Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 45–49, January 1993
Medical Sciences

Autoxidation of lipids and antioxidation by α-tocopherol and ubiquinol in homogeneous solution and in aqueous dispersions of lipids: Unrecognized consequences of lipid particle size as exemplified by oxidation of human low density lipoprotein

(atherosclerosis/ascorbate/superoxide/protein thiols)

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Contributed by Cheves Walling, August 5, 1992

ABSTRACT **Recent studies on the initial stages in oxidation of low density lipoprotein (LDL) have revealed certain previously unrecognized similarities to emulsion polymerization and some quite unexpected features including the following: (*i*) ascorbate is an extremely effective antioxidant for LDL containing α-tocopherol (α-TOH); (*ii*) in the presence of α-TOH and in the absence of both ascorbate and ubiquinol 10 ($Q_{10}H_2$), oxidation of LDL occurs via a free radical chain; (*iii*) $Q_{10}H_2$ is a much better antioxidant for LDL than α-TOH, although the reverse is true in homogeneous systems. We show here that these problems can be solved on the basis of three simple hypotheses, each of which is based on known chemistry: (*i*) α-TOH in LDL can be regenerated from its radical, α-TO•, by ascorbate; (*ii*) in the absence of ascorbate and $Q_{10}H_2$, the α-TOH in LDL acts as a chain-transfer agent rather than as a radical trap; (*iii*) $Q_{10}H_2$ is a much more effective chain-breaking antioxidant than α-TOH in LDL because the semiquinone radical $Q_{10}H^•$ exports its radical character from the LDL into the aqueous phase. Our conclusions imply that the search for better antiatherosclerotic drugs might profitably focus on antioxidants capable of exporting radicals from LDL particles or otherwise increasing the traffic of radicals between particles.**

There is growing clinical and biochemical evidence that free radical-mediated oxidative modification of low density lipoproteins (LDL) may be involved in the early stages in development of atherosclerotic lesions (1). Radical-trapping antioxidants will tend to protect LDL against oxidative modification and hence may diminish the risk and severity of atherosclerosis. The importance of this subject to human health has produced a veritable explosion of research (2–14). In this paper, it is shown that many hitherto inexplicable results from *in vitro* studies of LDL oxidation are a necessary consequence of autoxidation and antioxidation in small lipid particles dispersed in water.

The autoxidation (peroxidation) of lipids (LH) in homogeneous solution is a free radical chain reaction, which can be represented by (15, 16)

Initiation: $\text{Precursor} \rightarrow R^• \xrightarrow{O_2} ROO^• \xrightarrow{LH} ROOH + L^•$ [1]

Propagation: $L^• + O_2 \rightarrow LOO^•$ [2]

$LOO^• + LH \rightarrow LOOH + L^•$ [3]

Termination: $LOO^• + LOO^• \rightarrow \text{inactive products}$ [4]

The hydrogen atoms abstracted from LH in reactions **1** and **3** come principally from the bisallylic methylene groups of the polyunsaturated fatty acids (PUFA) (—CH=CH—CH_2—CH=CH—) since these C—H bonds in lipids are by far the most readily cleaved by attacking peroxyl radicals (17).

Most free radical-trapping antioxidants (AH) capture peroxyl radicals by the transfer of a hydrogen atom (16)

$$R(L)OO^• + AH \rightarrow R(L)OOH + A^• \quad [5]$$

The resultant antioxidant radical, A•, is generally too unreactive to continue the chain and so it waits around until it encounters a second peroxyl radical with which it reacts very rapidly to give nonradical products

$$R(L)OO^• + A^• \rightarrow \text{inactive products} \quad [6]$$

The initiation systems used to induce LDL oxidation have included $Cu^{(II)}$ (3–5, 8–11), γ-rays (6, 7), cultured cells that produce active oxygen (3, 8–10), and even air (apparently) alone (2). Many of these initiation systems are poorly reproducible (4) and do not yield the initiating radical, R•, at a known rate. They have generally been used to study the later stages of LDL oxidation (2–11). However, understanding the very earliest stages of LDL oxidation is arguably more important since atherosclerosis might be eliminated by total prevention of LDL oxidation.

Quantitative studies on the early stages in oxidation of aqueous lipid dispersions became possible with the introduction of the water-soluble (18, 19) and lipid-soluble (20–22) azo initiators 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN), respectively, which yield radicals at known, reproducible rates on thermolysis (reaction **7**). These compounds opened

$$R—N{=}N—R \xrightarrow{\Delta} 2R^• + N_2 \quad [7]$$

R—N=N—R = AAPH, $R^• = H_2NC(=NH_2^+)\dot{C}(CH_3)_2$

R—N=N—R = AMVN, $R^• = (CH_3)_2CHCH_2\dot{C}(CH_3)C{\equiv}N$

the door to direct investigations of the peroxyl radical-induced consumption of antioxidants and formation of lipid hydroperoxides under controlled conditions in systems such as human blood plasma (23, 24) and isolated human lipoproteins (12–14). Some interesting chemical problems were observed during the very earliest stages of LDL oxidation.

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Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); α-TOH, α-tocopherol; LDL, low density lipoprotein(s); $Q_{10}H_2$, ubiquinol 10; PUFA, polyunsaturated fatty acid(s).

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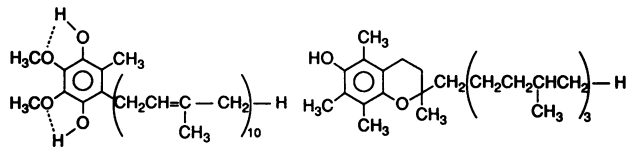
We show here that most of these problems arise from the same fact that makes emulsion polymerization such an efficient industrial process (15)—namely, individual radicals are isolated within individual lipid particles and are unable to diffuse through the solution from particle to particle (15).

The first firm indications that there were problems are contained in two 1990 reports (12, 13). Sato *et al.* (12) and Stocker *et al.* (13) both reported that when LDL was subjected to controlled attack by peroxyl radicals at 37°C the antioxidant ubiquinol 10 (reduced coenzyme Q_{10} or $Q_{10}H_2$) was consumed much more rapidly than α-tocopherol (α-TOH).



Ubiquinol-10, $Q_{10}H_2$ α-Tocopherol, α-TOH

Furthermore, during the α-TOH-induced induction period the LDL was actually being oxidized (12, 13) and this oxidation was a chain reaction (12). Thus, in one experiment (12) in which LDL oxidation was initiated with 11.3 mM AAPH, the chain length, ν (number of O_2 molecules absorbed or LH molecules oxidized per initiating radical), was 4.6 during the induction period (i.e., $\nu_{\alpha\text{-TOH}} = 4.6$) and increased to 10 ($\nu_{\text{uninhib}}$) after the α-TOH was consumed, while in an experiment initiated with 1.91 mM AMVN, $\nu_{\alpha\text{-TOH}} = 6.5$ and $\nu_{\text{uninhib}} = 22$ (12). [There is also evidence from other initiation systems that lipid peroxides are formed in LDL well before all the α-TOH is consumed (5, 8).]

A very much more detailed study of the early stages of LDL oxidation at 37°C was reported the following year by Stocker *et al.* (14), who uncovered an even more intriguing problem—namely, in the *very* earliest stages, LDL is not oxidized in a chain reaction (i.e., $\nu < 1$), but oxidation does become a chain process ($\nu > 1$) after consumption of a good fraction (≈50%) of the $Q_{10}H_2$ initially present in the LDL.

General Background to These Problems

Antioxidant Activities of $Q_{10}H_2$ and α-TOH. It is perfectly reasonable that different peroxyl radical-trapping antioxidants should have different activities and hence should confer different degrees of protection on the LDL. However, $Q_{10}H_2$ would be expected *a priori* to be a poorer antioxidant than α-TOH because its ability to donate a phenolic hydrogen to an attacking peroxyl radical (reaction 5) will be reduced both by internal hydrogen bonding and by the electron-withdrawing inductive effect of the meta-substituted methoxyl group (25) (i.e., meta-substituted with respect to the phenolic group that is under attack by an LOO• radical). Consistent with this view are several studies demonstrating that $Q_{10}H_2$ has ≈10% of the antioxidant activity of α-TOH in homogeneous solution (26–28). [The oxidized form of $Q_{10}H_2$—i.e., ubiquinone 10 or Q_{10}—has little or no antioxidant activity (26–28).] Similarly, two 7,8-dimethoxytocopherols are only ≈10% as reactive toward a persistent phenoxyl radical as α-TOH (29). The complexity of lipid oxidation systems inhibited by $Q_{10}H_2$ or α-TOH grows when we consider that ubiquinols are about as effective as antioxidants as α-TOH in arachadonic acid emulsions (30) and in phosphatidylcholine liposomal membranes (27, 31, 32). In summary, the relative antioxidant activities of $Q_{10}H_2$ vs. α-TOH are $Q_{10}H_2 >$ α-TOH in LDL, $Q_{10}H_2 <$ α-TOH in homogeneous solution, and $Q_{10}H_2 \approx$ α-TOH in aqueous lipid dispersions.

In contrast to the variations in relative antioxidant activities of $Q_{10}H_2$ and α-TOH when tested separately, it would appear that $Q_{10}H_2$ is always consumed more rapidly than α-TOH when both antioxidants are present together. This is true for LDL (12–14), for plasma (33), for phosphatidylcholine liposomes (27, 32), and for homogeneous solutions (27). In liposomes and in homogeneous solution the consumption of α-TOH began only after all the $Q_{10}H_2$ had been consumed (27, 32) so it was suggested that the $Q_{10}H_2$ regenerated α-TOH by reducing the α-tocopheroxyl radical α-TO•

$$\text{LOO}^\bullet + \alpha\text{-TOH} \rightarrow \text{LOOH} + \alpha\text{-TO}^\bullet \quad [8]$$

$$\alpha\text{-TO}^\bullet + Q_{10}H_2 \rightarrow \alpha\text{-TOH} + Q_{10}H^\bullet \quad [9]$$

Regeneration of α-TOH by $Q_{10}H_2$ has been shown to occur (26, 34) with $k_9 = 3.7 \times 10^5$ and 2.2×10^5 $M^{-1}\cdot s^{-1}$ at 25°C in benzene and ethanol, respectively (34). Regeneration may be important in liposomes containing equimolar concentrations of $Q_{10}H_2$ and α-TOH (27, 32). However, Stocker *et al.* (14) concluded that regeneration *per se* (i.e., a simple maintaining of the initial α-TOH concentration) could not explain their results in oxidizing LDL (in which $[Q_{10}H_2]/[\alpha\text{-TOH}]$ ratios ranged from 1:27 to 1:47) because the chain length increased 35-fold, from 0.2 at the beginning of oxidation to 7 after all the $Q_{10}H_2$ was consumed despite the fact that >95% of the initial α-TOH remained at this point.

The unimportance of $Q_{10}H_2$/α-TOH regeneration in LDL (14) is consistent with a report (35) that α-TO• radicals in a microsomal membrane suspension in water were not destroyed by the addition of $Q_{10}H_2$.

Suggestions that the sparing action of $Q_{10}H_2$ on α-TOH in liposomes (32) and the higher antioxidant activity of $Q_{10}H_2$ in LDL (14) are due to differences in the physical location of these two antioxidants ignore the internal dynamic motion of molecules in fluid lipid particles and are unnecessary.

Chain Reaction During Oxidation of LDL Containing α-TOH. A free radical chain oxidation of LDL containing α-TOH is not consistent with the usual picture of inhibited lipid autoxidations (see Introduction). That is, an average LDL particle contains ≈6.0 molecules of α-TOH and ≈1500 readily oxidizable, bisallylic methylene groups (—CH=CH—CH_2—CH=CH—) (4, 9–11). In a nonpolar medium at 30°C the rate constant, k_8, for abstraction of the phenolic hydrogen atom from α-TOH by a peroxyl radical is $\approx 3 \times 10^6$ $M^{-1}\cdot s^{-1}$ (36) and the rate constant for hydrogen abstraction from a bisallylic methylene group, k_3, is ≈60 $M^{-1}\cdot s^{-1}$ (17). Thus, in the nonpolar region of LDL the relative rates of peroxyl radical attack on α-TOH to PUFA are $(3 \times 10^6 \times 6):(60 \times 1500) = 200:1$, and so a chain reaction involving L• and LOO• radicals could not occur if the usual α-TOH inhibited autoxidation scheme were applicable to LDL.

In a more polar medium k_8 declines dramatically—e.g., k_8 has a value of $\approx 5 \times 10^5$ $M^{-1}\cdot s^{-1}$ at 37°C in homogeneous alcoholic solutions (20), $4–6 \times 10^4$ $M^{-1}\cdot s^{-1}$ at 37°C–40°C in SDS micelles (37, 38), and $3–6 \times 10^3$ $M^{-1}\cdot s^{-1}$ in dilinoleoylphosphatidylcholine (DLPC) bilayers at 37°C (39, 40). In both the SDS micelles and the DLPC bilayers, k_3 also decreases but only to about half its value in a homogeneous lipid environment—to 30–40 $M^{-1}\cdot s^{-1}$ at 30°C–37°C (39, 40). Nevertheless, even if the most unfavorable kinetic conditions were to apply to the α-TOH-inhibited oxidation of LDL (i.e., those of DLPC bilayers) the relative rates of peroxyl radical attack on α-TOH to PUFA would be roughly equal so the LOO• radicals could not carry a chain reaction.

Relevant Characteristics of LDL. LDL particles have a density of ≈1.05, a molecular weight of $\approx 2.5 \times 10^6$ (4, 10), a diameter of ≈20 nm, and a volume of $\approx 4 \times 10^{-18}$ cm^3. The composition of LDL from different donors varies quite widely in composition (3, 4, 9, 11). Esterbauer *et al.* (11) have

most recently estimated that an average LDL particle contains ≈1200 PUFA molecules, ≈6.0 α-TOH molecules, and ≈0.53 γ-TOH molecule. Other compounds currently listed (11) as antioxidants (see below) are β-carotene (≈0.33 molecule per LDL) and lycopene (≈0.18 molecule per LDL). Rapid oxidation of LDL has been reported to commence only after depletion of the tocopherols and carotenoids (see below) (4, 6, 8, 9, 12).

Irrelevant Characteristics of LDL. Conventional wisdom maintains that carotenoids are antioxidants because they are oxidized more readily than the PUFA (8–11). (The reader will recognize that according to this argument PUFA would be an antioxidant for fully saturated lipids!) While it is true that β-carotene can retard the oxidation of organic compounds under some conditions, there is only about one carotenoid molecule for every two LDL particles. Moreover, β-carotene is a very poor antioxidant relative to α-TOH (41). Fortunately, the antioxidant activities of carotenoids in LDL are irrelevant to the present paper.

Summary of Problems Uncovered in Studies of the Early Stages of LDL Oxidation (12–14)

(*i*) Ascorbate is an extremely effective antioxidant for LDL containing α-TOH. (*ii*) LDL oxidation occurs via a free radical chain in the presence of α-TOH and in the absence of ascorbate and $Q_{10}H_2$. (*iii*) $Q_{10}H_2$ is consumed much more rapidly than α-TOH in LDL. (*iv*) $Q_{10}H_2$ is a much better antioxidant than α-TOH for LDL (but not for other systems; see above).

These problems can be solved by three simple hypotheses each of which is based on known chemistry.

Hypothesis 1. α-TOH in LDL can be regenerated from its radical, α-TO•, by ascorbate. Justification: Kinetic studies on autoxidation of phosphatidylcholine liposomes have demonstrated that α-TO• radicals in the liposomes are rapidly and efficiently regenerated by ascorbate ($AscH^-$) (20, 22)

$$(\alpha\text{-TO}^\bullet)_{lipid} + (AscH^-)_{aqu} \rightarrow (\alpha\text{-TOH})_{lipid} + (Asc^-)^\bullet_{aqu} \quad [10]$$

Ascorbate traps the AAPH-derived, water-soluble peroxyls very efficiently but not the AMVN-derived, lipid-soluble peroxyl radicals (20, 22). Thus, in the absence of α-TOH, ascorbate acts as an excellent liposomal antioxidant when initiation is via AAPH but only as a very poor antioxidant when initiation is via AMVN (20, 22). However, in the presence of α-TOH ascorbate acts as an antioxidant with either initiation system (20, 22). The same situation would appear to hold for the AAPH and AMVN (and γ-ray) (6)-initiated oxidation of LDL (12–14). For example (12), the addition of ascorbate to an AAPH-initiated LDL oxidation produced an immediate, although temporary, increase in α-TOH concentration and its addition to an AMVN-initiated system temporarily halted the decline in concentration of α-TOH. In the latter system, ascorbate increased the induction period when α-TOH was present but it no longer suppressed oxidation once the α-TOH had been completely depleted.

Provided ascorbate is present, α-TOH is a very effective inhibitor of LDL oxidation when peroxyl radicals are generated initially either in the aqueous (12, 14) or in the lipid (12) phase. However, α-TOH is not a very effective inhibitor in the absence of ascorbate (14). The α-TOH/ascorbate combination provides a means whereby a radical within a LDL particle can be exported into the surrounding aqueous environment for disposal (i.e., reaction with a second radical, which may be in the water or in a different LDL particle).

Hypothesis 2. In the absence of ascorbate and $Q_{10}H_2$ the α-TOH in LDL acts as a chain-transfer agent rather than as a radical trap. Justification: There is abundant evidence that α-TOH can act as a prooxidant in homogeneous PUFA autoxidation systems (42–45) via chain-transfer reactions in which the α-TO• radical abstracts hydrogen from a PUFA molecule to form a L• radical (reaction **11**) or from a preformed PUFA hydroperoxide to form a LOO• radical (reaction **12**) (which is simply the reverse of reaction **8**).

$$\alpha\text{-TO}^\bullet + \underset{(LH)}{\text{—CH=CH—CH}_2\text{—CH=CH—}} \rightarrow \alpha\text{-TOH} + \underset{(L^\bullet)}{\text{—CH—CH—CH—CH—CH—}} \quad [11]$$

$$\alpha\text{-TO}^\bullet + LOOH \rightarrow \alpha\text{-TOH} + LOO^\bullet \quad [12]$$

To understand why chain transfer occurs in the absence of ascorbate, it is necessary to consider three factors: (*i*) the concentration of LDL particles, (*ii*) the rates of radical formation from the azoinitiator, and (*iii*) the magnitude of the rate constants for chain transfer relative to other possible reactions of a LDL-bound α-TO• radical.

(*i*) In the Stocker *et al.* experiments (14), the concentration of LDL was 2–4 × 10^{-6} M (1.2–2.4 mg of protein per ml with roughly 22% protein in the LDL) (3), which is similar to the concentration of LDL in plasma (4). [Concentrations were not given by Sato *et al.* (12).]

(*ii*) (*a*) To take a specific experiment (14), an ascorbate-free LDL oxidation was initiated with 1 mM AAPH for which the calculated rate of generation of water-soluble peroxyl radicals, R_g, was 10^{-9} M·s^{-1}. [A measurement of this rate by Sato *et al.* (12) under similar conditions implies that R_g would be only 5.7 × 10^{-10} M·s^{-1}.] If we were to assume that every encounter between a LDL particle and an AAPH-derived peroxyl, $H_2NC(=NH_2^+)C(CH_3)_2OO^\bullet$, led to incorporation of a radical within the LDL, then a LDL particle would encounter a water-soluble peroxyl only at intervals of 2–4 × $10^{-6}/10^{-9}$ = 2000–4000 s!

It is very improbable that the reaction between a water-soluble peroxyl and a LDL particle would be 100% efficient—i.e., each effective hit may be preceded by many unreactive encounters. Indeed, the phospholipid molecules at the LDL surface are probably rather impervious to attack by positively charged, water-soluble peroxyls because their reactive bisallylic methylene units are presumably buried within the LDL particle. In fact, it seems probable that effective hits will generally involve molecules containing hydrophilic groups with readily donatable hydrogen atoms that float on the LDL surface—e.g., $Q_{10}H_2$, α-TOH, LOOH. In the case of $Q_{10}H_2$, this does not lead to lipid oxidation (see below), but it is likely to be important for LDL particles containing α-TOH and LOOH. We therefore make the amusing prediction that a LDL particle that does not contain α-TOH will, under certain experimental conditions, be more resistant to lipid peroxidation induced by AAPH-derived radicals than a LDL particle that does contain α-TOH!

Despite uncertainty regarding the efficiency of reaction between water-soluble peroxyls and LDL particles, it is clear that when an α-TO• radical is formed in a LDL particle under the experimental conditions used (12–14) (and, in the absence of ascorbate, under most other conceivable conditions), it will have to wait around for a considerable period of time before it encounters a second radical. Furthermore, we can be fairly certain [thanks to some clever experiments on phosphatidylcholine liposomes by Niki *et al.* (20)] that it will wait around in the LDL particle in which it was formed.

(*b*) The situation for AMVN-initiated LDL oxidations is surprising. AMVN is believed to generate radicals within the LDL particles [because the oxidation was not inhibited by urate (12), nor was urate significantly consumed (14)]. There

can be no doubt that LDL oxidation initiated by AMVN is a chain reaction both in the presence of α-TOH ($\nu = 6.5$) (12) and after the α-TOH has been consumed ($\nu = 22$) (12). Furthermore, AMVN (1.9×10^{-3} M) was a fairly effective initiator at 37°C since the measured rate of chain generation (5.1×10^{-10} M·s^{-1}) (12) was ≈10% of the value found earlier for the same concentration of AMVN in homogeneous solution (4.9×10^{-9} M·s^{-1}) (20). Since the AMVN must make a singlet pair of radicals (reaction 7), LDL oxidation could not involve a chain reaction if both radicals always remained in the LDL particle in which they were formed. That is, it is well known that the thermal decomposition of lipid-soluble initiators is an ineffective method of inducing emulsion polymerization because, for a singlet radical pair in a confined space, fast radical–radical reactions (with $k \approx 10^{10}$ M^{-1}·s^{-1}) will completely dominate the much slower radical–molecule reactions (15). Rough calculations suggest that LDL particles contain 4–5 molecules of oxygen on average and that conversion of the two AMVN-derived carbon-centered radicals to peroxyl radicals ($R^\bullet + O_2 \rightarrow ROO^\bullet$) may be competitive with their bimolecular self-reaction ($R^\bullet + R^\bullet \rightarrow$ products). The results of Sato *et al.* (12) and Stocker *et al.* (14) are most readily explained by assuming that for every 10 radical pairs ($R^\bullet + R^\bullet$) generated from the AMVN in the LDL particles there is one, $R^\bullet$ (or its more water-soluble product, $ROO^\bullet$), that escapes from the LDL particle in which it was formed and successfully attacks another (nonradical containing) LDL particle. Since these neutral radicals will be more lipid than water soluble they will be better able to initiate the oxidation of another LDL particle in competition with their trapping in the aqueous phase by urate (12, 14) (or ascorbate) (12).

(*iii*) The rate constants for reaction **11** have been measured by ESR spectroscopy in benzene at 50°C (46): for methyl linoleate, $k_{11} = 0.075$ M^{-1}·s^{-1}, and for methyl linolenate, $k_{11} = 0.082$ M^{-1}·s^{-1}. Taking the mean value per active bisallylic methylene group of 0.058 M^{-1}·s^{-1} and assuming a preexponential factor of 10^8 M^{-1}·s^{-1} yield $E_{11} \approx 13.6$ kcal/mol (1 cal = 4.184 J), and hence $k_{11} \approx 0.024$ M^{-1}·s^{-1} at 37°C. This value is reasonably consistent with a mean value for k'_{11} in benzene at 25°C of 0.018 M^{-1}·s^{-1} for reaction of a variety of PUFA ethyl esters with the 5,7-diisopropyltocopheroxyl and 0.05 M^{-1}·s^{-1} with the 5,7-diethyltocopheroxyl radicals (47), which, since α-TO$^\bullet$ is sterically less hindered, implies that $k_{11} \gtrsim 0.05$ M^{-1}·s^{-1} at 25°C. Thus, at 37°C, k_{11} will have a value of ≈0.1 ± 0.05 M^{-1}·s^{-1}.

The rate constants for a reaction analogous to reaction **12** (reaction **12'**) between the 5,7-diisopropyltocopheroxyl radical and three butyl hydroperoxides lie in the range 0.13–0.37 M^{-1}·s^{-1} in benzene at 25°C (48)—i.e., $k'_{12} \approx 10\ k'_{11}$. It therefore seems probable that $k_{12} \approx 10\ k_{11}$ for α-TO$^\bullet$. Hence, except for an extremely heavily peroxidized LDL, only reaction **11** need be considered further.

There would appear to be no other reactions of α-TO$^\bullet$ in a LDL particle that could compete with reaction **11** (apart from reaction with another radical or hydrogen atom exchange with $Q_{10}H_2$, γ-TOH, etc.). Even the reaction of α-TO$^\bullet$ with oxygen is extremely slow (49).

$$\alpha\text{-TO}^\bullet + O_2 \xrightarrow{k^{23°C} < 6.5\ M^{-1}\cdot s^{-1}} \text{products} \qquad [13]$$

We can therefore ask what is the lifetime of an α-TO$^\bullet$ radical in a LDL particle? The concentration of bisallylic methylene units in an average LDL particle can be estimated to be ≈0.8 M from their number ($n = 1500$), the lipid volume of a LDL particle (≈3×10^{-21} liter), and Avogadro's number. Taking k_{11} to be 0.1 M^{-1}·s^{-1} at 37°C, the pseudo-first-order rate constant for loss of α-TO$^\bullet$ will be ≈0.08 s^{-1}—i.e., the mean lifetime of an α-TO$^\bullet$ radical in an average LDL particle will be ≈12.5 s.

At low radical fluxes, and in the absence of ascorbate and $Q_{10}H_2$, chain transfer by reaction **11** will be significant and hence LDL containing α-TOH must be oxidized in a chain reaction, just as has been observed (12–14).

Hypothesis 3. $Q_{10}H_2$ is a much more effective chain-breaking antioxidant than α-TOH in LDL because the semiquinone radical $Q_{10}H^\bullet$ exports its radical character from the LDL into the aqueous phase. Justification: The high antioxidant efficiency of the α-TOH/ascorbate couple relies on the export of radicals from the LDL into the aqueous phase (reaction **10**). Any LDL-bound, radical-trapping antioxidant must also rely on export if it is to be highly efficient and thus avoid the perils of chain transfer. The lipophilic hydroquinone $Q_{10}H_2$ can do this irrespective of the presence or absence of ascorbate because the semiquinone radical $Q_{10}H^\bullet$ will react with oxygen to form the quinone and superoxide:

$$ROO^\bullet\ (LOO^\bullet) + (Q_{10}H_2)_{lipid} \rightarrow ROOH\ (LOOH) + (Q_{10}H^\bullet)_{lipid} \qquad [14]$$

and either

$$(Q_{10}H^\bullet)_{lipid} + O_2 \rightarrow (Q_{10})_{lipid} + (H^+ + O_2^{-\bullet})_{aqu}(\equiv HOO^\bullet) \qquad [15]$$

or, more probably,

$$(Q_{10}H^\bullet)_{lipid} \rightarrow (Q_{10}^{-\bullet})_{lipid} + (H^+)_{aqu} \qquad [16]$$

$$(Q_{10}^{-\bullet})_{lipid} + O_2 \rightarrow (Q_{10})_{lipid} + (O_2^{-\bullet})_{aqu} \qquad [17]$$

Although there would appear to be no kinetic data on reactions **15** and **17**, it is known that semiquinone radicals react fairly rapidly with oxygen (15)—e.g., p-$HOC_6H_4O^\bullet + O_2 \rightarrow O{=}C_6H_4{=}O + HOO^\bullet$, $k \approx 300$ M^{-1}·s^{-1} at 50°C in chlorobenzene (50). The $O_2^{-\bullet}$ radical formed in these reactions is very unreactive in hydrogen atom abstractions and is probably trapped by $ROO^\bullet$ radicals derived from the initiator and by α-TO$^\bullet$ radicals trapped in the LDL.

$$(O_2^{-\bullet})_{aqu} + (\alpha\text{-TO}^\bullet)_{lipid} \xrightarrow{H^+} O_2 + (\alpha\text{-TOH})_{lipid} \qquad [18]$$

An interesting analogy can be drawn between the inhibiting action of $Q_{10}H_2$ on LDL oxidation and the manufacture of H_2O_2, which involves air oxidation of alkylanthrahydroquinones in organic solvents with countercurrent extraction of the H_2O_2 using water (51).

Conclusion and Implications

This paper has pointed out several previously unrecognized differences between homogeneous solutions of lipids and their aqueous dispersions in small particles when it comes to their oxidation and the effect and efficiencies of different lipophilic radical-trapping antioxidants. Although we have concentrated on PUFA oxidation and its prevention in LDL, similar differences in the behavior of homogeneous solutions and fine aqueous dispersions will be found for any lipid (e.g., cholesterol) yielding peroxyl radicals, which are essentially water insoluble. The analogy with the differences between vinyl polymerizations in bulk and in emulsions is most striking (15).

Under normal conditions, we expect the radical flux *in vivo* will be no higher than, and probably considerably lower than, the fluxes used by Sato *et al.* (12) and Stocker *et al.* (14). This leads us to conclude that LDL in the plasma of healthy humans is probably very effectively protected from oxidation due primarily to the α-TOH/ascorbate couple and, to a lesser extent (see below), to its content of $Q_{10}H_2$. Thus, if LDL oxidation is, indeed, wholly responsible for the development of atherosclerosis any antiatherosclerotic effect of vitamin E

probably arises from chemistry that occurs after the LDL particle has left the plasma. This conclusion is consistent with the view (1) that oxidation of LDL, which causes its atherogenicity, occurs more within the artery wall than in the circulation. Probucol, a lipid-soluble, radical-trapping antioxidant, which has an antiatherosclerotic effect even in the absence of changes in cholesterol levels (52), may also become active only after a LDL particle has left the circulation.

The efficiency of $Q_{10}H_2$ as a LDL antioxidant might lead one to suppose that each LDL particle would contain at least one molecule of $Q_{10}H_2$. However, this is not the case; only 50–60% of LDL particles contain a molecule of $Q_{10}H_2$ (53). Thus, under normal physiological conditions, the α-TOH/ascorbate couple is the major antioxidant system that suppresses excessive LDL oxidation in plasma. This couple is supplemented by $Q_{10}H_2$ and possibly by other antioxidants that yield $O_2^{-\cdot}$ for export following their sequential reaction with a peroxyl radical and oxygen. The three free and accessible thiol groups in the LDL apoprotein (55) could provide such a supplementary antioxidant system since, for nonproteinaceous thiols at least, reactions **20** and **21** are very fast with k values $\approx 3 \times 10^9$ and 4×10^8 $M^{-1} \cdot s^{-1}$, respectively (54).

$$ROO^{\cdot}\ (LOO^{\cdot}) + \text{protein-SH} \rightarrow ROOH\ (LOOH) + \text{protein-S}^{\cdot} \quad [19]$$

$$\text{Protein-S}^{\cdot} + \text{X-S}^{-}\text{(X-SH)} \rightarrow [\text{protein-S-S-X}]^{-\cdot} + (H^{+}) \quad [20]$$

$$[\text{Protein-S-S-X}]^{-\cdot} + O_2 \rightarrow \text{protein-S-S-X} + O_2^{-\cdot} \quad [21]$$

We suggest that future studies of potential methods for reducing lipoprotein oxidation by therapeutic intervention concentrate on a search for lipophilic or ambiphilic antioxidants that either export $O_2^{-\cdot}$ or themselves carry radicals away from a lipid particle into the aqueous phase and, hence, to another (radical containing) lipid particle.

We thank a reviewer for drawing our attention to the potential protective role of an apoprotein's thiol groups. One of us (R.S.) thanks the Australian National Health and Medical Research Council (Grant 910284).

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