Biochimica et Biophysica Acta 1820 (2012) 777–784

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Review

Nitroalkylation — A redox sensitive signaling pathway ☆

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ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 17 May 2011
Accepted 16 June 2011
Available online 24 June 2011

Keywords:
Nitrated fatty acids
Nitroalkylation
Electrophile
Nitric oxide

ABSTRACT

Redox-sensitive posttranslational modification emerged as important signaling mechanisms. Besides other posttranslational modifications nitroalkylation by nitrated fatty acids mediate favorable anti-inflammatory effects. This review gives an overview of the generation and the reactivity of nitrated fatty acids. Additionally, it provides insights into the so far described pathways regulated by nitrated fatty acids. This article is part of a Special Issue entitled Regulation of Cellular Processes by S-nitrosylation.

1. Introduction

Nitric oxide (·NO) has been identified as an important mediator in physiology and pathophysiology. Although ·NO itself is a relatively stable, highly diffusible free radical, its radius of action is limited since ·NO has a half-life time of only a few seconds. Over the last decade different ·NO-derived signaling mediators like peroxynitrite and nitrogen dioxide have been described influencing a variety of biochemical pathways. Recently, one class of ·NO-derived signaling mediators has been identified which is based on unsaturated fatty acids: nitrated fatty acids (NO_2-FA).

This review summarizes the current knowledge of the formation and signaling mechanisms of this group of lipid mediators.

Nitrated fatty acids are formed endogenously under oxidative and nitrosative conditions. Since they are chemically stable and have a longer half-life time they well extend ·NO-dependent signaling. Given their formation under inflammatory conditions and their potential to mediate anti-inflammatory effects they can be viewed as endogenous signaling mediators to resolve inflammation.

2. Formation of nitrated fatty acids

Initially considered as toxic and inflammatory byproducts, reactive oxygen (ROS) and nitrogen species (RNS) are now appreciated as signaling mediators that promote a wide range of biomolecule modifications on modulatory proteins, DNA bases, amino acids and unsaturated fatty acids [1–4].

Dictated by the biochemical milieu as well as the concentration of ROS and RNS, a variety of oxidized or nitrated lipid products are formed under physiological and pathophysiological conditions yielding a broad spectrum of reactive pluripotent signaling mediators [5,6].

Probably the best described species of oxidized lipid signaling mediators are eicosanoids – derivatives of essential unsaturated fatty acids such as arachidonic acid – that mainly mediate pro-inflammatory reactions [7,8]. The discovery of eicosanoids shed a new light on lipids, which had been solely viewed as energy carriers and cell membrane components for a long time. The formation of 'classic eicosanoids' like prostanoids, thromboxanes or leukotrienes generally depends on enzymatic catalysis (via cyclooxygenase-1 and -2 or 5-lipoxygenase). These eicosanoids transmit their effects mainly via G-protein coupled eicosanoid receptors resulting in either an inhibition or stimulation of adenylate cyclase [9–11].

Beside these classic enzymatically formed eicosanoids 'non-classic' eicosanoids such as isoprostanes have been described which are formed non-enzymatically by free radical-catalyzed peroxidation of predominantly arachidonic acid in vivo under the condition of oxidative stress. Depending on their chemical structure isoprostanes

Abbreviations: AP-1, activator protein-1; AT_1R, angiotensin 1 receptor; HO-1, heme oxygenase-1; HSF1/2, heat shock factor 1/2; HSR, heat shock response; IκB, NF-kappa-B inhibitor; Keap1, Kelch like ECH associating protein 1; LNO_2, linoleic acid; LPS, lipopolysaccharides; MKP-1, MAPK phosphatase 1; MMPs, matrix metalloproteinases; NFκB, nuclear factor of kappa light polypeptide gene enhancer in B-cells; NO, nitric oxide; NO_2, nitrogen dioxide; NO_2^-, nitrite; NO_2-FA, nitrated fatty acids; Nrf2, nuclear factor erythroid 2-related factor 2; O_2^-, superoxide anion; OA-NO_2, nitrated oleic acid; $ONOO^-$, peroxynitrite; PPAR-γ, peroxisome proliferator-activated receptor gamma; RNS, reactive nitrogen species; ROS, reactive oxygen species; STAT-1, signal transducer and activator of transcription-1

☆ This article is part of a Special Issue entitled Regulation of Cellular Processes by S-nitrosylation.

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doi:10.1016/j.bbagen.2011.06.014

mediate their pro-inflammatory effects either via classic eicosanoid receptors or via posttranslational modification, which is based on the electrophilicity of some classes of isoprostanes [12].

The recent discovery of anti-inflammatory lipid derivatives added a new dimension to the lipid field. Resolvins, protectins, lipoxins and maresins – all enzymatically derived from polyunsaturated fatty acid – are one class of lipid mediators that are formed under inflammatory conditions in order to resolve inflammation [13]. The molecular mechanisms by which these lipids operate are still a matter for controversy, but receptor mediated signaling seems to play an important role [14,15].

This concept of regulated inflammation resolution is a very recently appreciated physiological principle.

NO_2-FA derivatives, a class of ·NO-derived signaling mediators, differ significantly from the above mentioned oxidation lipid products in terms of formation and signaling mechanisms. Since they display potent anti-inflammatory properties they resemble the recently described protective lipid mediators. Regarding the multitude of NO_2-FA that have been detected so far, formation seems to be favored by free radical substitution reactions rather than enzymatic catalyzation.

Thus, formation of nitrated fatty acids basically includes reactions with ·NO, RNS (e.g. nitrogen dioxide ($\cdot NO_2$) and peroxynitrite ($ONOO^-$)) and ROS (superoxide anions ($\cdot O_2^-$), hydrogen peroxide (H_2O_2) and lipid peroxyl radicals (LOO·)). The addition of $\cdot NO_2$ to the alken double bonds is viewed as the major mechanism of in vivo formation, that can be proceeded in different possible ways: (a) via homolytic attack of nitrogen dioxide ($\cdot NO_2$) (yielding reactive nitroalkyl radicals that can be further modified by $\cdot NO_2$), (b) reactions of $\cdot NO_2$ with pre-existing lipid carbon centered radicals, and (c) ionic addition reactions like an electrophilic substitution of NO_2^+ at unsaturated double bonds [16–18].

Involving ·NO as a precursor molecule and $\cdot NO_2$ as the proximal reactant, unsaturated fatty acid nitration can be proceeded via diverse chemical mechanisms, depending on the local redox environment in hydrophobic tissue compartments, the concentration of reactive species or secondary target molecules (e.g. transition metals, thiols or glutathione). Of note, the concentration of ROS and RNS are significantly influenced by the specific activities of enzymes with the ability to generate (e.g. nitric oxide synthase, xanthine oxidase, NADPH oxidases and peroxidases) or degrade free radicals (e.g. superoxide dismutase and catalases) [19–22].

To date $ONOO^-$, which is formed during host inflammatory responses by the reaction of ·NO with $\cdot O_2^-$, is viewed as the main source of $\cdot NO_2$. In its protonated form (ONOOH) it undergoes homolytic scission and reveals hydroxyl radical (·OH) and $\cdot NO_2$, which can further oxidize, nitrate or nitrosate proteins and lipid species [23–26]. Other important mechanisms for $\cdot NO_2$ generation imply peroxidase-catalyzed oxidation of ·NO or nitrite (NO_2^-), respectively (e.g. by glutathione- or myeloperoxidase) [27]. Finally, transition metal-induced oxidation of NO_2^- via fenton chemistry reveals $\cdot NO_2$.

Regarding the conditions that are required for the formation of NO_2-FA (pH, oxygen tension, certain concentration of ROS and RNS), plasma levels under inflammatory conditions, where oxidation/reduction reactions frequently occur, are expected to be much higher than under physiological situations.

Recently, quantitative analyses of two unesterified regioisomers of nitrated oleic acid (OA-NO_2) revealed concentrations in a range of 1–3 nM in healthy individuals [28]. As expected from the biochemistry, current data provided evidence that concentrations of NO_2-FA increase significantly under inflammatory conditions: in particular, elevated in vivo generation of OA-NO_2 and nitrated linoleic acid (LNO_2) derivatives has been observed in response to vascular injury and myocardial ischemia and reperfusion [29–31]. Additionally, in vitro studies revealed an increase of nitrated cholesteryl linoleate in macrophages upon exposure to lipopolysaccharides (LPS) and interferon-γ [32]. The fact that nitrated cholesteryl-linoleate as one component of net esterified LNO_2 also has been detected in human blood reckons a similar mechanism in vivo [33].

A wide spectrum of endogenous NO_2-FA including nitroalkenes, their nitrohydroxy- and keto-derivatives and multiple stereo- and positional isomers has been identified in human plasma and urine, suggesting that fatty acid nitration is a principal adaptive mechanism to inflammatory stimuli that occurs to all unsaturated fatty acids present in vivo [34]. This variety is also due to the fact that NO_2-FA undergo β-oxidation [35], saturation and desaturation [16].

Nitrated oleic acid (18:1), linoleic acid (18:2) (Fig. 1), linolenic acid (18:3), palmitoleic acid (16:1), arachidonic acid (20:4) as well as eicosapentaenoic acid (20:5) have been detected in healthy individuals so far, whereat OA-NO_2 is the most prevalent NO_2-FA derivative in vivo [1,34].

3. 2. Reactivity of nitrated fatty acids

3.1. Release of ·NO

Since NO_2-FA – in particular LNO_2 and nitrohydroxy derivatives of arachidonic acid – were capable of inducing relaxation of smooth muscle cells and pre-constricted rat aortic vessel segments [36–38] in a soluble guanylate cyclase-dependent manner, the capacity of NO_2-FA to release ·NO has long been viewed as their main signaling mechanism rendering NO_2-FA as potent ·NO carriers.

Basically two mechanisms have been proposed for ·NO release under aqueous conditions, including (a) a modified Nef reaction that mainly involves the formation of a nitroso intermediate that rapidly decays to release either HNO or ·NO or (b) nitroalkene-rearrangement to a nitrite ester followed by N—C bond homolysis to form ·NO and the corresponding enol group [37,39]. However, while only low yields of ·NO have been detected under aqueous conditions so far and stabilization of NO_2-FA in hydrophobic environments prevents a subsequent ·NO release, signaling via ·NO release is considered to be of minor significance in vivo [39–41].

3.2. Nitroalkylation reactions

Corroborating the assumption that NO_2-FA display more functional capacities than simply serving as a pool for ·NO it has been recently shown that NO_2-FA predominantly act via posttranslational modification. As a potent electrophile with a high rate constant to react with glutathione, NO_2-FA covalently modify different molecules like proteins and amino acids influencing their structure and finally their function [35,42,43]. The strong electrophilicity is based on the chemical structure of NO_2-FA: due to the electron withdrawing nitro ($-NO_2$) substituent the beta carbon represents an attractive target for nucleophilic addition [44,45]. This reaction termed Michael addition involves a nucleophile donating a pair of electrons to the electrophile to form a covalent bond [46]. This reaction is termed S-alkylation when an alkyl group is involved as electrophile. In the case of NO_2-FA this covalent binding reaction is specifically termed nitroalkylation (Fig. 2) [47]. Current improvements in detection strategies including mass spectrometry and affinity chemistry reactions identified this chemical adduction to be reversible in vivo suggesting that nitroalkylation represents a selective signaling pathway as response to a stressful environment [35,42,48]. The reversibility of nitroalkylation in this context is a very important observation, since irreversible posttranslational modification by electrophiles usually leads to protein damage and permanent loss of function resulting in degradation of the protein. Only a reversible posttranslational modification can be viewed as regulated signaling pathway [48].

To date several electrophile-sensitive proteins and transcription factors involved in the defense or resolution of inflammation have been identified as targets of NO_2-FA. According to the current knowledge cysteine thiol residues are the main nucleophils NO_2-FA