UDC 577.118:577.1
BIBLID 0021-3225 (1998) 34 p. 463-477
Review article

REGULATION OF CELL PROCESSES BY REACTIVE OXYGEN AND NITRIC OXIDE SPECIES - MECHANISMS OF REACTIONS

Vesna NIKETIĆ[1*], Srđan STOJANOVIĆ[1] and Mihajlo B. SPASIĆ[2]

[1]Department of Chemistry, University of Belgrade, Belgrade, Yugoslavia
[2]Institute for Biological Research, Belgrade, Yugoslavia

Niketić Vesna, Stojanović Srđan and Spasić B. Mihajlo (1998): *Regulation of cell processes by reactive oxygen and NO species - mechanisms of reactions.* - Iugoslav. Physiol. Pharmacol. Acta, Vol. 34, 463-477.

Recent evidence revealed that reactive oxygen species (ROS) such as superoxide (O^{2-}) and hydrogen peroxide (H_2O_2), which are conventionally viewed as unwanted and toxic by-products of life in an aerobic environment, have physiological roles. Appraisal of the roles of reactive NO species (RNOS), such as nitrosonium (NO^+) and nitroxyl (NO^-) ions, peroxynitrite ($OONO^-$), and higher nitrogen oxides (NO_x) in NO mediated processes, is growing at a rapid rate. ROS and RNOS may evoke a variety of cellular responses, ranging from major changes in mammalian cell gene expression to apoptotic death. The greater prevalence and reactivity of thiols over other biological nucleophiles makes them targets for both ROS and RNOS. Any essential protein containing cysteine residue that is strategically located at either active or allosteric site should be considered as a target for regulation by ROS and/or RNOS. Candidate molecules include proteins that are

[*]Corresponding author: Prof.Vesna Niketić, Department of Chemistry, University of Belgrade, Studentski trg 16, POB 158, YU-11001 Belgrade, Yugoslavia,
fax +381 (11) 635-425; e-mail: *vniketic@chem.bg.ac.yu*

themselves involved in signal transduction processes, ion channels, receptors, G-proteins, protein-kinases, protein phosphatases, and transcription-activating factors. In this paper the mechanisms of generation of ROS and RNOS and their reactions with thiols are briefly described. The regulation of cell processes by ROS and RNOS is illustrated by the interference of ROS and RNOS effects with receptor tyrosine kinases signaling.

Key words: NO-dismutation, reactive oxygen species (ROS), reactive NO species (RNOS), redox signaling, thiols

INTRODUCTION

Nitric oxide (NO) has gained wide attention for its role as an ubiquitous intra- and intercellular messenger and cytotoxin. A variety of physiological as well as pathophysiological roles of NO have been elucidated in recent years (Moncada *et al.*, 1991; Bredt and Snyder, 1994). The heme group of soluble guanilate cyclase is one of the most sensitive and important sites of action of NO, based on the well established role of cGMP in many NO-mediated responses (Bredt and Snyder, 1994). Indeed, evidence for a physiological role of NO was preceded by studies demonstrating cGMP formation in response to nitroglycerin and other NO-donors (Milovanović *et al.*, 1984, 1985; Bredt and Snyder, 1994). The reversible reactions of NO with a number of other heme proteins have been studied extensively. It was found that NO activates cyclooxygenase (Gross and Wolin, 1995) and inhibits a host of other heme proteins such as cytochrome oxidase, lipoxygenases, peroxidases (Stamler, 1994; Radi, 1996) and catalase (Brown, 1995; Dusinović *et al.*, 1998). In addition to its reactions with heme proteins, it is well established that in the biological milieu NO takes part in diverse reactions yielding highly reactive species such as nitrosonium (NO^+) and nitroxyl (NO^-) ions, peroxynitrite, and higher nitrogen oxides (NO_x) that are more reactive than NO itself. Appraisal of the roles of these reactive NO species (RNOS) in NO mediated processes is growing at a rapid rate (Stamler *et al.*, 1992; Stamler, 1994; Gross and Wolin, 1995; Wink *et al.*, 1996).

Recent evidence demonstrate that reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), which are conventionally regarded as unwanted and toxic by-products of life in an aerobic environment, may play physiological roles in diverse cell events. Observation that stimuli to specific cell-surface receptors are associated with a production of ROS puts the action of ROS into an even more persuasive physiological context (Suzuki *et al.*, 1997; Nakamura *et al.*, 1997; Finkel, 1998; Kourie, 1998).

Both ROS and RNOS function in two discrete fashions: high amounts are cytotoxic, and when produced by macrophages and other immune-effector cells, they play a role in host defense. However, when produced at low, subtoxic level they serve physiological functions. Effects of RNOS resemble those of ROS in evoking a variety of cellular responses ranging from major changes in mammalian cell gene expression to apoptotic death (Herlich and Rahmsdorf, 1994; Polyak *et al.*, 1997; Kroemer *et al.*, 1998, Brune *et al.*, 1998; Marquis and Demple, 1998).

The greater prevalence and reactivity of thiols over other biological nucleophiles makes them targets for both ROS and RNOS. Any essential protein containing cysteine residue that is strategically located at either active or allosteric site should be considered

a target for regulation by ROS and/or RNOS. Candidate molecules include proteins that are themselves involved in signal transduction processes, ion channels, receptors, G-proteins, protein-kinases, protein phosphatases, and transcription-activating factors. A number of protein-kinases were shown to be activated by ROS or RNOS induced modifications of their cysteine residues (Gopalakrishna and Gundimeda, 1993; Konishi *et al.*, 1997; Gotoh and Cooper, 1998). On the other hand, activation of certain tyrosine kinases seems to be due to the inhibition of specific tyrosine phosphatases caused by ROS or RNOS modifications of cysteine residue in their active centres (Knebel *et al.*, 1996; Duarte, 1997). Nitrosylation/ thiolation of critical Cys-residue in $p21^{ras}$ was shown to activate this small G-protein, which was suggested to represent the central mechanism by which a variety of redox stress stimuli transmit their signal to nuclei (Lander *et al.*, 1995). The ROS/RNOS induces redox-sensitive transcription factors AP-1 and NF- κB (Schreck *et al.*,1991; Peunova and Enikolopov, 1993; Lander *et al.*,1993).

In this paper, mechanisms of generation of ROS and RNOS and their reactions with thiols will be briefly discussed. Since our primary aim is to interpret rather than exhaustively summarize the large body of accumulated literature, only the examples of ROS and RNOS interference with receptor tyrosine kinases (RTK) signaling will be described in more detail. Specific ligand stimuli to RTKs were shown to be associated with ROS generation. ROS and RNOS activate RTKs and affect also downstream components of RTKs cell signaling complex.

SOURCES OF ROS

Reactive oxygen species (ROS) include oxygen free radicals and molecules that are more strongly oxidizing than molecular oxygen itself. These are superoxide anion radical ($O^{2\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\cdot}$)[*]. There is a large number of intracellular and extracellular sources of ROS. $O_2^{\cdot-}$ and H_2O_2 are produced in large amounts by cells of the immune systems, whereas other cell types, appear to produce significantly lower amounts of these molecules (Simović *et al.*, 1995). The vast network of intracellular and extracellular anti-oxidant defenses point out that the level of ROS must be tightly regulated for the cell survival. Antioxidant defense in mammals is a rather complex system, being species-, organ-, and tissue-specific (Buzadžić *et al.*, 1990a; 1990b; Spasić *et al.*, 1993; Simović *et al.*, 1995), and its regulation is closely connected to the regulation of the principal metabolic functions (Petrović *et al*, 1980; 1982; 1983; 1991). The principal sources of ROS and mechanisms of antioxidant defense are summarized in Fig. 1.

SOURCES OF RNOS

Mammalian cells possess at least three genes encoding distinct isoforms of NO

[*]*Other oxygen free radicals and oxidants that can be formed in living systems include lipid ($L^{\cdot}$) and other (X) peroxy radicals ($LOO^{\cdot}$ and $XOO^{\cdot}$) lipid peroxide (LOOH), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl) and other N-chloramine compounds.*

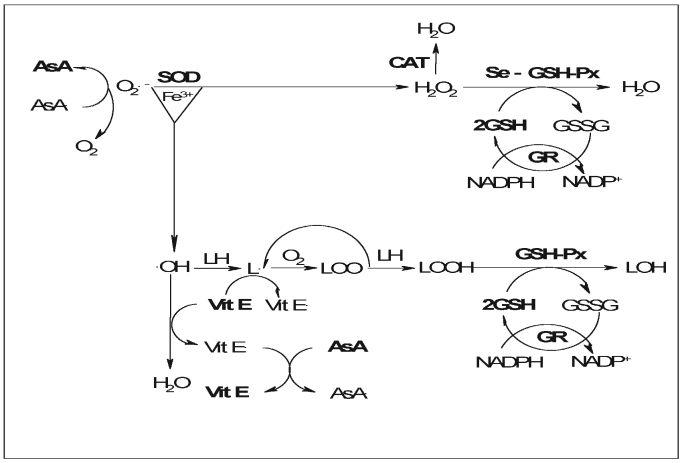

Fig 1. Reactive oxygen species and antioxidant defense: Vitamin C (AsA) acts as a cytosolic antioxidant; vitamin E acts as membrane antioxidant, reduced glutathione (GSH) protect both cytosol and membranes against ROS. Also present are the glutathione-containing enzymes glutathione peroxidase (Se-GSH-Px), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD). Some of these enzymes exist in several forms. Membrane, cytosolyc and plasma forms of GSH-Px have all been reported. Similarly, there are mitochondrial, cytosolic, and extracellular forms of SOD (modified from Spasić, 1993).

synthase (NOS). Two NOS gene products are constitutively expressed: one (eNOS) in endothelial cells, and second (nNOS) in the neuronal and number of other cell types. The third species of NOS is inducible by immunological stimuli in virtually all nucleated mammalian cells examined (iNOS). Both eNOS and nNOS produce a small, physiological increase of NO concentration in response to transient elevation of intracellular calcium, whereas iNOS produces a large and continuous flux of NO until substrate becomes limiting. NOS is one of the most regulated enzymes in biology. All three NOS isoforms oxidize the guanidine group of L-arginine in a process that consumes five electrons and results in the formation of NO with stochiometric formation of L-citruline (Bredt and Snyder, 1994; Gross and Wolin, 1995).

Nitric oxide is a relatively stable gas, that after a currently accepted paradigm, readily diffuses into cells and cell membranes (Lancaster, 1997; Chen *et al.*, 1998) where it reacts with molecular targets: transition metal centers, superoxide anion, and molecular oxygen (Fig. 2).

The reactions of NO with transition metal centers constitute the basis of NO actions in biological systems (Moncada *et al.*, 1991). In the majority of metal-nitrosyl complexes, bonding of the NO group to the central metal atom may be regarded as covalent, formed between NO^+ and a metal ion that has been reduced by one oxidation state unit. NO^- complexes may be formally regarded as resulting from bonding between NO^- and the metal atom that has been oxidized by one unit. Indeed, metal-catalysed reduction of NO has been postulated as a possible source of NO^- (Bonner and Pearsall, 1982). In a limited number of instances nytrosyl complexes are best described as

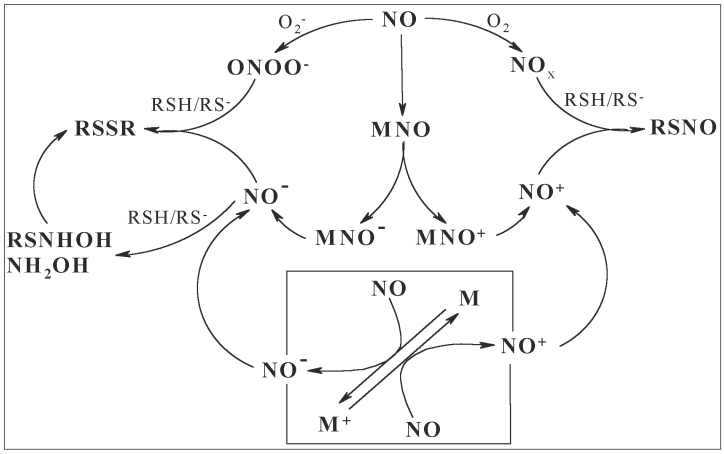

Fig. 2. Summary of NO reactions in biological systems that reactions with cell thiols to form RS-NO, disulphides, and a number of intermediates such as RSNHOH (for details see text).

containing a neutral NO molecule (Bonner and Stedman, 1996).

Our recent *in vitro* study demonstrated that NO-treatment of Mn and Fe SOD, but not Cu/Zn SOD gives rise to reactive nitrosyl complexes that generate both NO^+ and NO- ions. NO reacts with Mn and FeSODs presumably according to the following reaction schemes (Niketić *et al.*, 1999):

$$\mathbf{E_{red} + NO \rightarrow E_{ox} + NO^-}$$

$$\mathbf{E_{ox} + NO \rightarrow E_{red} + NO^+}$$

The generation of NO^+ and NO^- by Mn and FeSODs is analogous to the well known dismutation reaction of $O_2^{\cdot-}$ by SODs to generate O_2 and H_2O_2 (Michelson, 1987). Therefore, we suggest the term *NO-dismutation* for the above described reactions. It seems that Mn and FeSOD induced NO-dismutation reaction may not be an exception in the biological milieu. Indeed, our recent *in vitro* studies demonstrated that in CSFs of both ALS patients and normal individuals, ill-defined low molecular Fe complexes exist which are able to dismutate NO efficiently (Niketić *et al.*, 1999).

Peroxynitrite ($ONOO^-$) is formed in the near-diffusion controlled reaction between NO and $O_2^{\cdot-}$. Thus the formation of $ONOO^-$ will predominate in any setting where NO and $O_2^{\cdot-}$ are simultaneously produced, *e.g.*, in an inflammatory lesion (Wink *et al.*, 1996).

The products formed from NO/O_2 reaction in gas phase and hydrophobic media *i.e.* NO_2, N_2O_3 and N_2O_4 are well characterized. However, the true nature of higher nitrogen oxides (NO_x) formed upon oxidation of NO with O_2 in aqueous media remains disputable. The reaction between NO and O_2 was shown to occur *via* third- order kinetics, with nearly identical rates in any biological medium. Therefore, under physiological conditions the interference from NO/O_2 reaction is considered minimal. However, under the conditions of higher NO concentrations the rate of its oxidation

exponentially increases (Wink *et al.*, 1996).

REACTIONS OF ROS AND RNOS WITH THIOLS

Sulphydryl groups in proteins, free cysteine, and GSH represent abundant sources of reduced thiols in biological systems. The concentration of protein SH (PSH) groups in the cell is far greater than that of the metabolically most important thiol GSH (Simplicio *et al.*, 1998). There is an increasing body of evidence on proteins having reactivities similar to or higher than that of GSH. The intrinsic reactivity of PSH with ROS and RNOS will depend not only on their pKa but also on other structural features, such as their accessibility; moreover in allosteric proteins the -SH reactivity may be expected to be influenced by ligands.

A feasible mechanism for $O_2^{\cdot-}$ and H_2O_2 induced disulphide formation involve an oxyradical initiated mechanism. By this mechanism protein sulphydryl groups are activated by reaction with $O_2^{\cdot-}/H_2O_2$, and then the activated proteins react with GSH or with other protein SH groups. The process is rapid, it does not require extensive oxidation of cellular glutathione, and it might occur at localized sites in close proximity to the site of $O_2^{\cdot-}/H_2O_2$ generation. Further support for this mechanism is found in recently observed hemoglobin thiyl radical in erythrocytes exposed to $O_2^{\cdot-}/H_2O_2$ (Maples *et al.*, 1990) followed by detection of glutathione adduct of hemoglobin *in vivo* (Niketić *et al.*,1992).

The free sulphydryl groups will be restored by the thiol-disulphide exchange catalyzed by thiol-transferases in the presence of excess of GSH. Because the thiol-transferase reaction is bidirectional, the equilibrium will be determined by the redox state of the cell, *i.e.* in the excess of GSH it will be shifted to the right (Wang and Ballatori, 1998):

Thiol transferases

$$\mathbf{RS\text{-}SR' + GSH \rightleftharpoons RS\text{-}SG + R'SH}$$

$$\mathbf{RS\text{-}SG + GSH \rightleftharpoons RSH + GSSG}$$

Under anaerobic conditions, NO reacts directly with low molecular weight thiols at physiological pH yielding stable end products: disulphide and nitrous oxide (Pryor *et al.*, 1982). Thiol proteins under the same conditions can yield sulfenic acid (DeMaster *et al.*, 1995). It has been suggested recently that NO may react directly with thiols to form S-nitrosothiol in the presence of an electron acceptor (Gow *et al.*, 1997).

Peroxynitrite is more reactive with sulphydryls than H_2O_2. Low molecular weight thiols are oxidised with peroxynitrite to predominantly disulphide forms (Fig. 2), while steric restrictions due to the size of the molecule or the location of SH groups may preclude disulphide formation in protein molecules. Peroxynitrite may oxidize protein SH groups beyond the disulphide (Radi *et al.*, 1991).

Under physiological conditions NO^- is rapidly converted to N_2O and reacts with O_2 to yield peroxynitrite (Stamler *et al.*, 1992). The competing two step reaction with thiol groups results in the formation of disulphide and hydroxylamine (Fig. 2) (Wink *et al.*, 1996).

S-nitrosothiols (RSNO) are efficiently formed upon reaction of thiols with

nitrosating agents (NO^+ donors) and NO_x (Fig. 2) (Stamler *et al.*, 1992; Stamler 1994.; Wink *et al.*, 1996). They show varying degrees of stability under physio-logical conditions. For example, S-nitrosoglutathione (GSNO) undergoes decompo-sition over hours, whereas S-nitrosocysteine has a half life of less than 2 min (Singh *et al.*, 1996). Their decomposition to yield disulphides is strongly catalyzed by transition metal ions (McAninly *et al.*, 1993). For any system containing thiol (R'SH) and S-nitrosothiol (RSNO) the rapid transnitrosation (exchange reaction) is characteristic, but the reaction seems to be more complex than might be inferred from the few final reaction products (Singh *et al.*, 1996).Glutathione readily reacts with S-nitrosothiols, forming the stable GSNO in cells . It was demonstrated recently that in restoration of free GSH reducing agents such as ascorbic acid (Iwatsuki, 1997) and the thioredoxin system (Nikitović and Holmgren, 1996) may be involved.

The biological activity of S-nitrosothiols is thought to be associated with both heterolytic and homolytic mechanisms of decomposition (Garley and Stedman, 1988). RSNOs retain NO-like bioactivity, but exhibit resistance to reactions with O_2 and $O_2^{\cdot-}$. For that reason, the S-nitrosated proteins, the most abundant of which is serum albumin, are thought to serve as a source and sink of NO, buffering the concentration of free NO (Stamler *et al.*, 1992).

The detection of S-nitrosated proteins *in vivo* and evidence that such chemical modification can alter protein activity have led to the view that nitrosation of protein thiols may be a fundamental to signal transduction process (Stamler *et al.*, 1992; Stamler, 1994). S-nitrosation of a protein can regulate its activity by several discrete mechanisms. It may bring about a conformational change by altering intramolecular hydrogen bonding or electrostatic interactions. If located in the immediate vicinity of an intramolecular thiol it may accelerate disulphide formation (Stamler, 1994).

INTERFERENCE OF ROS AND RNOS WITH RECEPTOR TYROSINE KINASES (RTKs) SIGNALING

Numerous studies have demonstrated that ligand stimulation of non-phagocytic cells results in an increase in intracellular ROS. This has been observed in a wide variety of cell types and is stimulated by a diverse collection of ligands, including growth factors acting through tyrosine kinases (Finkel, 1998). ROS were shown to activate RTKs and regulate downstream tyrosine phosphorylation (Sundaresan *et al.*, 1995; Bae *et al.*, 1997). The primary source of ligand activated ROS-generating system seems to be NADPH oxidase functionally similar to the neutrophil NADPH oxidase (Finkel, 1998). The intracellular pathway in non-phagocytic cells leading from ligand activation to ROS generation appears to require also small GTP-binding proteins Ras and Rac1. This further supports the hypothesis that ROS may serve as intracellular second messengers and suggest that small GTP-binding proteins regulate redox-sensitive signal-transduction pathways (Sundersan *et al.*, 1996). These findings suggest that ligand stimulation of cells may activate two separate but interrelated pathways. One well cha-racterized pathway leads to the modification of protein by phospho-rylation of critical tyrosine residues. The other pathway, also con-trolled by small GTP-binding proteins, presumably would result in the generation of transient, highly local-ized, burst of ROS which will lead to the modification of protein functions by the oxidation of critical cysteine residues.

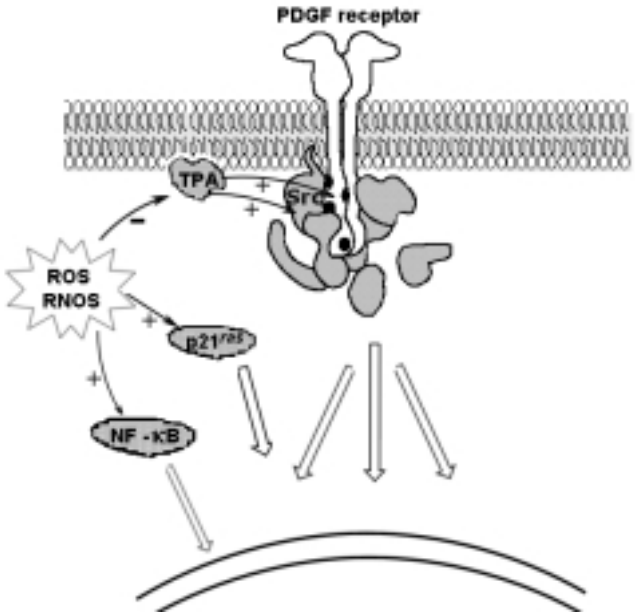

Figure 3. Interference of ROS and RNOS with RTKs signaling. (+) indicates activation and (-) inhibition. Black patches on the cytoplasmic domain of the receptor indicate the tyrosine phosphates to which signaling proteins bind. Increase in ROS causes oxidation of cysteine residue at the active center and consequent transient inhibition of tyrosine phosphatases (TPA), which temporarily permits a burst of kinase activity. Phosphorylation of RTKa triggers the assembly of an intracellular signaling complex on the receptor tail. The newly phosphorylated tyrosines serve as binding sites for different intracellular signaling molecules. The complex then broadcasts signals along multiple routes to many destinations inside the cell, activating and coordinating the numerous biochemical changes needed to trigger a complex response such as a cell proliferation. Each activating receptor produces multiple outputs (for details see: Bray, 1998).

ROS were shown to stimulate activities of various receptor and non-receptor tyrosine kinases (Suzuki *et al.*,1997; Finkel, 1998). Indeed, patterns of H_2O_2 induced epidermal growth factor (EGF) and insulin RTKs phosphorylation were found to be identical with those induced by the natural ligands (Gamou and Shimizu, 1995). Responses by RNOS closely resemble those of ROS (Lander *et al.* 1993; Stamler, 1994; Martin *et al.*, 1998). However, whereas ligand stimulation results in transient, highly localized burst of ROS which activates only specific RTKs, RNOS and ROS produced by other sources may induce a multitude of RTKs which lead to the activation of a numerous downstream signaling pathways. It is likely that the stimulation by specific growth factor may activate strong signaling to the nucleus while part of the signaling induced by ROS or RNOS may be neutralized and not reach the nucleus (Knebel *et al.*, 1996) (Fig. 3).

How do oxidants activate tyrosine kinases? It is not yet clear whether reactive species cause direct activation of tyrosine kinases or the observed increase in tyrosine phosphorylation is due to inhibition of tyrosine phosphatases. Because all protein-tyrosine phosphatases have reactive cysteine residues in their active centers (Fischer *et al.*, 1991), the inhibition of tyrosine phosphatases may account for the mechanisms of stimulation of tyrosine phosphorylation by ROS and RNOS (Fig. 3).

Receptor tyrosine kinase activation requires homo- or heterodimerization (Spivak-Kroizman *et al.*, 1994). It was documented that a considerable fraction of EGFR and platelet derived growth factor receptors (PDGFR) are in close association in the absence of ligand and exhibit intrinsic tyrosine kinase activity. However, under basal conditions tyrosine phosphatase activity predominates, since the specific activity of tyrosine phosphatases is several orders of magnitude greater than that of the corresponding tyrosine kinases. Increase in ROS causes oxidation of cysteine residue at the active center and consequent transient inhibition of tyrosine phosphatases, which temporarily permits a burst of kinases activity. It seems that RTKs as well as cytoplasmic tyrosine kinases are under the control of different phosphatases, which can be differently affected with ROS, and thus the signal transduction may vary (Knebel *et al.*, 1996).

The effects of ROS/RNOS and antioxidants on steps further downstream are less predictable. It was shown that H_2O_2 induces tyrosine phosphorylation of the Src family protein tyrosine kinases (Nakamura *et al.*, 1993), nitrosation/thiolation of critical Cys-residue activates $p21^{ras}$ (Lander *et al.*, 1995) and the ROS/RNOS may activate redox-sensitive transcription factors AP-1 and NF-κB (Devary *et al.*, 1993; Lander, 1993; Stamler, 1994)

CONCLUDING REMARKS

ROS and NO fulfill several criteria as possible signaling molecules. They are small diffusible species that are ubiquitously present and can be rapidly synthesized and destroyed. Contrary to conventional biosignaling ligands that act by binding to specific receptor molecules, the biological actions of NO and ROS are dictated by the reactions they undergo with target molecules in cells, membranes, and the extracellular milieu. The ROS and NO initiated processes are rapid enough to be of metabolic significance. They are very site directed since the reactivity of radicals suggests that they do not diffuse far before participating in chemical reactions.

Oxidation/reduction and nitrosation of critical SH-groups in proteins caused by ROS and/or RNOS may be more widely exploited as a regulatory principle than is currently assumed. In other words, continuum of ROS and RNOS (reversible) modifications of cysteine residues may constitute biological signaling events. The alteration of protein function by ROS and RNOS may be in many ways analogous to phosphorylation except that protein modification no longer occurs on specific serine or tyrosine residues but instead on redox sensitive cysteine residues.

The protein kinases and protein phosphatases seem to be well suited for the regulation *via* oxidation/reduction and/or S-nitrosation. But, significant work needs to be done in identifying direct targets of ROS and RNOS, the molecular basis of ROS and RNOS sensitivity in proteins, and the relevant sources of their production. Such work will hopefully provide some understanding of how specificity can be achieved

using ROS and RNOS as signal transducers. The relative importance of other post-translational modifications of proteins, which arise from direct reaction with ROS and RNOS species such as tyrosine nitration, which are expected to occur under toxic conditions, are yet to be realized.

ROS and RNOS have been postulated to be contributors to the pathogenesis of a number of diseases, including arteriosclerosis, cancer, and Alzheimer's disease. Interestingly, clinical and epidemiologic studies have, in some cases, indicated that antioxidant nutrients may be effective in disease prevention (Palmer and Paulson, 1997). Discoveries that ROS/RNOS and antioxidants can directly affect the cellular signaling apparatus and, consequently, the control of gene expression may provide the link between ROS/RNOS and antioxidant chemistries and the mechanisms of disease processes and prevention.

Acknowledgments. - This work was supported by the Ministry of Science and Technology of Serbia.

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Received February 16, 1999
Accepted February 20, 1999

РЕГУЛАЦИЈА ЋЕЛИЈСКИХ ПРОЦЕСА РЕАКТИВНИМ ВРСТАМА КИСЕОНИКА И АЗОТ МОНОКСИДА - МЕХАНИЗМИ РЕАКЦИЈА

Весна НИКЕТИЋ[1], Срђан СТОЈАНОВИЋ[1] и Михајло Б. СПАСИЋ[2]

[1]Хемијски факултет, Универзитет у Београду; [2]Институт за биолошка истраживања, Одељење за физиологију, Београд, Југославија

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Најновија сазнања указују да реактивне кисеоничне (*reactive oxygen species - ROS*) врсте као што су супероксид ($O_2^{\cdot-}$) и водоник пероксид (H_2O_2), који се уобичајено посматрају као нежељени и токсични споредни производи живота у аеробним условима, имају физиолошке улоге. Великом брзином долази се и до сазнања да реактивне *NO* врсте (*reactive nitric oxide species - RNOS*), као што су нитрозониум (NO^+) и нитроксил (NO^-) јони, пероксинитрит ($ONOO^-$) и виши азотови оксиди (NO_x) имају важне улоге у процесима у којима учествује *NO*. *ROS* и *RNOS* могу да изазову различите ћелијске одговоре, од измена у експресији гена до апоптотичне смрти ћелије. С обзиром да су тиоли у ћелији заступљенији и реактивнији од осталих нуклеофила, реакције *ROS* и *RNOS* са њима биће највише изражене. Сваки протеин који садржи остатак цистеина у активном или алостерном центру може да буде погодан за регулацију са *ROS* и/или *RNOS*. Кандидати су протеини који учествују у трансдукцији сигнала, јонски канали, рецептори, Г-протеини, протеинске киназе и фосфатазе и транскрипциони фактори. У овом раду укратко су описани механизми настајања *ROS* и *RNOS* и њихове реакције са тиолима. Улога *ROS* и *RNOS* у регулацији ћелијских процеса илустрована је на примеру тирозин-киназних рецептора.

Примљено 16.02.1999.
Прихваћено 20.02.1999.