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Biochemical and Biophysical Research Communications 338 (2005) 668–676
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Review

Lipid peroxidation: Mechanisms, inhibition, and biological effects ☆

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Received 16 July 2005
Available online 19 August 2005

Abstract

In the last 50 years, lipid peroxidation has been the subject of extensive studies from the viewpoints of mechanisms, dynamics, product analysis, involvement in diseases, inhibition, and biological signaling. Lipids are oxidized by three distinct mechanisms; enzymatic oxidation, non-enzymatic, free radical-mediated oxidation, and non-enzymatic, non-radical oxidation. Each oxidation mechanism yields specific products. The oxidation of linoleates and cholesterol is discussed in some detail. The relative susceptibilities of lipids to oxidation depend on the reaction milieu as well as their inherent structure. Lipid hydroperoxides are formed as the major primary products, however they are substrates for various enzymes and they also undergo various secondary reactions. Phospholipid hydroperoxides, for example, are reduced to the corresponding hydroxides by selenoproteins in vivo. Various kinds of antioxidants with different functions inhibit lipid peroxidation and the deleterious effects caused by the lipid peroxidation products. Furthermore, the biological role of lipid peroxidation products has recently received a great deal of attention, but its physiological significance must be demonstrated in future studies.

Keywords: Lipid peroxidation; Antioxidant; HODE; Linoleic acid; Cholesterol; Oxysterols; Vitamin E

Lipid peroxidation has been the subject of extensive studies for several decades, and its mechanisms, dynamics, and products are now fairly well established. It was first studied in relation to the oxidative deterioration of foods. In 1955, the oxygenase enzyme was discovered by Hayaishi et al. [1] and Mason et al. [2] independently, and since then lipid peroxidation by lipoxygenases and cyclooxygenases has been studied extensively. Lipid oxidation by cytochrome P450 has also been studied and is well documented. The free radical-mediated peroxidation of lipids has received a great deal of attention in connection with oxidative stress in vivo. The oxidation hypothesis for atherosclerosis [3] has stimulated extensive studies on the oxidative modification of low density lipoprotein (LDL). Researchers have also focused their attention on lipid peroxidation by non-enzymatic, non-radical mechanisms. Singlet oxygen and ozone are examples of molecules that induce such oxidation. More recently, the role of lipid peroxidation products as cellular regulators and signaling messengers has been a growing subject. In this article, the oxidation of lipids, particularly polyunsaturated fatty acids (PUFA) and cholesterol, and their inhibition will be briefly reviewed, aiming specifically at elucidating the effects of milieu.

Mechanisms and dynamics of lipid peroxidation

Both PUFA and cholesterol are oxidized by enzymatic and non-enzymatic pathways. The oxidation of arachidonates by lipoxygenases and cyclooxygenases has been studied extensively [4], however, in this article, linoleates are considered as the model substrate, since they are the most abundant PUFA in vivo and their oxidation proceeds by a straightforward mechanism to give much simpler products

☆ *Abbreviations:* DPPP, diphenylpyrenylphosphine; HNE, 4-hydroxynonenal; H(P)ODE, hydro(pero)xyoctadecadienoate; IsoF, isofuran; IsoP, isoprostane; KCh, ketocholesterol; LDL, low density lipoprotein; O(O)HCh, hydro(pero)xycholesterol; PUFA, polyunsaturated fatty acid; NP, neuroprostane; TOH, tocopherol.

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doi:10.1016/j.bbrc.2005.08.072

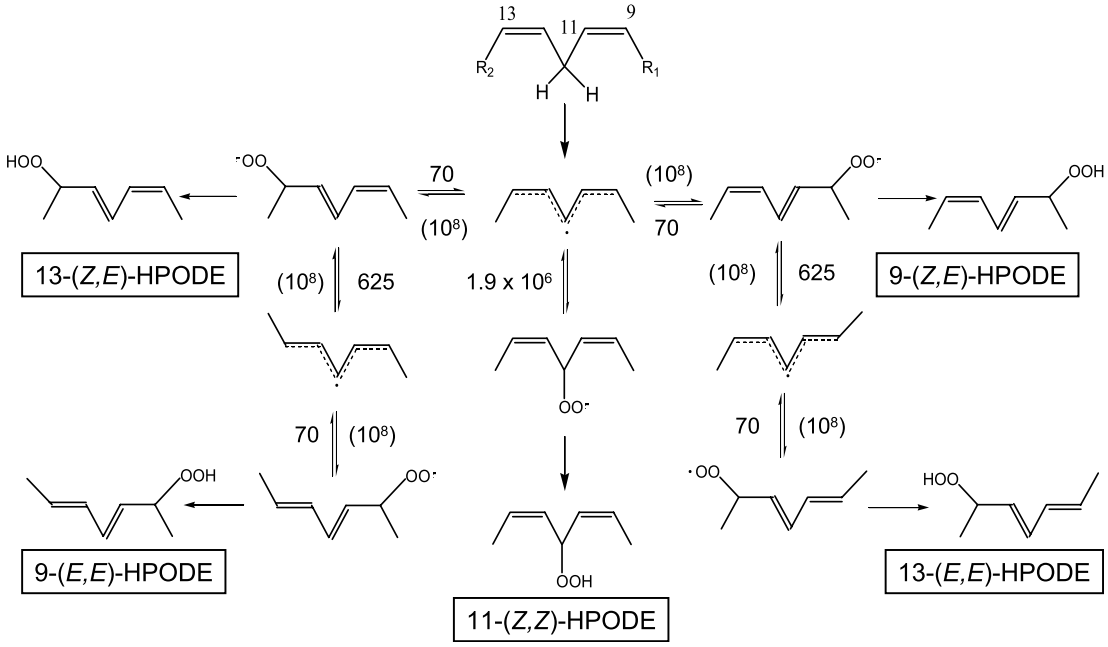
than arachidonates and more highly unsaturated fatty acids such as docosahexaenoates. The characteristics and specific products of the oxidation of linoleates by different mechanisms are given in Table 1. The oxidation of linoleate by lipoxygenase proceeds catalytically to give regio-, stereo-, and enantio-specific hydroperoxy octadecadienoates (HPODEs). The specificity depends on the type of enzyme, substrate, and reaction milieu. Additionally, the enantiospecificity varies considerably, which decreases with the type of substrate in the order of free linoleic acid > methyl linoleate > phospholipids > cholesteryl esters, and with the milieu, aqueous solution > lipoproteins > plasma [5,6]. Lipoxygenases undergo suicide inactivation [7] to liberate iron, which induces free radical-mediated lipid peroxidation, resulting in a decrease in specificity.

The free radical-mediated peroxidation of PUFA proceeds by five elementary reactions: (1) hydrogen atom transfer from PUFA to the chain initiating radical or chain carrying peroxyl radicals to give a pentadienyl carbon-centered lipid radical, (2) reaction of the lipid radical with molecular oxygen to give a lipid peroxyl radical, (3) fragmentation of the lipid peroxyl radical to give oxygen and a lipid radical (a reverse reaction of the above reaction (2)), (4) rearrangement of the peroxyl radical, and (5) cyclization of the peroxyl radical [8]. Reaction (5) is important only for PUFA having more than three double bonds, and it does not take place during the oxidation of linoleates. The reaction steps for linoleate oxidation are shown in Scheme 1 (modified based on [9]). The pathway and dynamics are determined primarily by thermochemistry. It has been confirmed that the oxidation of linoleic acid and its esters in an organic solution gives four isomeric conjugated diene hydroperoxides quantitatively. A typical example of the oxidation of methyl linoleate in an organic solution induced by an azo initiator is given in Scheme 2, and it can be noted that the amounts of oxygen consumption, substrate consumption, and formation of conjugated diene, peroxide, and HPODE are in good agreement [10].

Cholesterol oxidation products, commonly referred to as oxysterols, have received increasing attention as diagnostic biomarker of oxidative stress, as intermediates in bile acid biosynthesis, and messengers for cell signaling and cholesterol transport [11]. Cholesterol is oxidized by both enzymatic and non-enzymatic mechanisms (Scheme

Table 1
Lipid peroxidation (products from linoleate)

Type	Characteristics	Isomers of HPODE		
		Regio	Stereo	Enantio
Enzymatic (15-LOX)	Specific catalytic	13	cis, trans	*S*
Non-enzymatic, free radical chain oxidation ($LO_2^{\cdot}$)	Random chain reaction	9.13	cis, trans, trans, trans 9-ct = 13-ct, 9-tt = 13-tt	*R* = *S* (racemic)
Non-enzymatic, non-radical oxidation ($^1O_2$)	Random stoichiometric	9, 10, 12, 13	cis, trans	



Scheme 1. Reaction pathways of the peroxidation of linoleate.

Initial			Final	Difference(consumption or formation)	
MeLH	871	50°C	831	40	
O_2	126	60min	90	36	
				Conjugated diene[a)]	34
				Peroxide[b)]	38
				Total HPODE[c)]	38
				9-(Z,E)	6.2
				9-(E,E)	12.7
				13-(Z,E)	6.1
				13-(E,E)	12.8

Scheme 2. Oxidation of methyl linoleate (MeLH) in acetonitrile [10]. Numbers in μmol. (a) by UV absorption at 234 nm, (b) by iodometric titration, and (c) by HPLC.

3). Several enzymes of the cytochrome P450 family oxidize cholesterol to give specific hydroxycholesterol (OHCh), as shown in Table 2. 24(*S*)-OHCh is formed exclusively in the brain in humans and there is a continuous flux of 24(*S*)-OHCh from the brain to the circulation. 7α-OHCh is a product of the hepatic enzyme CYP7Al, but it is also formed by the non-enzymatic oxidation of cholesterol. Side-chain oxidation products such as 20-, 22-, 24-, 25-, and 27-OHCh are formed by an enzymatic mechanism.

The free radical-mediated oxidation of cholesterol gives 7α- and 7β-hydroperoxycholesterol (7α-OOHCh and 7β-OOHCh), 7α-OHCh, 7β-OHCh, 5α,6α- and 5β,6β-epoxycholesterol, and 7-ketocholesterol (7-KCh) as the major products [11]. The conversion of 7-KCh to 7β-OHCh in vivo was reported [12]. The oxidation of 7-OHCh either by the enzyme 7α-hydroxycholesterol dehydrogenase [13] or by non-enzymatic autoxidation gives 7-KCh. Singlet oxygen oxidizes cholesterol by a non-enzymatic and non-radical mechanism to give 5α-OHCh with a lower quantity of 6-OHCh [14]. 5α-OHCh undergoes isomerization to 7α-OHCh. 5-OHCh is a specific marker for singlet oxygen oxidation. 7β-OHCh may be regarded as a marker for free radical-mediated oxidation. Oxysterols are present in vivo

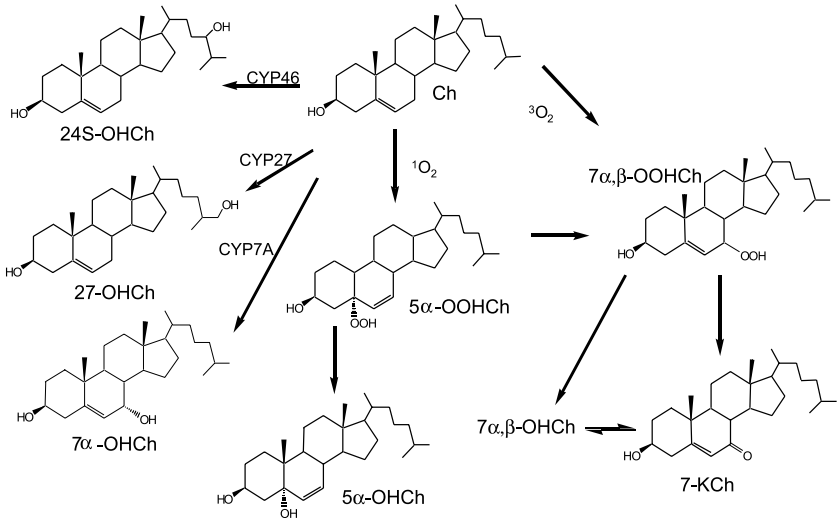
Table 2
Enzymatic oxidation of cholesterol

Enzyme	Abbreviation	Principal product[a]
Cytochrome P450 3A4	CYP3A4	4β-OHCh
Cholesterol 7α-hydroxylase	CYP7A1	7α-OHCh
Cytochrome P450 scc	CYP11A1	20α-OHCh, 22-OHCh
Cholesterol 24-hydroxylase	CYP46	24(*S*)-OHCh
Cholesterol 25-hydroxylase		25-OHCh
Sterol 27-hydroxylase	CYP27A1	27-OHCh

[a] OHCh, hydroxycholesterol.

in different forms, namely, the esterified, sulfated, and conjugated forms as well as free oxysterols [15].

It has been observed that saturated and mono-olefinic fatty acids are much less susceptible to free radical-mediated oxidation than PUFA and that the oxidizability of PUFA increases with an increase in the number of double bonds [16]. Cholesterol has one cyclic double bond, but no bisallylic hydrogen. As expected, it was found that the relative rate of oxidation of fatty acids in cholesterol esters in the free radical-mediated oxidation of LDL decreases in the order of 22:6 > 20:4 > 18:2 ≫ 18:1 > 16:0 ∼ free cholesterol (Fig. 1) [17]. Initially, only PUFA were consumed



Scheme 3. Oxidation pathway of cholesterol.

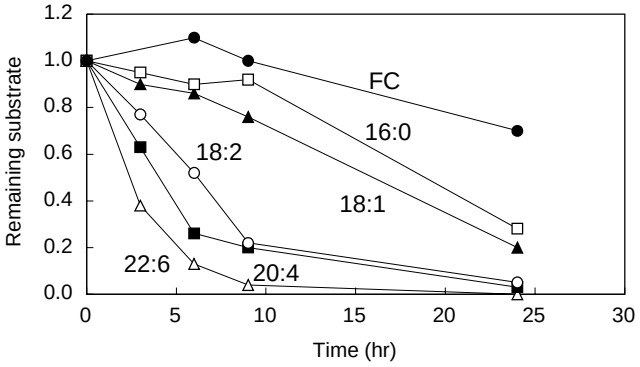


Fig. 1. Consumption of cholesterol esters and cholesterol in the oxidation of LDL Human LDL (0.25 mg protein/ml) was oxidized by AAPH (2 mM) and the decrease of each cholesteryl ester and free cholesterol was followed with time.

and cholesteryl oleate, cholesteryl palmitate, and free cholesterol were not appreciably oxidized.

LDL is a spherical particle with a diameter of 22 nm and an average molecular weight of 2.5 million. Neutral lipids such as cholesterol esters and triglycerides form a hydrophobic core, while amphipathic phospholipids and free cholesterol form a surface monolayer and surround the core. The approximate number of molecules of phospholipids, free cholesterol, cholesteryl esters, and triglycerides is 700, 400, 1600, and 100 per LDL particle, respectively [18]. The outer monolayer containing a large amount of cholesterol is rigid, while the core is fluid. The relative rate of formation of cholesteryl ester hydroperoxide to that of phospholipid hydroperoxide was 3.4 times higher in LDL particles than in a *tert*-butyl alcohol solution [17]. This different susceptibility to oxidation can be attributed to the physical properties of the outer surface and core where the two lipid classes reside.

Detection, identification, and measurement of lipid peroxidation

The detection of reactive oxygen species, reactive nitrogen species, and other active oxidants has been performed by using various probes and techniques such as fluorescence probes, chemiluminescence probes, and the ESR spin trapping technique. Their detection has been successfully accomplished in several in vitro systems, including cell cultures, however, the real-time in vivo measurement of the active species is rather difficult. On the other hand, the detection and identification of lipid peroxidation products are easier and more reliable. Coordination ion-spray mass spectrometry (CIS-MS) and electrospray ionization (ESI) or matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry have been found to be powerful tools for the detection and identification of complex mixtures of lipid peroxidation products [19].

Hydroperoxides are formed as the primary products in lipid peroxidation. They can be determined with high sensitivity by the HPLC-chemiluminescence method [20,21], wherein the hydroperoxides are first separated by HPLC and then reacted with an iron complex in the presence of a chemiluminescence probe such as luminol. Care should be taken in the quantitation of hydroperoxides, since the chemiluminescence intensity, that is the sensitivity, depends markedly on the type of hydroperoxide and iron complex and also on physical factors such as flow rate of the eluent and dead volume from the mixing cell to the detector [22].

Diphenylpyrenylphosphine (DPPP) is an interesting probe that reacts with hydroperoxides to give a strong fluorescent DPPP oxide [23]. DPPP reacts with lipid hydroperoxides selectively and stoichiometrically, and the resulting DPPP oxide can be measured in a solution, cultured cells, and tissues (Fig. 2) [24–26]. It was also measured by the thin-layer chromatography blotting technique [27] and a flow injection analysis system [28].

Lipid hydroperoxides are not stable end products of lipid peroxidation, but they are good substrates for several enzymes such as glutathione peroxidases and phospholipases, and they undergo secondary reactions. Therefore, the level of lipid hydroperoxides does not represent the extent of lipid peroxidation in vivo. In fact, it has been found that the in vitro oxidation of human plasma induced by a free radical initiator gives cholesteryl ester hydro(pero)xide and phosphatidylcholine hydro(pero)xide as the major products; most of the products from the cholesteryl ester were found to be hydroperoxide, whereas those from phosphatidylcholine gave hydroxides [29]. On the other hand, the oxidation of lipids extracted from the plasma in an organic solution gave hydroperoxides from both the cholesteryl ester and phosphatidylcholine, with little concomitant formation of hydroxides.

With increasing evidence indicating the involvement of lipid peroxidation in various disorders and diseases, the biomarkers for lipid peroxidation have gained increasing attention. Various markers have been proposed and applied. Isoprostanes (IsoPs), which are prostaglandin F2-like compounds, and neuroprostanes (NPs) that are formed by the non-enzymatic, free radical-mediated oxidation of arachidonates and docosahexaenoates, respectively, are now regarded as the ''gold standard'' for assessing oxidative stress in vivo [30]. Similar products that are characterized by a substituted tetrahydrofuran ring structure and termed isofurans (IsoFs) have also been measured and found to increase with increasing oxygen tension, in contrast to IsoPs [30].

Linoleates and cholesterol are abundant lipids in vivo and their free radical-mediated oxidation gives HPODE and 7-OOHCh as the major products with high selectivity. We have recently proposed the measurement of total hydroxyoctadecanoic acid (tHODE) and total 7-hydroxycholesterol (t7-OHCh) as biomarkers of oxidative stress in vivo [31]. In this method, biological samples such as plasma, erythrocytes, urine, and tissues are first reduced by sodium borohydride, followed by saponification with potassium hydroxide. The hydroperoxides and ketones as

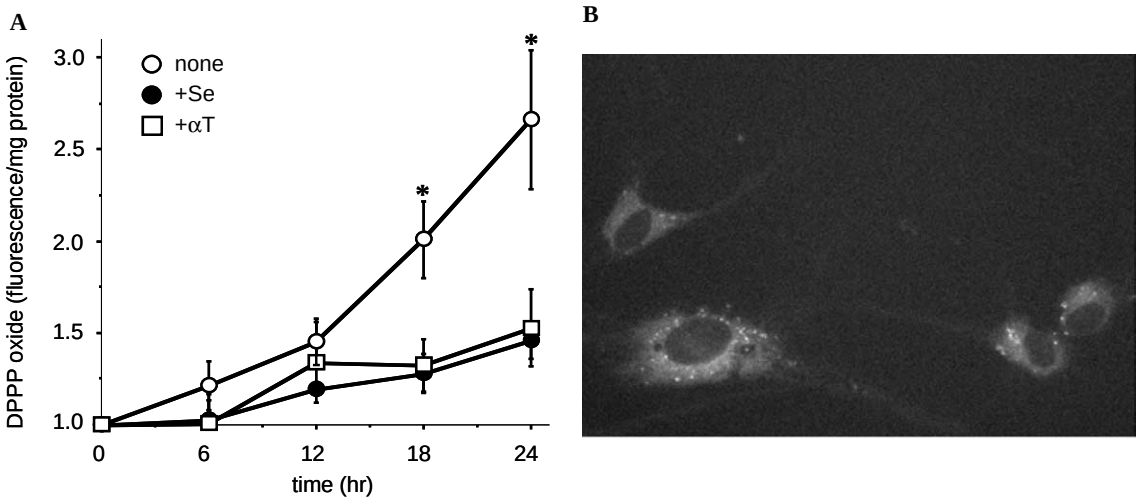


Fig. 2. Detection of DPPP oxide as a marker for lipid hydroperoxides. (A) Formation of DPPP oxide in Jurkat cells cultured in a selenium-deficient medium. The DPPP-labeled cells were cultured in a selenium-deficient medium, without (none) and with 100 nM sodium selenite (+Se) or 2 μM α-tocopherol (+αT) for the indicated times, and fluorescence intensities of the cell samples were measured as described previously [26]. $^{*}P < 0.05$ when compared with selenium-sufficient and α-tocopherol-sufficient cells. Mean values of DPPP oxide fluorescence per total protein relative to that of DPPP-labeled control cells (time 0; 3600 fluorescence/mg protein) are shown with SE ($n = 3$). (B) Fluorescence from DPPP oxide in human endothelial cell. Human umbilical vein endothelial cell was incubated with DPPP═O containing LDL for 4 h.

Table 3
Plasma levels of biomarkers in human, rat, and mouse

	n	Age	tHODE (nM)	HODE ratio ZE/EE	isoPs (nM)	t7-OHCh (nM)
Human	45	57.2 ± 15.1	207 ± 15	0.63 ± 0.21	0.72 ± 0.60	
	17	64.3 ± 6.5				161 ± 73
Rat	3	11 Weeks	666 ± 240	0.59 ± 0.10	1.63 ± 0.06	
Mouse	4	11 Weeks	2517 ± 615	0.52 ± 0.06	8.47 ± 2.85	

well as hydroxides of both free and ester forms of linoleic acid and cholesterol are measured as tHODE and t7-OHCh, respectively. Furthermore, the ratio of stereo-isomers of HODE, (Z,E)/(E,E), which is a good measure of antioxidant capacity, can also be measured. The levels of tHODE and t7-OHCh have been measured along with total IsoP in healthy volunteers, patients with several diseases, and experimental animals. It has been found that their levels are elevated under oxidative stress. Some examples are shown in Table 3. It may be noteworthy that the level of lipid peroxidation products in vivo is determined by the balance between their formation, metabolism, secondary reactions, and excretion. These biomarkers are also useful for evaluating the beneficial effects of antioxidant foods, spices, beverages, supplements, and drugs.

Reduction of lipid hydroperoxides

The reduction of lipid hydroperoxides is necessary not only for the prevention of further oxidation and decomposition with concomitant formation of free radicals but also for cell signaling in a regulated and compartmented manner. Cells have a system for the reduction of lipid hydroperoxides that is composed of diverse antioxidant enzymes with different structures, substrate specificity, and localization. In this reduction system, selenocysteine-containing proteins, such as glutathione peroxidase (GPx), function as a major player [32]. Four types of selenium-containing GPxs, namely, cellular GPx (cGPx), gastrointestinal GPx (GIGPx), extracellular GPx (eGPx), and phospholipid hydroperoxide GPx (PHGPx), have been identified. All members of the GPx family reduce variable hydroperoxides at the expense of glutathione and/or other reducing equivalents [33,34]. Plasma selenoprotein, selenoprotein P (SeP), has also been reported to catalyze the reduction of phospholipid hydroperoxide by GSH and thioredoxin [34,35].

Inside the cells, hydroperoxides of both the free and ester forms of fatty acids and hydroperoxycholesterol in the biomembrane are considered to be cellular lipid hydroperoxides. For the hydroperoxide of free fatty acid, there are many candidate reductants including three kinds of intracellular GPx (cGPx, GIGPx, and PHGPx), thioredoxin reductase (TR), peroxiredoxine 6 (Prx 6), and glutathione transferase (GST) [32,33]. On the other hand, for the hydroperoxides of esterified fatty acids such as phospholipid hydroperoxide, PHGPx is the sole reductant in the intracellular GPx family. Although it has been reported that non-selenoproteins such as Prx 6 and GST can directly reduce phospholipid hydroperoxide (reviewed in [32]),

PHGPx exhibits the highest reducing activity against phospholipid hydroperoxide and it is considered to act as a universal intracellular reductant against cellular phospholipid hydroperoxide. It is known that PHGPx can reduce hydroperoxycholesterols such as 5α-, 7α-, and 7β-OOHCh [36]. However, it has been reported that the reduction of hydroperoxycholesterols by PHGPx is at least six times slower than that of phospholipid hydroperoxides [36].

In the extracellular fluid, hydroperoxides of both the free and ester forms of fatty acids and cholesterol in the outer plasma membrane and lipoprotein are considered to be extracellular lipid hydroperoxides. For these hydroperoxides, two kinds of selenoproteins, namely, eGPx and SeP might act as major reductants although under a relatively lower glutathione concentration (5 μM) compared with cellular glutathione (2 mM). The hydroperoxides of both free fatty acid and cholesterol are substrates for eGPx [37], but are not known as substrates for SeP. It has also been reported that albumin and three kinds of apolipoproteins (A-I, A-II, and B-100) have reducing activity against phospholipid hydroperoxide [38]. There are many candidates for reducing phospholipid hydroperoxide. On the other hand, for cholesterol ester hydroperoxide (CE-OOH), it has been demonstrated that only apolipoprotein A and B-100, but not eGPx, reduce CE-OOH in a methionine-dependent manner [39,40].

Inhibition of lipid peroxidation

As mentioned above, lipid peroxidation has been implicated in various diseases and aging, including atherosclerosis, cataract, rheumatoid arthritis, and neurodegenerative disorders. Consequently, the role of antioxidants has received extensive attention. Antioxidant defenses may be divided into four categories: prevention of the formation of active oxidants, scavenging, quenching and removal of active oxidants, repair of damage and excretion of toxic oxidation products, and adaptive responses. The inhibition of enzymatic lipid oxidation may be achieved by inhibition of either the activation or reaction of an enzyme. Free radical-mediated lipid peroxidation may be inhibited by the inhibition of chain initiation and chain propagation and/or acceleration of chain termination. Lipid peroxidation induced by singlet oxygen may be inhibited by the inhibition of its formation by, for example, quenching of ultraviolet light and quenching of the singlet oxygen itself by carotenoids. Foods contain various radical-scavenging antioxidants. Many natural and synthetic supplements and drugs with radical-scavenging capacity have been explored. The antioxidant activity in vivo is determined by several factors, such as reactivity toward radicals, fate of antioxidant-derived radicals, absorption, distribution, localization and mobility of antioxidant, and interaction with other antioxidants [40]. Functions that are independent of antioxidant action, such as induction of phase II enzymes, may also be important in the total defense network against oxidative stress.

One of the interesting characteristics of the biological system is its heterogeneity. The antioxidants are localized in the aqueous or lipid phase. The lipophilic antioxidants are distributed in the lipophilic compartment of membranes and lipoproteins. Vitamin E and vitamin C are typical lipophilic and hydrophilic antioxidants, respectively. Vitamin C resides and scavenges radicals in the aqueous phase. By using a doxyl stearic acid spin probe, it was confirmed experimentally that the rate of scavenging of radicals within the membrane [41] and LDL [42] became slower as the radical goes deeper into the interior of the membranes and LDL particles from the surface (Fig. 3). Interestingly, cholesterol 16-doxylstearate incorporated into the LDL core was not reduced by vitamin C, whereas it was reduced by 6-*O*-palmitoylascorbic acid. Another important function of vitamin C is to reduce the vitamin E radical to regenerate vitamin E and also to inhibit the prooxidant action of vitamin E. This interaction proceeds efficiently for the vitamin E radical in the membrane [43,44] and LDL [45].

Vitamin E, a major lipophilic antioxidant in vivo, has been most extensively studied and is suitable for exploring antioxidant action in vivo. It scavenges lipid peroxyl radicals, chain-carrying species in the lipid peroxidation, to break chain propagation. A structural characteristic of vitamin E is the phytyl side chain, which is required for incorporation and retainment in the membranes and lipoproteins; however, the side chain reduces the mobility within and between the membranes along with the radical-scavenging efficacy [46]. There are eight isoforms of vitamin E; however, the α-tocopherol transfer protein in the liver binds only α-tocopherol (α-TOH) preferentially and enhances its bioavailability [47]. The subcellular

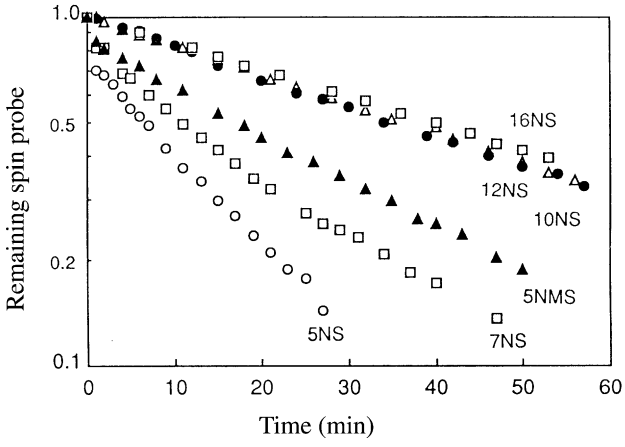


Fig. 3. Reduction of doxylstearic acid incorporated into LDL particle by ascorbic acid. The spin probes doxylstearic acid and methylstearate (final concentration 30 μM) were incorporated into LDL (1.3 mg protein/ml) by adding its ethanol solution, followed by incubation. The 5-doxylstearic acid methyl ester was also incorporated into LDL similarly. Ascorbic acid (5.2 mM) was then added to the LDL suspensions and the disappearance of doxyl state was followed with ESR at 37 °C.

distribution of both α-tocopherol and α-tocotrienol in nuclear, mitochondrial, microsomal, and cytosolic fractions was found to be directly proportional to the lipid distribution [48]. 2,2,5,7,8-Pentamethyl-6-chromanol (PMC), an analogue of α-TOH that has a methyl side chain in place of the phytyl group, exerts substantially the same antioxidant activity as α-TOH in an organic solution, but higher activity against lipid peroxidation in the membranes and LDL due to higher mobility within and between membranes and particles [42,46,49].

The active site of α-TOH, the phenolic hydrogen at position 6, is located at the water–lipid interface. It was found that the efficacy of radical scavenging decreases as the radical goes deeper into the interior of the membrane and LDL [42]. Recently, it was reported that α-TOH inhibited the cholesteryl ester oxidation in the LDL core more effectively than it did phospholipid oxidation at the surface monolayer [50].

In 1992, it was discovered that vitamin E acted as a prooxidant against LDL oxidation [51], and Stocker and his colleagues have extensively argued this action that has been termed tocopherol mediated peroxidation (TMP). In this scheme, α-TOH translocates radicals from the aqueous phase into the LDL particle (phase transfer effect), and the resulting α-tocopheroxyl (α-TO) radical attacks the bisallylic hydrogen of PUFA in LDL (chain transfer effect) to continue the chain reaction. In fact, many studies on the in vitro oxidation of LDL have shown the function of TMP and α-TOH acts as a prooxidant [52]. However, it should be pointed out that this TMP may be important for isolated LDL oxidation, but not in plasma, where vitamin C reduces the α-TO radical rapidly. In agreement with this, it has been shown that the lipid peroxidation of LDL and plasma is efficiently inhibited by a combination of vitamin E and vitamin C, although plasma oxidation does proceed even in the presence of vitamin E alone. It may be safely stated that α-TOH does not act as a prooxidant in vivo.

A study using stopped-flow ESR showed that the stability of the aryloxyl radicals derived from phenolic antioxidants depends to a large extent on the substituents at the two *ortho* positions [53]. The two *tert*-butyl substituents at the *ortho* positions sterically hinder the reaction of the aryloxyl radical with the PUFA substrate and diminish the prooxidant action of phenolic antioxidants [54].

Although there is now a consensus that the oxidative modification of LDL is an important initial event in the progression of atherosclerosis and that antioxidants should be effective for prevention of atherosclerosis, the results of many large-scale, long-term intervention studies do not always support the beneficial effect of vitamin E and have cast some doubts on the oxidative modification hypothesis [55]. It may be added that the oxidative modification of LDL can be induced by many oxidants by different mechanisms, and that vitamin E effectively inhibits some pathways, but not others [56].

Biological role of lipid peroxidation products

It has been known that lipid peroxidation gives complex products including hydroperoxides, cleavage products such as aldehydes, and polymeric materials, and that these products exert cytotoxic and genotoxic effects [57]. Lipid peroxidation products and modified proteins have been found in human atherosclerotic lesions, although their pathological significance, such as cause or consequence, has not yet been fully elucidated. More recently, the role of lipid peroxidation products as signaling messengers has received a great deal of attention [58]. For example, 9- and 13-HODE have been shown to act as activators and ligand of PPARγ, leading to the induction of CD36 scavenger receptors and foam cell formation [59]. Oxysterols are involved in the regulation of gene expression, and cholesterol metabolism and homeostasis [60,61]. Furthermore, it has been found that cyclopentenone prostaglandins, 15-deoxy-Δ12,14-prostaglandin J2 in particular, induce phase II detoxification enzymes [62], and exert a complex array of neurodegenerative, neuroprotective, and anti-inflammatory effects [63].

Cells exhibit a broad spectrum of responses to oxidative stress, depending on the stress type and level encountered. Oxidative stress exceeding the antioxidant capacity level may induce oxidative damage, but low-level stress may enhance the defense capacity. Such an adaptive response has been observed in several instances, particularly in low-dose irradiation [64]. It was reported that the pretreatment of human umbilical vein endothelial cells with 15d-PGJ2 protected the cells from subsequent 4-HNE-induced apoptosis [65]. It was recently found that a sublethal level of 4-HNE exerted a cytoprotective effect primarily through the induction of thioredoxin reductase 1 against subsequent oxidative stress [66]. The physiological significance of these biological effects of lipid peroxidation products observed in vitro including cell culture systems has to be established in future studies.

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