NADPH OXIDASE: STRUCTURE AND ACTIVATION MECHANISMS (REVIEW). NOTE I

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NADPH OXIDASE: STRUCTURE AND ACTIVATION MECHANISMS (Review). NOTE I (Abstract) NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase), with its generically termed NOX isoforms, is the major source of ROS (reactive oxigen species) in biological systems. ROS are small oxygen-derived molecules with an important role in various biological processes (physiological or pathological). If under physiological conditions some processes are beneficial and necessary for life, under pathophysiological conditions they are noxious, harmful. NADPH oxidases are present in phagocytes and in a wide variety of nonphagocytic cells. The enzyme generates superoxide by transferring electrons from NADPH inside the cell across the membrane and coupling them to molecular oxygen to produce superoxide anion, a reactive free-radical. Structurally, NADPH oxidase is a multicomponent enzyme which includes two integral membrane proteins, glycoprotein $gp91^{phox}$ and adaptor protein $p22^{phox}$, which together form the heterodimeric flavocytochrome b558 that constitutes the core of the enzyme. During the resting state, the multidomain regulatory subunits $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ are located in the cytosol organized as a complex. The activation of phagocytic NADPH oxidase occurs through a complex series of protein interactions. **Keywords:** REACTIVE OXYGEN SPECIES, NADPH OXIDASES, NOX, STRUCTURE, LOCALIZATION, FUNCTIONS.

Oxidative stress (SO) is essentially the result of an imbalance between the endogenous production of free radicals derived from oxygen (ROS) or nitrogen (RNS), and the ability of the body to counteract or detoxify their harmful effects through neutralization by antioxidants (1, 2, 3). This molecular imbalance in ROS metabolism has been proven both at the cellular level and throughout the body. ROS have an opposing, context-depending dual role.

Under normal circumstances, the production and removal of ROS, that occurs in distinct extracellular or (sub)cellular spaces, are strictly controlled by effective endogenous defense mechanisms that block their excessive production. Some reactive oxygen species are necessary for life (especially superoxide anion radical O^{2-}, hydrogen peroxide H2O2) (4) having a pivotal role in mediating important functions in normal cellular physiology by their contri-

bution to regulating different physiological processes such as cellular defense, signaling mechanisms, steroid synthesis, activation of G protein-coupled receptors, regulation of transcription factors, gene expression (5).

Overproduction of ROS results in oxidative stress with various pathological consequences such as atherosclerosis, hypertension, vascular remodeling after angioplasty, myocardial infarction, asthma, stroke, etc. (6, 7, 8, 9).

Sources of ROS. Of the many sources of ROS we mention the redox enzymes NADPH oxidase, mitochondrial respiratory chain, xanthine oxidase, lipoxygenase, and cyclooxygenase, which are continuously interacting with each other. In biological systems, ROS have the superoxide radical anion O^{2-} as one of the most important representative. A typical enzyme belonging to this redox group that generates ROS is NADPH oxidase, with important biological functions (10). NADPH oxidases are present in the higher life forms (animals, plants), but also in fungi, but not in prokaryotes and most unicellular eukaryotes. The enzyme is expressed in professional phagocytic cells, multiple types of blood cells (such as neutrophils, monocytes, eosinophils, macrophages, mast cells) (11) involved in antibacterial, antifungal, antimicrobial defense by reactions with free radicals; the enzyme is also involved in innate immunity (12). Phagocytic NADPH oxidase becomes active upon stimulation and then generates superoxide anions. An alternative scenario in that phagocytic NADPH oxidase directs the ions inside and outside the vacuole formed by endocytosis producing changes in pH and ion flow (13). NADPH oxidase and NADPH oxidase-like enzyme also described in non-phagocytic cells have been identified in almost every type of tissue, but functionally distinct from phagocytic oxidases (14). Non-phagocytic NADPH oxidases are constitutively active enzymes that produce superoxide radical anion intracellularly in a slow and sustained manner and act as a signaling molecule influencing the transcription factors, molecules involved in inflammation, cell growth etc (15).

The cellular compartments in which NADPH oxidases are expressed include: the nucleus, endoplasmic reticulum, endosomes, phagosomes, mitochondria, and extracellular space (16).

Structure of NADPH oxidase. In mammals, NADPH oxidase is a plasma membrane-bound multicomponent complex (heteroprotein) that faces the extracellular space, consisting of 7 isoforms (homologues). ROS is produced by NADPH oxidase through NOX protein, thus differentiating the enzyme from other oxidases. NADPH oxidases are collectively referred to as the NOX family: NOX 1, NOX 2 (identical to membrane glycoprotein $gp91^{phox}$, also known as a subunit β, suffix phox refers to **ph**agocyte **ox**idase) (17), NOX 3, NOX 4, NOX5 and associated dual oxidases – Duox 1 and Duox 2 (18, 19, 20). The catalytic subunits of NOX 1, NOX 2, NOX3 and NOX 4 form a macromolecular complex with $p22^{phox}$ (known as subunit α) as a common enzyme activator subunit. NOX 5 Duox1 and Duox2 are $p22^{phox}$-independent isoforms. Duox 1 and 2 have additional peroxidase domains termed this way due to homology to peroxidases which catalyze dismutation of superoxide anion to hydrogen peroxide – H2O2 (13). Therefore, each of these isoforms has a core catalytic

subunit, the so-called NADPH-oxidase (NOX), two dual oxidase subunits (Duox) and 5 regulatory subunits.

Isoenzymes of NADPH-oxidase (NOX) are distinguished by the following features: subcellular localization, tissue and subcellular patterns of expression, structure (the nature of the catalytic subunits determines the adaptation of regulatory subunits), mechanism of enzyme activation, ROS-generating catalytic mechanism and functions (1, 10,21).

Physiological functions of NADPH oxidases: the enzymes catalyze the transfer of electrons in cytosol across the membrane from NADPH, which oxidizes, to molecular oxygen, which is reduced to produce the superoxide radical anion (O^{2-}), generator of other ROS species. The NADPH oxidases was originally identified as a component of innate host defence. In phagocytes, this complex enzyme is activated to produce superoxid anion and other secondarily derived ROS, wich promote killing of invading micro-organisms. Another non phagocytic NADPH oxidases system is present in almost every body organ being physiologically involved in signal transduction, cell proliferation and differentiation, posttranslational processing of proteins (4, 18).

Mechanism of action of NADPH oxidase Although all NOX enzymes have the same function in ROS generation, the activation and control mechanisms, the nature of the ROS produced, subunit requirements, and intracellular distribution varies between isoforms (22). Some NOX are membrane proteins with catalytic functions, other have cytosolic localization and regulatory functions (23). All NOX family members share a common core structure made up of 6 transmembrane domains. The catalytic core of the classic phagocytic enzyme – NADPH-oxidase (NOX 2) as of the other isoforms consists of two membrane-integrated proteins (subunits): $gp91^{phox}$, glycosylated protein (24), (considered the patriarch of NOX family) and $p22^{phox}$ (non-glycosylated adapter protein) which together form a large subunit, the flavocytochrome b558 complex (heterodimeric flavohemoprotein b558). $Gp91^{phox}$ subunit consists of 6 transmembrane domains and the cytoplasmic C-terminal region which contains two binding sites for FAD (flavin adenine dinucleotide) and for the electron donor NADPH. All NADPH oxidases except NOX 5, Duox 1 and Duox 2 have similar topological structure of the catalytic core $gp91^{phox}$. The N-terminal domain of NOX 5 binds calcium, Duox1 and Duox 2, in addition to the NOX 5 structure present at the N-terminal transmembrane α-helix a domain homologous to peroxidase (25).

The regulatory subunits play important roles in: maturation and expression of NOX and Duox subunits in biological membranes, and in enzyme activation and spatial organization of the various components of the enzyme complex (26). Some isoforms of NADPH oxidase contain GTP-ase (guanine triphosphate hydrolase), Rac 1 and Rac 2, which modulate their activity (27).

The activation of phagocyte NADPH oxidase occurs through a series of complex protein interactions in response to numerous agonists (27). In unstimulated cells, components $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ are grouped in the cytosol into a complex (25).When the resting cell is exposed to a variety heterogeneous stimuli (chemical, physical, environmental, biological factors)

(24), the peripheral cytosolic component becomes highly phosphorylated able to form a complex which migrates to the membrane where it associates with flavocytochrome b558 and together with other components – $p40^{phox}$, $p47^{phox}$ and $p67^{phox}$ – forming the active oxidase which transfers electrons from the substrate (NADPH) to molecular oxygen with formation of superoxide radical anion (27, 28), precursor of other toxic ROS (17). The activation process is strictly controlled, involving in addition to phosphorylation multiple conformational changes. This enzymatic complex is involved in many key biological functions such as cell growth, proliferation, differentiation, migration, defense, signaling and regulation of gene expression. Excessive ROS production attributed to the increased NOX activity, causing cellular oxidative stress, may contribute to the occurrence of a wide variety of diseases: vascular disease, inflammation, endothelial dysfunction, cell proliferation, fibrosis, angiogenesis, cardiovascular remodeling, cardiac aging, renal remodeling in hypertension, atherosclerosis, diabetes, heart failure, myocardial ischemia-reperfusion injury.

CONCLUSIONS

NADPH-oxidases is a proteic complex that, being stimulated, is capable to produce O^{2-}, by transfering an electron from NADPH to O2. It is a multicomponent family enzyme, with a complex structure made of membrane proteine complex, with cathalitic properties; in the same time, the enzyme has citosolic structures with regulatory properties. The tisulary distribution of NADPH-oxidases and their activating mechanisms are of large variety.

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