Mechanisms of Ageing and Development xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

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

Original Article

Phagocyte NADPH oxidase, oxidative stress and lipids: Anti- or pro ageing?

Laura Baciou, Rawand Masoud[1], Hager Souabni[2], Xavier Serfaty, Gilda Karimi[3], Tania Bizouarn, Chantal Houée Levin*

Laboratoire de Chimie Physique, Université Paris Sud, UMR 8000, CNRS, 91405, Orsay Cedex, France

ARTICLE INFO

Keywords:
NADPH oxidase
Oxygen free radicals
Lipids
Cholesterol

ABSTRACT

The role of NADPH oxidase in ageing is debated because of the dual roles of free radicals, toxic though necessary. In this paper we summarize some results about two aspects linked to the regulation of the activity of phagocyte NADPH oxidase (Nox2), encountered frequently in elderly people: inflammation and hypercholesterolemia. In the presence of a high amount of reactive oxygen species (ROS) created by itself or by any other source, the enzyme activity is mostly lowered. Oxidation of the membrane and/or of one of the cytosolic partners could be responsible for this loss of activity. However using a cell free system, we had also shown that a low amount of ROS could activate this enzyme. Similarly, cholesterol has a similar dual role, either activating or inhibiting. In *in vitro* cell free system with neutrophil membranes from healthy donors, the addition, as well as the removal of cholesterol, diminishes the Nox2 activity. The activity of Nox2 is lowered in neutrophils of untreated hypercholesterolemic patients. Finally oxysterols (25-hydroxy-cholesterol or 5α, 6α − epoxy-cholesterol) do not induce effects different from that of non-oxidized cholesterol. These findings are in agreement with the Janus role of NADPH oxidase, the main source of non-mitochondrial ROS.

1. Introduction

More than 60 years ago Prof. D. Harman emitted the hypothesis of the key role of free radicals in ageing (Harman, 1956). Since this pioneering hypothesis, a wealth of knowledge has been accumulated concerning the formation, reactions, deleterious and/or beneficial effects of free radicals in cells. It is now obvious that the so-called "reactive oxygen species (ROS)", which comprise $O_2^{\bullet -}$ and ·OH free radicals and H_2O_2, are produced in living organisms (Aruoma and Halliwell, 1998). They come from respiration and metabolism. The roles of these free radicals are numerous. In particular, they are formed inside macrophages and neutrophils and act as oxidants during the phagocytosis. In these cells, they are key actors in the defence of living organisms towards all pathogens (bacteria, viruses, fungi…).

The major source of ROS in neutrophils is due to the activity of the enzyme NADPH oxidase Nox2 (Leto and Geiszt, 2006). More generally, the ubiquitous enzyme NADPH oxidase was recognized as the major source of non-mitochondrial superoxide anions in cells. Its beneficial role in the destruction of pathogens in neutrophils was first enlightened through increased knowledge about the chronic granulomatous disease (CGD) (Gabig and Lefker, 1984; Curnutte and Babior, 1987), due to dysfunction of phagocyte NADPH oxidase (Heyworth et al., 2003). To date, six human homologs of Nox2 (Nox1, Nox3, Nox4, Nox5, Duox1 and Duox2) have been identified in a variety of non-phagocytic cells. All of them produce superoxide ions and/or hydrogen peroxide in various organs and cell types and are involved in redox pathophysiology of major diseases (cancer, neurodegenerative diseases etc.) and in ageing. Despite these harmful effects, positive signalling roles of ROS (redox signalling) have been well documented. Superoxide ions or hydrogen peroxide and/or other compounds coming from their reactions, can induce signal transduction (Forman, 2016; Frey et al., 2009; Ushio-Fukai, 2006), which is essential in physiological homeostasis. Alterations in redox signalling are observed in aging, and sustained deviations from redox homeostasis result in disease.

In this paper we explore some aspects linked to the regulation of the NADPH oxidase Nox2 from neutrophils, taken as a model, thanks to a cell-free system. We focussed on the following: i) the self-regulation of the NADPH oxidase by its own product, the superoxide ions and by their by-products, ROS; ii) the enzyme regulation by its environment, lipids and especially cholesterol. The first aspect is part of the self-

* Corresponding author.
E-mail address: Chantal.houee@u-psud.fr (C. Houée Levin).
[1] Present address: Centre de Recherche en Cancérologie de Marseille (CRCM) Campus de Luminy, 13273 Marseille Cedex09, France.
[2] Present address: Laboratoire de Biologie Physico-chimique des protéines membranaires, UMR7099 CNRS Université Paris Diderot, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France.
[3] Present address: Department of Biological Sciences, Kharazmi University, Tehran, Iran.

https://doi.org/10.1016/j.mad.2017.11.001
Received 15 May 2017; Received in revised form 29 September 2017; Accepted 1 November 2017

modulation of NADPH oxidase concomitant with ROS formation in inflammation, which is a situation frequently encountered with age. The second point refers to the regulation by lipids and especially by sterols of the rate of superoxide production. In previous studies with Nox2 heterologously expressed in yeast we have correlated low NADPH oxidase activity with high sterol-content membrane (e.g. plasma membranes) and high activity with low cholesterol content (e.g. endoplasmic reticulum) (Souabni et al., 2014). We have also provided evidence that the dynamics imposed by the cholesterol-protein interactions was determinant for the regulation of the NADPH oxidase activity (Souabni et al., 2017a). Here we have examined more closely the problem of high level of cholesterol and oxidized cholesterol on the Nox2 activity. High cholesterol level is linked to an elevated risk of cardiovascular disease (James and Underwood, 2009), through a high concentration of LDL-cholesterol in blood (Brown and Goldstein, 1984; Bhatnagar et al., 2008). The effect of hypercholesterolemia on non −specific immune defence has been little explored.

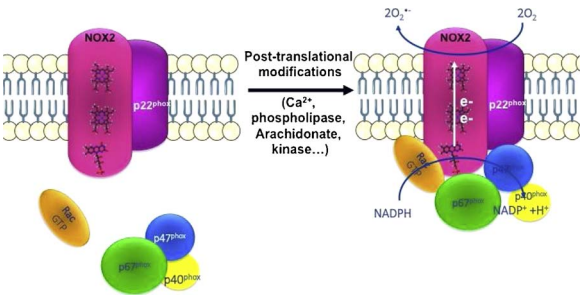
2. The phagocyte NADPH oxidase NOX2

The structure and function of the NADPH-oxidase have been extensively reviewed (Brown and Griendling, 2009). The NADPH oxidase is a protein complex made of two membrane proteins (Nox2 also called gp91^{phox} and p22^{phox}) and at least 4 cytosolic partners (Scheme 1).

In resting cells, the cytosolic proteins p47^{phox}, p67^{phox}, p40^{phox} and Rac are dispersed in the cell. Upon infection signals, all these subunits associate to the membrane partners in a stimulus-dependent manner to form the active state of the enzyme. Activation of the system consists of phosphorylation events namely of p47^{phox} and/or liberation of arachidonic acid by phospholipase A2, which triggers the assembly of proteins p47^{phox}, p67^{phox} and Rac to their membrane partners (Nauseef, 2004). The following Reaction (1) takes place. Superoxide ions are formed thanks to intramolecular electron transfers from NADPH to dioxygen in the Nox2 membrane subunit.

$$NADPH + 2\ O_2 \rightarrow NADP^+ + 2\ O_2^{\bullet -} + H^+ \quad (1)$$

In this article, we used a cell-free system for activity measurements. In this system, the membrane fractions were extracted from human neutrophils from healthy or untreated hypercholesterolemic donors, while the cytosolic proteins were obtained as recombinant proteins in our laboratory (Karimi et al., 2014; Baciou et al., 2009). The different cell free methods have been described elsewhere (Ostuni et al., 2010; Souabni et al., 2017b). Activation was obtained by addition of arachidonic acid (AA) inducing structural modifications of the cytosolic proteins that we have investigated using spectroscopic approaches (Bizouarn et al., 2016). It seems that, in cells, there is a connection between the activities of NADPH oxidase and phospholipase A2. The product of this enzyme, AA, would activate the NADPH oxidase *in vivo* albeit with an unclear mechanism (Krishnaiah et al., 2013; Bromberg and Pick, 1983). Activation takes place during a 5 min incubation of the membrane and cytosolic partners in the presence of AA in Phosphate Buffer Saline (PBS) supplemented with 10 mM $MgSO_4$ at room temperature (25 °C). During this period, required to reach a maximal production rate of superoxide anions, conformational changes occur that we have investigated using diverse techniques (Karimi et al., 2014; Ostuni et al., 2010; Bizouarn et al., 2016). The $O_2-^{\bullet}$ production was initiated by addition of NADPH (250 μM) and its rate of production was quantified by the reduction rate of cytochrome *c* (50 μM).



Scheme 1. The phagocyte NADPH oxidase Nox2 in its resting (left) and activated (right) forms.

3. Reactive oxygen species

The appellation of ROS covers several species coming from an one-electron reduction or an excitation of dioxygen. As shown by Reaction (1), *in vivo* the first formed species is the superoxide ion. Strangely enough, superoxide ions have a low reactivity, summarized some years ago in (Bielski et al., 1985) and more recently in (Abreu and Cabelli, 2010). Actually, the fastest reactions are those with other free radicals including itself (Bielski et al., 1985), with metal ions embedded in proteins or not, and with NO· (Abreu and Cabelli, 2010). Its lifetime is thus function of the amount of metal ions and on the production of NO· in its vicinity.

The decay of $O_2^{\bullet -}$ leads to hydrogen peroxide by disproportionation, which in biological media takes place by reaction with its acidic form HO_2· (Reaction (2)). This reaction is rendered very fast thanks to superoxide dismutase.

$$HO_2\cdot + O_2^{\bullet -} + H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

The lifetime of hydrogen peroxide is much longer than that of $O_2^{\bullet -}$, since it is not a free radical. Its most important reaction in the absence of enzymatic catalysts is certainly the Fenton one (Reaction (3))

$$H_2O_2 + Fe^{II} \rightarrow Fe^{III} + OH\cdot + OH^- \quad (3)$$

This reaction can take place with any transition metal ion (iron, copper, manganese etc.). However many other reactions, which must be taken into account, are catalysed by peroxidases. These regulatory systems are extremely important. Actually such reactions might be at the origin of the role of ROS as signalling agents.

The Fenton reaction (Reaction (3)) leads to OH· radicals. Their high reactivity toward most of organic compounds is well known. Actually the roles of OH· radicals in signalisation are highly improbable and most of their oxidation properties participate in the deleterious effect of ROS. Finally let us mention singlet oxygen, formed principally by decomposition of hydroperoxides and especially lipid hydroperoxides (Miyamoto et al., 2014; Miyamoto and Di Mascio, 2014) and/or by the effect of light thanks to sensitizers. It is a powerful oxidant.

In conclusion, the actual role of superoxide is probably attributable to its decay products and one should keep in mind that it is at the origin of the noxious effect of ROS as well as their signalling properties without being itself the agent.

4. Effect of reactive oxygen species on NADPH oxidase

The deregulation of NADPH oxidase is associated to several pathologies involving inflammatory processes like in traumatic events (Bao et al., 2009) and various cardiovascular diseases (atherosclerosis, vascular inflammation, angiogenesis, etc.) (Cave, 2009). All these diseases are mostly age-dependent.

The over-activation of NADPH oxidase might be related to over-expression of the cytosolic fractions, however a feed-forward mechanism of activation by hydrogen peroxide has been shown in vascular cells (Li et al., 2001a). Indeed, we have shown that some proteins might also be activated by free radical oxidation (Sicard-Roselli et al., 2004).

Our aim was to evaluate the resistance of the NADPH oxidase components to oxidation by ROS. For this study free radicals were created by ionising radiation. It is well known that the selection of OH· and superoxide free radicals is easily obtained through the following reactions (Spinks and Woods, 1990).