15-Lipoxygenase has been implicated in the in vivo oxidation of low density lipoprotein (LDL) a process thought to be important in the origin and/or progression of human atherosclerosis. We have suggested previously that oxidation of LDL's cholesteryl esters (CE) and phospholipids by soybean (SLO) or human recombinant 15-lipoxygenase (rhLO) can be ascribed largely to α-tocopherol (α-TOH)-mediated peroxidation (TMP). In this study we demonstrate that addition to LDL of unesterified linoleate (18:2), other free fatty acid (FFA) substrates, or phospholipase A₂ (PLA₂) significantly enhanced the accumulation of CE hydro(pero)xides (CE-O(O)H) induced by rhLO, whereas the corresponding CE and nonsubstrate FFA were without effect. The enhanced CE-O(O)H accumulation showed a dependence on the concentration of free 18:2 in LDL. In contrast, addition of 18:2 had little effect on LDL oxidation induced by aqueous peroxyl radicals or Cu²⁺ ions. Analyses of the regio- and stereoisomers of oxidized 18:2 in SLO-treated native LDL demonstrated that the small amounts of 18:2 associated with the lipoprotein were oxidized enzymically and within minutes, whereas cholesteryl linolate (Ch18:2) was oxidized nonenzymically and continuously over hours. α-Tocopheroxyl radical (α-TO) formed in LDL exposed to SLO was enhanced by addition of 18:2 or PLA₂. With rhLO and 18:2-supplemented LDL, oxidation of 18:2 was entirely enzymic, whereas that of Ch18:2 was largely, though not completely, nonenzymic. The small extent of enzymic Ch18:2 oxidation increased with increasing enzyme to LDL ratio. Ascorbate and the reduced form of coenzyme Q₁₀ ubiquinol-10, which are both capable of reducing α-TO and thereby preventing TMP, inhibited nonenzymic Ch18:2 oxidation induced by rhLO. Trolox and ascorbyl palmitate, which also inhibit TMP, ameliorated both enzymic and nonenzymic oxidation of LDL's lipids, whereas probucol, a radical scavenger not capable of preventing TMP, was ineffective. These results demonstrate that rhLO-induced oxidation of CE is largely nonenzymic and increases with LDL's content of FFA substrates. We propose that conditions which increase LDL's FFA content, such as the presence of lipases, increase 15-LO-induced LDL lipid peroxidation and that this process requires only an initial, transient enzymic activity.

Oxidation of low density lipoprotein (LDL) in the arterial intimal space, by an as yet undefined process, is widely believed to participate in the process of atherogenesis (1–5). 15-Lipoxygenase (15-LO) has been implicated as an in vivo oxidant of LDL based on the presence in human atherosclerotic lesion of its mRNA, protein (6), and stereospecific lipid oxidation products (7–9). Furthermore, 15-LO has been described to oxidize LDL in vitro and transfer of the 15-LO gene into rabbit iliac arteries results in the appearance of lipid-protein adducts characteristic of oxidized LDL (10).

Much of the literature ascribes oxidation of LDL by 15-LO to a direct reaction with esterified fatty acid substrates, including phospholipids (PL) and cholesteryl esters (CE) (11–14). In LDL exposed to 15-LO, or to cells overexpressing this enzyme (15, 16), the major oxidation products found are CE hydroperoxides and hydroxides (collectively referred to as CE-O(O)H). This may be expected as CE, particularly cholesteryl linolate (Ch18:2), are the major oxidizable lipid in LDL. However, the vast majority of CE are normally buried in the core of the lipoprotein particle and hence may be largely inaccessible to 15-LO. In addition, certain aspects of 15-LO catalysis, such as the rapid oxidation and characteristic suicidal inactivation of the enzyme observed with free fatty acid (FFA) substrates, are notably absent from, or distinct to, 15-LO-induced LDL oxidation (11–14).

Recently, we have obtained results suggesting that α-tocopherol (α-TOH)-mediated peroxidation (TMP) (17, 18), initiated and promoted by the formation of α-tocopheroxy radical (α-TO), largely accounts for the persistent lipid oxidation observed in LDL induced by soybean (SLO) (19) and human recombinant 15-LO (rhLO) (20). To reconcile these observations with previous reports, we have hypothesized (20) that 15-LO-mediated oxidation of FFA associated with LDL stimulates TMP of esterified lipids in LDL via initial, enzyme-induced formation and release of FFA peroxyl radicals. The latter have been demonstrated previously by electron paramagnetic resonance (EPR) spectroscopy (21). If formed, FFA peroxyl radicals would be expected to be scavenged by LDL's α-TOH, resulting in the formation of FFA hydroperoxides and α-TO; the latter which could subsequently initiate and propagate TMP.

The abbreviations used are: LDL, low density lipoprotein; apoB, apolipoprotein B-100; AAPH, 2,2'-azobis(2-amidinopropane); CE, cholesteryl esters; CE-O(O)H, cholesteryl ester hydro(pero)xides; Ch18:2, cholesteryl linoleate; Ch18:2-O(O)H, cholesteryl linoleate hydro(per)oxygenes; CoQ₉-H, ubiquinol-10; ETYA, eicosatetraenoic acid; FFA, free fatty acid(s); FFA-O(O)H, free fatty acid hydro(pero)xides; 13-Z,E-H/P/O2E, 13-hydroxy-peroxy-9Z,11E-octadecadienoic acid; 15-LO, 15-lipoxygenase(s); NP-HPLC, normal phase high performance liquid chromatography; PBS, phosphate-buffered isotonic saline; PL, phospholipids; PLA₂, phospholipase A₂; rhLO, recombinant human 15-lipoxygenase; SLO, soybean 15-lipoxygenase; α-TOH, α-tocopherol; TMP, tocopherol-mediated peroxidation; α-TO, α-tocopheroxy radical.
Herein we demonstrate that increasing the levels of FFA in LDL enhances the accumulation of hydroperoxides and hydroxides of Ch18:2 (Ch18:2-OH) in LDL exposed to 15-LO. The oxidation products of Ch18:2, formed over prolonged periods of time, display an entirely (in the case of SLO) or predominantly (rhLO) nonenzymic profile, whereas those of 18:2, which are formed within minutes, are entirely enzymic. These results demonstrate, for the first time, that two mechanisms contribute to 15-LO-induced LDL oxidation, i.e., rapid and direct enzymic interaction of 15-LO with LDL lipid and ongoing, nonenzymic CE oxidation that is both initiated and promoted by α-TO. Our findings have major implications for the inhibition of 15-LO-induced LDL oxidation.

EXPERIMENTAL PROCEDURES

Materials—rhLO, prepared as described in Ref. 22, was a generous gift from Dr. Vitaly Roginsky (Russia). Anal reagent-grade chemicals were of the highest available purity. Nanopure water (Millipore, Bedford, MA). The nitroxide spin label 2,2,5,5-tetramethyl-4-phenyldimiazolin-3-oxide-1-oxyl was a gift from Dr. Vitaly Roginsky (Russia). Sephadex G-25M columns were from Pharmacia Biotech Inc. (Uppsala, Sweden) and Centriprep-30 concentrator tubes were from Amicon Inc. (Beverly, MA). The nitroxide spin label 2,2,5,5-tetramethyl-4-phenylimidazolin-3-oxide-1-oxyl was a gift from Dr. Vitaly Roginsky (Russian Academy of Sciences, Moscow) and was used without further purification. Organic solvents of HPLC quality were obtained from Polysciences (Warrington, PA). Hydroxy 13-(Z,E)-9-(S,E,Z)-Ch18:2 and 13-(Z,E)-H(P)ODE, 18:2, ilomelane, and arachidonate were purchased from Cayman Chemicals (Ann Arbor, MI).

Authentic standards of racemic Ch18:2-OH were prepared by vitamin E-controlled autoxidation of Ch18:2 followed by NaBH₄ reduction (23). Ubiquinol-10 (CoQ₁₀H₂) was produced by reduction of coenzyme Q₁₀ in phosphate-buffered (50 mM, pH 7.4) saline (PBS) at 4 °C. CE, ascorbate, Trolox, sodium borohydride (NaBH₄), ascorbyl palmitate, eicosatetraenoic acid (ETYA), SLO (6.3 × 10⁵ units/mg of protein), where 1 unit is defined as 18:2/min at 25 °C, (pH 9) and porcine pancreatic PLAs (760 units/mg of protein, where 1 unit hydrolyzes 1 µmol of phosphatidylcholine/min at 37 °C, pH 8) were obtained from Sigma. Coenzyme Q (50 mg) capsules were a generous gift from Blackmores Ltd (Sydney, Australia). dl-α-TOH was purchased from Eastman Kodak Co., and probucol was a gift from Marion-Merrell Dow Inc. (Cincinnati, OH). 2,2-Azobis(2-aminopropane) dihydrochloride (AAPH) was purchased from Polysciences (Warrington, PA). Hydroxy 13-(Z,E)-9-(S,E,Z)-Ch18:2 and 13-(Z,E)-H(P)ODE, 18:2, ilomelane, and arachidonate were purchased from Cayman Chemicals (Ann Arbor, MI).

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Experiments using LDL from three donors. The SLO used in these EPR studies was concentrated 10 times using Ultracentrifugation under identical spectrometer conditions in the absence of LDL. 

![Graph A](image1.png)  
**Graph A:** Specific fatty acids enhance rhLO-initiated LDL oxidation. Isolated, human LDL (1.0 ± 0.25 μM in apoB) was incubated with rhLO (0.3 μM) for 8 h in the absence or presence of free fatty acids or cholesteryl esters. At various times 50-μl aliquots were removed and analyzed for CE-O(O)H (A) and α-TOH (B) as described under “Experimental Procedures.” Lipids (10 μM) used were: 18:2 ( ), 18:3 ( ), 18:1 ( ●), 20:4 ( ●), and Ch20:4 ( ●). Reaction mixtures containing no added lipid (but containing 0.5%, v/v, solvent) are represented by ■. The results represent mean values ± S.D. derived from three separate experiments using LDL from three donors.

and carrying out the EPR analysis was consistently 1–2 min. The time-dependent increase in α-TOH intensity was measured using the following parameters: magnetic field scan, 60 G; power, 20 milliwatts; modulation amplitude, 1.0 G; modulation frequency, 12.5 kHz; gain, 2 × 105; time constant, 163 ms; and sweep time, 20.5 s. Employing these EPR parameters and averaging the output of five cumulative scans gave an acceptable signal to noise ratio that allowed the determination of time-dependent changes in concentration of the α-TOH signal. Radical concentrations were estimated by comparison of the total peak area for the α-TOH signal with that obtained from a solution of the nitroxide, 2,2,5,5-tetramethyl-4-phenylimidazolin-3-oxide-1-oxyl (5 μM) used were: 18:2 ( ), 20:4 ( ●), 18:3 ( ), 18:1 ( ●), Ch18:2 ( ●), and Ch20:4 ( ●). Reaction mixtures containing no added lipid (but containing 0.5%, v/v, solvent) are represented by ■. The results represent mean values ± S.D. derived from three separate experiments using LDL from three donors.

Results
We have proposed recently (20) that 15-LO-induced oxidation of LDL-associated FFA stimulates TMP of esterified lipids in the lipoprotein via initial, enzyme-induced formation and release of peroxyl radicals of FFA. To test this hypothesis, we first examined the effect of supplementation of LDL with various FFA (10 μM; oleate, 18:2, linolenate, and arachidonate), and some of the corresponding CE, on CE-O(O)H accumulation induced by rhLO. Fig. 1A shows that of the various lipids tested only FFA containing a 1,4-pentadienyl group, and that have been described to be substrates for 15-LO (i.e., 18:2, linolenate and arachidonate (29)), significantly enhanced CE-O(O)H accumulation in LDL. Both 18:2 and arachidonate also appeared to accelerate α-TOH consumption (Fig. 1B).

The increase in initial rate of rhLO-induced CE-O(O)H accumulation in LDL (1 μM in apoB) showed a dose dependence with increasing amounts of 18:2, which became saturated at ~200 μM 18:2 (Fig. 2). In contrast, 18:2 supplementation of LDL had no significant effect on the rate of CE-O(O)H accumulation when LDL oxidation was induced nonenzymically by aqueous peroxyl radicals (AAPH) or Cu2+ ions (Fig. 2). These results demonstrate that the enhancing effect of supplemented 18:2 on CE peroxidation is specific for rhLO as the oxidant. The data are also consistent with 18:2 supplementation of LDL increasing rhLO-induced CE-O(O)H accumulation by increasing the formation of FFA peroxyl radicals from the enzymic reaction.

As PL2a cleaves FFA in PL to generate free FFA substrates for 15-LO, we examined its effect on rhLO-induced CE-O(O)H accumulation in LDL. In the presence of 2.9 μM PL2a, the LDL content of LDL increased to approximately 90 μM. Similarly to exogenous addition of FFA, PL2a significantly increased CE-O(O)H accumulation and α-TOH loss in LDL following incubation with rhLO (Fig. 3). These results suggest that substrates for rhLO are released from PL in LDL by PL2a action and that rhLO conversion of these substrates increases the radical flux to which the lipoprotein is exposed.

The direct detection of FFA peroxyl radicals by EPR requires large amounts of materials (21), that greatly exceed the amounts of human LDL readily available to us. For this reason, we did not attempt to measure FFA peroxyl radicals in LDL undergoing rhLO-induced oxidation. However, the FFA peroxyl radicals demonstrated to be released by 15-LO (21) would be expected to be scavenged by LDL’s α-TOH, quantitatively the major and most important peroxyl radical scavenger in LDL. We therefore assessed the effect of 10 μM 18:2 supplementation, or addition of 2.5 μM PL2a on the formation of α-TOH, the product of such a putative reaction. As expected, both conditions led to an enhanced rate of formation of α-TOH in LDL exposed to 15-LO, as measured by EPR spectroscopy (Fig. 4). As 15-LO was required in large amounts for these experiments, it was necessary to use the more readily available, commercial

Fig. 1. Specific fatty acids enhance rhLO-initiated LDL oxidation. Isolated, human LDL (1.0 ± 0.25 μM in apoB) was incubated with rhLO (0.3 μM) for 8 h in the absence or presence of free fatty acids or cholesteryl esters. At various times 50-μl aliquots were removed and analyzed for CE-O(O)H (A) and α-TOH (B) as described under “Experimental Procedures.” Lipids (10 μM) used were: 18:2 ( ), 20:4 ( ●), 18:3 ( ), 18:1 ( ●), Ch18:2 ( ●), and Ch20:4 ( ●). Reaction mixtures containing no added lipid (but containing 0.5%, v/v, solvent) are represented by ■. The results represent mean values ± S.D. derived from three separate experiments using LDL from three donors.

![Graph B](image2.png)  
**Graph B:** The level of 18:2 influences the initial rate of CE-O(O)H accumulation in rhLO-induced LDL oxidation. LDL (1 μM in apoB) was incubated with various concentrations of 18:2 (0–200 μM) in the presence of rhLO (0.3 μM, ■), AAPH (1 mM, ○), or Cu2+ ions (1.5 μM, ○) for 8 h. At various times 50-μl aliquots were removed and analyzed for CE-O(O)H. 18:2 was added to the LDL preparation prior to addition of the enzyme. The results show mean values ± S.D. and are derived from three separate LDL preparations for each oxidant.

![Graph C](image3.png)  
**Graph C:** The level of 18:2 influences the initial rate of CE-O(O)H accumulation in rhLO-induced LDL oxidation. LDL (1 μM in apoB) was incubated with various concentrations of 18:2 (0–200 μM) in the presence of rhLO (0.3 μM, ■), AAPH (1 mM, ○), or Cu2+ ions (1.5 μM, ○) for 8 h. At various times 50-μl aliquots were removed and analyzed for CE-O(O)H. 18:2 was added to the LDL preparation prior to addition of the enzyme. The results show mean values ± S.D. and are derived from three separate LDL preparations for each oxidant.
LDL (1 μM apoB) was incubated with rhLO (0.23 μM) for 9 h in the absence (○, □) or presence (●, ■) of PLA₂ (2.9 μM) and aliquots analyzed at various times for α-T OH (○, ●) and CE-OH (□, ■). The results show mean values ± S.D. of three experiments, and the extent of variation is indicated by the error bars.

SLO. In addition, it was also necessary to concentrate the LDL to ~6 μM apoB to afford α-T OH concentrations sufficient for quantitation (30). The rate of α-T OH accumulation in control LDL following exposure to SLO (1.74 μM) increased with time, with a steady-state level reached after ~3 h (Fig. 4); this corresponded to 0.36–0.45 μM in different pooled preparations. FFA supplementation of LDL significantly shortened the time required to reach steady-state α-T OH levels from 3 to 1 h with 18:2 or to 2 h for PLA₂, although the steady-state α-T OH concentration remained unaffected (Fig. 4).

The above results suggest that enzymic oxidation of 18:2 results in α-T OH formation during 15-LO-induced LDL oxidation. We therefore exposed native LDL (~1 μM apoB) to SLO (1.1 μM) and analyzed for enzymic and nonenzymic lipid oxidation products after reduction of lipid hydroperoxides to the corresponding hydroxides, but without saponification of the samples (see “Experimental Procedures”). SLO was used for these studies due to the large amount of enzyme required to detect the small amounts of FFA oxidation products by UV 234 nm (20). As can be seen in Fig. 5, formation of 18:2-OH was rapid and reached a maximum after 30 min. Both the regio- and stereoisomers of 18:2-OH were determined: at 15 and 60 min 13-(Z,E)-18:2-OH corresponded to 73 ± 10 and 62 ± 13% (n = 3, mean values ± S.D.), respectively, of the total 18:2-OH detected. At the same time points, 94 ± 9 and 100 ± 10%, respectively (n = 3, mean values ± S.D.), comprised the enzyme S stereoisomer. Thus, 18:2 oxidation in native LDL exposed to SLO is largely enzymic.

Analysis of the regio- and stereoisomers of Ch18:2-OH formed in the same LDL exposed to SLO demonstrates that these products were formed primarily via nonenzymic processes (Fig. 6). For enzymatic oxidation, the expected product of Ch18:2 is 13-(S,Z,E)-Ch18:2-OH (26). As shown in Fig. 6A, however, equal amounts of the 13-(Z,E)- and 9-(E,Z)-Ch18:2-OH accumulated even at the earliest time points (15 min) studied, with little 13- or 9-(E,E) isomers detectable. These results are inconsistent with SLO oxidizing LDL’s CE enzymically. Rather they suggest that CE peroxidation is nonenzymic and controlled kinetically by a hydrogen donor (31). Indeed, α-T OH, the single most important H-donor for LDL’s lipids, remained present throughout the oxidation (Fig. 6A). In addition to the apparent α-T OH-controlled formation of Ch18:2-OH regioisomers, analysis of the chiral isomers of 13-(Z,E)-Ch18:2-OH showed that equal amounts of both the R and S forms accumulated throughout the time course of oxidation (Fig. 6B). The chirality of this regioisomer is commonly used as an indicator of 15-LO action, as only the S form is produced during enzymic oxidation (29). Thus, these results rule out the possibility that Ch18:2 peroxidation in LDL exposed to SLO proceeds enzymically, while they are fully consistent with 15-LO-induced peroxidation of the lipoprotein’s CE proceeding via TMP.

Previous studies by others demonstrated differences in the substrate specificity of different types of 15-LO (12). We therefore compared the extent of enzymic and nonenzymic lipid peroxidation in human LDL oxidized with SLO versus rhLO. As the availability of rhLO was restricted, we supplemented LDL with 10 μM 18:2 so that the possible enzymic production of 13-(S,Z,E) 18:2-OH (measured by UV 234 nm) could be monitored. Fig. 7 shows that in such LDL exposed to rhLO, the 13-(S,Z,E)-18:2-OH isomer is the major oxidation product of 18:2, with maximal concentrations obtained at ~15 min (the earliest time point measured) and this stereospecificity persists throughout the time course of oxidation. In contrast, in the
same LDL preparation, predominantly, though not exclusively, regio- and stereo-random Ch18:2-0H isomers were formed (Fig. 8). In particular, at the earliest time points measured (15 min), the enzyomic product, 13-(S)(Z,E)-Ch18:2-0H, comprised ~75% of the total 13-(Z,E)-Ch18:2-0H products (Fig. 8B). Thus, in the earliest stages of oxidation rhLO appeared to act directly on LDL’s Ch18:2, in contrast to SLO. With increasing duration of oxidation, the relative concentration of stereo-random products increased, so that after 4 h the S and R isomers of 13-(Z,E)-Ch18:2-0H reached similar concentrations (Fig. 8B). These results demonstrate a small but significant enzyomic oxidation of LDL’s Ch18:2 by rhLO. They also suggest the occurrence of a rapid loss of enzyomic activity, yet prolonged CE oxidation in LDL via a nonenzymic process.

To further distinguish between enzyomic and nonenzymic CE oxidation by rhLO, and to assess the importance of TMP in the latter process, we tested the effect of various agents on 18:2-supplemented LDL oxidation induced by rhLO. Of the reagents tested, ascorbate (10 µM), Trolox (5 µM), and ascorbyl palmitate (5 µM), inhibited CE-O(O)H accumulation by 79, 63, and 59%, respectively, over 4 h (Fig. 9A). However, none of these reagents significantly inhibited the concentrations of CE-O(O)H detected at the earliest time point. In fact, following this initial time point, ascorbate almost completely inhibited CE-O(O)H accumulation. In contrast, probucol (50 µM) had no effect, and ETYA (50 µM), an inhibitor of 15-LO, strongly inhibited overall CE-O(O)H accumulation (Fig. 9A). ETYA also completely prevented, and ascorbyl palmitate and Trolox inhibited the forma-
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within the same time period of enzymic CE oxidation (<30 min, Fig. 11B).

DISCUSSION

The results presented demonstrate directly that SLO- and rhLO-induced oxidation of LDL’s CE occurs predominantly via nonenzymic, prolonged processes in contrast to the rapid and exclusively enzymic oxidation of FFA associated with the lipoprotein. These findings are based on the kinetics and patterns of accumulating positional, regio- and stereospecific oxidation products of free and esterified 18:2, the major readily oxidizable lipid of LDL. The findings are supported by the observation that supplementation of LDL with FFA substrates for 15-LO, but not the corresponding CE, increased the extent of enzymic lipid oxidation. In contrast, the addition of ascorbate, which does not inhibit 15-LO activity, failed to affect nonenzymic oxidation of CE in LDL exposed to SLO and rhLO.

We have demonstrated recently that the extent to which SLO and rhLO peroxidize esterified lipids (CE and PL) depends on, and directly relates to, LDL’s α-TOH content (19, 20). These findings of α-TOH-controlled oxidation of the majority of LDL’s lipids are fully supported by the present findings, implying TMP as the major process responsible for the prolonged and extensive peroxidation of LDL’s CE induced by 15-LO. Thus, α-TO’ was formed rapidly in LDL exposed to SLO, and this is likely the result of indirect oxidation of the vitamin, as α-TOH is not a substrate for 15-LO. Once formed, α-TO’ in LDL has the propensity to both initiate and propagate lipid peroxidation via TMP, resulting in equal fractional peroxidation of the lipoprotein’s PL and CE (17–20). In agreement with this, the extent of nonenzymic CE-O(O)H accumulation in 15-LO-exposed LDL correlated with the rate of α-TO’ formation, as indicated by the stimulatory effect of FFA supplementation on these two processes. The regiosomers of 18:2-O(O)H produced in oxidizing LDL (Figs. 6 and 8) were those expected to be formed in the presence of α-TOH, the most significant hydrogen donor in LDL (33), all inhibited rhLO-induced nonenzymic oxidation of LDL’s CE. By contrast, probucol, which shows some radical scavenging activity (35), is unable to prevent rhLO-induced CE oxidation in LDL. Exposure of native and 18:2-supplemented LDL to SLO and rhLO, respectively, resulted in the formation of FFA-O(O)H with kinetics and isomer specificity markedly different to that of the lipoprotein’s CE (Figs. 5–8). Foremost, practically all of...
the 13-(Z,E)-H(P)ODE detected comprised the enzymic S stereoisomer, demonstrating that 15-LO reacts directly with FFA associated with LDL. Modeling and mutagenesis studies employing rhLO (36) are consistent with FFA being the primary substrate for mammalian 15-LO. Thus, the activity of wild-type rhLO for the methyl ester of arachidonate is only 7% of that of free arachidonate.

In addition to its enzymic nature, 15-LO-induced oxidation of FFA in native and 18:2-supplemented LDL was observed only during the earliest stages of LDL oxidation and ceased, while CE oxidation continued to proceed. This absence of ongoing accumulation of enzymic FFA-O(O)H by SLO and rhLO (Figs. 5 and 7) suggests that enzyme activity was lost during the early stages of LDL oxidation and was not required throughout the whole period of CE oxidation. Mammalian 15-LO is known to undergo self-inactivation in the presence of substrate FFA (29, 37). In support of this, addition of 18:2 to LDL previously exposed to rhLO for 60 min failed to result in further accumulation of 13-(S)(Z,E)-H(P)ODE.

Earlier studies noted that LDL lipid oxidation induced by various 15-LO did not display this characteristic self-inactivation (11–14). It is well established that FFA peroxyl radicals are released from the active site of 15-LO during enzymic formation of 13-(S)(Z,E)-HPODE (21, 38) and that this can lead to the co-oxidation of various compounds, including phenolic antioxidants, PL, cholesterol, and proteins (37, 39). As the major peroxyl radical scavenger of LDL, α-TOH is expected to preferentially react with FFA peroxyl radicals released from active 15-LO. The resulting α-TOH remains present at nearly unchanged concentration for prolonged periods of time, well beyond the initial phase during which 15-LO activity is apparent (compare Figs. 4 and 5). This is fully consistent with the TMP model of lipid peroxidation, where α-TOH in LDL is the chain-carrying radical and its elimination, in the absence of co-antioxidants, is dependent on a second radical species entering an oxidizing lipoprotein particle containing α-TOH. The probability of the latter event decreases as 15-LO, the source of FFA peroxyl radicals, becomes inactivated. Together, the above provide a rationale as to how prolonged LDL lipid peroxidation can occur even if 15-LO becomes self-inactivated.

The present findings are, in part, inconsistent with the view that exposure to mammalian 15-LO results in direct oxidation LDL’s esterified lipids (11–14). SLO-1 (the isoenzyme used in the experiments described herein) shows similarities to mammalian 15-LO in general properties, enzyme kinetics and the active site (40, 41), but requires the presence of bile salts to oxidize esterified lipids such as PL or biomembranes (42). The present results, which demonstrate that the oxidation of the LDL’s Ch18:2 by SLO (in the absence of detergent) is nonenzymic throughout the entire time course (Fig. 6), are fully consistent with this and further support the notion that α-TOH rather than SLO is responsible for CE oxidation.

Previous support for the direct, enzymic oxidation of LDL’s esterified lipids by mammalian 15-LO includes the observation that commercial CE and CE isolated from human plasma are oxidized by purified rabbit reticulocyte 15-LO (12). During LDL oxidation by mammalian 15-LO, 13-(S)(Z,E)-H(P)ODE was reported as the predominant lipid oxidation product and present largely in esterified lipids, although the regio- and stereospecificity observed was notably lower than that of FFA (11, 12). During the earliest phase of rhLO-induced oxidation of 18:2-supplemented LDL, we also observed some enzymic oxidation of Ch18:2 oxidation (Fig. 8), and the extent of this enzymic oxidation of LDL’s CE appeared to increase with increasing rhLO to LDL ratios used (Fig. 10). This contrasts with SLO, which does not appear to directly oxidize CE. However, for a given rhLO to LDL ratio, the proportion of the enzymic product was rapidly overcome by significant amounts of the nonenzymic-
rhLO as the oxidant (Fig. 2). Therefore, we propose that enzymatic oxidation was observed (Figs. 1 and 3), and this was specific to 15-LO, as indicated in the present study.

The present studies provide mechanistic information on the oxidation of free and esterified lipids in human LDL by 15-LO, in vitro may be useful in establishing its in vivo function(s). Our studies indicate that in addition to direct inhibitors of 15-LO, agents that prevent TMP, such as the endogenous antioxidants ascorbate and ubiquinol-10, also warrant consideration as potential pharmacological prevention regimens in atherogenesis.

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