteins, with several of these approaches also allowing the reactive cysteine residue to be defined.

A free thiol labeling method has been used to quantify free thiols within proteins. The method uses thiol-reactive chemicals such as N-ethylmaleimide (NEM), iodoacetamide (IAM) or iodoacetate coupled with a reporter such as biotin, fluorophore or radiolabel, for detection and quantification. However, due to their chemical properties and molecular size, these labeling probes preferentially react with free thiols on the surface of the protein. This tendency to label surface thiol groups may be very helpful since ROS may also preferentially target the same pool of accessible cysteines. In addition to free thiol labeling, many methods are available to detect specific thiol oxidation. Biotin-labeled GSH was first used to detect S-glutathiolation, and can also be captured on solid-phase avidin, to allow target protein identification. Dimedone is used to measure sulfenic acid in proteins, specifically for *in vitro* studies [102, 103]. Recently, biotin-labeled dimedone and sulfenic-acid-specific antibody were generated to allow the selective identification and monitoring of sulfenic acid formation *in vivo* [7, 104, 105]. S-Nitrosylation can also be detected using an indirect labeling method. SNO adducts are selectively reduced by ascorbate, with subsequent labeling of the free thiol with tagged IAM. A related method uses a resin-assisted capture (SNO-RAC) to measure and identify the site of protein S-nitrosylation [106–108]. However, lack of specificity may generate false positive results, since ascorbate may also reduce sulfenic acid. Modified resin-assist capture to identify targets of protein oxidation (Ox-RAC) eliminates SNO by adding ascorbate before blocking unmodified and ascorbate-reduced thiol groups from modification. Oxidized cysteines are reduced by DTT and subsequently captured by tagged resin, allowing quantification and identification of the site of cysteine oxidation. By combining the SNO-RAC and Ox-RAC procedures, one can easily distinguish and compare SNO and other oxidation levels. The analysis of IPC samples has shown that SNO, induced by IPC, prevents cysteine oxidation induced by IR injury [109].

Proteomics has become a useful tool for monitoring and analyzing global protein oxidation status and identifying unknown redox-sensitive molecules in complex systems, such as whole cell lysate or tissue samples. Typically, proteomics couples the separation of proteins with high resolution methods, for example, by using 2-D electrophoresis with mass spectrometry (MS) and data base search for protein identification. However, one issue is that MS analysis preferentially identifies abundant molecules, most of which are not redox-sensitive, causing less abundant redox-active proteins to be missed. Tagged thiol reactive

chemicals, such as biotinylated IAM and GSH, can purify oxidation-modified proteins from unmodified pools using an avidin-based purification method, thereby eliminating noise from high abundance proteins and enriching modified proteins.

Perhaps the isotopic-coded affinity tag (ICAT) approach is one of the most convenient methods to rapidly screen oxidative posttranslational modification in redox-sensitive cysteine residues in response to various interventions [110, 111]. The ICAT method uses two different reagents, which bind specifically to thiol active groups. Both reagents are labeled with biotin affinity tags, allowing rapid purification. The only difference between these two reagents is that they include isotopically different atoms to yield a light or heavy variant of the affinity tag so that MS can distinguish them by molecular weights. Typically the control is labeled with one isotopic variant and the oxidative stress sample with the other. Oxidative stress is anticipated to reduce the amount of free thiol labeling compared to the control. The control and treated sample pairs are then typically combined as a single sample, and the labeled proteins are captured using solid-phase avidin and then analyzed by MS. The ratio of MS peak intensities of a pair of labels can directly measure the relative extent of cysteine oxidation. The limitation of this approach is that ICAT cannot determine the specific forms of cysteine oxidation and provides no absolute quantification. However, methods for detecting cysteine oxidative modifications have been evolving very quickly, and they will soon likely contribute to a deeper understanding of the cysteine oxidative effects involved in signal regulation.

7. Concluding remarks

Posttranslational oxidative-reductive modification is an integral mechanism for regulating the activity of the signaling molecules in the heart. However, spatial and temporal regulation of ROS and the specificity of ROS-mediated signaling are not yet fully understood. New techniques are required to precisely evaluate the local level and the identity of ROS in the heart *in vivo*. Although different kinds of ROS, such as O_2^- and H_2O_2 , are expected to exert distinct functions, surprisingly little is known regarding the specificity of their effects upon signaling mechanisms. Uncovering targets of ROS at subcellular compartments will help us better understand the biological effects of ROS and redox signaling in both physiological and pathological conditions in the heart. Oxidative stress is intimately involved in cardiac hypertrophy and heart failure. Although inhibition of oxidative stress often attenuates cardiac hypertrophy and dysfunction, it is often difficult to determine the direct cause-and-effect relationship between ROS and alterations in the signaling mechanism and other end points in the heart. One should evaluate when and where ROS are produced and which signaling molecules are subjected to direct posttranslational modification by ROS. The knowledge obtained from such experiments would be useful to the development of effective and specific treatments for heart disease. It is expected that posttranslational redox modification of signaling molecules will become a much more important area of research during the next decade, since it will clearly be a key to better understanding of heart disease.

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Abbreviations

AngII	angiotensin II
ASK1	apoptosis signal-regulating kinase 1
BDNF	brain-derived neurotrophic factor
BH4	tetrahydrobiopterin
CaMKII	Ca ²⁺ /calmodulin-dependent protein kinase II
cGMP	cyclic guanosine monophosphate
ETC	electron transport chain
Grx1	glutaredoxin1
GSH	glutathione
GSSG	glutathione disulfide
HDAC	histone deacetylase
IAM	iodoacetamide
ICAT	isotopic-coded affinity tag
IPC	ischemic preconditioning
Msr	methionine sulfoxide reductase
MS	mass spectrometry
NEM	N-ethylmaleimide
NOS	NO synthase
Nox	NADPH oxidase
Ox-RAC	oxidation resin-assist capture
PKA	protein kinase A
PKG	cGMP-dependent protein kinase
PTOM	post translational oxidation modification
PTP1B	protein tyrosine phosphatase 1B
ROS	reactive oxygen species
SCOT	succinyl-CoA:3-oxoacid CoA transferase
SNO-RAC	SNO resin-assist capture
SOD	superoxide dismutase
Trx	thioredoxin
XO	xanthine oxidase

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Highlights

- Intracellular redox status is tightly controlled by oxidant and anti-oxidant systems.
- Redox posttranslational modification is an important signal transduction process in cardiovascular systems.
- Various alterations of protein function are dependent upon the specificity of redox posttranslational modification.
- Modern techniques have been successfully applied to determine redox-sensitive proteins.

Figure 1.

(A) The general mechanism of oxidative-stress-induced cell dysfunction. Oxidative stress modified protein structure and triggered protein dysfunction, thereby mediating cell damage.

(B) The two different consequences of oxidants in mediating intracellular signaling.

Oxidants can modulate protein structure reversibly, thereby mediating adaptive responses under stress conditions, in order to prevent cell damage. Too many antioxidants will block this modification and cause adverse effects. On the other hand, oxidants can also modify protein structure irreversibly under severe conditions. Protein dysfunction may affect its aggregation or degradation process, which negatively affects cell function and induces cell injury. However, antioxidants can prevent oxidant accumulation and inhibit oxidant-induced protein PTMs, thereby providing a protective effect.

- 1) $O_2 \xrightarrow{+e} O_2^{\cdot -}$
- 2) $O_2^{\cdot -} \xrightarrow[2H^+]{+e} H_2O_2$
- 3) $H_2O_2 + Fe^{2+} \longrightarrow OH^{\cdot} + OH^- + Fe^{3+}$
- 4) $H_2O_2 + Fe^{3+} \longrightarrow OOH^{\cdot} + H^+ + Fe^{2+}$
- 5) $O_2^{\cdot -} + NO \longrightarrow ONOO^-$

Figure 2.

(A) The chemical reactions involved in production of (1) superoxide, (2) hydrogen peroxide, (3) hydroxyl radicals and (5) peroxynitrite. In the Fenton reaction (3 and 4), ferrous iron is oxidized by hydrogen peroxide to ferric iron, a hydroxyl radical and a hydroxyl anion. Ferric iron is reduced back by hydrogen peroxide to ferrous iron, a peroxide radical and a proton. (B) Schematic figure representing the major sources of reactive oxygen species (ROS) and the antioxidant system inside cells. ROS are usually generated by electron transport chain (ETC) leakage and Nox family proteins, which are neutralized by antioxidants including SOD family proteins, catalase, GSH and Trx systems.

Figure 3.

Structural redox modification of amino acid side chains. Typically, thiolated cysteine can be oxidized by ROS to form sulfenic acid, which can be stabilized or undergo reversible modification by forming a disulfide bond or sulfonamide. Further oxidation of sulfenic acid to sulfinic acid is now thought to be a reversible process. In severe conditions, sulfenic acid can be irreversibly oxidized to sulfonic acid. Reactive nitrogen species (RNS) usually mediate the S-nitrosation process. (A) Modification of the thiol group on cysteine. (B) Oxidation of methionine to form methionine sulfoxide. (C) The structure of oxidized tyrosine mediated by RNS. (D) The structural modification of tryptophan. (E) The common protein carbonylation on cysteine residues.