Biochemical Pharmacology 80 (2010) 778–785

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Peptide-based inhibitors of the phagocyte NADPH oxidase

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ARTICLE INFO

Article history:
Received 30 March 2010
Accepted 17 May 2010

Keywords:
Phagocytes
NADPH oxidase
Inhibitors
gp91phox/NOX2
p22phox
p47phox

ABSTRACT

Phagocytes such as neutrophils, monocytes and macrophages play an essential role in host defenses against pathogens. To kill these pathogens, phagocytes produce and release large quantities of antimicrobial molecules such as reactive oxygen species (ROS), microbicidal peptides, and proteases. The enzyme responsible for ROS generation is called NADPH oxidase, or respiratory burst oxidase, and is composed of six proteins: gp91phox, p22phox, p47phox, p67phox, p40phox and Rac1/2. The vital importance of this enzyme in host defenses is illustrated by a genetic disorder called chronic granulomatous disease (CGD), in which the phagocyte NADPH oxidase is dysfunctional, leading to life-threatening recurrent bacterial and fungal infections. However, excessive NADPH oxidase activation and ROS over-production can damage surrounding tissues and participate in exaggerated inflammatory processes. As ROS production is believed to be involved in several inflammatory diseases, specific phagocyte NADPH oxidase inhibitors might have therapeutic value. In this commentary, we summarize the structure and activation of the phagocyte NADPH oxidase, and describe pharmacological inhibitors of this enzyme, with particular emphasis on peptide-based inhibitors derived from gp91phox, p22phox and p47phox.

1. Introduction

Reactive oxygen species (ROS) produced by phagocytes are one of the most powerful host defenses against bacteria, yeasts and fungi [1,2]. ROS produced by phagocytes include superoxide anion ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\bullet$) and hypochlorous acid (HOCl). These ROS are produced in large quantities when phagocytes are stimulated by pro-inflammatory agents or by particles such as bacteria. This process, known as the "oxidative burst" or "respiratory burst", is characterized by a rapid, cyanide-insensitive increase in oxygen uptake and glucose consumption [1,2]. Superoxide anion ($O_2^{\bullet-}$), the precursor of the other ROS, is first produced by an enzyme called NADPH oxidase, as follows [2,3]:

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

Once produced, $O_2^{\bullet-}$ is immediately transformed into H_2O_2 by spontaneous dismutation at acid pH in the phagosome, or through enzymatic dismutation by superoxide dismutase in the cytosol. Interaction between H_2O_2 and $O_2^{\bullet-}$ can, through the Haber–Weiss reaction in the presence of a transition metal (or the Fenton reaction in the presence of iron), give rise to the hydroxyl radical ($OH^\bullet$), one of the most powerful oxidants. H_2O_2 is also a substrate of myeloperoxidase, an enzyme stored in neutrophil azurophilic granules and released during neutrophil activation. Myeloperoxidase catalyses the transformation of H_2O_2, in the presence of a halogen (Cl^-, Br^-, I^-), into highly toxic molecules such as hypochloric acid ($HOCl^-$). Other reactions between OCl^- and H_2O_2 can lead to the formation of singlet oxygen ($^1O_2$). Most of the hypochlorous acid (OCl^-) thus generated is converted into toxic chloramines. MPO can also use H_2O_2 to oxidize tyrosines into tyrosyl radicals. HOCl, chloramines and tyrosyl radicals, which are toxic species that serve to kill bacteria and other pathogens. The crucial role of phagocyte NADPH oxidase in host defenses against microbial pathogens is illustrated by a human genetic disorder called chronic granulomatous disease, which is associated with life-threatening recurrent bacterial and fungal infections [4]. However, excessive ROS production can damage healthy bystander tissues. ROS hyper-production by neutrophils is believed to cause direct tissue insult in a broad range of inflammatory diseases, including rheumatoid arthritis, inflammatory bowel diseases, acute respiratory distress syndrome, sepsis, diabetic complications, cardiovascular disease, ischemic tissue injury and neurodegenerative diseases [5,6]. Pharmacological

Abbreviations: DPI, diphenylene iodonium; CGD, chronic granulomatous disease; fMLF, formyl-methionyl-leucyl-phenylalanine; MPO, myeloperoxidase; phox, phagocyte oxidase; ROS, reactive oxygen species.

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doi:10.1016/j.bcp.2010.05.020

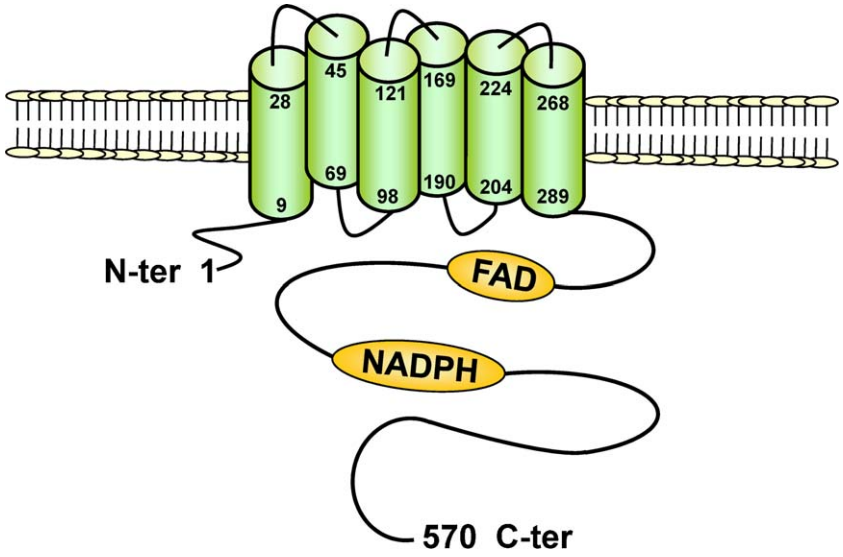

Fig. 1. Predicted structure of gp91phox/NOX2. The protein has a short N-terminal cytosolic sequence, 6 transmembrane helices, and one long C-terminal cytosolic tail containing the FAD binding site and one NADPH binding site.

NADPH oxidase inhibition might therefore be beneficial in patients with these disorders. Currently, there are no specific inhibitors of the phagocyte NADPH oxidase. Peptide-based inhibitors derived from specific subunits of the enzyme could provide a certain degree of specificity. In the first part of this manuscript, we review the principal features of NADPH oxidase in order to provide the information necessary to understand the action of the inhibitory peptides described in the second part.

2. Components of the phagocyte NADPH oxidase

The phagocyte NADPH oxidase is a multicomponent enzyme complex comprising six proteins, namely p22phox (phox: phagocyte oxidase), gp91phox, p47phox, p67phox, p40phox and the small G-protein Rac1 or Rac2. In resting cells the NADPH oxidase is inactive because its components are distributed between the cytosol (p47phox, p67phox, p40phox and Rac1/2) and the plasma membrane and membranes of specific granules (p22phox and gp91phox/NOX2, which form the flavocytochrome b_{558}). When cells are activated, the cytosolic components migrate to the membranes, where they associate with the membrane-bound components to assemble the catalytically active oxidase [7,8].

Flavocytochrome b_{558} is the central membrane-bound component of NADPH oxidase [9]. It is composed of a 1:1 complex between a glycosylated 91-kDa protein subunit (gp91phox) of 570 amino acids (Fig. 1), and a nonglycosylated 22-kDa subunit (p22phox) of 195 amino acids (Fig. 2). Flavocytochrome b_{558} contains one FAD and two hemes and forms the NADPH oxidase electron transfer chain [9]. It serves as the central docking station for the cytosolic components, via numerous interaction sites [8]. P22phox is phosphorylated on threonine residues by a phosphatidic acid-activated kinase and PKC [10]. Gp91phox phosphorylation in human neutrophils enhances its enzymatic activity and its binding to p47phox, p67phox and Rac1 [11].

P47phox is a cytosolic protein composed of 390 amino acids. Its COOH-terminal sequence is very basic and rich in serine and arginine [12,13]. The amino acid sequence of p47phox also contains two src-homology 3 (SH3) domains, one phox homology (PX) domain, a proline-rich region and one auto-inhibitory region (AIR) (Fig. 3). P47phox binds to the flavocytochrome b_{558} during

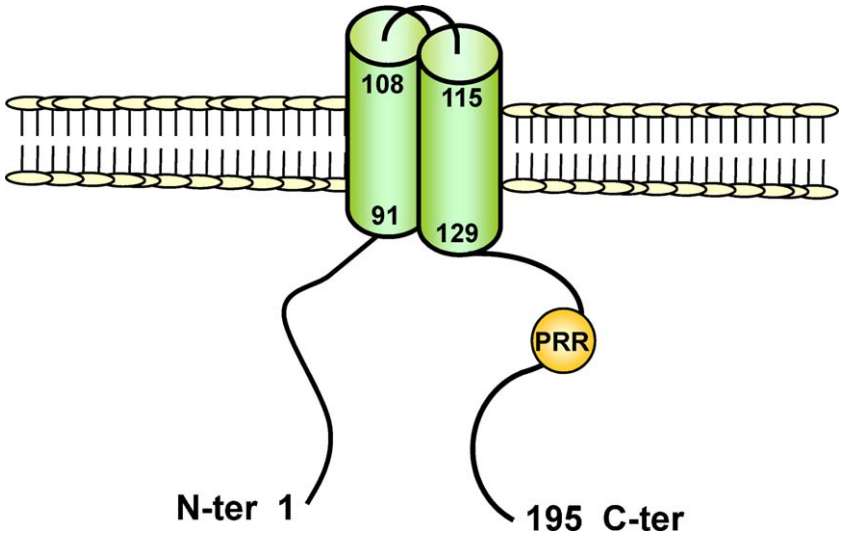

Fig. 2. Predicted structure of p22phox. The protein has an N-terminal cytosolic sequence, 2 transmembrane helices, and one long C-terminal cytosolic tail containing a proline-rich region (PRR) which binds to p47phox SH3 domains.