Biosensors and Bioelectronics 58 (2014) 359–373

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Nitro-oxidative species *in vivo* biosensing: Challenges and advances with focus on peroxynitrite quantification

Serban F. Peteu [a,c,*], Rabah Boukherroub [b], Sabine Szunerits [b,**]

[a] Michigan State University, Chemical Engineering & Materials Science, 3523 Engineering Bldg, 428S. Shaw Lane, East Lansing, MI 28824-1226, United States
[b] Institut de Recherche Interdisciplinaire (IRI, USR 3078), Université Lille1, Parc de la Haute Borne, 50 Avenue de Halley, BP 70478, 59658 Villeneuve d'Ascq, France
[c] National Institute for Research and Development in Chemistry and Petrochemistry, 202 Splaiul Independentei, 060021 Bucharest, Romania

ARTICLE INFO

Article history:
Received 18 November 2013
Received in revised form
20 January 2014
Accepted 10 February 2014
Available online 2 March 2014

Keywords:
Peroxynitrite
Reactive nitrogen and oxygen species
Biosensors
in vivo sensing
Sensitivity
Selectivity

ABSTRACT

The importance of the so-called reactive nitrogen and oxygen species (RNOS) in biology and food technology has been widely recognized. However when these species are in excess, the steady-state maintained by physiological processes is disturbed. At this point, the *nitro oxidative metabolic stress* develops and its action *in vivo* over time leads to nitro-oxidative reactions in food and in living organisms, but also results in chronic degenerative diseases. Analytical methods enabling the assessment of the total antioxidant activity of a biological sample or a plant extract is therefore largely sought after. The ability of biosensors for rapid and real-time analysis that decreases the assay time and the possibility of automated and multi-analyte analysis at low cost has also allowed the quantitative and qualitative detection of RNOS. Among these RNOS, peroxynitrite ($ONOO^-$) is a well-known inflammatory mediator during a number of physiological and pathological processes. Consequently, many efforts are underway to detect peroxynitrite in the biomedical field. This urgent demand makes the development of $ONOO^-$ specific probes of great interest. Not only they can be useful for the detection of disease states, but they will also allow for a screening-type analysis of potential signal transduction pathways in the cells.

This invited review will critically discuss for the first time the very latest advancements and the challenges in the field of peroxynitrite biosensors and probes for *in vivo* and *in vitro* studies. Also, the main trends will be extracted, in order to chart the future directions and hence create an instrumental outlook.

Contents

* Corresponding author at: Michigan State University, Chemical Engineering & Materials Science, 3523 Engineering Bldg, 428S. Shaw Lane, East Lansing, MI 28824-1226, United States
** Corresponding authors.
E-mail addresses: Peteu@msu.edu (S.F. Peteu), sabine.szunerits@iri.univ-lille1.fr, sabine.szunerits@gmail.com (S. Szunerits).

http://dx.doi.org/10.1016/j.bios.2014.02.025
0956-5663

1. Introduction

Nitric oxide (NO), one of the smallest molecules found in nature, plays several roles as intracellular messenger and cytotoxic agent in biological systems. NO is highly reactive and interacts with a number of molecules one of them being superoxide radical anion ($O_2^{\bullet -}$) formed during oxidative stress. The reaction of NO and $O_2^{\bullet -}$ *in vivo* is extremely fast and results in the formation of peroxynitrite ($ONOO^-$) by a diffusion-limited reaction (k=0.4–1.9 × 10^{10} M^{-1} s^{-1}) (Fig. 1) (Beckman et al., 1990).

Intracellular peroxynitrite formation is thus associated with elevated NO production and its diffusion to sites of superoxide formation. This reaction is indeed about three times faster than the reaction between superoxide and superoxide dismutase. While stable at basic pH, $ONOO^-$ undergoes three main degradation routes under physiological conditions: it decomposes directly into nitrite radical ($(NO_2)^{\bullet}$) and nitrate anion (NO_3^-) or through the intermediate formation of its conjugated acid (peroxynitrous acid (O=N–O–OH), pK_a=6.8) (Kurz et al., 2005) and the subsequent formation of NO_2 and $OH^{\bullet}$ radicals and interacts rapidly with CO_2, glutathione, hemoproteins, peroxi-redoxin, metals, thiols, *etc.* (Sieracki et al., 2013). Although the $ONOO^-$ half-life time is about 1 s at pH 7.4, it can damage a wide array of molecular components in cells, including DNA and proteins, due to its high oxidizing and nitrating properties. Moreover, peroxynitrite abnormal levels are clearly correlated with pathogenic effects, including neurodegenerative, cardiovascular, or inflammatory diseases and diabetes (Fig. 1).

Considering this urgent demand and the great interest to develop $ONOO^-$ specific quantification tools, the main objectives of this review are to critically discuss, arguably for the first time, the challenges and very latest advancements in peroxynitrite sensitive and selective chemical sensors and probes, with focus on the *in vitro*-relevant conditions. Also, the main trends will be extracted so as to chart future directions.

2. Challenges in peroxynitrite formation and quantification

Since the pioneering work of Clark, biosensors have been considered adequate means useful for environmental and biological monitoring. The detection and quantification of $ONOO^-$ in biological systems are thus a challenging task, but highly important for the understanding of the implication of $ONOO^-$ in oxidative injury. While a well-controlled level of peroxynitrite is essential for cellular integrity and organ homeostasis, under pathological conditions such as bacterial infection, lipopolysaccharides induce high expression of nitric oxide synthase in macrophages and neutrophiles to produce large amounts of peroxynitrite in addition to other reactive oxygen and nitrogen species. Already established as a powerful *nitrating, nitrosating and oxidative "triple agent"* for cellular constituents, clinical studies suggest that aberrant peroxynitrite levels are correlated with acute cytotoxicity and may contribute to many human diseases, including septic shock, stroke, inflammatory bowel disease, cancer and several neurodegenerative diseases (Koppenol et al., 1992; Beckman and Koppenol, 1996; Pacher et al., 2007; Szabo et al., 2007; Amatore et al., 2008a, 2008b).

It was also hypothesized that peroxynitrite is generated in plant cells (Fig. 2), especially after revealing that NO cooperates with reactive oxygen species (ROS) during plant response to biotic stress (Alamillo and Garcia-Olmedo, 2001; Garcia-Olmedo et al., 2001; Bolwell, 1999; Durner and Klessig, 1999; Delledonne et al.,

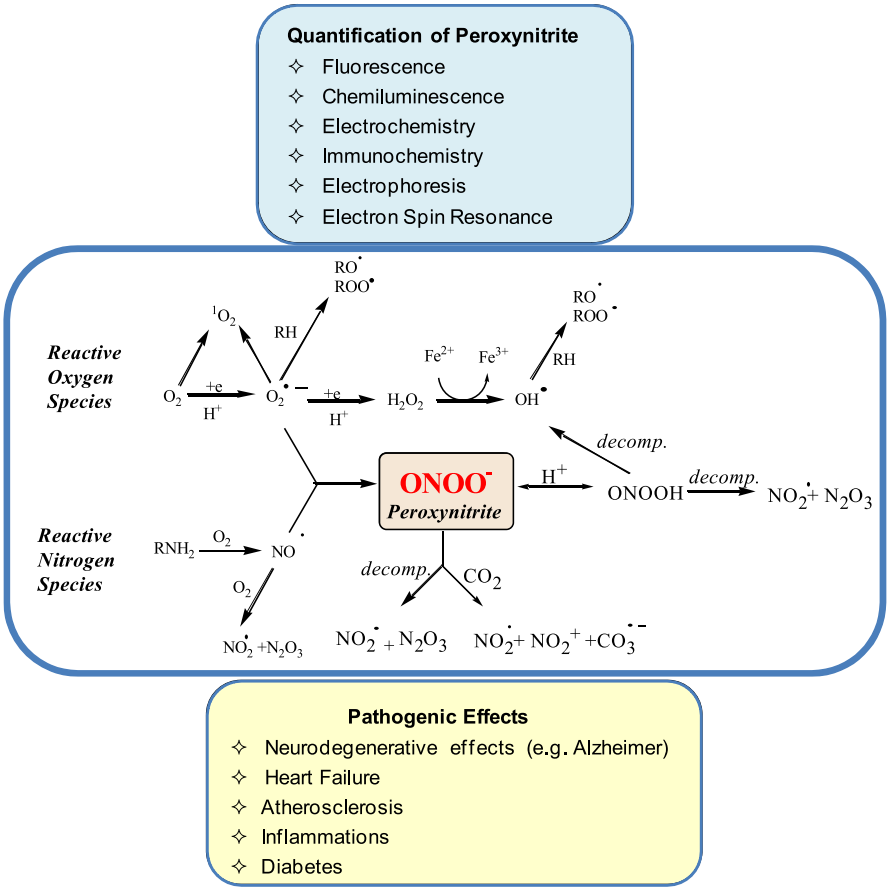

Fig. 1. Peroxynitrite: formation, reactivity, quantification and pathogenic effects.