Biochimie xxx (2011) 1–9

Contents lists available at SciVerse ScienceDirect

Biochimie

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

Mini-review

Lipid peroxidation modifies the picture of membranes from the “Fluid Mosaic Model” to the “Lipid Whisker Model”

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ARTICLE INFO

Article history:
Received 15 June 2011
Accepted 27 September 2011
Available online xxx

Keywords:
Lipid peroxidation
Fluid Mosaic Model
Lipid rafts
Lipid Whisker Model

ABSTRACT

The “Fluid Mosaic Model”, described by Singer and Nicolson, explain both how a cell membrane preserves a critical barrier function while it concomitantly facilitates rapid lateral diffusion of proteins and lipids within the planar membrane surface. However, the lipid components of biological plasma membranes are not regularly distributed. They are thought to contain “rafts” – nano-domains enriched in sphingolipids and cholesterol that are distinct from surrounding membranes of unsaturated phospholipids. Cholesterol and fatty acids adjust the transport and diffusion of molecular oxygen in membranes. The presence of cholesterol and saturated phospholipids decreases oxygen permeability across the membrane. Alpha-tocopherol, the main antioxidant in biological membranes, partition into domains that are enriched in polyunsaturated phospholipids increasing the concentration of the vitamin in the place where it is most required. On the basis of these observations, it is possible to assume that non-raft domains enriched in phospholipids containing PUFAs and vitamin E will be more accessible by molecular oxygen than lipid raft domains enriched in sphingolipids and cholesterol. This situation will render some nano-domains more sensitive to lipid peroxidation than others. Phospholipid oxidation products are very likely to alter the properties of biological membranes, because their polarity and shape may differ considerably from the structures of their parent molecules. Addition of a polar oxygen atom to several peroxidized fatty acids reorients the acyl chain whereby it no longer remains buried within the membrane interior, but rather projects into the aqueous environment “Lipid Whisker Model”. This exceptional conformational change facilitates direct physical access of the oxidized fatty acid moiety to cell surface scavenger receptors.

1. Introduction

According to the Fluid Mosaic Model described by Singer and Nicolson [1], a biological membrane is a two dimensional fluid of oriented proteins and lipids. The lipid bilayer is the fundamental structure of all cell and organelle membranes and the basis of cell biology. Cell membranes are dynamic, fluid structures, and the majority of their molecules are able to move in the plane of the membrane. In biology, the membrane fluidity refers to the viscosity of the lipid bilayer of a cell membrane. The membrane phospholipids incorporate fatty acids of varying length and unsaturation. Shorter-chain fatty acids, and ones with greater saturation, are less stiff, less viscous and have lower melting points. Fluidity is the ease of movement and symbolizes the reciprocal value of the membrane viscosity. Fluid properties of biological membranes are decisive for many cell functions. Even small changes in membrane fluidity may cause atypical function and pathological processes. Fluid properties are established mainly by the occurrence of polyunsaturated fatty acids in phospholipids molecules located in both sides of the lipid bilayer.

The adaptability of biological membranes is dependent on their structures and biophysical properties, which are determined by the types of lipids and proteins that make up the membranes.

Abbreviations: C16:0, palmitic acid; C18:0, stearic acid; C18:2 *n*-6, linoleic acid; C18:3 *n*-3, α-linolenic acid; C20:4 *n*-6, arachidonic acid; CD36, “pattern recognition” receptor; FAT, fatty acid translocase; C22:6 *n*-3, docosahexaenoic acid; HHE, 4-hydroxy-2-hexenal; HNE, 4-hydroxy-2-nonenal; KOdiA-PC, 1-palmitoyl-2-(5-keto-6-octene-dioyl) phosphatidylcholine; L•, lipid radical; LO•, lipid alkoxyl radical; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide; LPC, lysophosphatidylcholine; MS, mass spectrometry; MDA, malondialdehyde; MS/MS, tandem mass spectrometry; OxPL, oxidized phospholipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Pls, phospholipids; PUFAs, polyunsaturated fatty acids.

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doi:10.1016/j.biochi.2011.09.025

The functions of membranes require a fluid plasticity which is attained through modification in lipid composition. Lipid composition is diverse, not only among different organisms, but also among different compartments within the same cells and between the two leaflets of the same membrane. Lipid composition is determined during regulation of de novo synthesis at chosen cellular sites, selective distribution or trafficking to new sites, and by localized remodeling reactions.

The "Fluid Mosaic Model", described by Singer and Nicolson, explain both how a cell membrane preserves a critical barrier function while it concomitantly facilitates rapid lateral diffusion of proteins and lipids within the planar membrane surface.

Some biological processes, however, are not compatible with a classic phospholipid orientation as described for a lamellar (bilayer) phase membrane structure, and instead engage other phospholipid conformations that are described ahead.

2. Lipid rafts

The lipid molecules of biological plasma membranes are not regularly distributed. They are thought to contain "rafts" – micro domains enriched in sphingolipids and cholesterol. In model membranes, the lipid raft phase is typically associated with a so-called "liquid-ordered" (lo) phase, while the non-raft phase has been identified as the "liquid-disordered" (ld) phase based on differences in the short-ranged lipid translational and conformational order [2].

Lipid rafts are dynamic and highly ordered membrane nanodomains rich in cholesterol and sphingolipids that are distinct from surrounding membranes of unsaturated phospholipids. The average size of lipid rafts is estimated to be 50 nm in diameter, although several distinct raft domains can exist in a cell, that are heterogeneous in size and life time [3]. Lipid rafts concentrate select proteins and serve as a platform for cellular processes such as cell signaling, pathogen entry, cell adhesion, motility, protein sorting and trafficking [3,4].

Although positive identification of rafts in cell membranes has proven elusive, they are usually thought to exist and to concentrate proteins in regulated behavior. When in rafts, proteins could rapidly interact with each other to realize their functions, and this is thought to occur in processes as varied and far reaching as signal transduction and membrane trafficking [5–8]. Most studies directed at characterizing rafts in cells have established that they are small, on the order of tens of nanometers [9–12]. But an ample range of raft sizes has been described [13,14] and rafts may comprise a huge fraction of the area of a cell membrane [15].

The eukaryotic cells utilize around 5% of their genes to produce thousands of varied lipids. Although we currently distinguish the exact functions of many lipids, the complete utility of the eukaryotic lipid variety remains unknown.

Lipids accomplish three general purposes. First, because of their reduced state, lipids are utilized for energy storage, mostly as triacylglycerol and steryl esters, in lipid droplets. These function mainly as anhydrous reservoirs for the efficient storage of caloric reserves and as supplies of fatty acid and sterol components that are required for membrane biogenesis. Second, the environment of cellular membranes is formed by polar lipids, which consist of a hydrophobic and a hydrophilic component. The propensity of the hydrophobic moieties to self-associate (entropically established by water), and the tendency of the hydrophilic moieties to interact with aqueous environments and with each other, is the physical origin of the spontaneous arrangement of membranes. This basic principle of amphipathic lipids is a chemical property that allowed the first cells to separate their internal constituents from the external environment. This similar principle is applied within the cell to generate distinct organelles. This compartmentalization facilitates separation of specific chemical reactions for the purposes of expanded biochemical effectiveness and limited spread of reaction products. In addition to the barrier reason, lipids provide membranes with the potential for budding, fission and fusion, characteristics that are indispensable for cell division, biological reproduction and intracellular membrane trafficking. Lipids also allow particular proteins in membranes to aggregate, and others to disperse. Finally, lipids can act as first and second messengers in signal transduction and molecular recognition processes. The degradation of amphipathic lipids allows for bipartite signaling phenomenon, which can be transmitted within a membrane by hydrophobic portions of the molecule and also spread through the cytoplasm by soluble (polar) portions of the molecule. Additionally, numerous lipids function to characterize membrane domains, which employ proteins from the cytoplasm that subsequently organize secondary signaling or effectors complexes.

3. Polyunsaturated fatty acids in membranes determine structural and functional properties

Phospholipids containing polyunsaturated fatty acids (PUFAs) have received increased attention in recent years, because they are of fundamental significance for a large number of biological functions [16]. Such PUFA-containing phospholipids are found as components of cellular membranes and play both a structural and functional role. The extent of desaturation in a fatty acid is straight related to its flexibility. Saturated fatty acids such as palmitic (16:0) or stearic acids (18:0) are straight and rigid. This rigidity allows saturated fatty acids to pack jointly firmly and form a solid at lower temperatures. Introduction of a double bond into the fatty acid causes a "kink" to arise in the fatty acids. Unsaturated fatty acids, such as C22:6 *n*-3, assume multiple conformations because the fatty acid can rotate around C–C bonds but not around the rigid C=C bonds [17]. The flexible nature of PUFAs will not permit phospholipids containing these fatty acids to pack jointly firmly, resulting in a significant augment in membrane fluidity relative to phospholipids comprised only of saturated fatty acids. Membranes high in PUFAs may also amplify the effectiveness of membrane fusion events [18]. Furthermore, augmented fluidity of the membrane appears to be significant for increasing the speed at which protein–protein interaction episodes take place within the phospholipid bilayer. This is especially correct in the outer segment of the photoreceptor, where activation of transducin by the rhodopsin-to-metarhodopsin conversion does not occur efficiently when the level of C22:6 *n*-3 in phospholipids is decreased [19]. It is also remarkable to note that the phospholipids of mitochondria are also augmented in C22:6 *n*-3 and high content of this fatty acid in mitochondrial membranes may enlarge the efficiency of electron transport by increasing the lateral movement of proteins within the bilayer, thus facilitating protein–protein interactions [20]. Moreover, there is a direct relationship between the C22:6 *n*-3 content of mitochondrial phospholipids and the permeability of the membrane to protons [21]. Thus, the C22:6 *n*-3 content of phospholipids influences mitochondrial function.

4. Cholesterol and fatty acids adjust the transport and diffusion of molecular oxygen in membranes

It has been known for some time that the membrane bilayer is a resistance factor to oxygen diffusive passage or to the diffusion of small soluble lipid molecules. Oxygen molecules pass through the lipid bilayer where rapid rotation and acyl chain motion (transbilayer movement) would facilitate oxygen diffusion. A variety of techniques have shown that the rigid steroid ring structure of

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cholesterol reduces the mobility of the phospholipid acyl chains observed in the liquid state [22].

For oxygen, significant chemical reactions occurring within the membrane include lipid peroxidation and the formation of reactive oxygen species. For these reactions the value of the oxygen diffusion-concentration product across the membrane is highly important. For instance, Barclay and Ingold [23] highlighted the consequences of lipid peroxyl radicals in the termination of lipid peroxidation chain reactions by vitamin E.

The demonstrated tendency of alkyl chains to undergo fast vertical fluctuations suggests that other processes involved in lipid peroxidation could also occur at the membrane–water interface, such as initiation, which requires hydrogen abstraction from unsaturated lipid carbon–carbon bonds, and secondary initiation by water-soluble hydroperoxides. Both are supported by reactions with certain metal ions and water-soluble free radicals [24]. These reactions can occur at the membrane–water interface during vertical fluctuations of lipid alkyl chains toward the membrane surface. They can also take place in the membrane core since metal ions and other polar molecules can, to a certain extent, enter the lipid bilayer. Both vertical fluctuations of lipid alkyl chains near the membrane surface and penetration of polar molecules into the bilayer should thus be considered in the examination of chemical reactions involved in lipid peroxidation.

Subczynski et al., have investigated the diffusion of molecular oxygen in phosphatidylcholine (PC)-cholesterol membranes and their molecular mechanism [25]. A special attention was paid to the molecular interaction involving unsaturated alkyl chains and cholesterol. Oxygen transport was evaluated by monitoring the bimolecular collision rate of molecular oxygen and the lipid-type spin probes, tempocholine phosphatidic acid ester, 5-doxylstearic acid, and 16-doxylstearic acid. The collision rate was determined by measuring the spin-lattice relaxation times (T1's) in the presence and absence of molecular oxygen with long-pulse saturation-recovery ESR techniques. It was observed that in the absence of cholesterol, incorporation of either a cis or trans double bond at the C9–C10 position of the alkyl chain reduces oxygen transport at all locations in the membrane. Intercalation of cholesterol in saturated PC membranes reduces oxygen transport in the headgroup region and the hydrophobic region near the membrane surface but little affects the transport in the central part of the bilayer. In unsaturated PC membranes, intercalation of cholesterol also reduces oxygen transport in and near the headgroup regions. In contrast, it increases oxygen transport in the middle of the bilayer.

On the basis of these observations, a model for the mechanism of oxygen transport in the membrane was proposed in which oxygen molecules reside in vacant pockets created by gauche-trans isomerization of alkyl chains and the structural nonconformability of neighboring lipids, unsaturated PC and cholesterol in particular, and oxygen molecules jump from one pocket to the adjacent one or move along with the movement of the pocket itself.

The presence of cholesterol decreases oxygen permeability across the membrane in all membranes used in that work in spite of the increase in oxygen transport in the central part of unsaturated PC-cholesterol membranes because cholesterol decreases oxygen transport in and near the headgroup regions, where the major barriers for oxygen permeability are located.

5. Alpha-tocopherol and PUFA-containing phospholipids co-localize in non-raft domains

Vitamin E (alpha-tocopherol) has long been identified as the main antioxidant in biological membranes, and yet several structurally related questions continue of how the vitamin functions. For example, the very low levels of alpha-tocopherol reported for whole cell extracts question how this molecule can effectively defend the relatively enormous quantities of PUFA-containing phospholipids found in membranes that are highly vulnerable to lipid peroxidation attack. Recently Atkinson et al. [26], have hypothesized that alpha-tocopherol partition into domains that are enriched in polyunsaturated phospholipids, increasing the concentration of the vitamin in the place where it is most required. These highly disordered domains depleted in cholesterol are analogous, but organizationally opposing, to the well-studied lipid rafts. Experimental proof in support of the formation of PUFA-rich domains in model membranes is presented, focusing upon docosahexaenoic acid that is one of the most unsaturated fatty acids commonly found. Physical methodologies are then described to elucidate the nature of the interaction of alpha-tocopherol with PUFA and to establish that the vitamin and PUFA-containing phospholipids co-localize in non-raft domains.

Atkinson et al. [26], have reviewed the status of α-tocopherol's organization in membranes, including vertical location or depth, and have proposed lateral segregation of the vitamin into PUFA-rich domains. These domains are postulated in correspondence with the very well-studied lipid raft domain that at present plays a significant role on membrane structure. While lipid rafts are SM-rich, *lo* domains that are held together by cholesterol and have functions relating to cell signaling events, the non-raft domains are PUFA-rich, *ld* domains in which α-tocopherol co-localizes and serves its antioxidant function. Structural and functional roles for α-tocopherol, thus, would fit together nicely in a reciprocally helpful mode. By tracking after PUFA, the vitamin is situated "in the correct position at the correct time to be an antioxidant". A series of diverse physical methodologies are proposed to test the nature of the hypothesized α-tocopherol/PUFA membrane domains.

6. A new hypothesis on the role of lipid peroxidation and lipidic vitamin E partitioning in promoting changes in membrane structure

On the basis of the observations described in items 4 and 5, it is possible to assume that non-raft domains enriched in phospholipids containing PUFAs and vitamin E will be more accessible by molecular oxygen than lipid raft domains enriched in sphingolipids and cholesterol. This situation will render some nano-domains more sensitive to lipid peroxidation than others.

7. The lipid peroxidation process perturb the assembly of the membrane

Oxidative stress that take place in the cells, because an imbalance between the prooxidant/antioxidant systems, causes damage to biomolecules such as nucleic acids, proteins, structural carbohydrates, and lipids [27]. Among these targets, the peroxidation of lipids is essentially damaging because the formation of lipid peroxidation products directs to spread of free radical reactions. The general process of lipid peroxidation consists of three stages: initiation, propagation, and termination [28–31]. The initiation phase of lipid peroxidation includes hydrogen atom abstraction. Several species can abstract the first hydrogen atom and include the radicals: hydroxyl (•OH), alkoxyl (RO•), peroxyl (ROO•), and possibly HO_2• but not H_2O_2 or O_2–• [32].

The membrane lipids, mostly phospholipids, containing polyunsaturated fatty acids are principally susceptible to peroxidation because abstraction from a methylene (–CH2–) group of a hydrogen atom, which holds only one electron, leaves at the back an unpaired electron on the carbon, –•CH–. The occurrence of a double bond in the fatty acid weakens the C–H bonds on the carbon atom near to the double bond and thus facilitates H• subtraction. The initial reaction of

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•OH with polyunsaturated fatty acids produces a lipid radical (L•), which in turn reacts with molecular oxygen to form a lipid peroxyl radical (LOO•). The LOO• can abstract hydrogen from an adjacent fatty acid to produce a lipid hydroperoxide (LOOH) and a second lipid radical [28]. The LOOH formed can undergo reductive cleavage by reduced metals, such as Fe^{++}, producing lipid alkoxyl radical (LO•). Both alkoxyl and peroxyl radicals stimulate the chain reaction of lipid peroxidation by abstracting other hydrogen atoms [33]. Peroxidation of lipids can perturb the assembly of the membrane, causing changes in fluidity and permeability, modifications of ion transport and inhibition of metabolic processes [34].

8. Lipid peroxidation of *n*-3 and *n*-6 polyunsaturated fatty acids generates hydroxy-alkenals

Lipids containing polyunsaturated fatty acids are vulnerable to free radical-initiated oxidation and can contribute in chain reactions that increase damage to biomolecules as described above. Lipid peroxidation often occurs in response to oxidative stress, and a great diversity of aldehydes is formed when lipid hydroperoxides break down in biological systems. Some of these aldehydes are highly reactive and may be considered as second toxic messengers, which disseminate and increase initial free radical events. The aldehydes most intensively studied up to now are 4-hydroxy-2-nonenal, 4-hydroxy-2-hexenal, and malondialdehyde. 4-Hydroxy-2-nonenal (HNE) is recognized to be the main aldehyde formed during lipid peroxidation of *n*-6 polyunsaturated fatty acids, such as linoleic acid C18:2 *n*-6 and arachidonic acid C20:4 *n*-6. On the other hand, lipid peroxidation of *n*-3 polyunsaturated fatty acids such as α-linolenic acid C18:3 *n*-3 and docosahexaenoic acid C22:6 *n*-3 generates a closely related compound, 4-hydroxy-2-hexenal (HHE), which is a possible mediator of mitochondrial permeability transition [35]. 4-Hydroxy-2-alkenals symbolize the most important aldehyde substances generated during lipid peroxidation. Among them, 4-hydroxy-2-nonenal (HNE) is known to be the main aldehyde formed during lipid peroxidation of *n*-6 polyunsaturated fatty acids, such as linoleic acid and arachidonic acid (Fig. 1).

HNE was identified three decades ago as a cytotoxic aldehyde formed during the NADPH–Fe^{2+} induced peroxidation of liver microsomal lipids [36]. Since then, an enormous number of reports have been accessible, which sustain a function for this compound in a variety of disease processes. HNE is considered as an indicator of oxidative stress and a probable contributing agent of several diseases.

The damaging effects of lipid peroxidation on membrane structure and function are well documented [29]. Among cellular macromolecules, polyunsaturated fatty acids (PUFAs) exhibit the highest sensitivity to oxidative damage. Many studies have shown that free radical damage and lipid peroxidation increase as a function of the degree of unsaturation of the fatty acids present in the phospholipids of biological membranes. In this regard it has been demonstrated that the number of bis-allylic positions contained in the cellular lipids of intact cells determines their susceptibility, i.e. oxidizability, to free radical mediated peroxidative events. Membrane phospholipids are particularly susceptible to oxidation not only because of their highly polyunsaturated fatty acid content but also because of their association in the cell membrane with non-enzymatic and enzymatic systems capable of generating prooxidative-free radical species. There are two broad outcomes to lipid peroxidation: structural damage to membranes and generation of secondary products. Membrane damage derives from the generation of fragmented fatty acyl chains, lipid–lipid cross-links, lipid–protein cross-links and endocyclization to produce isoprostanes and neuroprostanes. These processes combine to produce changes in the biophysical properties of membranes that can have profound effects on the activity of membrane-bound proteins. The consequence of peroxidation of unsaturated fatty acids is severe: damage of membranes function, enzymatic inactivation, toxic effects on the cellular division, etc [29,30].

9. The lipid peroxidation process produces oxidized phospholipids that acquire new biological activities not characteristic of their unoxidized precursors

Biomembranes contains different phospholipid classes (headgroup heterogeneity), subclasses (acyl, alkyl chains) and species (chain length and unsaturation degree). PC is the major phospholipid in all mammalian cells (40–50%) and thus, most oxidized phospholipids detected in mammalian tissues have the choline moiety. However, recently oxidized PE has been found in the retina, a tissue that contains very high quantities of ethanolamine lipids [37] enriched in docosahexaenoic acid [38]. In addition, there are also reports providing evidence for the presence of oxidized PS in the surface of apoptotic cells [39]. Furthermore, oxidized phospholipids have been demonstrated to act as signals in monocyte activation, programmed cell death, and phagocytotic clearance of apoptotic cells [40–44].

In eukaryotic phospholipids, the *sn*-1 position is either linked to an acyl residue via an ester bond or an alkyl residue via an ether bond, whereas the *sn*-2 position almost exclusively contains acyl residues. The highly oxidized (*n*-3 and *n*-6 polyunsaturated fatty acids) are preferably bound to the *sn*-2 position of glycerophospholipids. Thus, most of the oxidized phospholipids are modified at this position. At the *sn*-1 position of glycerol a saturated fatty acid is frequently bound. Plasmalogens (alkenylacylglycerophospholipids) contain a vinyl ether bond in position *sn*-1 and, as a result, they are also susceptible to oxidative modifications at the *sn*-1 position.

The divergences in chemical structure of diverse types of phospholipids determine the physical properties of the membrane. PC tends to form bilayers with small curvature, while PE imposes a negative curvature on these lipid bilayers [44]. Conversely, introduction of the micelle-forming LPC into a PC membrane results in a positive curvature. In addition to the polar headgroups the polarity, length and unsaturation of the phospholipid acyl chains have also an impact on physical membrane properties. Thus, phospholipid oxidation products are very likely to change the properties of biological membranes, because their polarity and shape may differ considerably from the structures of their parent molecules. Thus, they may modify lipid–lipid and lipid–protein interactions and, as a consequence, also membrane protein functions.

When the *sn*-2 fatty acids of phospholipids are oxidized by radicals, numerous different types of oxidative products is formed [30]. These comprise phospholipids containing fatty acid oxidation products (usually referred to as oxidized phospholipids), lysophospholipids, and fragmentation products of fatty acid oxidation. Some of these products, lysophosphatidic acid or lysophospholipids, can be formed both enzymatically and non-enzymatically. Work from several laboratories have identified a diversity of phospholipid oxidation products and confirmed bioactivities of these phospholipids on vascular wall cells, leukocytes, and platelets. Because of the great number of fatty acid oxidation products that have been identified, it is almost sure that many other bioactive oxidized phospholipids will be find out. There are several significant issues that confront investigators concerned in bioactive phospholipids, including preparation, identification, quantification, and biological checking.

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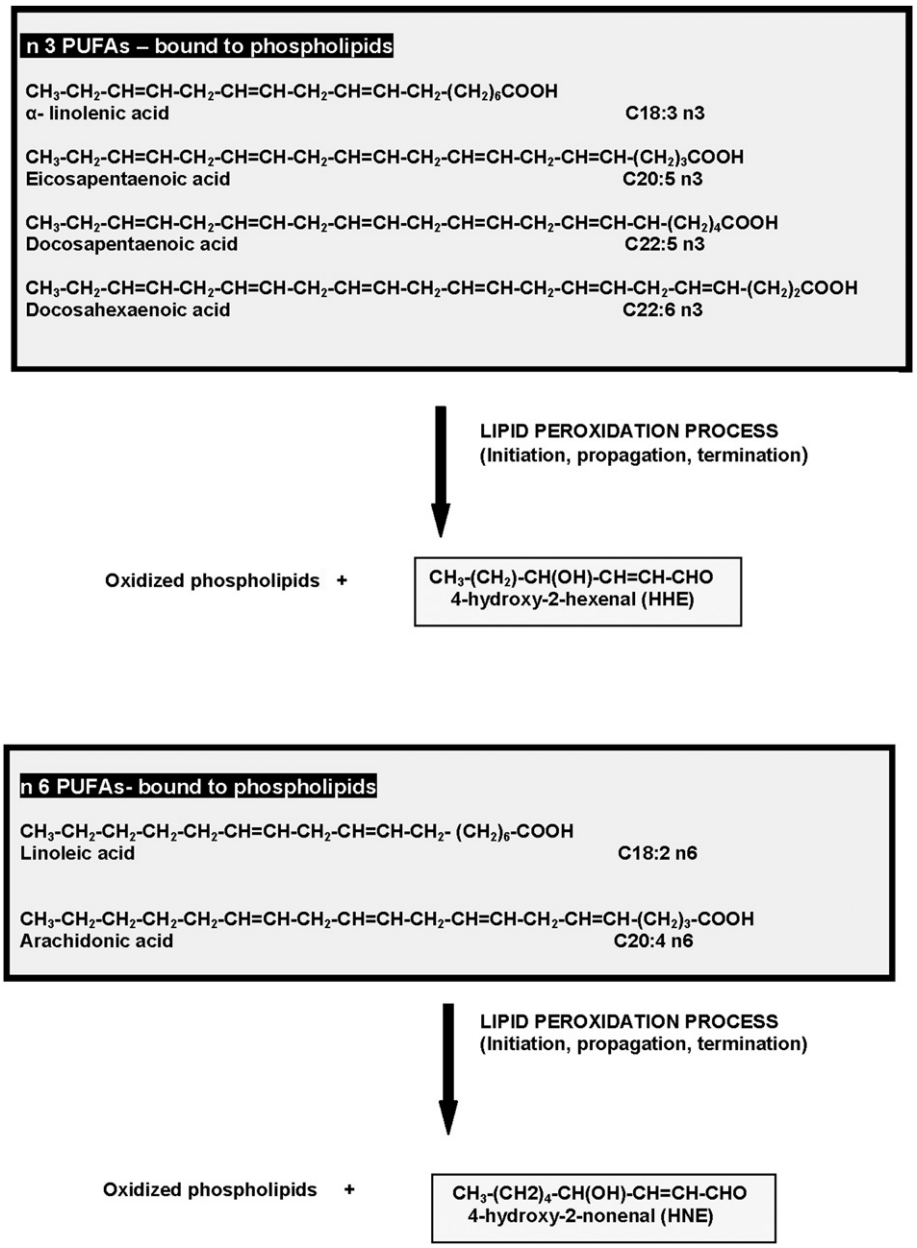

Fig. 1. Schematic diagram of reactive hydroxy-alkenals generated during lipid peroxidation of *n*-3 and *n*-6 polyunsaturated fatty acids.

Changes in phospholipid-induced oxidation reactions generates a large number of structurally dissimilar oxidation products, which difficult their isolation and characterization. Mass spectrometry (MS) and tandem mass spectrometry (MS/MS) using the soft ionization methods (electrospray and matrix-assisted laser desorption ionization) are the optimum approaches for the study of oxidized phospholipids. Product ions in tandem mass spectra of oxidized phospholipids allows identify changes in the fatty acyl chain and specific features such as existence of new functional groups in the molecule and their position along the fatty acyl chain [45].

10. Repair of damaged membranes

The best way for repair of lipoperoxidised membranes is the cleavage of the peroxidized fatty acid residues and their subsequent replacement by native fatty acids (Fig. 2). In membrane phospholipids, the PUFAs are preferentially located in the *sn*-2 position which is especially prone to lipoxygenation or lipid peroxidation. A number of observations were in line with a repair function of PLA_2. Indeed, lipid peroxidation has been found to stimulate PLA_2-mediated liberation of fatty acids [45–50].

The oxidative damage of cellular structures is always connected with the formation of oxidized proteins. The 20S proteasome is responsible for recognition and degradation of oxidatively damaged proteins (Fig. 2).

Recently Jung et al. [51] analyzed the distribution of oxidized proteins and proteasome in HT22 cells during oxidative stress.

Distribution of the proteasome and the total protein content revealed the highest concentration of both in the nucleus. The normalized ratio of protein carbonyls to protein content was analyzed, indicating the highest concentration of oxidized proteins in the cytosolic region near the cell membrane.

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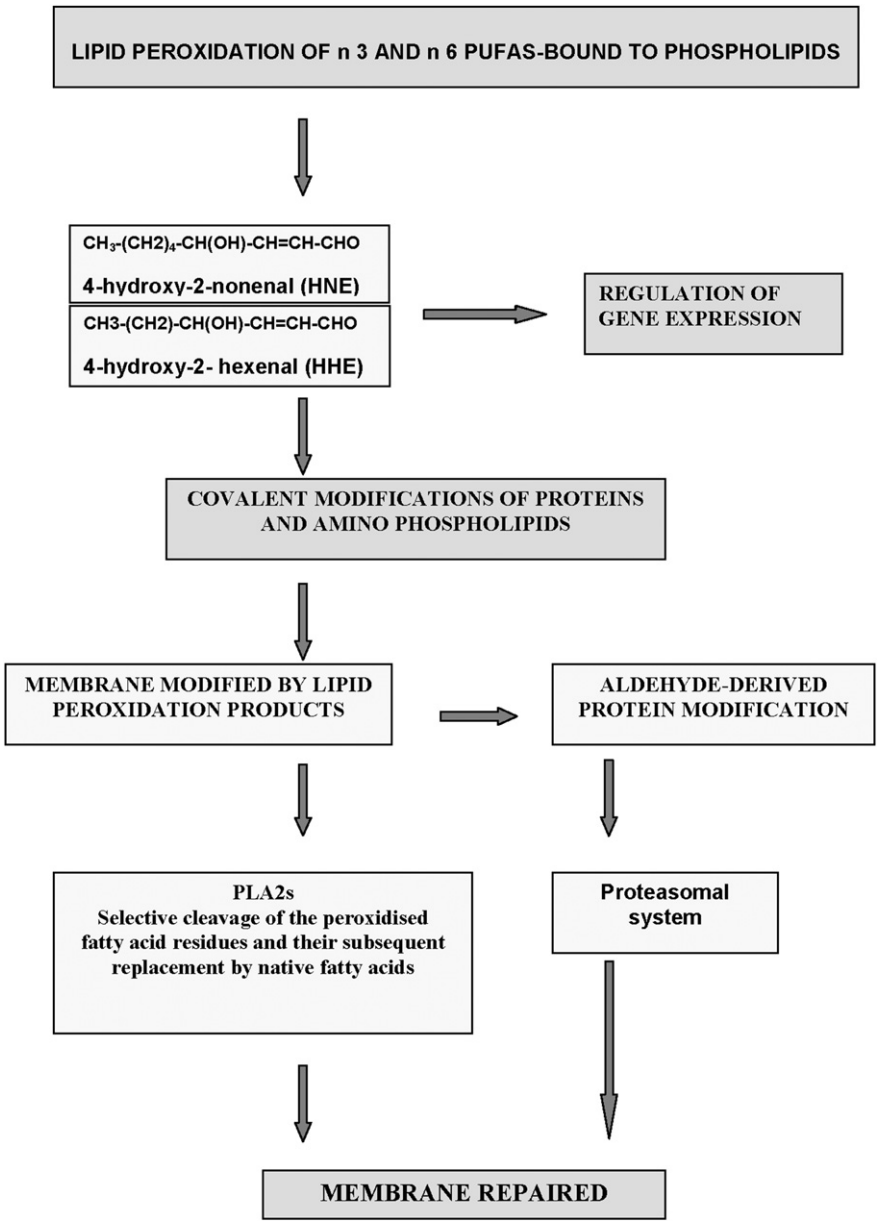

Fig. 2. Scheme of the formation and modifications at the level of membranes by the lipid peroxidation products HNE and HHE. The scheme shows the mechanisms by which damaged membranes are repaired.

11. The structure of oxidized cell membranes is better explained by the "Lipid Whisker Model"

Recent studies into the conformation of oxidized phospholipid (OxPL) species recognized by CD36 within model membranes have led to the development of the Lipid Whisker Model [52], an improvement to the classic Fluid Mosaic Model first suggested by Singer and Nicolson.

A key feature of the Fluid Mosaic Model is that amphipathic phospholipids are oriented into a lamellar mesophase organization, with hydrophobic fatty acyl chains buried within the membrane interior and hydrophilic polar headgroups oriented toward the aqueous environment. This lipid organization allows rapid lateral diffusion of lipid and transmembrane protein alike within the planar membrane surface. It also determines the character of cell membranes impermeable to hydrophilic species. X-ray studies confirm this structural organization of lipids within cell membranes. However, current data suggest that after peroxidation of cell membranes, many of the OxPL species assume a singular conformation.

Lipid peroxidation of membrane lipids is accompanied by addition of numerous polar moieties on fatty acid chains. Thus, as cell membranes and lipoproteins age and undergo oxidation, if not remodeled through the actions of phospholipases, they may "grow whiskers" comprised of an assortment of protruding oxidized *sn*-2 fatty acids of varied structures.

In the Lipid Whisker Model, the orientation of many OxPL within cell membranes differs from the characteristic architecture of adjacent non-OxPL. Biophysical studies have corroborated the conformation of structurally defined synthetic oxPC within per-deuterated membranes. Addition of a polar oxygen atom to numerous peroxidized fatty acids reorients the acyl chain whereby

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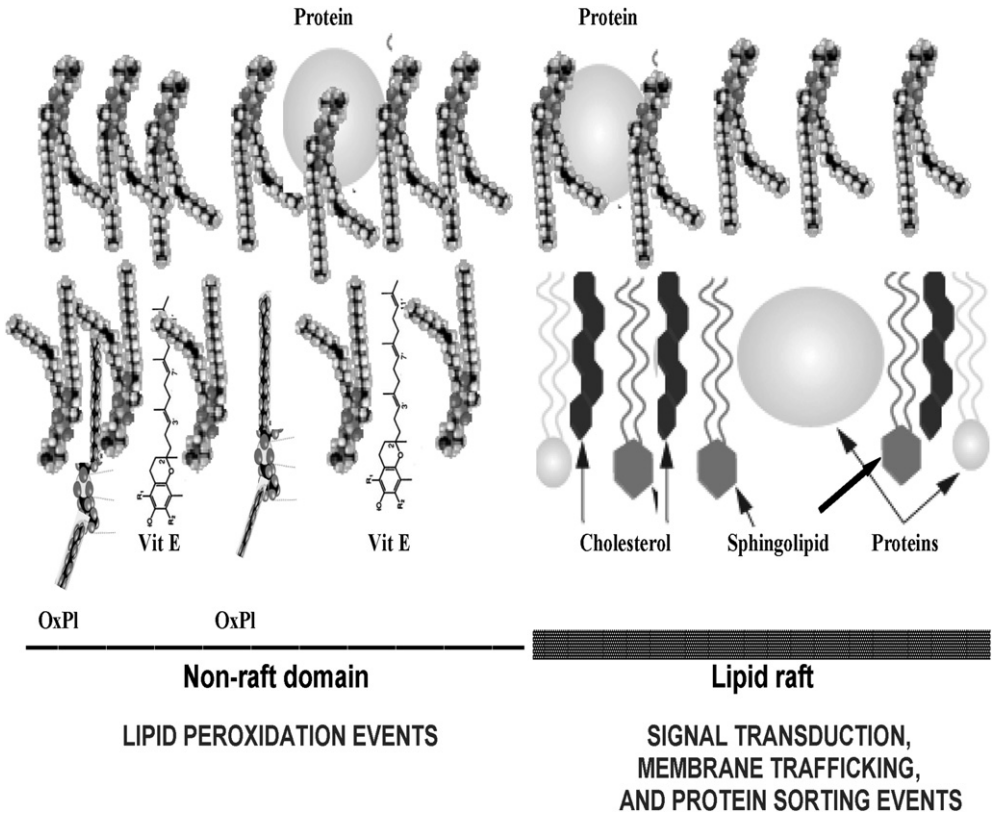

Fig. 3. Hypothetical model of the plasma membrane. In terms of lipids, the heterogeneous membrane is believed to consist of a mixture of a dispersed "lipid raft" phase, enriched in cholesterol, raft-associated proteins, and saturated lipids (such as sphingolipids), and the "non-raft" matrix phase enriched with phospholipids containing PUFAs and vitamin E. Vitamin E, partition into domains that are enriched in polyunsaturated phospholipids increasing the concentration of the vitamin in the place where take place the lipid peroxidation process and oxidized phospholipids (OxPL) are formed.

it no longer remains buried within the membrane inside, but rather projects into the aqueous milieu (Fig. 3) [52].

This notable conformational change facilitates direct admission of the oxidized fatty acid moiety to cell surface scavenger receptors in macrophages.

The conformation of individual lipids was inferred by determining multiple critical internuclear distances using nuclear Overhauser effect spectroscopy of individual structurally defined phospholipid molecular species within perdeuterated lipids in model membrane bilayers [52].

The conformation of the lipids thus determined proposes the following global phenomenon: as cell membranes suffer lipid peroxidation, such as during inflammation, senescence, or apoptosis, previously hydrophobic portions of fatty acids will move from the interior of lipid bilayers to the aqueous exterior. This conformational change may facilitate physical contact between pattern recognition receptor and molecular pattern ligand. From the point of view of the cell surface, membranes will in fact "grow whiskers" as phospholipids undergo peroxidation, and several of their oxidized fatty acids project at the surface. The conformational shift in a fatty acyl chain upon oxidation within a membrane bilayer may serve as the triggering event for numerous downstream biological activities.

12. The functional consequences of "whisker" formation

The functional consequences of "whisker" formation, is well documented. In a recent series of studies, Greenberg et al. [52] demonstrated that CD36-specific binding and uptake of PS-containing vesicles are mediated by oxPS (not non-oxPS) species. A significant role for oxPS species in apoptotic cell recognition by macrophage CD36 was supported by numerous independent lines of evidence. Liposomes containing oxPS (but not non-oxPS) were shown to preferentially bind to CD36-transfected cells, whereas complementary studies employing wild-type and CD36 knock-out peritoneal macrophages confirmed a requirement for oxidation of PS for cell binding and phagocytosis via endogenously expressed CD36 in macrophages [53]. Moreover, incorporation of oxPS (but not PS) into viable non-apoptotic cell membranes was shown to confer CD36 binding activity and capacity to be phagocytosed by CD36-bearing cells. Finally, studies employing numerous distinct cells and apoptotic triggers confirmed a critical role for oxPS versus PS as a recognition ligand for CD36-mediated phagocytosis, with multiple MS-based approaches showing that oxPS species possessing the structurally conserved CD36 recognition pattern are formed within apoptotic membranes and thus may play a function in CD36-dependent recognition of apoptotic cells [41].

13. Conclusions

It is clear that the adaptability of biological membranes is dependent on their structures and biophysical properties, which are determined by the types of lipids and proteins that make up the membranes. In terms of lipids, the heterogeneous membrane is believed to consist of a mixture of a dispersed "lipid raft" phase, enriched in cholesterol, raft-associated proteins, and saturated lipids (such as sphingolipids), and the "non-raft" matrix phase enriched with phospholipids containing PUFAs and vitamin E. Alpha-tocopherol partition into domains that are enriched in polyunsaturated phospholipids, increasing the concentration of the

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vitamin in the place where it is most required, where the lipid peroxidation process is carried out and oxidized phospholipids are formed.

Lipid peroxidation of membrane lipids is accompanied by addition of numerous polar moieties on polyunsaturated fatty acyl chains present in the "non-raft" matrix phase. Thus, phospholipid oxidation products are very likely to alter the properties of biological membranes, because their polarity and shape may differ significantly from the structures of their parent molecules. Thus, they may modify lipid–lipid and lipid–protein interactions and, as a consequence, also membrane protein functions.

Thus, as cell membranes undergo oxidation, if not remodeled through the actions of phospholipases, they may "grow whiskers" comprised of a variety of projecting oxidized *sn*-2 fatty acids of diverse structures. It will be especially interesting to determine functions and mechanisms regulating the recently identified, non-cholesterol-dependent nano-domains and how the lipid peroxidation process affects it's remodeling and provide insights into their organization.

Acknowledgments

Studies in the author laboratory were supported by PIP-0157 National Research Council (CONICET).

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