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Lipid nitration and formation of lipid-protein adducts: biological insights

Review Article

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Summary. Lipid-protein adducts are formed during oxidative and nitrative stress conditions associated with increasing lipid and protein oxidation and nitration. The focus of this review is the analysis of interactions between oxidative-modified lipids and proteins and how lipid nitration can modulate lipid-protein adducts formation. For this, two biologically-relevant models will be analysed: a) human low density lipoprotein, whose oxidation is involved in the early steps of atherogenesis, and b) α-synuclein/lipid membranes system, where lipid-protein adducts are being associated with the develop of Parkinson disease and other synucleinopathies.

Keywords: Lipid oxidation – Nitrated lipid – Peroxynitrite – Lipid-protein adduct – Nitric oxide – Free radicals – α-Synuclein – Low density lipoprotein

Oxidative modification of low density lipoprotein (LDL) and scavenger receptors

Human LDL has a unique polypeptide forming its protein moiety (apolipoprotein B-100), showing many oxidizable residues including histidine, lysine, cysteine and tyrosine. This protein interacts with the lipid milieu of LDL formed mainly by cholesteryl esters (i.e. cholesteryl linoleate) and tryglicerides in the hydrophobic core, surrounded by a monolayer of phospholipids and unesterified cholesterol. More than 50% of the total fatty acids are unsaturated and susceptible to be oxidized by reactive oxygen species (Esterbauer et al., 1992). In fact, one of the major changes that occurs during LDL oxidation is the formation of lipid-protein adducts as a consequence of the extensive oxidative breakdown of polyunsaturated fatty acids. This yields hydroperoxides that decompose to aldehydes and other bioactive products that cross link with free amino groups, making the LDL particle more electronegative (Fruebis et al., 1992; Itakura et al., 2000; Requena et al., 1997; Steinbrecher, 1987a; Tsai et al., 1998). Lipid-protein adducts represent ligands for macrophage scavenger receptors that contribute to foam cells formation and the subsequent develop of atherosclerotic lesions (Boullier et al., 2000; Horkko et al., 1999; Steinbrecher et al., 1989). The relevance of these adducts formation in vivo has been supported by specific antibodies that recognize antigenic epitopes in human atherosclerotic lesions (Horkko et al., 1999; Kato and Osawa, 1998; Kim et al., 1997; Tsai et al., 1998).

α-Synuclein and lipid membranes

α-Synuclein (α-syn) is a soluble protein having 140 amino acid residues, thermo stable, characterized by acidic stretches toward the C-terminal and six repetitive amino acid sequences of the prototype KTKEGV. α-Synuclein does not assume a stable globular structure as typical globular proteins, instead it has a disordered conformation or random coil structure (Weinreb et al., 1996). However, in association with small unilamellar vesicles (20–25 nm diameter) rich in acidic phospholipid, i.e. phosphatidylserine and phosphatidyc acid (Davidson et al., 1998; Jo et al., 2000), the helical structure of the protein is stabilized (Davidson et al., 1998). The degenerated repeats, reminiscent of those in the class A_2 apolipoproteins (Davidson et al., 1998; Perrin et al., 2000; Segrest et al., 1992) and projected in a helical wheel having an hydrophilic and an

hydrophobic face, are involved in the interaction with lipids. The suppression of α-syn expression in primary culture of hippocampus cells induces a decrease in the number of presinaptic vesicles, strongly supporting that α-syn interaction with phospholipids occurs in vivo (Murphy et al., 2000).

Mechanism of lipid oxidation and nitration by reactive nitrogen species (RNS)

Several pathways promote lipid oxidation and nitration by RNS that include peroxynitrite ($ONOO^-$) and nitrogen dioxide ($^{\bullet}NO_2$). We will discuss a potential mechanism of lipid oxidation and nitration by RNS and how nitrated lipids may affect lipid-protein adducts formation. Peroxynitrite represents a biologically relevant oxidizing and nitrating agent that is formed from the diffusion-limited reaction between nitric oxide ($^{\bullet}NO$) and superoxide ($O_2^{\bullet -}$) (Radi et al., 2000). While the in vivo mechanism of lipid oxidation and nitration by $ONOO^-$ still to be defined, it involves free radical chain reactions (Botti et al., 2004, 2005; Trostchansky et al., 2001). In fact, we have previously shown that when added as a continuous infusion, $ONOO^-$-derived radicals ($^{\bullet}NO_2$ and hydroxyl radical,

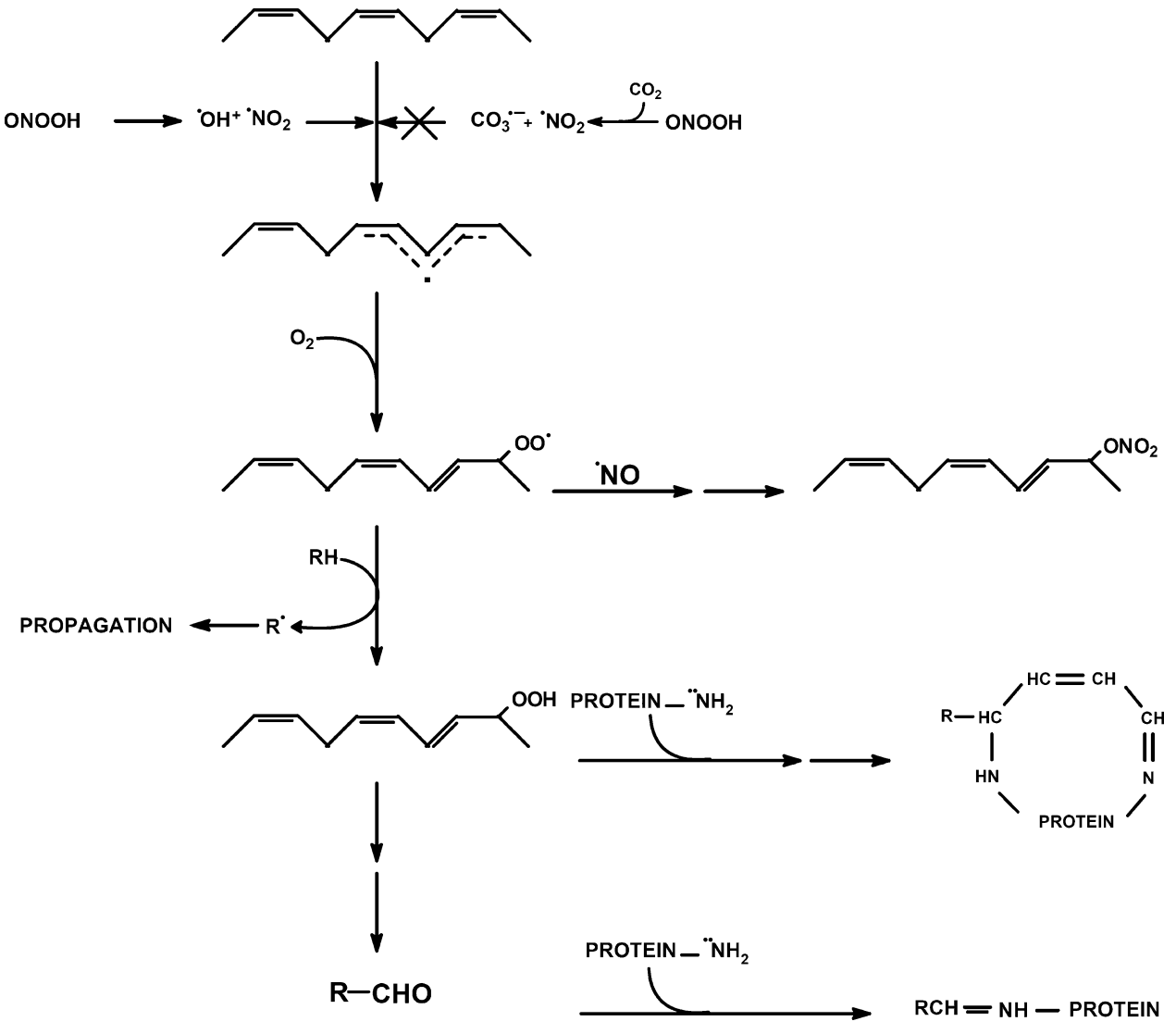

Fig. 1. Proposed mechanism for $ONOO^-$ – mediated lipid-protein adducts formation and the inhibitory role of $^{\bullet}NO$. Lipid hydroperoxides generated in LDL from lipid oxidation by fluxes of $ONOO^-$ can react with free amino groups (e.g. apo B-100 lysine residues) to form fluorescent cyclic lipid-protein adducts. Alternatively, aldehydes formed from hydroperoxides decomposition react with LDL amino groups, yielding both cyclic and non-cyclic fluorescent derivatives. Nitric oxide inhibits lipid-protein adduct formation through its radical–radical diffusion-limited termination reaction with lipid radicals (e.g. peroxyl radicals), preventing both hydroperoxides and aldehydes formation. Adapted from Trostchansky et al. (2001) with permission

$^{\bullet}OH$) can abstract a *bis*-allilic hydrogen atom from a double bond of unsaturated fatty acids, initiating lipid oxidation (Fig. 1). Bicarbonate at physiologically relevant concentrations, in equilibrium with CO_2, inhibited lipid oxidation due to the limited diffusion of the resultant carbonate radical to LDL hydrophobic compartments, diverting $ONOO^-$ reactivity from lipid to surface-exposed apoB-100 residues (Botti et al., 2004). The propagation and termination phases of lipid oxidation yields lipid hydroperoxides (LOOH) as well as aldehydes (i.e. malondialdehyde; 4-hydroxynonenal) in a process that can be prevented by $^{\bullet}NO$ due to its well known chain breaking antioxidant activity (Fig. 1; O'Donnell et al., 1999a; Rubbo et al., 1995; Trostchansky et al., 2001).

Lipid nitration

The ability of $^{\bullet}NO$ and $^{\bullet}NO$-derived species to oxidize, nitrosate or nitrate (addition of a nitro group, $-NO_2$) biomolecules serves as the molecular basis for understanding how $^{\bullet}NO$ influences the synthesis and reactions of bioactive lipids (Rubbo et al., 1994; Schopfer et al., 2003). Reactions between RNS and unsaturated fatty acids yield a spectrum of lipid oxidation and nitration products (Rubbo et al., 1994). In addition to its oxidizing properties, $ONOO^-$ mediates nitration of free as well as esterified unsaturated fatty acids (Lima et al., 2003; O'Donnell et al., 1999b, and unpublished results). Recently, the nitroalkene derivative of linoleic acid (LNO_2) was detected and quantified in human blood plasma and lipoproteins, representing the largest pool of bioactive oxides of nitrogen in the vascular compartment (Baker et al., 2005; Lima et al., 2002, 2003; Schopfer et al., 2005a, b). Nitrolinoleic acid exerts in general anti-inflammatory cell signalling properties including cGMP-mediated vasorelaxation, inhibition of neutrophil degranulation and superoxide production, inhibition of platelet activation, release of $^{\bullet}NO$ in aqueous conditions and activation of heme oxygenase 1 expression (Baker et al., 2005; Coles et al., 2002; Lim et al., 2002; Schopfer et al., 2005a; Wright et al., 2006). These biological actions are related with the ability of LNO_2 to be an endogenous ligand and activator of peroxisome proliferator-activated receptors (PPARs) (Baker et al., 2005; Schopfer et al., 2005b).

Multiple mechanisms likely account for lipid nitration in vivo (Fig. 2) including: a) $^{\bullet}NO$ autooxidation to $^{\bullet}NO_2$, which has strong oxidant and nitrating properties (Radi et al., 2000); b) peroxynitrous acid (ONOOH) homolysis to $^{\bullet}NO_2$ and $^{\bullet}OH$; c) nitronium ion (NO_2^+) electrophilic addition to unsaturated fatty acids; d) a possible caged radical rearrangement of lipid peroxyl radical ($LOO^{\bullet}$) by $^{\bullet}NO$ intermediates (O'Donnell et al., 1999a; Rubbo et al., 1994); and e) LOOH reaction with N_2O_4 (in equilibrium with $^{\bullet}NO_2$) to give an unstable alkyl peroxynitrite adduct (ROONO) that rearranges by cage recombination of the $LO^{\bullet}/^{\bullet}NO_2$ radical pair to give primarily an organic nitrate ($LONO_2$) or, to a lesser extent, $LO^{\bullet}$/epoxyallylic ($L(O^{\bullet})$) radicals and $^{\bullet}NO_2$ (O'Donnell et al., 1999a; Rubbo et al., 1994).

Modification of tyrosine by RNS

One of the key molecular footprints of RNS reactions with biomolecules is the nitration of tyrosine residues to 3-nitrotyrosine, representing a posttranslational modification associated to acute and chronic diseases (Radi, 2004). Although it was first proposed that 3-nitrotyrosine arise from $ONOO^-$, alternative pathways including formation of $^{\bullet}NO_2$ from heme peroxidases and nitrite (NO_2^-) (Eiserich et al., 1998) or the reaction of $^{\bullet}NO$ and tyrosyl radical (Goodwin et al., 1998) were also proposed. Protein nitration is not the unique oxidative modification induced by RNS on tyrosine residues: nitrating agents can also mediate dityrosine formation which in the case of α-syn leads to the crosslinking and stabilization of α-syn filaments both in vitro and in vivo (Souza et al., 2000).

Lipid-protein adducts formation: Schiff's bases adducts and Michael addition reactions

The formation of lipid-protein adducts is a consequence of the extensive oxidative breakdown of polyunsaturated lipids yielding LOOH and aldehydes that may react with lysines or histidines in the protein milieu through mechanisms that yield different end products: Schiff's bases or Michael addition reactions (Fruebis et al., 1992; Itakura et al., 2000; Requena et al., 1997; Steinbrecher, 1987a; Tsai et al., 1998). A series of oxidative reactions between LOOH or $LOO^{\bullet}$ with the amino group of lysines leading to the formation of a fluorescent cyclic Schiff's base is shown in Fig. 1. In fact, we demonstrated the presence of these adducts during the oxidation of LDL by fluxes of $ONOO^-$, leading to the conversion of native LDL to a more negatively charged form (Trostchansky et al., 2001). Schiff's bases adducts formed in the LDL-$ONOO^-$ system showed similar spectral properties (excitation and emission maxima at 365 and 420 nm, respectively) than the reported in lipid models including linoleic acid hydroperoxides plus bovine serum albumin (Fruebis et al., 1992), LDL oxidized by copper (Steinbrecher,

1987a) or malondialdehyde or 4-hydroxynonenal-modified LDL (Itabe et al., 1996; Itakura et al., 2000; Requena et al., 1997; Uchida, 2000). Lipid-protein adducts formation was also confirmed by the decrease of LDL free amino groups content during $ONOO^-$ infusion (Trostchansky et al., 2001) in agreement with a progressive decrease in the number of reactive lysine ε-amino groups (Fruebis et al., 1992; Requena et al., 1997; Steinbrecher, 1987a; Steinbrecher et al., 1987b).

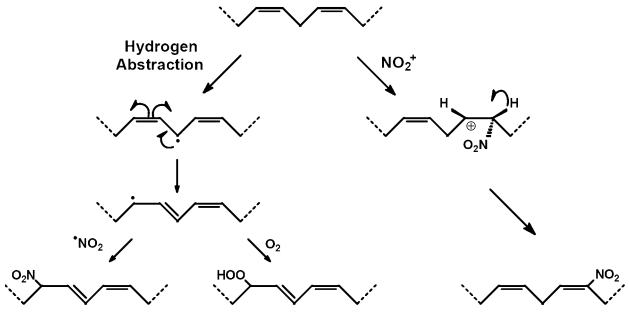

Fig. 2. Pathways for unsaturated fatty acid oxidation and nitration. Linoleic acid hydrogen abstraction by RNS results in free radical reactions following conjugated dienes formation with the nitro group bonded at C9 and C13. In contrast, NO_2^+ perform electrophilic addition reactions yielding positional isomers that, for linoleate, would be C10- and C12 nitro derivatives. Participation of O_2 in radical reactions will give COOH with extents of oxidized vs. nitrated products depending on relative local concentrations of oxygen, other free radical and nitrating species, catalysts and oxidant scavengers. Reprinted with permission from O'Donnell et al. (1999b)

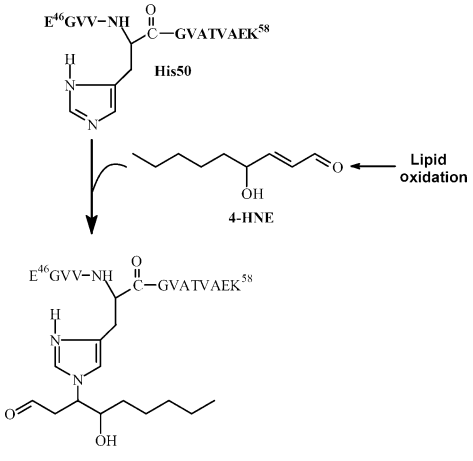

Fig. 3. 4-Hydroxynonenal modifies His50 in α-syn. The reaction of 4-hydroxynonenal with His occurs through a Michael addition mechanism as reported for other lipoprotein systems. This adducts exhibit characteristic fluorescence spectra ($\lambda_{exc} = 365$ nm, $\lambda_{em} = 440$ nm) characteristic of aldehydes-protein adducts (Uchida, 2000). Most of the biological consequences of 4-hydroxynonenal have been ascribed to its capacity to react with the nucleophilic sites in proteins and peptides to form covalently modified molecules (Uchida et al., 1994). Reprinted with permission from Trostchansky et al. (2006)

Although aldehydes should react with lysines to form Schiff's bases adducts, its major reactions occur through Michael addition reactions forming hemiacetals adducts with histidines (Figs. 1 and 3; Requena et al., 1997). 4-hydroxynonenal has been implicated as a key mediator in the onset and progression of several cardiovascular as well as neurodegenerative diseases (Uchida, 2000). In fact, wild type α-syn is a major component of Lewy bodies in sporadic Parkinson's disease and α-syn modified by 4-hydroxynonenal may play a major pathogenic role. In this context we have recently demonstrated the formation of 4-hydroxynonenal-protein adducts in a α-syn/liposome model (Trostchansky et al., 2006). α-Syn has a resemblance to the A_2 apolipoprotein family through the 11-residue periodicity showing amphipathic α-helices that participate in a variety of lipid and protein interactions, including its binding to membranes (Davidson et al., 1998; Perrin et al., 2000; Segrest et al., 1992). This cluster is characterized by the presence of lysine and histidine residues in the nonpolar/polar interface, stabilizing lipid-protein interactions (Davidson et al., 1998; Perrin et al., 2000). In the presence of unsaturated fatty acids, $ONOO^-$ mediates accumulation of lipid oxidation products which in turn can react with α-syn leading to fluorescent lipid-protein adducts formation (Trostchansky et al., 2006). Mass spectrometry analysis demonstrated that 4-hydroxynonenal could modify α-syn at His50 within the lipid-binding domain of the protein amino acid sequence (Fig. 3). From this, we postulate that α-syn nitration and oxidation by $ONOO^-$ can be modulated by the lipid environment, which may promote the formation of α-syn-lipid adducts as a novel posttranslational α-syn modification (Trostchansky et al., 2006).

Can nitrated lipids modulate lipid-protein adduct formation?

As discussed above, a direct consequence of oxidative damage in a lipoprotein milieu is the formation of lipid-protein adducts leading to pro-inflammatory as well as pro-oxidant events. However, in oxidative and nitrative stress conditions, RNS also mediate nitrated lipid formation. Nitrated lipids could modulate oxidative pathways through: a) ''NO like'' termination of lipid oxidation processes by chain breaking antioxidant reactions, preventing the propagation of damage as well as lipid-protein adduct formation (Fig. 1; O'Donnell et al., 1999b; Trostchansky et al., 2001), and b) representing novel footprints of tissue oxidative damage that can counteract the pro-inflammatory activities of lipid and protein oxidized products; this

includes the ability of nitrated lipids to exert a variety of anti-inflammatory and cell-signaling properties through PPARγ dependent-and independent-mechanisms (Baker et al., 2005; Schopfer et al., 2005b). Finally, we would like to emphasize that another biologically-relevant Michael addition reaction is the addition of nucleophiles to electron deficient nitroalkenes (Wang et al., 2005). In the case of nitrated fatty acids, the β-carbon proximal to the alkenyl NO_2 group is strongly electrophilic and reacts with H_2O via a Michael addition-like mechanism to generate nitrohydroxy adducts (Baker et al., 2005). As an example of relevance, nitrohydroxylinoleic acid levels were found greater in lipid extracts from hypercholesterolemic than normal human plasma (Lima et al., 2002). Moreover, recent preliminary data show that nitroalkenes can modify α-syn in His50 leading to a decrease in protein aggregation compared to 4-hydroxynonenal (unpublished). These examples illustrate a novel capacity of nitrated fatty acids to exert anti-inflammatory actions through Michael addition adducts that could down-modulate pro-inflammatory lipid-protein adducts formation. Future work will focus on the presence of lipid-modified α-syn in vivo and its potential involvement in the pathogenesis of α-synucleinopathies.

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