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NADPH Oxidase, The Macrophage Killing Weapon

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Abbreviations

CGD, chronic granulomatous disease
EPO, eosinophil peroxidase
fMLP, N-formyl-Met-Leu-Phe
GTP, guanosine triphosphate
MPO, myeloperoxidase
NADPH, nicotinamide adenine dinucleotide phosphate
PMA, phorbol 12-myristate 13-acetate

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I. Abstract

The human neutrophil NADPH oxidase is a multi-component complex composed of membrane-bound and cytosolic proteins. During activation, cytosolic proteins translocate to the plasma membrane and associate with flavocytochrome b to form the active superoxide-generating system. The low-spin state of the iron of the heme in cytochrome b is essential to generate $O_2^{\bullet-}$ in the NADPH oxidase system. NADPH oxidase is involved in human diseases like CGD. CGD is an inherited immune deficiency in which phagocytes from affected patients are unable to manufacture $O_2^{\bullet-}$. This paper discusses the unique structure of NADPH oxidase, its activity, and the important role of the enzyme in the defense mechanisms of body macrophages.

II. Introduction

Leukocytes are important mediators of innate immunity through the generation of reactive oxygen species that are capable of killing invading microorganisms. The source of these deleterious oxygen species is superoxide ($O_2^{\bullet -}$) produced by the NADPH oxidase, a large, membrane-associated enzyme complex. In humans, the NADPH oxidase constitutes the primary defense mechanism against microbial infections. However, the highly reactive chemical species produced by NADPH oxidase may also cause severe tissue damage and induce inflammatory responses (*e.g.* rheumatoid arthritis or asthma) emphasizing the need for a tight regulation of this multi-protein complex. This is achieved by maintaining the oxidase in a dormant, inactive state in which the component proteins are partitioned between the cytosol and the cell membrane. This report explores the different mechanisms involved in NADPH oxidase activation and control.

III. NADPH oxidase complex

THE NADPH oxidases are a group of plasma membrane-associated enzymes found in a variety of cells of mesodermal origin. The most thoroughly studied of these is the leukocyte NADPH oxidase (E.C. 1.23.45.3), which is found in professional phagocytes and B-lymphocytes. It catalyzes the production of superoxide ($O_2^{\bullet -}$) by the one-electron reduction of oxygen, using NADPH as the electron donor [1]:

$$2\ O_2 + NADPH \text{ ---> } 2\ O_2^{\bullet -} + NADP^+ + H^+$$

The NADPH oxidase of phagocytes is a multi-component electron transport system, in which activation requires the assembly of five components: $p40^{PHOX}$ (PHOX for PHagocyte OXidase), $p47^{PHOX}$, $p67^{PHOX}$, $p22^{PHOX}$ and $gp91^{PHOX}$. In the resting cell,

three of these five components: $p40^{PHOX}$, $p47^{PHOX}$ and $p67^{PHOX}$ exist in the cytosol as a complex. The other two components: $p22^{PHOX}$ and $gp91^{PHOX}$ are located in the membranes of secretory vesicles and specific granules, where they occur as a heterodimeric flavohemoprotein known as cytochrome b_{558}. Separating these two groups of components by distributing them between distinct subcellular compartments guarantees that the oxidase is inactive in the resting cell [2].

When the resting cell is exposed to any of a very wide variety of stimuli, the cytosolic component $p47^{PHOX}$ becomes heavily phosphorylated and the entire cytosolic complex migrates to the membrane, where it associates with cytochrome b_{558} to assemble the active oxidase (Figure 1). Cytochrome b_{558} is a flavocytochrome with a six-coordinated low spin heme and FAD as redox centers. The electrons provided by NADPH are thought to be transferred in a linear sequence as follows:

NADPH ---> Flavin (FAD) ---> Heme (Fe^{3+})---> O_2 ---> $O_2^{\bullet-}$

The heme in cytochrome b_{558} is assumed to be the terminal electron donor in the production of $O_2^{\bullet-}$ from molecular oxygen due to its unusually low reduction potential, 245 mV [3].

Activation requires the participation, not only of the core subunits, but of two low-molecular-weight guanine nucleotide-binding proteins: Rac2, which in the resting cell is located in the cytoplasm in a dimeric complex with Rho-GDI (Guanine nucleotide Dissociation Inhibitor), and Rap1A, which is located in membranes from which it can be co-purified with the cytochrome. During activation, Rac2 binds guanosine triphosphate

(GTP) and migrates to the membrane along with the core cytosolic complex. At the same time, cytochrome b_{558} and Rap1A are delivered to the cell surface by fusion of the secretory vesicle membranes and later the specific granule membranes with the plasma membrane of the cell. This fusion event also releases the contents of the organelles to the exterior. When phagocytosis takes place, the plasma membrane is internalized as the wall of the phagocytic vesicle, with what was once the outer membrane surface now facing the interior of the vesicle. From this location, the enzyme pours $O_2^{\bullet-}$ into the vesicle, and the rapid conversion of this $O_2^{\bullet-}$ into its successor products bathes the internalized target in a lethal mixture of corrosive oxidants [4].

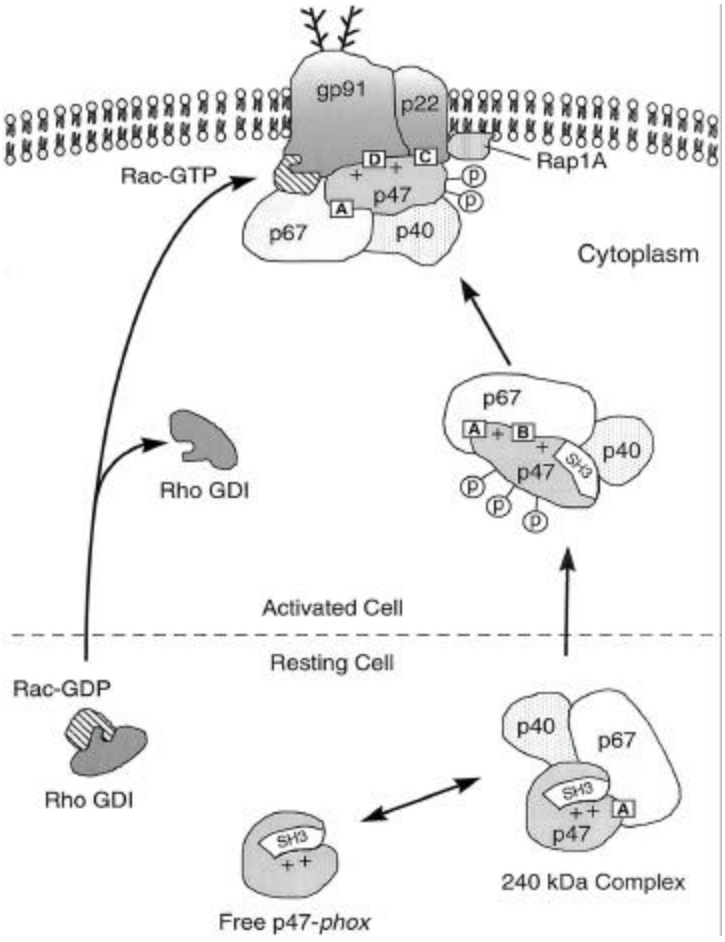

Figure 1 Assembly of the NADPH oxidase in phagocytic cells. In resting phagocytes, the cytochrome b_{558} subunits p22-^{phox} and gp91-^{phox}, in association with Rap1A, are located in the membranes of specific granules and of secretory vesicles. Upon cell activation, these organelles fuse with the plasma membrane, which results in expression of cytochrome b_{558} in this membrane. At the same time, a complex of p47-^{phox}, p67-^{phox} and p40-^{phox} translocates from the cytosol to the plasma membrane and forms a complex with cytochrome b_{558}. This translocation is facilitated by simultaneous redistribution of *rac* to the plasma membrane (Adapted from [5]).

IV. Superoxide-generating activity and the spin state

To clarify the relationship between the spin state of the heme in cytochrome b_{558} and the $O_2^{\bullet-}$ generating activity of the NADPH oxidase system, Fujii H *et al*. (1995) performed an experiment where the $O_2^{\bullet-}$ generating activity was measured in a reconstituted cell-free system consisting of the purified cytochrome b_{558} with different ratios of the low-spin to high-spin heme prepared by changing the pH. The optimum $O_2^{\bullet-}$ generating activity was found in the vicinity of pH 7. On lowering pH in cytochrome b, the $O_2^{\bullet-}$ generating activity of the NADPH oxidase system decreased concomitant with the decrease in percent of the low-spin heme in cytochrome b. These results indicate that cytochrome b is in the low-spin state when the NADPH oxidase system produce $O_2^{\bullet}$ in the cell-free system (Figure 2) [6].

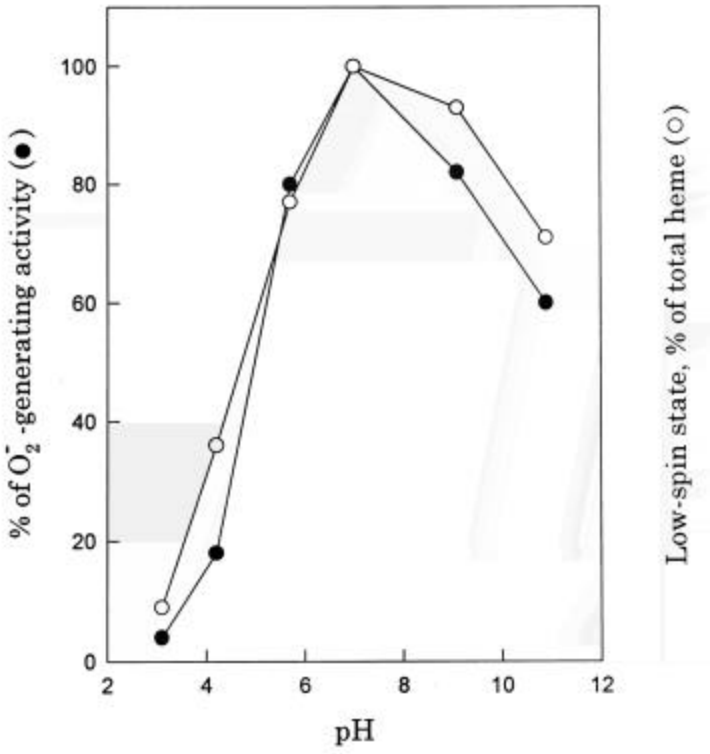

Figure 2 Effect of pH on percent of the low-spin state of the heme (°) in cytochrome b_{558} and $O_2^{\bullet-}$ generating activity (?) of NADPH oxidase system. pH in purified cytochrome b_{558} was adjusted to form various different ratios of the low-spin to high-spin heme. The $O_2^{\bullet-}$ generating activity of the purified cytochrome b_{558} with different ratios of the low-spin to high-spin heme was measured in a reconstituted cell-free system. Iron is in the ferric state. (Adapted from [6]).

V. NADPH oxidase in the phagocytic system

The $O_2^{\bullet-}$ generated by NADPH oxidase serves as the starting material for the production of a vast assortment of reactive oxidants, including oxidized halogens, free radicals, and singlet oxygen. These oxidants are used by phagocytes to kill invading microorganisms, but they also cause a lot damage to nearby tissues, so their production has to be tightly regulated to make sure they are only generated when and where required (Figure 3) [7].

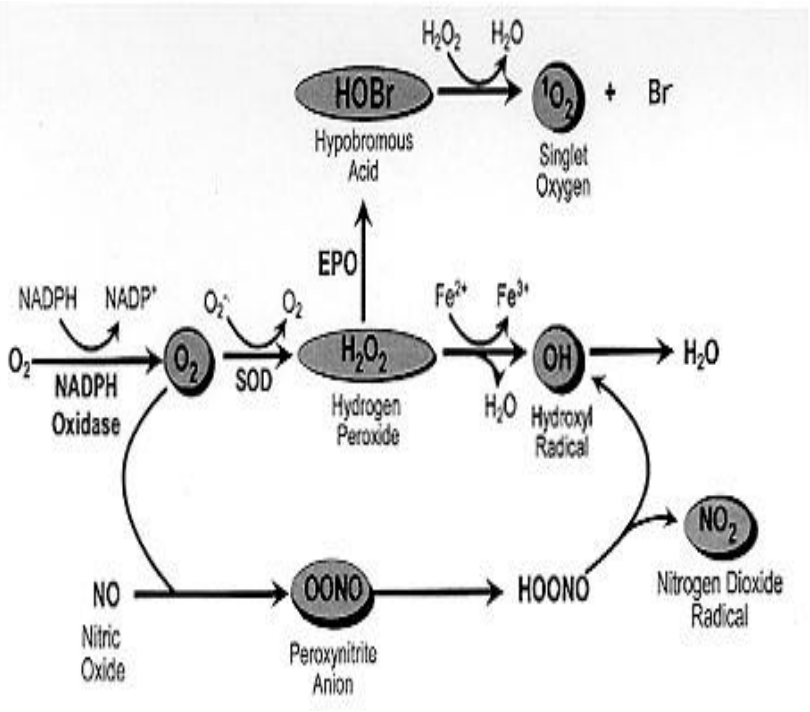

Figure 3 generation of reactive oxygen species in eosinophils. The superoxide produced by NADPH oxidase is subsequently converted to hydrogen peroxide H_2O_2, and hypobromous acid (HOBr) by eosinophil peroxidase (EPO), {and hypochlorous acid (HOCl) in the neutrophils using myeloperoxidase (MPO)}. Other microbicidal products (oxidants) like HOONO are also produced as seen in the figure (Adapted from [7]).

The oxidase can be activated by receptor-mediated and by receptor-independent mechanisms. Typical receptor-dependent stimuli are the complement fragment C5a, the chemotactic tripeptide N-formyl-Met-Leu-Phe (fMLP), and immune complexes [8]. Receptor-independent stimuli include long-chain unsaturated fatty acids and phorbol 12-myristate 13-acetate (PMA). Oxidase activation by receptor-mediated stimuli usually lasts less than 5 min, while receptor-independent stimuli activate the enzyme for a much longer period, but only when the stimulus remains present. It appears that in intact cells the activated oxidase is undergoing a continuous process of activation and deactivation [2].

VI. NADPH oxidase and human diseases

The importance of the NADPH oxidase in human host defense is exemplified by patients suffering from chronic granulomatous disease (CGD). CGD is an immunodeficiency syndrome characterized clinically by severe recurrent bacterial and fungal infections. Biochemically, CGD is characterized by the inability of phagocytic leukocytes (neutrophils, eosinophils, monocytes and macrophages) to activate the NADPH oxidase and to generate the reactive oxygen compounds needed for the killing of phagocytosed micro-organisms [9,10]. Therefore, the most common pathogens encountered in CGD patients are catalase-positive organisms, because catalase prevents the CGD phagocytes from using microbial hydrogen peroxide for killing these pathogens. CGD is a rare disease, with an estimated incidence of 1 in about 250,000 individuals, without any ethnic preference. It usually manifests itself in early childhood and is predominantly found in boys [11].

VII. Summary

In summary, we can see that the complex mechanism of activation of NADPH oxidase enzyme plays an important role in the process of killing pathogens by the phagocytes. The most likely reason for this complexity is the toxicity of the oxygen radicals produced by the active NADPH oxidase; these compounds are not only harmful to the invading pathogens, but also to the surrounding tissues. Characterizing the mutations that lead to CGD is not only important for improved diagnosis and treatment of CGD patients, but also provides a better understanding of the functional domains within the oxidase components.

VIII. References

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