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Original Contribution

Formation of 4-hydroxynonenal from cardiolipin oxidation: Intramolecular peroxyl radical addition and decomposition

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ABSTRACT

We report herein that oxidation of a mitochondria-specific phospholipid tetralinoleoyl cardiolipin (L_4CL) by cytochrome *c* and H_2O_2 leads to the formation of 4-hydroxy-2-nonenal (4-HNE) via a novel chemical mechanism that involves cross-chain peroxyl radical addition and decomposition. As one of the most bioactive lipid electrophiles, 4-HNE possesses diverse biological activities ranging from modulation of multiple signal transduction pathways to the induction of intrinsic apoptosis. However, where and how 4-HNE is formed in vivo are much less understood. Recently a novel chemical mechanism has been proposed that involves intermolecular dimerization of fatty acids by peroxyl bond formation; but the biological relevance of this mechanism is unknown because a majority of the fatty acids are esterified in phospholipids in the cellular membrane. We hypothesize that oxidation of cardiolipins, especially L_4CL, may lead to the formation of 4-HNE via this novel mechanism. We employed L_4CL and dilinoleoylphosphatidylcholine (DLPC) as model compounds to test this hypothesis. Indeed, in experiments designed to assess the intramolecular mechanism, more 4-HNE is formed from L_4CL and DLPC oxidation than 1-palmitoyl-2-linoleoylphosphatydylcholine. The key products and intermediates that are consistent with this proposed mechanism of 4-HNE formation have been identified using liquid chromatography–mass spectrometry. Identical products from cardiolipin oxidation were identified in vivo in rat liver tissue after carbon tetrachloride treatment. Our studies provide the first evidence in vitro and in vivo for the formation 4-HNE from cardiolipin oxidation via cross-chain peroxyl radical addition and decomposition, which may have implications in apoptosis and other biological activities of 4-HNE.

Free radical-induced oxidation products of polyunsaturated fatty acids (PUFAs) have been implicated as a major upstream component in signal transduction cascades in many cellular functions including apoptosis, proliferation, inflammatory responses, stimulating adhesion molecules, and chemoattractant production [1–3]. Fatty acid hydroperoxides are the primary products in enzymatic and nonenzymatic oxidation of fatty acids. These lipid hydroperoxides not only have potent biological activities related to human physiology and pathophysiology, but also can serve as precursors to form other highly oxidized lipid oxidation products or decompose to generate an array of reactive lipid electrophiles [4]. Over the past 2 decades, 4-hydroxy-2-nonenal (4-HNE) has become one of the most studied reactive lipid electrophiles [5–9]. As a highly reactive α,β-unsaturated aldehyde, 4-HNE exhibits a variety of biological activities including inhibition of protein and DNA synthesis and inactivation of enzymes. 4-HNE can also form protein adducts with amino acid residues such as cysteine, histidine, arginine, and lysine because of its strong electrophilic character [10–12]. Moreover, the presence of these protein adducts can serve as biomarkers for lipid peroxidation and oxidative stress [5]. 4-HNE can also trigger multiple signaling events in a physiological context [8].

In contrast to the biology of 4-HNE, the mechanisms for its formation are much less understood [13–15]. Several mechanisms have been proposed that may account for the formation of 4-HNE, which include free radical-induced decomposition of lipid hydroperoxides such as 13-hydroperoxyoctadecadienoic acid (13-HPODE) or 15-hydroxyeicosatetraenoic acid (15-HETE)

Abbreviations: ALDH, aldehyde dehydrogenase; C-0, a water-soluble azo initiator; CID, collision-induced dissociation; CL, cardiolipin; DLPC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; DNPH, 2,6-dinitrophenylhydrazine; ESI, electrospray ionization; 4-HNE, 4-hydroxy-2-nonenal; 4-HPNE, 4-hydroperoxy-2-nonenal; HPLC, high-performance liquid chromatography; HODE, hydroxyoctadecadienoic acid; HPODE, hydroperoxyoctadecadienoic acid; HETE, hydroxyeicosatetraenoic acid; IsoP, isoprostane; L_4CL, tetralinoleoyl cardiolipin; MeOAMVN, 2,2′-azobis-(4-methoxy-2,4-dimethylvaleronitrile); MS, mass spectrometry; O_4CL, tetraoleoyl cardiolipin; 4-ONE, 4-oxo-2-nonenal; PLPC, 1-palmitoyl-2-linoleoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PC, phosphatidylcholine; PHGPX, phospholipid hydroperoxide glutathione peroxidase; PPh_3, triphenylphosphine; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SRM, selective reaction monitoring; UPLC, ultrapressure liquid chromatography.

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[16,17]. Ferrous iron (Fe^{2+})- or vitamin C-induced decomposition of lipid hydroperoxides has also been proposed [18,19]. Recently, an alternative mechanism was suggested, which involves an intermolecular cross-linking of a peroxyl radical [13,17]. This mechanism has precedence in styrene–oxygen polymerization and depolymerization [20]. There is also evidence to support the decomposition of these cross-linked peroxides of linoleic acid as initiators of free radical lipid oxidation and formation of reactive lipid aldehydes in vitro [21–25]. It should be noted that all these studies were carried out using linoleic acid or methyl ester as models and thus the biological relevance of this mechanism remains unclear because a majority of linoleic acids are esterified on phospholipids in cellular membranes.

Cardiolipin (CL) is a unique class of phospholipids containing four fatty acyl side chains and three glycerol moieties (Fig. 1A). In most mammalian tissues the predominant form of CL is tetralinoleoyl CL (L_4CL), and typically the distribution of linoleate mitochondrial CL is around 85–90% [26–28]. Recently, cardiolipin oxidation has attracted much attention because it is involved in regulation of programmed cell death (apoptosis) initiated in mitochondria [29]. During apoptosis, CL interacts with cytochrome *c* to form a peroxidase complex that catalyzes CL oxidation, and accumulating evidence indicates that the oxidation products of CL play a critical role in the mitochondrial stage of the execution of the cell death program [30,31].

We hypothesize that L_4CL is the best model compound with biological relevance to test the idea of interchain peroxyl radical addition and decomposition to form 4-HNE. In this case, the addition of a peroxyl radical on one linoleic acid to another fatty acid side chain occurs *intra*molecularly in L_4CL instead of *inter*molecularly, as in the model systems that have been studied previously. Moreover, the intramolecular addition of a peroxyl radical in L_4CL is favored kinetically

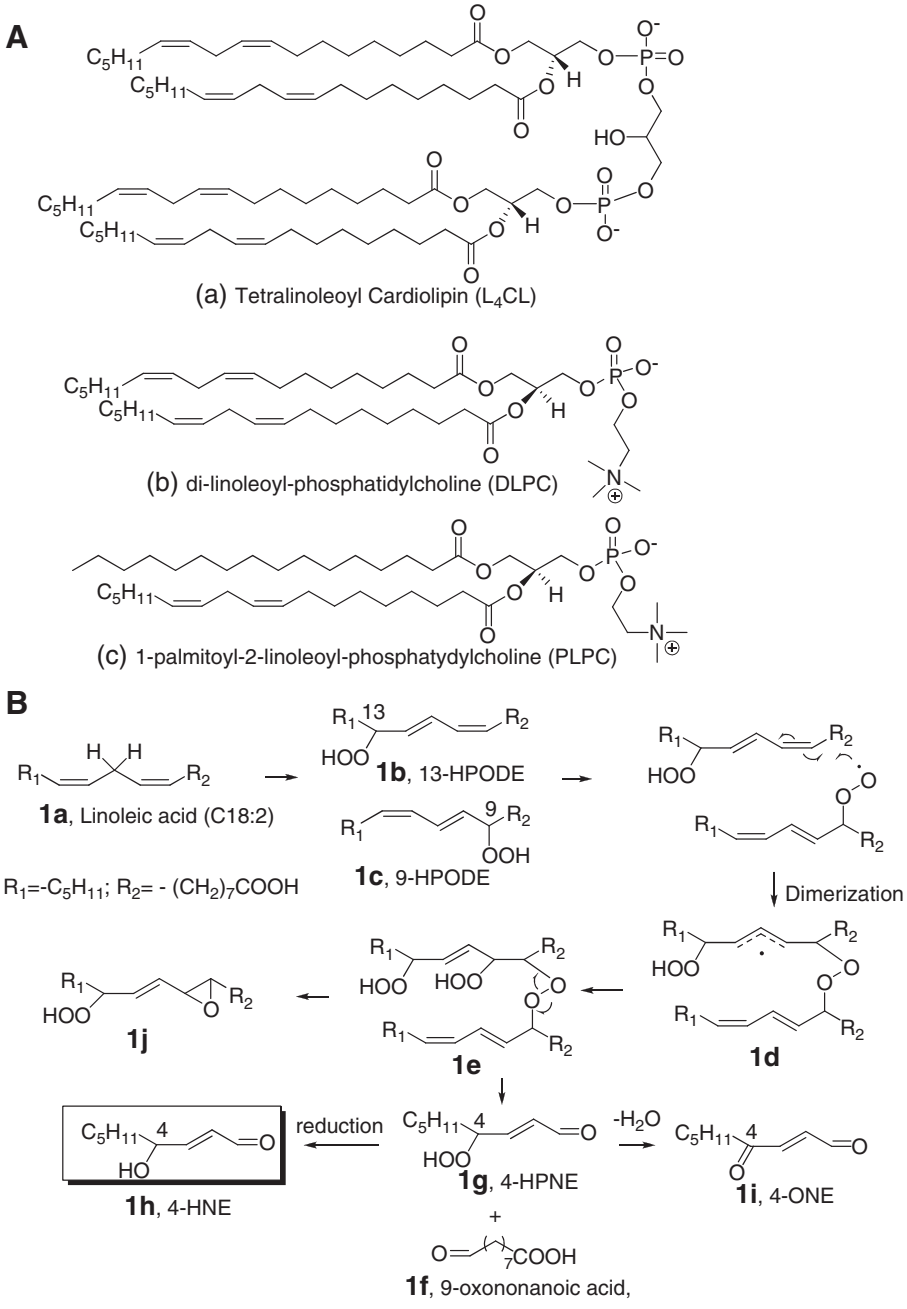

Fig. 1. Chemical structures of linoleoylphospholipids and proposed chemical mechanism for 4-HNE formation. (A) Chemical structures of L_4CL, DLPC, and PLPC. (B) Formation of 4-HNE from dimeric peroxides of two linoleic acids under free radical conditions. *Note*. Other isomers of **1e** can be formed during the process, but only the structures that are relevant to the proposed mechanism are illustrated for simplicity.