The Leukocyte NADPH Oxidase

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Abstract

The leukocyte NADPH oxidase catalyzes the reduction of oxygen to O_2- (superoxide) at the expense of NADPH. The O_2- then dismutes to H_2O_2, which serves to oxidize Cl- to HOCl, a potent microbicidal agent that is used by leukocytes to kill invading microorganisms. This oxidation is catalyzed by myeloperoxidase. O_2 is also used to make other microbicidal oxidants, some in reactions with nitric oxide. The oxidase itself is highly complex, consisting of four unique subunits and Rac2. In the resting cell, two of the subunits, p22PHOX and gp91PHOX, are located in the membrane, and the other two, p47PHOX and p67PHOX, are in the cytosol. The electron-carrying components of the oxidase are located in gp91PHOX; the NADPH binding site is generally regarded to be in gp91PHOX as well, but there is some evidence that it may be in p67PHOX. When the oxidase is activated, p47PHOX is phosphorylated at specific sites, and the cytosolic components plus Rac2 migrate to the membrane to assemble the active oxidase.

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NADPH oxidase is an enzyme that catalyzes the reduction of oxygen to superoxide at the expense of NADPH [1]

$$NADPH + O_2 \rightarrow NADP^+ + H^+ + O_2^-$$

The O_2^- then reacts with itself, either spontaneously or under catalysis by superoxide dismutase, to form hydrogen peroxide and oxygen [2]

$$2O_2^- \rightarrow O_2 + H_2O_2$$

In the neutrophil and the monocyte, H_2O_2 is then used to oxidize Cl^- in a reaction catalyzed by myeloperoxidase, a green heme enzyme of unusual structure [3], to form HOCl (hypochlorite) [4], which according to most researchers is used to kill the bacteria. (An alternative hypothesis is held by the Segal laboratory in London, that the secretion of O_2^- from the phagocytic vesicle, an electrogenic process, results in the influx of K^+ into the vesicle accompanied by the entry of microbicidal peptides, which do the killing [5]. Eosinophils also contain the NADPH oxidase [6], but have their own peroxidase that is unable to catalyze the oxidation of Cl^- but instead catalyzes the oxidation of Br^- to HOBr (hypobromite) [7], another microbicidal agent. Additional reactive oxidants include $^1O_2$ (singlet oxygen) [8], produced by the reaction between H_2O_2 and a hypohalite, and OH (hydroxyl radical) [9], made when H_2O_2 oxidizes Cu^+ or Fe^{++}:

$$H_2O_2 + Fe^{++} \rightarrow Fe^{+++} + OH^- + OH$$

Hydroxyl radical in turn reacts with other compounds to produce a whole panoply of radicals, for example,

$$OH + RH \rightarrow H_2O + R$$

In addition, nitric oxide plays a role in killing. Little NO is made by neutrophils, but abundant quantities are produced by activated macrophages [10]. NO, a radical, reacts with O_2^- to produce peroxynitrite ($ONOO^-$) [11], which combines with CO_2 to form the reactive O_2N-O-CO_2 [12]. NO also oxidizes to NO_2, which in turn oxidizes to the toxic NO_2^-. NO_2^- can react with HOCl to produce NO_2Cl [13], another highly toxic compound. This partial listing of the reactive products of phagocytes indicates the wide variety of agents employed by these cells to kill bacteria (though of course not according to Segal).

The oxidase itself is highly complex, not only in the number of subunits required by the enzyme but also in the structure and interaction of the various subunits. Four subunits are unique to the enzyme: $p22^{PHOX}$ (^{PHOX} for phagocyte oxidase) [14], $p47^{PHOX}$ [15], $p67^{PHOX}$ [16] and $gp91^{PHOX}$ (gp because it is a glycoprotein) [17]. $gp91^{PHOX}$ and $p22^{PHOX}$ are associated with the membrane, the association occurring through the N-terminal half of $gp91^{PHOX}$, which has four transmembrane loops that are glycosylated facing out. The combination of $gp91^{PHOX}$ and $p22^{PHOX}$ is known as cytochrome b_{558}, and resides in the membranes of the secretory vesicles and the specific granules [18]. (If either $gp91^{PHOX}$ or $p22^{PHOX}$ is lacking, neither appears in the membrane.) On $gp91^{PHOX}$ are two hemes [19] whose 5th and 6th coordination positions are occupied by histidine residues, and a flavin binding site [20]. There is also a sequence alleged to be homologous to NADPH binding sites, but there is some question as to whether such homologies exist. $p47^{PHOX}$ and $p67^{PHOX}$ are cytosolic proteins, the former containing 2 SH3 domains in the middle of the polypeptide and the latter containing an SH3 domain in the middle and an SH3 domain near the N-terminus of the polypeptide. $p67^{PHOX}$ also contains a binding site for NADPH [21]. There is another cytosolic protein, $p40^{PHOX}$, that co-purifies with $p67^{PHOX}$, but its function is not clear.

Finally, the small G protein rac1 or rac2 is necessary for oxidase activity [22]. G proteins are proteins that bind GTP in their active form but inactivate themselves by slowly hydrolyzing GTP to GDP. They are acted on by catalysts that activate the G proteins by exchanging GTP for GDP (guanidine nucleotide exchange factors, or GEFs) and inactivating the G proteins by accelerating the hydrolysis of GTP to

NO = nitric oxide

GDP (GTPase accelerating proteins, or GAPs). Rac1 has many functions besides activating the oxidase, but Rac2 chiefly activates the oxidase. Rac2 is the major oxidase-activating protein in humans. Rac appears to be necessary for the transfer of electrons through cytochrome b_{558}, the electron-carrying component of the oxidase.

Activation of the oxidase is a complex process. Looked at simply, the two cytosolic components move to the plasma membrane where they bind to cytochrome b_{558} [23]. Looked at in more detail, however, the key to the activation of the oxidase is the phosphorylation of $p47^{PHOX}$ [24]. When phosphorylated by protein kinase C (i.e., by activation of the neutrophil with phorbol myristate acetate), the protein is phosphorylated on serines S303 or S304 and serines S359 or S370. Phosphorylation of one member of each of the two groups is necessary for oxidase activation. In unpublished work, we showed that Akt is able to activate the oxidase, phosphorylating serines S304 and S328. Calmodulin kinase is also able to activate the oxidase (unpublished), but casein II kinase has no effect. These findings indicate that the NADPH oxidase can be activated by many routes, as would be expected for an enzyme involved in host defense.

Phosphorylation undoubtedly causes a change in the conformation of $p47^{PHOX}$. The nature of the change in conformation has not been proven experimentally. Because of a polyproline domain near the N-terminus of $p47^{PHOX}$, however, it has been postulated that the resting $p47^{PHOX}$ is folded over so that one of its SH3 domains is associated with the polyproline domain, and that when the protein is phosphorylated it flops open, exposing the formerly occupied SH3 and polyproline domains as well as a PX domain that binds to phosphoinositide-3-phosphates [25] and may help to hold the cytosolic components of the oxidase to the membrane. The final player is $p67^{PHOX}$, which contains two SH3 domains one of which may bind to the polyproline domain liberated by the phosphorylation of $p47^{PHOX}$, an activation domain (amino acids 200-210) whose function is unknown, and the very interesting but as yet poorly understood NADPH binding site [21]. (I would like to think that the NADPH binding site carries NADPH to the flavin of cytochrome b_{558}, but evidence is lacking.) Alternatively, the liberated polyproline site may bind to the SH3 domain of $p22^{PHOX}$. It is this fully assembled enzyme that manufactures O_2^-.

References

1. Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* 1973;52:741–4.
2. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein. *J Biol Chem* 1969;244:6049–55.
3. Fenna R, Zeng J, Davey C. Structure of the green heme in myeloperoxidase. *Arch Biochem Biophys* 1995;316:65–6.
4. Rosen H, Klebanoff SJ. Hydroxyl radical generation by polymorphonuclear leukocytes measured by electron spin resonance spectroscopy. *J Clin Invest* 1979;64:1725–9.
5. Reeves EP, Lu H, Jacobs HL, et al. Killing activity of neutrophils is mediated through activation of proteases by K^+ flux. *Nature* 2002;416:291–7.
6. Bolscher BGJM, Koenderman L, Tool ATJ, et al. NADPH:O_2 oxidoreductase of human eosinophils in the cell-free system. *FEBS Lett* 1990;268:269–73.
7. Mayeno AN, Curran AJ, Roberts RL, Foote CS. Eosinophils preferentially use bromide to generate halogenating agents. *J Biol Chem* 1989;264:5660–8.
8. Maugh TH. Singlet oxygen: a unique microbicidal agent in cells. *Science* 1973;182:44–5.
9. Liochev SI. The role of iron-sulfur clusters in *in vivo* hydroxyl radical production. *Free Radic Res* 1996;25:369–84.
10. Green SJ, Chen T-Y, Crawford RM, et al. Cytotoxic activity and production of toxic nitrogen oxides by macrophages treated with IFN-gamma and monoclonal antibodies against the 73-kDa lipopolysaccharide receptor. *J Immunol* 1992;149:2069–75.
11. Beckman JS, Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol Cell Physiol* 1996;271:C1424–37.
12. Denicola A, Freeman BA, Trujillo M, Radi R. Peroxynitrite reaction with carbon dioxide/bicarbonate: Kinetics and influence on peroxynitrite-mediated oxidations. *Arch Biochem Biophys* 1996;333:49–58.
13. Eiserich JP, Cross CE, Jones AD, et al. Formation of nitrating and chlorinating species by reaction of nitrite with hypochlorous acid. A novel mechanism for nitric oxide-mediated protein modification. *J Biol Chem* 1996;271:19199–208.
14. Dinauer MC, Pierce EA, Bruns GAP, et al. Human neutrophil cytochrome b light chain (p22-phox). Gene structure, chromosomal location, and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease. *J Clin Invest* 1990;86:1729–37.
15. Volpp BD, Nauseef WM, Donelson JE, et al. Cloning of the cDNA and functional expression of the 47-kilodalton cytosolic component of human neutrophil respiratory burst oxidase. *Proc Natl Acad Sci USA* 1989;86:7195–9. Published erratum appears in *Proc Natl Acad Sci USA* 1989 Dec;86(23):9563.
16. Leto TL, Lomax KJ, Volpp BD, et al. Cloning of a 67-kD neutrophil oxidase factor with similarity to a noncatalytic region of $p60^{c\text{-}src}$. *Science* 1990;248:727–30.
17. Dinauer MC, Orkin SH, Brown R, et al. The glycoprotein encoded by the X-linked chronic granulomatous disease locus is a component of the neutrophil cytochrome b complex. *Nature* 1987;327:717–20.
18. Borregaard N, Lollike K, Kjeldsen L, et al. Human neutrophil granules and secretory vesicles. *Eur J Haematol* 1993;51:187–98.
19. Cross AR, Rae J, Curnutte JT. Cytochrome b245 of the neutrophil superoxide-generating system contains two nonidentical hemes. Potentiometric studies of a mutant form of gp91phox. *J Biol Chem* 1995;270:17075–7.
20. Cross AR, Jones OTG, Garcia R, Segal AW. The association of FAD with the cytochrome b245 of human neutrophils. *Biochem J* 1982;208:759–63.
21. Smith RM, Connor JA, Chen LM, Babior BM. The cytosolic subunit p67phox contains an NADPH-binding site that participates in catalysis by the leukocyte NADPH oxidase. *J Clin Invest* 1996;98:977–83.
22. Bokoch GM. Regulation of the human neutrophil NADPH oxidase by the Rac GTP-binding proteins. *Curr Opin Cell Biol* 1994;6:212–18.
23. Heyworth PG, Curnutte JT, Nauseef WM, et al. Neutrophil nicotinamide adenine dinucleotide phosphate oxidase assembly. Translocation of p47phox and p67phox requires interaction between p47-phox and cytochrome b558. *J Clin Invest* 1991;87:352–6.
24. El Benna J, Faust LP, Johnson JL, Babior BM. Phosphorylation of the respiratory burst oxidase subunit p47phox as determined by 2-dimensional phosphopeptide mapping. Phosphorylation by protein kinase C, protein kinase A and a mitogen-activated protein kinase (MAP-kinase). *J Biol Chem* 1996;271:6374–8.
25. Bravo J, Karathanassis D, Pacold CM, et al. The crystal structure of the PX domain from $p40^{phox}$ bound to phosphatidylinositol 3-phosphate. *Mol Cell* 2001;8:829–39.

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