Biochimica et Biophysica Acta 1840 (2014) 768–780

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Kinetic and mechanistic considerations to assess the biological fate of peroxynitrite☆

Sebastián Carballal [a,b], Silvina Bartesaghi [a,b,c], Rafael Radi [a,b,*]

[a] Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[b] Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[c] Departamento de Educación Médica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

ARTICLE INFO

Article history:
Received 3 May 2013
Received in revised form 25 June 2013
Accepted 4 July 2013
Available online 18 July 2013

Keywords:
Free radical
Nitrotyrosine
Peroxynitrite
Oxidative stress
Superoxide radical
Nitric oxide

ABSTRACT

Background: Peroxynitrite, the product of the reaction between superoxide radicals and nitric oxide, is an elusive oxidant with a short half-life and a low steady-state concentration in biological systems; it promotes nitroxidative damage.
Scope of review: We will consider kinetic and mechanistic aspects that allow rationalizing the biological fate of peroxynitrite from data obtained by a combination of methods that include fast kinetic techniques, electron paramagnetic resonance and kinetic simulations. In addition, we provide a quantitative analysis of peroxynitrite production rates and conceivable steady–state levels in living systems.
Major conclusions: The preferential reactions of peroxynitrite *in vivo* include those with carbon dioxide, thiols and metalloproteins; its homolysis represents only <1% of its fate. To note, carbon dioxide accounts for a significant fraction of peroxynitrite consumption leading to the formation of strong one-electron oxidants, carbonate radicals and nitrogen dioxide. On the other hand, peroxynitrite is rapidly reduced by peroxiredoxins, which represent efficient thiol-based peroxynitrite detoxification systems. Glutathione, present at mM concentration in cells and frequently considered a direct scavenger of peroxynitrite, does not react sufficiently fast with it *in vivo*; glutathione mainly inhibits peroxynitrite-dependent processes by reactions with secondary radicals. The detection of protein 3-nitrotyrosine, a molecular footprint, can demonstrate peroxynitrite formation *in vivo*. Basal peroxynitrite formation rates in cells can be estimated in the order of 0.1 to 0.5 μM s^{-1} and its steady-state concentration at ~1 nM.
General significance: The analysis provides a handle to predict the preferential fate and steady-state levels of peroxynitrite in living systems. This is useful to understand pathophysiological aspects and pharmacological prospects connected to peroxynitrite. This article is part of a Special Issue entitled Current methods to study reactive oxygen species - pros and cons and biophysics of membrane proteins. Guest Editor: Christine Winterbourn.

© 2013 Elsevier B.V. All rights reserved.

1. Peroxynitrite biochemistry

1.1. Peroxynitrite formation pathways

The reaction between the free radicals nitric oxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet-}$) leads to the diffusion-controlled formation of peroxynitrite,[1] a potent oxidizing and nitrating agent formed *in vivo* [1].

$$^{\bullet}NO + O_2^{\bullet-} \rightarrow ONOO^- \quad (1)$$

Nitric oxide is a relatively stable and mildly reactive free radical mainly generated enzymatically from L-arginine, NADPH and oxygen in a reaction catalyzed by several isoforms of nitric oxide synthase (NOS). Nitric oxide is a ubiquitous intracellular messenger, which mediates multiple physiological processes, including regulation of blood pressure, neurotransmission, immune response and platelet aggregation [2–4]. Its consumption in biological systems is determined by reactions preferentially with other paramagnetic species, such as

Abbreviations: $ABTS^{2-}$, 2,2′-azinobis (3-ethylbenzthiazoline-6-sulfonic acid); DTPA, diethylenetriaminepentaacetic acid; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; MPO, myeloperoxidase; EPO, eosinophil peroxidase; NT, 3-nitrotyrosine; NOS, nitric oxide synthase; SOD, superoxide dismutase; GSH, glutathione; Prx, peroxiredoxin

☆ This article is part of a Special Issue entitled Current methods to study reactive oxygen species - pros and cons and biophysics of membrane proteins. Guest Editor: Christine Winterbourn.

* Corresponding author at: Departamento de Bioquímica, Facultad de Medicina, Universidad de la República Avda. Gral. Flores 2125, 11800 Montevideo, Uruguay. Tel.: +598 29249561; fax: +598 29249563.
E-mail address: rradi@fmed.edu.uy (R. Radi).

[1] The term peroxynitrite is used to refer to the sum of peroxynitrite anion ($ONOO^-$) and peroxynitrous acid (ONOOH). IUPAC recommended names are oxoperoxonitrate (1−) and hydrogen oxoperoxonitrate, respectively.

0304-4165/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbagen.2013.07.005

organic radicals (tyrosyl and peroxyl radicals) or transition metal centers, most remarkably with oxyhemoglobin, which represents an important fate of $^{\bullet}NO$ in the vasculature [5,6]. Another relevant reaction responsible for $^{\bullet}NO$ depletion is with $O_2^{\bullet-}$ to yield peroxynitrite [2,4,7,8]. The sources of $O_2^{\bullet-}$, the one electron reduction product of molecular dioxygen, include enzymes such as NAD(P)H oxidase, xanthine oxidase and uncoupled NOS, the electron leakage in the mitochondrial respiratory chain, and through redox cycling of xenobiotics, among other possible mechanisms. $O_2^{\bullet-}$ can react with iron sulfur clusters, transition metal centers and thiols but the main route for its consumption in biological systems is the reaction with superoxide dismutases (SOD), which are extremely efficient in catalyzing $O_2^{\bullet-}$ dismutation to hydrogen peroxide (H_2O_2) and dioxygen (rate constant $> 10^9\ M^{-1}\ s^{-1}$) [9–11]. This preferential reaction with SODs, which are abundant (concentration > 10 μM) and ubiquitous enzymes, keeps the concentration of $O_2^{\bullet-}$ at a low steady-state level (10^{-9}–10^{-12} M range) [12–14]. However, this value can increase several-fold under conditions of altered cellular homeostasis and during inflammatory processes.

An early evidence of the biological formation of peroxynitrite was obtained in experiments that showed that SOD prolonged the half-life and biological effects of the $^{\bullet}NO$ [15,16]. The rate constant of $O_2^{\bullet-}$ reaction with $^{\bullet}NO$ to form peroxynitrite (Eq. (1)), has been reported within the range of $(4–16) \times 10^9\ M^{-1}\ s^{-1}$ [17–20], which is a higher value than that of the reaction with Mn or Cu,ZnSOD ($1–2 \times 10^9\ M^{-1}\ s^{-1}$) [10,11]. Therefore, $^{\bullet}NO$ can be produced at a sufficient concentration and react fast enough to outcompete SOD for its reaction with $O_2^{\bullet-}$. In fact, it can be estimated that in the presence of physiological concentrations of SOD ~10 to 20 μM, $^{\bullet}NO$ produced at micromolar concentrations will be able to outcompete SOD and trap a significant fraction of the $O_2^{\bullet-}$ formed.

Nitric oxide is a hydrophobic uncharged radical which can easily permeate cell membranes and diffuse outside the cell, while $O_2^{\bullet-}$ is much more short-lived and due to its anionic character ($pK_a \sim 4.8$) has restricted diffusion across biomembranes. For this, peroxynitrite formation, which requires the simultaneous generation of both radicals, will be spatially circumscribed to the sites of $O_2^{\bullet-}$ formation. The steady-state concentrations of $^{\bullet}NO$ and $O_2^{\bullet-}$ also command the rate of peroxynitrite formation. Considering a cellular scenario where diverse competitive scavenging occurs, particularly direct competition between SOD and $^{\bullet}NO$ for the reaction with $O_2^{\bullet-}$, from a combination of experimental values and the known rate constants it was estimated that the maximal rate of peroxynitrite formation in endothelial cell mitochondria under basal metabolic conditions is 0.3 μM s^{-1} [21,22]. In this sense, from a reported kinetic model that included the effects of multiple cellular targets, intracellularly steady-state concentrations of peroxynitrite in the nanomolar range were estimated [21,23]. Even though the levels of $^{\bullet}NO$ and $O_2^{\bullet-}$ *in vivo* are regulated efficiently by the scavengers and disposal systems, the concentration could increase several-fold under altered cellular homeostasis and therefore influence the rate of peroxynitrite formation. Thus, peroxynitrite flux can be increased when other scenarios are considered, such as in inflammatory cells, where both $^{\bullet}NO$ and $O_2^{\bullet-}$ production rates are largely enhanced. For example, in selected cellular compartments such as the phagosome, fluxes of peroxynitrite produced by immuno-stimulated (*i.e.* cytokine exposure leading to iNOS expression) and activated (*i.e.* trigger of the respiratory burst) macrophages were estimated as ~0.83–1.66 μM s^{-1} in the murine cell line J774A.1 [24].

1.2. Physicochemical properties of peroxynitrite

Peroxynitrite is more reactive than its precursors $^{\bullet}NO$ and $O_2^{\bullet-}$. With one- and two-electron reduction potentials of $E^{\circ\prime}(ONOO^-, 2H^+/{}^{\bullet}NO_2, H_2O) = +1.4$ V and $E^{\circ\prime}(ONOO^-, 2H^+/NO_2^-, H_2O) = +1.2$ V, respectively [25,26], peroxynitrite is a relatively strong biological oxidant and nitrating agent able to react with a wide range of biomolecules. The anionic form of peroxynitrite ($ONOO^-$) exists in equilibrium with its conjugated acidic form (ONOOH) ($pK_a \sim 6.8$, Eq. (2)). Thus, under biological conditions both species will be present at ratios depending on the local pH. For example, at the physiological pH of 7.4, peroxynitrite anion will be present in a proportion of 80%.

$$ONOOH \rightleftharpoons ONOO^- + H^+ \quad \left(K_a = 1.58 \times 10^{-7} M^{-1}\right) \tag{2}$$

The coexistence of the anionic and protonated forms of peroxynitrite is also relevant since they have different reactivity [21] and diffusional [27,28] properties.[2]

The anionic form of peroxynitrite displays a moderate absorbance near the ultraviolet region. The absorption spectrum in aqueous alkaline solution consists of a single band with a maximum centered at 302 nm, which has been used to quantify peroxynitrite and follow its reactions, using the reported extinction coefficient ($\varepsilon_{302} = 1670\ M^{-1}\ cm^{-1}$) [30].

2. Kinetics and reaction mechanisms of peroxynitrite

Peroxynitrite biochemistry is dictated by the kinetics of its formation and decay together with its partial limitation to diffuse through biological membranes: most importantly the availability of targets in different cell compartments critically modulates its biological fate. In biological systems, peroxynitrite promotes the oxidative modification of target molecules by different types of reactions involving: a) peroxynitrite-derived radicals from the homolytic cleavage of peroxynitrous acid or secondary to the reaction with carbon dioxide or b) by direct oxidation reactions.

2.1. Peroxynitrite reactivity

2.1.1. Homolytic cleavage of ONOOH

In the absence of direct targets peroxynitrite anion is relatively stable. However, peroxynitrous acid decays rapidly by homolysis of its peroxo bond ($k = 0.9\ s^{-1}$ at 37 °C and $0.26\ s^{-1}$ at 25 °C and pH 7.4) leading to the formation of nitrogen dioxide ($^{\bullet}NO_2$) and hydroxyl radicals ($^{\bullet}OH$) in ~30% yield whereas the rest of peroxynitrous acid directly isomerizes to nitrate (NO_3^-) [31–33]. Hydroxyl radical is a much stronger oxidant than $^{\bullet}NO_2$, however it reacts very rapidly with most biomolecules ($\sim 10^9\ M^{-1}\ s^{-1}$) in a non-selective manner, with addition reactions predominating over one-electron abstractions. In contrast, $^{\bullet}NO_2$ reacts at slower rates but represents a more selective one-electron oxidant. Thus, these peroxynitrite-derived radicals ($^{\bullet}OH$ and $^{\bullet}NO_2$) can mediate several reactions that may lead to the oxidation or nitration of different targets, such as tyrosine nitration and lipid peroxidation.

Considering the relative slowness of ONOOH homolysis compared to the reaction of peroxynitrite with multiple cellular targets that react directly with relatively high rate constants (*vide infra*), it can be estimated that in biological systems, most peroxynitrite formed will be consumed by direct reactions, with <1% evolving to $^{\bullet}NO_2$ and $^{\bullet}OH$, so the homolytic route is just a modest component of peroxynitrite reactivity in the aqueous compartment [34]. However, the homolytic decomposition of ONOOH may be a relevant process in hydrophobic compartments initiating radical-dependent processes such as lipid peroxidation and even protein and lipid nitration [35–39].

[2] Peroxynitrous acid can cross biological membranes through the lipid bilayer by passive diffusion, whereas the anionic form can penetrate cells through anion channels [27,28]. Despite the short biological half-life of peroxynitrite at physiological pH (~10 ms, [28]), due to a multiplicity of reactions with biotargets, the ability to cross cell membranes implies that peroxynitrite generated by a cellular source could influence surrounding target cells within one or two cell diameters (~5–10 μm) [29]. In fact, considering the peroxynitrite targets in different compartments, it has been estimated that it can traverse a mean distance of 0.5, 3 and 5.5 μm in erythrocytes, mitochondria and blood plasma, respectively, during one half-life [21].

Download English Version:

https://daneshyari.com/en/article/10800170

Download Persian Version:

https://daneshyari.com/article/10800170

Daneshyari.com