Dynamics of Antioxidant Action of Vitamin E

ETSUO NIKI*,† AND NORIKO NOGUCHI‡
Human Stress Signal Research Center, National Institute of Advanced Industrial Science and Technology, Ikeda, Osaka 563-8577, Japan, and Research Center for Advanced Science and Technology, University of Tokyo, 4-6-1, Komaba, Tokyo 153-8904, Japan

Received March 20, 2003

ABSTRACT
Vitamin E is the major lipophilic, radical-scavenging antioxidant in vivo and protects humans from the oxidative stress mediated by active oxygen and nitrogen species. The mechanisms of the inhibition of oxidation by vitamin E in vitro are now fairly well understood, but the dynamics of antioxidant action of vitamin E in vivo have not been well elucidated yet, primarily because of the inherent heterogeneity of biological systems. In this Account, the factors which determine the antioxidant capacity of vitamin E are discussed, and the importance of its localization and mobility in the membranes and lipoproteins is emphasized.

Introduction

Organic materials and foods are oxidatively damaged and deteriorated by air oxidation. There is now increasing evidence which suggests the involvement of oxidative damage of lipids, proteins, and DNA in a variety of disorders, diseases, cancer, and aging.[1] As a consequence, the role of antioxidants has received much attention. We aerobic organisms are protected against oxidative stress by an array of defense systems composed of versatile antioxidants with different functions such as reduction of peroxides, sequestration of metal ions, scavenging of free radicals, detoxification of oxidation products, and clearance and repair of damage. The radical-scavenging antioxidants play a pivotal role in the inhibition of oxidative damage of DNA, proteins, and lipids by scavenging radicals before they attack the substrates and by breaking chain propagation. Some of the radical-scavenging antioxidants are hydrophilic, while others are lipophilic. The former are present in the cytosol and in extracellular fluids, while the latter are localized in the lipophilic domain of the membranes and lipoproteins. This localization of the antioxidants is characteristic of biological systems and is quite different from that of foods and oil products. Vitamin C and vitamin E are the typical hydrophilic and lipophilic radical-scavenging antioxidants, respectively, in vivo. It is quite important to elucidate the dynamics of antioxidant action of vitamin E in heterogeneous system in order to understand the role of vitamin E in vivo and also to develop antioxidant drugs. It is necessary to try to fill in the gap between the in vitro and in vivo systems.

Vitamin E, carotenoids, and ubiquinol, a reduced form of coenzyme Q, are known as natural, lipophilic radical-scavenging antioxidants. Considering its chemical reactivity toward radicals and physiological concentration, it may be said that vitamin E is the most important lipophilic antioxidant in vivo. In this Account, the action of vitamin E as a radical-scavenging antioxidant is discussed on the basis of in vitro studies by our group and others,[2] aiming specifically at elucidating the effects of various factors which determine antioxidant capacity in heterogeneous systems. The action of vitamin E as an antioxidant has been the subject of extensive studies, reported in numerous reviews as well as original papers.[3,4] However, the effects of environment on the dynamics of action of vitamin E in heterogeneous system, the key factor which determines its biological activity, have not received as much attention as they should.

Vitamin E includes eight different related homologues, that is, α, β, γ, and δ forms, depending on the number and site of methyl substituents on the chroman ring and corresponding tocopherols and tocotrienols. Tocopherols have a phytyl side chain, while tocotrienols have a similar chain but with three double bonds at positions 3′, 7′, and 11′. α-Tocopherol (α-TOH) is the most abundant and active form in vivo, and, unless otherwise stated, vitamin E refers to α-TOH in this Account.

The chemical reactivity of antioxidants toward radicals is apparently an important factor which determines antioxidant activity. However, in biological systems, where the environment is quite heterogeneous, many other factors also contribute in determining the antioxidant activity, for example, the localization, concentration, and mobility at the microenvironment, the fate of the antioxidant-derived radical, and interaction with other antioxidants. These factors will be considered below in this Account.

Factors Which Determine Antioxidant Activity of Vitamin E

Reactivity toward Radicals. There are numerous reports which show that vitamin E acts as a radical-scavenging antioxidant, and thereby it inhibits lipid peroxidation both in vitro and in vivo. Since vitamin E is localized in the lipophilic domain of the membranes and lipoproteins, where the polyunsaturated lipids are abundant, the major

Etsuo Niki obtained a B.Sc. in 1963 and then a Ph.D. in 1968 from the University of Tokyo in applied chemistry. He spent two years, from 1969 to 1971, as a Visiting Scientist at Stanford Research Institute in California under the supervision of Dr. Frank R. Mayo. He returned to the University of Tokyo in 1971, and worked there until retirement in 2000. He became the Director of the present Research Center in April 2001.

Noriko Noguchi obtained a B.Sc. in biology in 1981, a master's degree in medical sciences in 1983, and then a Ph.D. in 1987 from Tsukuba University. She worked as a Guest Researcher at the U.S. National Institute of Standards and Technology from 1990 to 1991. She obtained a position at the University of Tokyo in 1991 and is now an Associate Professor.

* Address correspondence to this author at Human Stress Signal Research Center, National Institute of Advanced Industrial Science and Technology, 1-8-31, Midorigaoka, Ikeda, Osaka 563-8577, Japan. Tel.: +81-72-751-9991. Fax: +81-72-751-9964. E-mail: etsuo-niki@aist.go.jp.
† National Institute of Advanced Industrial Science and Technology.
‡ University of Tokyo.

10.1021/ar030069m CCC: $27.50
Published on Web 11/19/2003

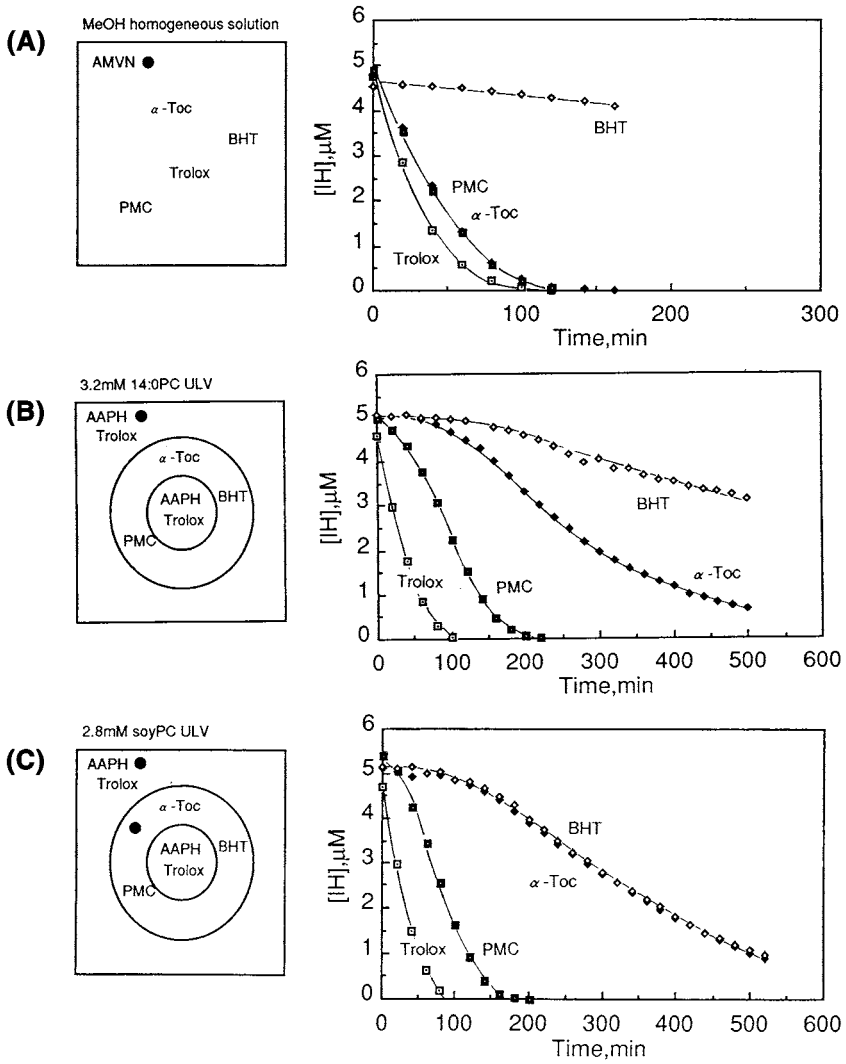

FIGURE 1. Rates of consumption of antioxidants. Equal concentrations of α-tocopherol (α-Toc), 2,2,5,7,8-pentamethyl-6-chromanol (PMC), 2-carboxy-2,5,7,8-tetramethyl-6-chromanol (Trolox), and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were treated with AMVN or AAPH in different media, and their consumption was followed with an HPLC: (A) in methanol; (B) in a dimyristoyl phosphatidylcholine liposomal membranes aqueous suspension; and (C) in soybean phosphatidylcholine liposome aqueous suspensions.

task for vitamin E in vivo must be the inhibition of lipid peroxidation. The antioxidant activity should be assessed by two factors: (1) how much does it reduce the rate of oxidation, and (2) how long does it suppress the oxidation? The former is determined primarily by the reactivity of the antioxidant toward radicals and the latter by the stoichiometric number of radicals that can be trapped by each antioxidant molecule. In addition to these inherent parameters, the concentration of antioxidant is, of course, important.

The rate constants for scavenging of radicals by α-TOH, that is, the hydrogen atom transfer from α-TOH to radicals, have been measured mostly in organic solutions. Hydroxyl and alkoxyl radicals are quite reactive and abstract hydrogen from lipids as well as from α-TOH rapidly. Considering the physiological concentrations of α-TOH and lipids, that is, [α-TOH]/[polyunsaturated lipids] < $1/10^2$, it is clear that α-TOH cannot act as an efficient scavenger for hydroxyl or even alkoxyl radicals in vivo. On the other hand, α-TOH reacts with peroxyl radicals much faster than lipids do, by a factor of 10^4, and thus it may scavenge more than 90% of peroxyl radicals before the radicals attack lipids, even when the molar ratio of α-TOH to lipids is 1/1000.

Localization. In contrast to the situation in organic solutions, the antioxidant efficacy in vivo depends more on the localization than on the chemical reactivity. A typical example which shows the significant effect of antioxidant localization on its activity is shown in Figure 1. Four antioxidants were compared, α-TOH, 2,2,5,7,8-pentamethyl-6-chromanol (PMC), 2-carboxy-2,5,7,8-tetramethyl-6-chromanol (Trolox), and 2,6-di-*tert*-butyl-4-methylphenol (BHT). α-TOH, PMC, and Trolox have the same chemical reactivity toward peroxyl radicals, while BHT is much less reactive than α-TOH, PMC, and Trolox. In the homogeneous solution, where the activity of scavenging radicals is determined primarily by the chemical reactivity toward radicals, α-TOH, PMC, and Trolox were consumed at the same rate and BHT was spared (Figure 1A). On the other hand, quite a different pattern

was observed in the oxidation of membranes. α-TOH, PMC, and BHT were incorporated into the dimyristoyl phosphatidylcholine (PC) liposomal membranes, while hydrophilic Trolox was localized in the aqueous phase.[5] When the radicals were generated in the aqueous phase, Trolox was consumed first, followed by lipophilic antioxidants in the order of PMC, α-TOH, and BHT. This order reflects the relative rate of scavenging aqueous peroxyl radicals outside the membranes. When soybean PC was used, in which about 70% of the fatty acids were polyunsaturated linoleic, Trolox was consumed first, followed by PMC, and α-TOH and BHT were consumed with a similar rate after essentially all of the Trolox and PMC had been exhausted. Thus, the relative antioxidant activities vary markedly, depending on the conditions. These results can be interpreted on the basis of the accessibility toward radicals and mobility of the antioxidant in the membranes in addition to the chemical reactivity toward radicals.

Similar effects have been observed in the competition between α-TOH and β-carotene toward peroxyl radicals.[6] α-TOH is about 30 times more reactive than β-carotene toward peroxyl radicals. When the radicals were generated in the aqueous region in the liposomal membrane suspensions, α-TOH was consumed faster than β-carotene, but interestingly, when the peroxyl radicals were generated in the interior of the membranes, β-carotene, despite its lower reactivity, was consumed faster than α-TOH.

The above two examples clearly show that the relative rate of scavenging of radicals by antioxidant, that is, the antioxidant activity, depends on the localization of the antioxidant and radicals as well as the inherent chemical reactivity of the antioxidant.

It has to be pointed out that the relative rate of consumption of antioxidants does not always reflect the antioxidant efficacy. It has been observed that the peroxyl radicals generated from the water-soluble azo initiator, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH, also abbreviated as ABAP), induce oxidative hemolysis of erythrocytes.[7] As shown above, Trolox scavenges the aqueous peroxyl radicals derived from AAPH more rapidly than α-TOH present in the membranes, but Trolox was found to be much less efficient than α-TOH in preventing the hemolysis induced by AAPH. The oxidative hemolysis induced by AAPH was compared between erythrocytes obtained from normal and vitamin E-deficient rats. The concentration of α-TOH contained in the vitamin E-deficient erythrocytes was less than 5% that of normal erythrocytes. Trolox added to the suspensions suppressed the hemolysis in a concentration-dependent manner. However, 50 times as much Trolox as α-TOH was required to suppress the hemolysis to the same extent. This is because, although Trolox can scavenge radicals in the aqueous phase more rapidly than α-TOH, it is not capable of scavenging the lipophilic radicals within the erythrocyte membranes efficiently, and hence it cannot break the chain propagation. Only a small fraction of radicals attacking the erythrocyte is sufficient to induce chain oxidation of many lipid molecules, which results in critical damage. Unlike Trolox, α-TOH in the membrane is capable of breaking the chain propagation and inhibits the hemolysis more efficiently than Trolox. It has been shown that the chain oxidation of lipids proceeds in erythrocytes membranes.[8] Another such case is observed in the oxidation inhibited by two antioxidants which interact with each other. For example, the oxidation of PC liposomal membranes and low-density lipoprotein (LDL) induced by lipophilic radicals is inhibited very efficiently by a combination of α-TOH and ascorbic acid (vitamin C). Ascorbic acid is consumed preferentially at first and α-TOH is spared. However, ascorbic acid itself is a poor antioxidant in this system, since it is not capable of efficiently scavenging radicals present in the lipophilic domain of membranes and LDL, and α-TOH is far more potent than ascorbic acid. However, ascorbic acid is consumed faster since it reduces α-tocopheroxyl radical (α-TO$^\bullet$), which is formed when α-TOH scavenges radicals in the membranes and lipoproteins. The interaction between α-TOH and ubiquinol also is noteworthy. Although ubiquinol reduces α-TO$^\bullet$ radical and is consumed faster than α-TOH, the apparent antioxidant activity of ubiquinol by itself is smaller than that of α-TOH.[9]

Thus, the preferential disappearance of one antioxidant over the other does not always mean that it is a more potent antioxidant than the other.

Mobility. The mobility of the antioxidant in the membranes and lipoproteins is another important factor which determines antioxidant activity. It is known that the phytyl side chain of α-TOH is required for incorporation and retention in the membranes and lipoproteins. This is a dilemma, because the side chain reduces the mobility and efficiency of radical scavenging in the domain.

The apparent antioxidant activity of α-TOH was found to be substantially smaller in the membranes than in homogeneous solution.[10−12] We found that the ratio of the rate constants k_{inh}/k_p for α-TOH in the membranes was much smaller than that in homogeneous solution by a factor of 10^2. Barclay[13] has also observed such diminution of the peroxyl radical-scavenging rate constant for α-TOH in the membranes as compared to that in solution. A smaller apparent activity for scavenging radicals has been also observed in micelle systems.[14,15]

$$X^\bullet + \alpha\text{-TOH} \xrightarrow{k_{inh}} XH + \alpha\text{-TO}^\bullet \qquad (I)$$

$$X^\bullet + \text{substrate (SH)} \xrightarrow{k_p} XH + S^\bullet \qquad (II)$$

The decrease in the reactivity of α-TOH in membrane and micelle aqueous systems was ascribed, in part, to a hydrogen bonding to the phenolic OH group and also to the para ether oxygen atom, which interferes in the reaction with the radicals and reduces its ability to conjugate with the aryloxyl radical, respectively. In fact, it has been shown that the rate constant k_{inh} varies significantly with solvent.[16] However, the marked effect of the side chain of α-TOH cannot be accounted for by the solvent effect (Figure 1). We have shown several experiments which strongly suggest that the mobility of α-TOH is important in determining its antioxidant activity in the membranes and lipoproteins.

The efficacy of scavenging radicals by α-TOH in the membranes was estimated by using a spin probe.[17] Doxyl stearic acid (NS) is a spin probe bearing a nitroxide radical along a stearic acid chain and is capable of acting as a radical scavenger by itself. The advantage of using NS is that the nitroxide radical can be placed at different depths from the surface of the membranes. When radicals are generated in the absence of α-TOH, NS scavenges radicals and is consumed. In the presence of α-TOH, NS and α-TOH compete in scavenging radicals, and from the extent of suppression of the rate of consumption of NS by α-TOH, the efficacy of α-TOH in scavenging radicals can be estimated. It was found that the apparent rate of scavenging radicals by α-TOH decreased as the radical went deeper into the interior of the membranes. Interestingly, such an effect was not observed for PMC, which could scavenge radicals more rapidly than α-TOH. Apparently, in order for α-TOH to scavenge radicals in the membrane, α-TOH has to go into the interior or the radicals have to float to the surface, as suggested first by Barclay and Ingold.[18]

Essentially the same results were obtained in the oxidation of LDL.[19] The effect of α-TOH and PMC on the consumption of 5-NS and 16-NS incorporated into LDL was studied. Both α-TOH and PMC, incorporated into LDL, spared 5-NS efficiently. Interestingly, however, α-TOH did not spare 16-NS efficiently, but PMC did (Figure 2).

Furthermore, in accordance with the above observation, the antioxidant activity against LDL oxidation, as judged from the formation of PC and cholesteryl ester hydroperoxides, increased with decreasing length of side chain of the chromanols at the 2-position.[19,20] These results strongly suggest that the mobility of antioxidant within the membranes and lipoprotein particles is an important factor which determines antioxidant activity.

The effect of length and number of side chains was also studied using fatty acid esters of ascorbic acid at either or both the 5- and 6-positions, the carbon numbers of the esters ranging from 3 to 22.[21] It was found that the mobility and the efficacy of sparing α-TOH in the membranes decreased with increasing length and number of side chains.

A significant effect of side chain has been also observed for intermembrane mobility.[7,22] α-TOH and PMC exerted the same antioxidant activities in solution, but α-TOH incorporated into dimyristoyl PC liposomal membranes could not suppress the oxidation taking place in the soybean PC liposomal membranes in the same aqueous suspensions, whereas PMC could. Furthermore, although PMC incorporated into multilamellar liposomal membranes reacted rapidly with galvinoxyl radical incorporated into different multilamellar liposomal membranes, α-TOH did not.[22]

Fate of Vitamin E Radical. When an antioxidant scavenges a radical, it is in general converted to a radical in turn. The fate of this antioxidant-derived radical is another factor which determines antioxidant activity. α-TOH is converted to α-TO• radical, which may undergo several reactions. It may scavenge another radical to give a stable product. It has been shown that α-TO• radical reacts with peroxyl radical to give a peroxo adduct at the para 8a-position.[23,24] It may react with another α-TO• radical to give a dimer. It may be reduced by a reductant, such as ascorbate and ubiquinol, to regenerate α-TOH. Under certain cases, it may react with the substrate and/or hydroperoxide to give active radicals, which may start a chain reaction. Apparently, in such cases, α-TOH does not act as an antioxidant but it acts as a chain-transfer agent. Stocker, Bowry, and Ingold have found that α-TOH acts as a phase-transfer agent to bring aqueous radicals into LDL particles and also as a chain transfer agent to propagate chain reactions in the oxidation of LDL.[20]

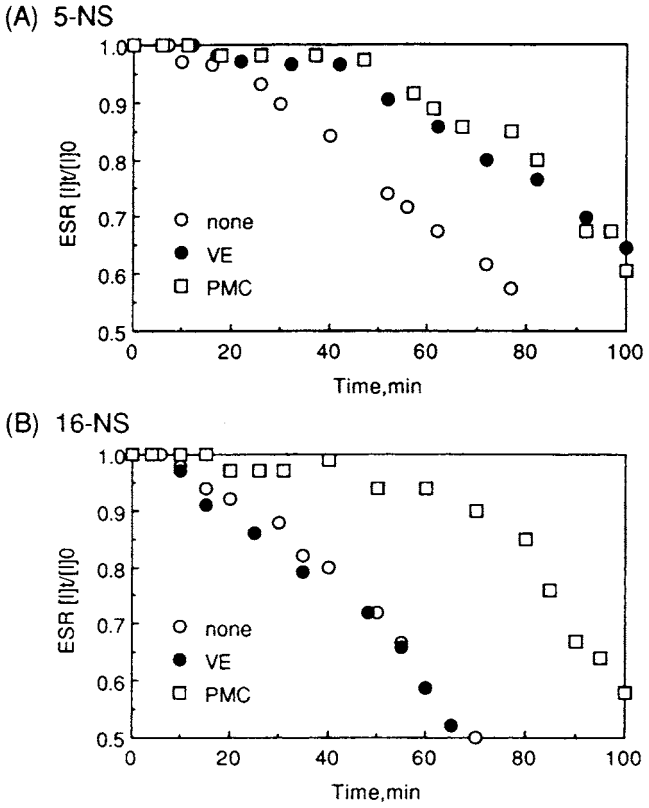

FIGURE 2. Effects of α-tocopherol (VE) and PMC on the consumption of 5-NS and 16-NS during the oxidation of LDL. The spin probe and α-tocopherol or PMC were incorporated into LDL, and then LDL (1.69 mg of protein/mL) was oxidized in the presence of 20 mM AAPH at 37 °C under air. The consumption of (A) 5-NS or (B) 16-NS was followed by ESR signal intensity. ○, without antioxidant addition; ●, with 10 μM α-tocopherol added exogenously; □, with 10 μM PMC.

To understand the fate of the α-TO• radical, the rate constants for competing reactions should be known. This measurement has been hampered by relatively fast bimolecular interactions of α-TO• radicals. In our recent study, the rate constants for the reaction of α-TO• radical with several substrates were measured by a stopped-flow ESR technique equipped with a rapid mixing device, taking both the first- and second-order reactions of α-TO• radicals into consideration.[25] The decay of α-TO• radicals formed by the reaction of excess α-TOH with galvinoxyl was followed continuously from an ESR signal intensity at a constant magnetic field of 334.54 mT. The time elapsed between the mixing of α-TOH and galvinoxyl and

Table 1. Fate of α-Tocopheroxyl Radical: Rate Constant and Physiological Importance

reaction substrate	rate constant k_s, M^{-1} s^{-1}	physiological substrate concn, M	apparent first-order rate constant, s^{-1}
α-TO• + AH_2	1×10^5	10^{-4}	10
α-TO• + LOO•	10^8	10^{-10}	10^{-2}
α-TO• + R-SH	170	5×10^{-6}	8.5×10^{-4}
α-TO• + α-TO•	1×10^3	10^{-7}	1×10^{-4}
α-TO• + NO	300	10^{-7}	3×10^{-5}
α-TO• + LH	3×10^{-2}	10^{-3}	3×10^{-5}
α-TO• + LOOH	41×10^{-1}	10^{-8}	4×10^{-4}

the beginning of recording operations (~1 s) corresponded to full consumption of galvinoxyl. α-TOH was first reacted with galvinoxyl and then mixed with substrate. The concentrations of α-TOH and galvinoxyl were chosen so that galvinoxyl was depleted by the reaction with α-TOH before introduction of a substrate.

The rate of reduction of α-TO• radical by ascorbic acid has been studied extensively. The pK_a of ascorbic acid is 4.2, and hence ascorbic acid is present predominantly as a monoanion under physiological conditions. It has been shown by Mukai[26] that the monoanion form of ascorbic acid reduces α-TO• radical most rapidly. The α-TO• radical localized in the membranes[27,28] and LDL[29,30] can also be reduced by ascorbate. The rate of reduction of radicals by ascorbate decreases as the radical goes deeper into the interior,[31] and it was found that the nitroxide radical on the fatty acid chain of cholesteryl ester localized in LDL particles was not reduced by ascorbate, suggesting that ascorbate is not able to reduce the radicals present in the LDL core.[19] Interestingly, the fatty acid esters of ascorbate reduced such radicals. The α-TO• radical can be reduced by other reductants, such as ubiquinol, α-tocopheryl hydroquinone, and polyphenolic compounds.

The rate constants obtained by a stopped-flow ESR method,[25] together with the biological concentrations of the substrates, enable us to estimate the relative importance of relevant reactions of α-TO• radical in vivo (Table 1). It can be seen that the major pathway for the α-TO• radical in vivo should be the reduction by ascorbate to regenerate α-TOH. It may be argued that the lipophilic substrates are condensed in the lipophilic domain together with α-TO• radical, and the actual concentration should be higher by a couple of orders of magnitude. However, even if the concentrations of polyunsaturated lipids are assumed to be 10^3 times greater than that shown in Table 1, the attack of α-TO• upon lipids to initiate chain oxidation should be much less important than the reduction by ascorbate. In accordance with this, the pro-oxidant effect of α-TOH has not been observed in the presence of ascorbic acid.

Synergistic Inhibition of Oxidation. α-TOH acts as an antioxidant not only by itself but also in collaboration with other antioxidants. A profound synergistic effect is observed when α-TO• radical is reduced by an antioxidant, which by itself does not act as an efficient antioxidant, to regenerate α-TOH and inhibit the attack on substrate by α-TO• radical. The combination of α-TOH and ascorbic acid is a typical example. Another possible case is a combination of α-TOH and antioxidant which undergoes autoxidation. α-Tocopheryl hydroquinone (TQH_2) and ubiquinol are examples of such antioxidants.[9]

The synergistic inhibition of oxidation of LDL by α-TOH and ascorbate is discussed below as an example. It was found that α-TOH and ascorbate inhibited synergistically the oxidation of LDL,[20,32,33] which has been accepted as an initial event of the progression of atherosclerosis. The effects of ascorbate and uric acid on the consumption of α-TOH in the oxidation of LDL are compared. Both ascorbate and uric acid are potent water-soluble, radical-scavenging antioxidants, but unlike ascorbate, uric acid is not capable of reducing α-TO• radical. When LDL was oxidized by water-soluble AAPH, both ascorbate and uric acid spared α-TOH. On the other hand, when LDL oxidation was induced by lipophilic AMVN incorporated into LDL particles, ascorbate spared α-TOH, but uric acid did not.[33] Apparently, when the oxidation was induced by AAPH, both ascorbate and uric acid scavenged the AAPH-derived radicals efficiently in the aqueous phase and spared α-TOH, whereas since neither vitamin C nor uric acid was capable of scavenging lipophilic radicals localized within LDL particle efficiently, uric acid was not capable of sparing α-TOH, but ascorbate could spare α-TOH by reducing α-TO• radical. The reduction of α-TO• radical in LDL particles by ascorbate has been shown by an ESR study.[29,30] Thus, the fact that a compound spares vitamin E does not necessarily mean the compound is a synergist for vitamin E. Vitamin E and uric acid act as antioxidants independently, and their combination results simply in an additive effect on the oxidation of LDL induced by AAPH.

Many hydroquinones and catechols can act as potent radical-scavenging antioxidants, but they are readily autoxidized, which results in diminution of antioxidant capacity, both in rate and duration.[9,34] α-TOH suppressed the autoxidation of hydroquinones and catechols, while they in turn reduced α-TO• radical. Thus, their combination may exert a synergistic effect.

Reduction of Metal Ions by Vitamin E. Transition metal ions are accepted to contribute in the generation of free radicals in vivo. Above all, iron and copper in their lower valency states, Fe(II) and Cu(I), decompose hydroperoxides and hydrogen peroxide faster than their corresponding higher valency state ions, Fe(III) and Cu(II), respectively. In general, a potent antioxidant is a good reducing agent and is capable of reducing metal ions, which results in a pro-oxidant action of the antioxidant. The combination of iron and ascorbate is well known and has been used for initiating chain oxidation. The iron/ascorbate ratio determines whether ascorbate acts as a pro-oxidant or an antioxidant.[35]

α-TOH is also capable of reducing metal ions such as Fe(III) and Cu(II) and acts under certain circumstances as a pro-oxidant, and a cytotoxic effect of α-TOH has also been also reported.[36] However, the metal ions are sequestered by proteins in vivo, and we believe there has been no clear evidence which shows the deleterious effect of reduction of metal ions by α-TOH in vivo.

Activities of Vitamin E Homologues. Among the vitamin E homologues, α-TOH is by far the most abundant form found in vivo, since α-tocopherol transfer protein (α-TTP) selectively transports α-TOH into plasma.[37] It has also been found that γ-TOH, a major form of vitamin E in many plants and diet, is metabolized faster than α-TOH by cytochrome P450.[38,39] However, the role of other tocopherols and tocotrienols has received attention recently. Above all, it has been reported that γ-TOH exerts a higher antioxidant capacity than α-TOH under certain circumstances.[40] Furthermore, the action of tocotrienol as compared with tocopherol has also received much attention. Inconsistent relative reactivities toward radicals have been reported for tocotrienols, but it was confirmed that the reactivities decrease in the order α > β ∼ γ > δ-tocotrienol and that the corresponding tocotrienols and tocopherols have the same reactivities toward peroxyl radicals.[41] It is also noteworthy that tocotrienols are more readily incorporated into the culture cells than the corresponding tocopherols, making tocotrienols more potent than tocopherols.[42,43] However, the potency is determined primarily by the cellular concentrations.[43]

As described above, α-TOH reduces Fe(III) and Cu(II) to Fe(II) and Cu(I), respectively. Interestingly, however, it was found that β-, γ-, and δ-TOH did not reduce Cu(II) as rapidly. Tocotrienols behaved similarly to tocopherols; that is, only α-tocotrienol reduced Cu(II), but other tocotrienols did not.[41] It may be added that the phenolic compounds having two bulky *tert*-butyl groups at both ortho positions, such as BHT and BO-653, do not reduce Cu(II).[44]

Future Perspectives

Eighty years have passed since the discovery of vitamin E by Evans and Bishop, in 1922, as an essential nutrient for reproduction. The role of vitamin E against atherosclerosis has been the subject of extensive studies.[45] It is now accepted that the oxidative modification of LDL plays a pivotal role in the pathogenesis of atherosclerosis. This implies that inhibition of LDL oxidation should be effective for the prevention of progression of atherosclerosis. In fact, many, if not all, epidemiological and intervention studies suggest that dietary intake of vitamin E reduces the incidence of cardiovascular disease. Vitamin E may exert its effect by acting as a radical-scavenging antioxidant to inhibit the oxidation of LDL, which results in the suppression of the uptake of LDL by macrophages and also of the proatherogenic properties associated with oxidized LDL. It may be also possible for vitamin E to function by a mechanism which is not directly related to inhibition of oxidation. Such non-antioxidant function of vitamin E may be a result of specific cell signaling and gene regulation.[46] The role of vitamin E in cellular signaling, especially its biological significance, is certainly an important subject for future studies.

Vitamin E supplements are provided usually as the ester of acetate, nicotinate, or succinate. The characteristic action of α-tocopheryl succinate (α-TS) has received much attention recently. It has been shown that α-TS has a unique ability to selectively kill tumor cells, but not normal cells.[47] The specific functions of the individual types, isomers, and esters of vitamin E analogues should also be the subjects of future studies.

The question that originated in the 1980s was how vitamin E and other antioxidants cope with the oxidative stress induced by oxygen radicals. The now-emerging issue is the role of reactive oxygen and nitrogen species in beneficial as well as detrimental effects and also the modulatory function of antioxidants, including vitamin E, against such effects. A solid chemical approach is warranted.

We would like to thank many scientists and colleagues for their invaluable comments, discussion, and experimental work. We are also grateful to Eisai Co. for the generous gift of natural forms of vitamin E homologues.

References

(1) Niki, E. Free Radicals in the 1900's: from in Vitro to in Vivo. *Free Radical Res.* **2000**, *33*, 693−704.
(2) Many important papers could not be cited due to space limitations.
(3) Niki, E. Antioxidants in Relation to Lipid Peroxidation. *Chem. Phys. Lipids* **1987**, *44*, 227−253.
(4) Burton, G. W.; Ingold, K. U. Vitamin E−Application of the Principles of Physical Organic Chemistry to the Exploration of Its Structure and Function. *Acc. Chem. Res.* **1986**, *19*, 194−201.
(5) The partition coefficient of Trolox was measured as log $P = -0.97$. Since more than 99% of the liposomal suspensions are composed of water, most of the Trolox is assumed to be present in the aqueous phase.
(6) Tsuchihashi, H.; Kigoshi, M.; Iwatsuki, M.; Niki, E. Action of β-Carotene as an Antioxidant against Lipid Peroxidation. *Arch. Biochem. Biophys.* **1995**, *323*, 137−147.
(7) Niki, E.; Komuro, E.; Takahashi, M.; Urano, S.: Ito, E.; Terao, K. Oxidative Hemolysis of Erythrocytes and Its Inhibition by Free Radical Scavengers. *J. Biol. Chem.* **1988**, *263*, 19809−19814.
(8) Miki, M.; Tamai, H.; Mino, M.; Yamamoto, Y.; Niki, E. Free-Radical Chain Oxidation of Rat Blood Cells by Molecular Oxygen and Its Inhibition by α-Tocopherol. *Arch. Biochem. Biophys.* **1987**, *258*, 373−380.
(9) Shi, H.; Noguchi, N.; Niki, E. Comparative Study on Dynamics of Antioxidative Action of α-Tocopheryl Hydroquinone, Ubiquinol, and α-Tocopherol against Lipid Peroxidation. *Free Radical Biol. Med.* **1999**, *27*, 334−346 and references therein.
(10) Niki, E.; Takahashi, M.; Komuro, E. Antioxidant Activity of Vitamin E in Liposomal Membranes. *Chem. Lett.* **1986**, 1573−1576.
(11) Barclay, L. R. C.; Vinqvist, M. R.; Antunes, F.; Pinto, R. E. Antioxidant Activity of Vitamin E Determined in a Phospholipid Membrane by Product Studies: Avoiding Chain Transfer Reactions by Vitamin E Radicals. *J. Am. Chem. Soc.* **1997**, *119*, 5764−5765.
(12) Cullbertson, S. M.; Antunes, F.; Havrilla C. M.; Mione, G. L.; Porter, N. A. Determination of the alpha-tocopherol inhibition rate constant for peroxidation in low-density lipoprotein. *Chem. Res. Toxicol.* **2002**, *15*, 870−876.
(13) Barclay, L. R. C. 1992 Syntex Award Lecture−Model Biomembranes−Quantitative Studies of Peroxidation, Antioxidant Action, Partitioning, and Oxidative Stress. *Can. J. Chem.* **1993**, *71*, 1−16.
(14) Castle, L.; Perkins, M. J. Inhibition Kinetics of Chain-Breaking Phenolic Antioxidants in SDS Micelles. *J. Am. Chem. Soc.* **1986**, *108*, 6381−6382.
(15) Pryor, W. A.; Stickland, T.; Church, D. F. Comparison of the Efficiencies of Several Natural and Synthetic Antioxidants in Aqueous Sodium Dodecyl-Sulfate Micelle Solutions. *J. Am. Chem. Soc.* **1988**, *110*, 2224−2229.
(16) Valgimigli, L.; Banks, J. T.; Lusztyk, J.; Ingold, K. U. Solvent Effects on the Antioxidant Activity of Vitamin E. *J. Org. Chem.* **1999**, *64*, 3381−3383 and references therein.
(17) Takahashi, M.; Tsuchiya, J.; Niki, E. Scavenging of Radicals by Vitamin E in the Membranes as Studied by Spin Labeling. *J. Am. Chem. Soc.* **1989**, *111*, 6350−6353.

(18) Barclay, L. R. C.; Ingold, K. U. Autoxidation of Biological Molecules. 2. The Autoxidation of a Model Membrane. A Comparison of the Autoxidation of Egg Lecithin Phosphatidylcholine in Water and in Chlorobenzene. *J. Am. Chem. Soc.* **1981**, *103*, 6478−6485.
(19) Gotoh, N.; Noguchi, N.; Tsuchiya, J.; Morita, K.; Sakai, H.; Shimasaki, H.; Niki, E. Inhibition of Oxidation of Low-Density Lipoprotein by Vitamin E and Related Compounds. *Free Radical Res.* **1996**, *24*, 123−134.
(20) Bowry, V. W.; Ingold, K. U. The Unexpected Role of Vitamin E (Alpha-Tocopherol) in the Peroxidation of Human Low-Density Lipoprotein. *Acc. Chem. Res.* **1999**, *32*, 27−34. In this Account, the authors suggest the importance of water solubility in the context of radical transport.
(21) Takahashi, M.; Komuro, E.; Niki, E.; Tanaka, K. Action of Fatty Acid Esters of L-Ascorbic Acid as Antioxidants in Phosphatidylcholine Liposomal Membranes. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 679−684.
(22) Niki, E.; Kawakami, A.; Saito, M.; Yamamoto, Y.; Tsuchiya, J.; Kamiya, Y. Oxidation of Lipids. X. Effect of Phytyl Side Chain of Vitamin E on Its Antioxidant Activity. *J. Biol. Chem.* **1985**, *260*, 2191−2196.
(23) Matsuo, M.; Matsumoto, S.; Iitaka, Y.; Niki, E. Radical Scavenging Reactions of Vitamin E and Its Model Compound, 2,2,5,7,8-Pentamethyl-6-Chromanol, in a *tert*-Butylperoxyl Radical-Generating System. *J. Am. Chem. Soc.* **1989**, *111*, 7179−7185.
(24) Yamauchi, R.; Noro, H.; Shimoyamada, M.; Kato, K. Analysis of Vitamin E and Its Oxidation Products by HPLC with Electrochemical Detection. *Lipids* **2002**, *37*, 515−522 and references therein.
(25) Watanabe, A.; Noguchi, N.; Fujisawa, A.; Kodama, T.; Tamura, K.; Cynshi, O.; Niki, E. Stability and Reactivity of Aryloxyl Radicals Devived from a Novel Antioxidant BO-653 and Related Compounds. Effects of Substituents and Side Chain in Solution and Membranes. *J. Am. Chem. Soc.* **2000**, *122*, 5438−5442.
(26) Mukai, K.; Nishimura, M.; Kikuchi, S. Stopped-flow Investigation of the Reaction of Vitamin C with Tocopheroxyl Radical in Aqueous Triton X-100 Micellar Solutions. *J. Biol. Chem.* **1991**, *266*, 274−278.
(27) Niki, E.; Kawakami, A.; Yamamoto, Y.; Kamiya, Y. Oxidation of Lipids. VIII. Synergistic Inhibition of Oxidation of Phosphatidylcholine Liposome in Aqueous Dispersion by Vitamin E and Vitamin C. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1971−1975.
(28) Doba, T.; Burton, G. W.; Ingold, K. U. Antioxidant and Co-Antioxidant Activity of Vitamin-C. The Effect of Vitamin C, Either Alone or in the Presence of Vitamin-E or A Water-Soluble Vitamin-E Analog, upon the Peroxidation of Aqueous Multilamellar Phospholipid Liposomes. *Biochim. Biophys. Acta* **1985**, *835*, 298−303.
(29) Kagan, V. E.; Serbinova, E. A.; Forte, T.; Scita, G.; Packer, L. Recycling of Vitamin E in Human Low-Density Lipoproteins. *J. Lipid Res.* **1992**, *33*, 385−397.
(30) Kalyanaraman, B.; Darley-Usmar, V. M.; Wood, J.; Joseph, J.; Parthasarathy, S. Synergistic Interaction between the Probucol Phenoxyl Radical and Ascorbic Acid in Inhibiting the Oxidation of Low-Density Lipoprotein. *J. Biol. Chem.* **1992**, *267*, 6789−6795.
(31) Takahashi, M.; Tsuchiya, J.; Niki, E.; Urano, S. Action of Vitamin E as Antioxidant in Phospholipid Liposomal Membranes as Studied by Spin Label Technique. *J. Nutr. Sci. Vitaminol.* **1988**, *34*, 25−34.
(32) Waldeck, A. R.; Stocker, R. Radical-Initiated Lipid Peroxidation in Low Density Lipoproteins: Insights Obtained from Kinetic Modeling. *Chem. Res. Toxicol.* **1996**, *9*, 954−964.
(33) Sato, K.; Niki, E.; Shimasaki, H. Free Radical-Mediated Chain Oxidation of Low-Density Lipoprotein and Its Synergistic Inhibition by Vitamin E and Vitamin C. *Arch. Biochem. Biophys.* **1990**, *279*, 402−405.
(34) Miyauchi, K.; Urakami, T.; Abeta, H.; Shi, H.; Noguchi, N.; Niki, E. Action of Pyrroloquinolinequinol as an Antioxidant against Lipid Peroxidation in Solution. *Antiox. Redox Signal.* **1999**, *1*, 547−554,
(35) Yamamoto, K.; Takahashi, M.; Niki, E. Role of Iron and Ascorbic Acid in the Oxidation of Methyl Linoleate Micelles. *Chem. Lett.* **1987**, 1149−1152.
(36) Burkitt, M. J. A Critical Overview of the Chemistry of Copper-Dependent Low-Density Lipoprotein Oxidation: Roles of Lipid Hydroperoxides, Alpha-Tocopherol, Thiols, and Ceruloplasmin. *Arch. Biochem. Biophys.* **2001**, *394*, 117−135 and references therein.
(37) Hosomi, A.; Arita, M.; Sato, Y.; Kiyose, C.; Ueda, T.; Igarashi, O.; Arai, H.; Inoue, K. Affinity for Alpha-Tocopherol Transfer Protein as a Determinant of the Biological Activities of Vitamin E Analogs. *FEBS Lett.* **1997**, *409*, 105−108.
(38) Swanson, J. E.; Ben, R. N.; Burton, G. W.; Parker, R. S. Urinary Excretion of 2,7,8-Trimethyl-2-(beta-Carboxyethyl-6-hydroxychroman is a Major Route of Elimination of Gamma-Tocopherol in Humans. *J. Lipid Res.* **1999**, *40*, 665−671.
(39) Birringer, M.; Drogan, D.; Brigelius-Flohe, R. Tocopherols are Metabolized in HepG2 Cells by Side Chain Omega-Oxidation and Consecutive Beta-Oxidation. *Free Radical Biol. Med.* **2001**, *31*, 226−232.
(40) Jiang, Q.; Christen, S.; Shigenaga, M. K.; Ames, B. N. γ-Tocopherol, the Major Form of Vitamin E in the US Diet, Deserves More Attention. *Am. J. Clin. Nutr.* **2001**, *74*, 714−722.
(41) Yoshida, Y.; Niki, E.; Noguchi, N. Comparative Study on the Action of Tocopherols and Tocotrienols as Antioxidant: Chemical and Physical Effects. *Chem. Phys. Lipids* **2003**, *123*, 63−75.
(42) Sen, C. K.; Khanna, S.; Roy, S.; Packer, L. Molecular Basis of Vitamin E Action. *J. Biol. Chem.* **2000**, *275*, 13049−13055.
(43) Noguchi, N.; Hanyu, R.; Nonaka, A.; Okimoto Y.; Kodama, T. Inhibition of THP-1 Cell Adhesion to Endothelial Cells by α-Tocotrienol is Dependent on Intracellular Concentration of the Antioxidants. *Free Radical Biol. Med.* **2003**, *34*, 1614−1620.
(44) Yoshida, Y.; Noguchi, N.; Watanabe, A.; Iwaki, Y.; Yanagisawa, M.; Kodama, T.; Cynshi, O.; Niki, E. Antioxidant Action of 2,2,4,6-tetra-Substituted 2,3-dihydro-5-hydroxybenzofuran against Lipid Peroxidation: Effects of Substituents and Side Chain. *Free Radical Res.* **2002**, *36*, 1171−1178.
(45) Pryor, W. A. Vitamin E and Heart Disease: Basic Science to Clinical Intervention Trials. *Free Radical Biol. Med.* **2000**, *28*, 141−164.
(46) Ricciarelli, R.; Zingg, J.-M.; Azzi, A. The 80th Anniversary of Vitamin E: Beyond Its Antioxidant Properties. *Biol. Chem.* **2002**, *383*, 457−465.
(47) Kogure, K.; Manabe, S.; Hama, S.; Tokumura, A.; Fukuzawa, K. Potentiation of Anti-cancer Effect by Intravenous Administration of Vesiculated α-Tocopheryl Hemisuccinate on Mouse Melanoma in Vivo. *Cancer Lett.* **2003**, *192*, 19−24.

AR030069M