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Dynamics of Action of Bisphenol as Radical-Scavenging Antioxidant against Lipid Peroxidation in Solution and Liposomal Membranes

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Probucol is used commercially as an antiatherogenic drug. Bisphenol is formed *in vivo* as a metabolite of probucol. The structure of bisphenol suggests the antioxidant function but its capacity has not been studied in detail. In the present study, dynamics of the antioxidant action of bisphenol were studied in several model systems and compared with those of probucol and α-tocopherol. The reactivity toward radicals and antioxidant activity of bisphenol *per se* were found to be much smaller than those of α-tocopherol or N,N′-diphenyl-*p*-phenylenediamine (DPPD) but stronger than probucol. However, bisphenol spared α-tocopherol in the oxidation of phosphatidylcholine liposomal membranes and it spared DPPD and acted as a synergist against the oxidant of methyl linoleate in solution. These results imply that bisphenol may act as a potent antioxidant in combination with other antioxidants.

Keywords: Antioxidant, bisphenol, probucol, α-tocopherol, lipid peroxidation, free radicals

Abbreviations: AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; AMVN, 2,2′-azobis(2,4-dimethylvaleronitrile); DPPD, N,N′-diphenyl-*p*-phenylenediamine; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ESR, electron spin resonance; HPLC, high pressure liquid chromatography; PC, phosphatidylcholine; PMC, 2,2,5,7,8-pentamethyl-6-chromanol; WHHL, Watanabe heritable hyperlipidemic

INTRODUCTION

There is an increasing amount of experimental and clinical evidence which suggests the involvement of active oxygen species in various diseases, cancer and aging, and consequently the role of antioxidants against such oxidative stress has received much attention. For example, it is now accepted that the oxidative modification of low density lipoprotein (LDL) is an important initial event in the pathogenesis of atherosclerosis and inhibition of such oxidation by antioxidant continues to be of major interest.[1] It has been shown that probucol, 4,4′-(isopropylidenedithio)bis(2,6-di-*tert*-butylphenol), prevents the development of atherosclerotic lesion in Watanabe heritable hyperlipidemic (WHHL) rabbit,[2–4] an animal model of familial hypercholesterolemia. It was originally prescribed for treatment of

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atherosclerosis based on its lipid-lowering activity, but it has been suggested that its efficacy may also be attributable to its antioxidant activity.[5–7] Furthermore, it has been reported that probucol prevents restenosis following angioplasty.[8,9] The oxidation of probucol is known to give diphenoquinone (3,3′5,5′-tetra-*tert*-butyl-4,4′-diphenoquinone) as a metabolite[5] and considerable amount of bisphenol (4,4′-bis(2,6-di-*tert*-butylphenol)), a reduced form of diphenoquinone, has been found in LDL isolated from rabbit and mouse.[10,11] We have also observed considerable concentrations of bisphenol and diphenoquinone in LDL isolated from WHHL and wild-type rabbits fed with probucol (unpublished results). From the chemical structure, bisphenol is anticipated to exert antioxidant function, but its activity has not been studied in detail. This prompted us to study the antioxidant action of bisphenol in several model systems. It was found that bisphenol, although less potent that α-tocopherol chemically exerted interesting mixing effect with α-tocopherol and N,N′-diphenyl-*p*-phenylenediamine (DPPD).

MATERIALS AND METHODS

Materials

Methyl linoleate purchased from Sigma Chemical Ind. (St. Louis, MO) was purified with an HPLC using semipreparative LC-18 column (10 × 250 mm, Supelco, Tokyo). Soybean phosphatidylcholine (PC) was also obtained from Sigma and purified with silica-gel and alumina columns before use. Dimyristoyl PC obtained from Sigma was used as received. Probucol was kindly supplied from Daiichi Pharmaceutical Co. Ltd. (Tokyo) and (2R,4′R,8′R)-α-tocopherol and 2,2,5,7,8-pentamethyl-6-chromanol (PMC) from Eisai Co., Ltd. (Tokyo), respectively. 2,2′-Azobis-(2-amidinopropane) dihydrochloride (AAPH) and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) used as a radical-generating initiator were obtained from Wako Pure Chemical Ind. (Osaka). All other chemicals were those of the highest grade available commercially and used without further purification.

Procedures

The reactivities of antioxidant compounds toward galvinoxyl and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were measured with a spectrophotometer equipped with a rapid-mixing stopped-flow apparatus (RX-1000, Applied Photophysics) by following the decrease in maximum absorption of galvinoxyl at 429 nm and of DPPH at 520 nm.

The oxidation of methyl linoleate and soybean PC was performed and lipid peroxidation products were analyzed as described previously.[12] α-Tocopherol was analyzed with an HPLC equipped with an electrochemical detector (set at 800 mV) using LC-18 column (Supelco, 4 × 250 mm, 5 μm) and methanol/*tert*-butyl alcohol (90/10 by vol) containing 50 mM $NaClO_4$ as an eluent at a flow rate of 1.0 ml/min. Probucol, bisphenol and diphenoquinone were analyzed with an HPLC by an absorption at 240 and 420 nm using LC-18 column (Supelco, 4 × 250 mm, 5 μm). The eluent was acetonitrile/H_2O (95/5 by vol) at a flow rate of 1.5 ml/min. In the oxidation of methyl linoleate, probucol and bisphenol were analyzed with an electrochemical detector (set at 1050 mV) under the same HPLC conditions as those for α-tocopherol.

The ESR spectra were recorded on an X-band JEOL FE1X spectrometer under the following conditions: magnetic field, 329 ± 5 mT; sweep time 8 mT/min; microwave power, 1 mW; modulation frequency, 100 kHz; and modulation amplitude, 0.02 mT.

RESULTS

Reactivities of Bisphenol, Probucol and α-tocopherol toward Galvinoxyl and DPPH

In order to estimate the potential of bisphenol, as a radical-scavenging antioxidant, the reactivities

toward galvinoxyl and DPPH were measured and compared with those of probucol and α-tocopherol. Galvinoxyl and DPPH are well-known stable radicals and have been often used to estimate the reactivities of various antioxidants. Both galvinoxyl and DPPH have a strong absorption in the visible region, 429 and 520 nm, respectively, which is bleached by the reaction with a hydrogen-donating antioxidant. The results shown in Figure 1 suggest that bisphenol is more reactive than probucol but much less reactive than α-tocopherol toward either galvinoxyl or DPPH.

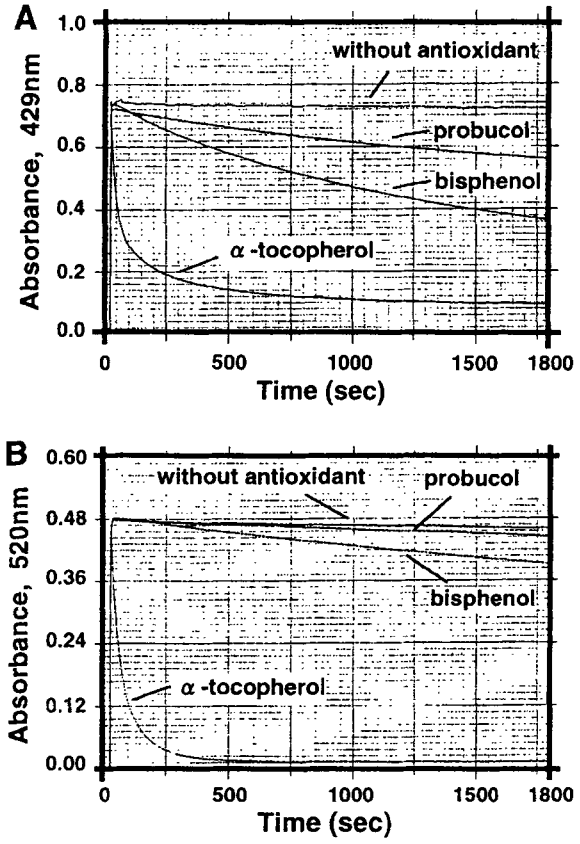


FIGURE 1 Reaction of bisphenol, probucol and α-tocopherol with (A) galvinoxyl and (B) DPPH. Bisphenol, probucol or α-tocopherol (5.0 μM) were incubated with galvinoxyl (5.0 μM) or DPPH (5.0 μM) in acetonitrile at 37°C in air and the decrease in absorption at 429 nm (A) and 520 nm (B) was followed by a stopped-flow spectrophotometer as described in Methods.

Inhibition of Lipid Peroxidation by Bisphenol, Probucol and α-Tocopherol in Organic Solution and in Aqueous Dispersions

The oxidation of methyl linoleate in solution induced by free radicals proceeds by a straightforward mechanism to give four kinds of conjugated diene hydroperoxides.[13] The rate of oxidation can be followed quantitatively by measuring either formation of methyl linoleate hydroperoxides, accumulation of conjugated diene, oxygen uptake, or substrate consumption. Therefore, this is a convenient and appropriate experimental model system for quantitatively assessing the antioxidant activities. In the present study, methyl linoleate was oxidized in acetonitrile at 37°C in air in the presence of AMVN with and without antioxidant. AMVN, a lipid-soluble radical initiator, generates chain-initiating radicals at a constant rate.[14] The effects of bisphenol, probucol, diphenoquinone, and α-tocopherol against the oxidation of methyl linoleate in acetonitrile at 37°C are shown in Figure 2. In Figure 3 is shown the effect of bisphenol concentration and the kinetic data are summarized in Table I. The data for DPPD are also included in Table I. In the absence of any antioxidant, methyl linoleate hydroperoxides increased at a constant rate without any induction period. Probucol and bisphenol retarded the oxidation, but their antioxidant effects were much smaller than that of α-tocopherol or DPPD, which inhibited the oxidation almost completely and produced a clear induction period. Bisphenol suppressed the oxidation in a dose-dependent manner (Figure 3). Diphenoquinone exerted little antioxidative effect and it was not consumed (data not shown). The rate of consumption of antioxidant decreased in the order of α-tocopherol > DPPD > bisphenol > probucol. The antioxidant potencies correlated well with their reactivities toward galvinoxyl or DPPH.

The reactivity of the antioxidant toward peroxyl radical determines the rate of oxidation, that is, how much does the antioxidant reduce the

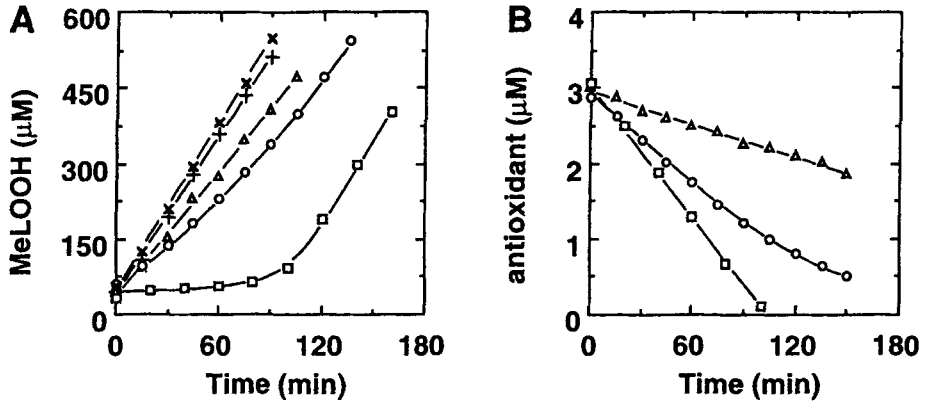


FIGURE 2 Inhibition of oxidation of methyl linoleate by antioxidant in solution. Methyl linoleate (75.5 mM) was oxidized with AMVN (0.20 mM) in acetonitrile at 37°C in air in the absence and presence of antioxidant (3.0 μM) and (A) accumulation of methyl linoleate hydroperoxide (MeLOOH) and (B) consumption of antioxidant were followed with an HPCL as described in Materials and Methods. ×: Without antioxidant; ○: bisphenol; △: probucol; □: α-tocopherol; +: diphenoquinone.

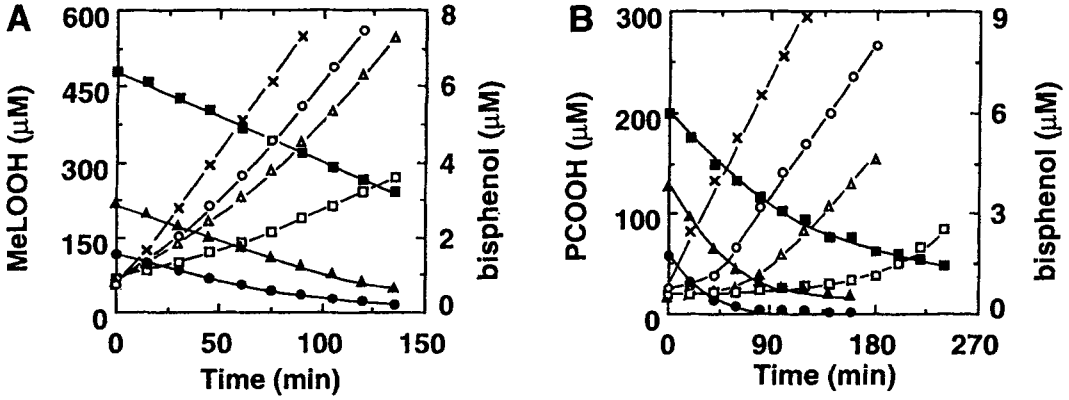


FIGURE 3 Effect of bisphenol on the oxidation of methyl linoleate in acetonitrile and soybean PC liposomes. (A) Methyl linoleate (75.5 mM) was oxidized with AMVN (0.20 mM) in the absence and presence of bisphenol and the formation of methyl linoleate hydroperoxide (open mark) and consumption of bisphenol (solid mark) were followed with an HPLC. Bisphenol: ×: 0; ○, ●: 1.5; △, ▲: 3.0; □, ■: 6.0 μM. (B) Soybean PC (2.83 mM) multilamellar vesicles containing AMVN (1.0 mM) and bisphenol were incubated in aqueous dispersions at 37°C in air. Open mark, PC hydroperoxides (PCOOH); solid mark, bisphenol; ×: 0; ○, ●: 2.0; △, ▲: 4.0; □, ■: 6.0 μM.

TABLE I Inhibition of oxidation of methyl linoleate (75.5 mM) by bisphenol, probucol, α-tocopherol and DPPD (3.0 μM) induced by AMVN (0.20 mM) in acetonitrile at 37°C in air

	Antioxidant				
	None	Probucol	Bisphenol	α-Tocopherol	DPPD
R_0, R_{IH}, nM/s	91.8	60.8	41.3	2.25	5.03
kcl	118	78.3	53.2	2.90	6.48
R_{IH}/R_0		0.66	0.45	0.025	0.055
$-d[IH]/dt$, nM/s		0.154	0.350	0.498	0.382
n		5.0	2.2	1.6	2.0

R_0 and R_{IH} denote the rate of methyl linoleate hydroperoxide formation without antioxidant and with antioxidant, respectively. $-d[IH]/dt$ is a rate of consumption of antioxidant. The stoichiometric number n was calculated from the ratio (rate of chain initiation)/(rate of antioxidant consumption). The rate of chain initiation was obtained as 7.76×10^{-10} M/s from the induction period produced by DPPD by assuming n for DPPD as 2.0.

rate of oxidation. It is also required for the antioxidant to suppress the oxidation for a long duration. This is determined by a stoichiometric number n, that is the number of free radicals scavenged by each antioxidant molecule. This stoichiometric number n can be determined from the rates of consumption of antioxidant and radical flux. The rate of consumption of antioxidant was measured in the presence of AMVN in acetonitrile at 37°C in air. It was confirmed that the rate of consumption of antioxidant was independent of its concentration and directly proportional to AMVN concentration (data not shown). The rate of radical flux from AMVN was measured from the induction period produced by DPPD assuming its stoichiometric number as 2.0.[15] The results are included in Table I. The stoichiometric numbers for bisphenol and probucol were obtained as 2.2 and 5.0, respectively, while that for α-tocopherol was 1.6.

The effects of bisphenol, probucol, α-tocopherol and PMC on the oxidation of soybean PC liposomal membranes are shown in Figures 3 and 4. Multilamellar and unilamellar vesicles were used for the oxidation induced by AMVN and AAPH, respectively. AMVN and antioxidant were incorporated into the membranes by dissolving them with PC, while AAPH was added as an aqueous solution after preparation of unilamellar vesicles. In contrast to the oxidation of

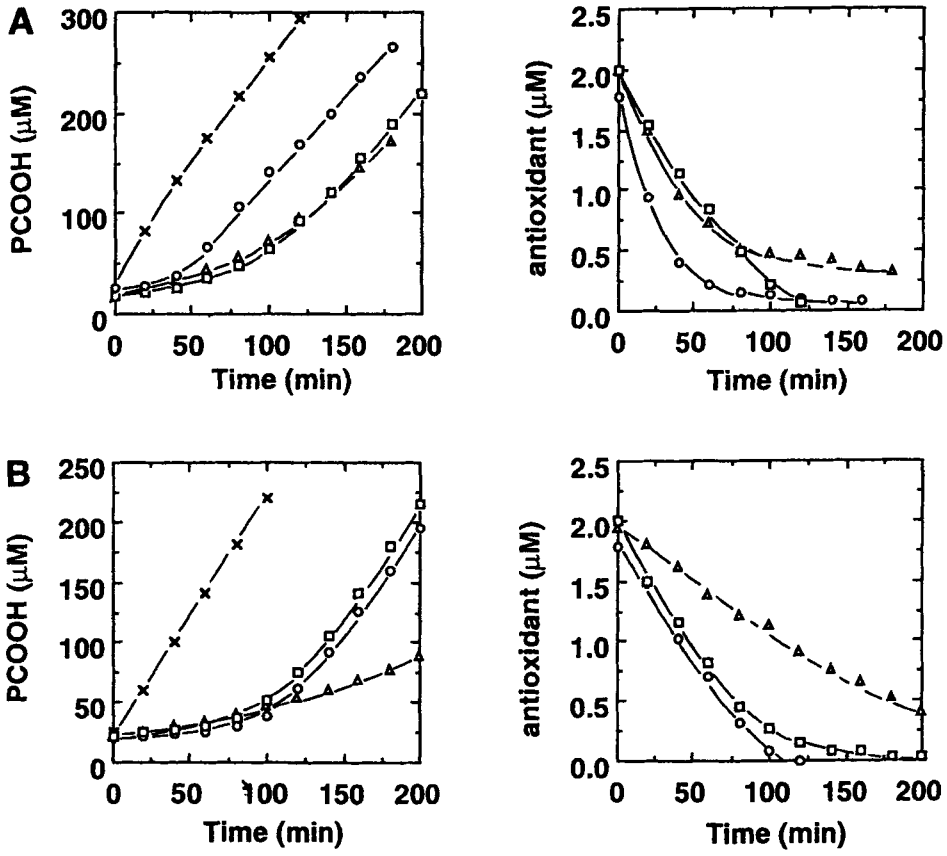


FIGURE 4 Inhibition of oxidation of PC liposomal membranes by antioxidant. Soybean PC (2.83 mM) liposomal membranes containing either bisphenol, probucol or α-tocopherol (2.0 μM) were incubated at 37°C in air and the formation of PC hydroperoxides (PCOOH) and consumption of antioxidant were followed with an HPLC. (A) Multilamellar vesicles. AMVN was incorporated into liposomal membranes. (B) Unilamellar vesicles. AAPH was added as an aqueous solution after preparation of vesicles. ×: Without antioxidant; ○: bisphenol; △: probucol; □: α-tocopherol.

methyl linoleate in homogeneous solution where α-tocopherol exerted by far the higher antioxidant activity than bisphenol and probucol, they exerted similar antioxidant capacity in liposomal membranes (Figure 4). Bisphenol inhibited the PC oxidation dose-dependently (Figure 3). PMC inhibited the oxidation almost completely (data not shown).

Inhibition of Lipid Peroxidation by a Combination of Antioxidants

The effect of mixing of antioxidants on the lipid peroxidation was then studied. The results of oxidation of methyl linoleate in acetonitrile inhibited by a combination of bisphenol or probucol with α-tocopherol are shown in Figure 5. The mixing effect was additive rather than synergistic. Only α-tocopherol was consumed at the initial stage, while bisphenol and probucol were spared and then decreased after α-tocopherol was almost depleted (Figure 5). The oxidation was inhibited efficiently by α-tocopherol and, after its disappearance, the oxidation proceeded and the antioxidant was consumed at the similar rates as those inhibited by bisphenol or probucol alone.

Interestingly however, quite a different results were observed in the liposomal membranes from

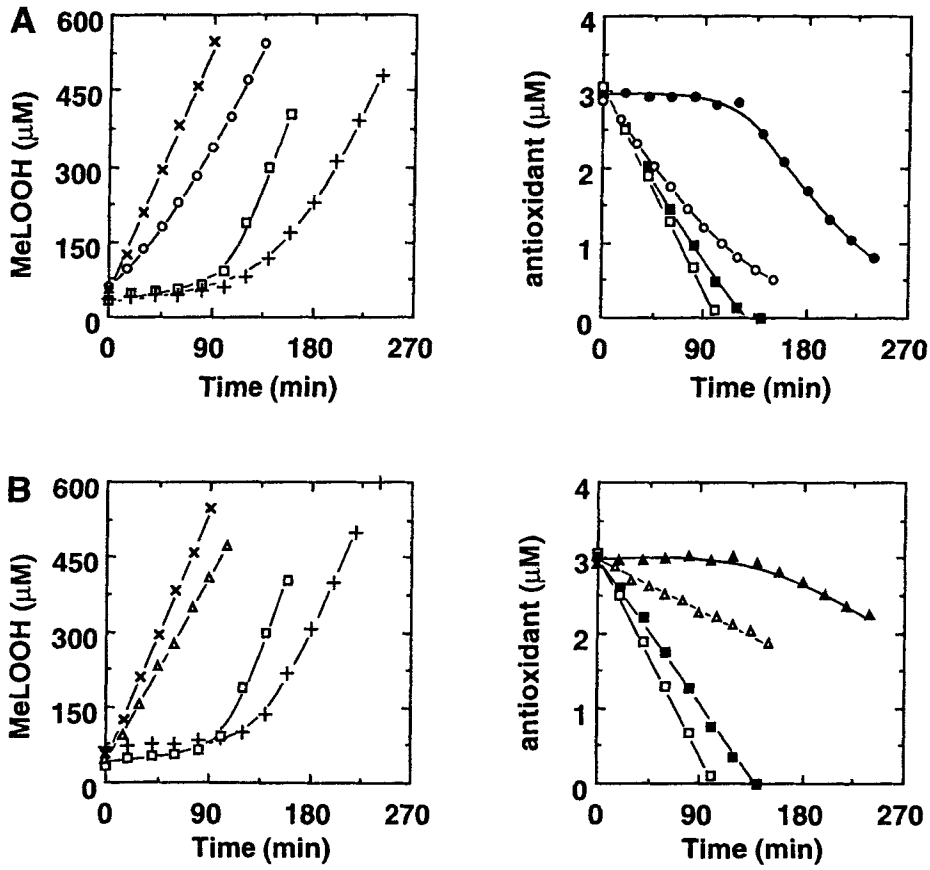


FIGURE 5 Inhibition of oxidation of methyl linoleate by a combination of antioxidant in solution. Methyl linoleate (75.5 mM) was oxidized with AMVN (0.20 mM) in the absence and presence of α-tocopherol with either (A) bisphenol or (B) probucol (3.0 μM each) in acetonitrile at 37°C in air and formation of methyl linoleate hydroperoxide (MeLOOH) and consumption of antioxidant were followed with an HPLC. ×: Without antioxidant; ○, ●: bisphenol; □, ■: α-tocopherol; △, ▲: probucol +: α-tocopherol and bisphenol (A) or probucol (B). The open and solid marks for the antioxidant consumption show the results when the antioxidant was used alone and in combination, respectively.

those in organic solution. When soybean PC liposomal membranes were oxidized in the presence of both bisphenol and α-tocopherol, bisphenol was consumed predominantly at first and α-tocopherol was spared independent of the site of initial radical generation (Figures 6 and 7). When the oxidation was inhibited by a combination of probucol and α-tocopherol, probucol was always spared by α-tocopherol, although the sparing efficacy was lower in membranes than in homogeneous solution. The results of oxidation in the presence of bisphenol, probucol and α-tocopherol all together in different media are shown in Figure 8. The antioxidant was consumed in acetonitrile solution in the order of α-tocopherol > bisphenol > probucol, while the order was bisphenol > α-tocopherol > probucol in soybean PC liposomal membranes independent of the site of initial radical production. That bisphenol was consumed faster than α-tocopherol in the liposomal membranes is interesting, considering that bisphenol is less reactive than α-tocopherol toward radicals and that bisphenol was spared by α-tocopherol in the homogeneous solution.

It was also found that the relative rate of consumption of antioxidants was dependent on the type of PC of the membranes. When bisphenol and α-tocopherol were incorporated simultaneously into dimyristoyl PC liposomal membranes

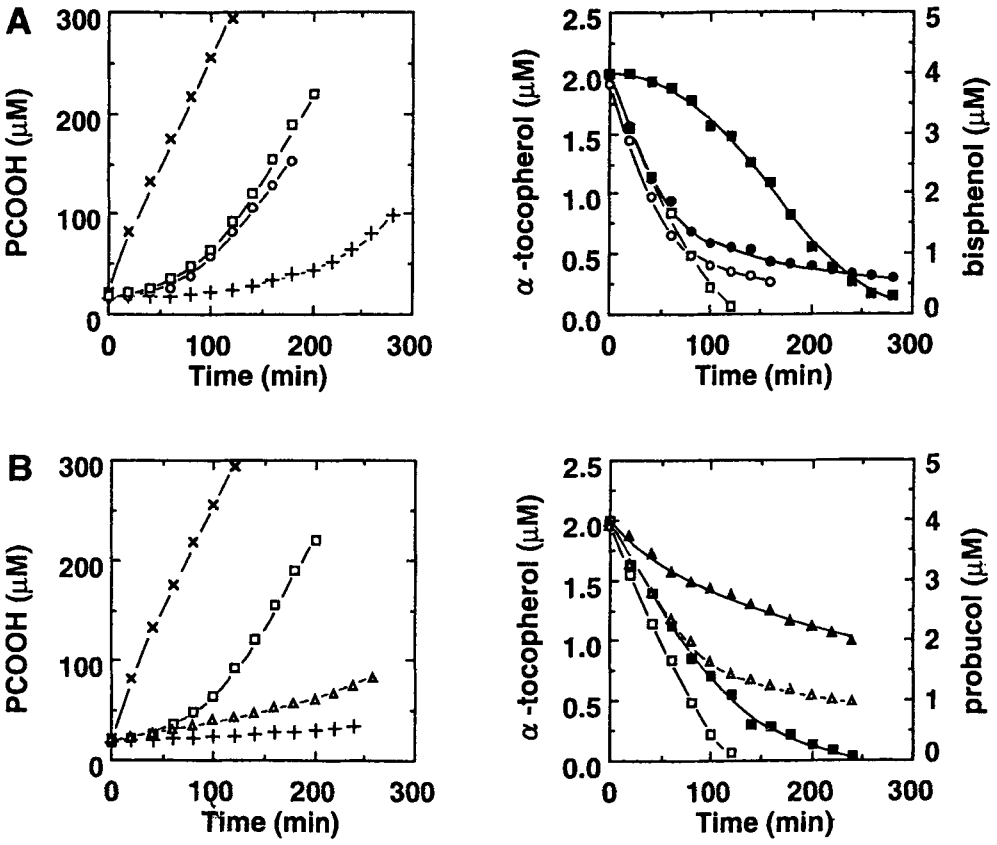


FIGURE 6 Inhibition of oxidation of soybean PC liposomal membranes by a combination of α-tocopherol and bisphenol or probucol. Soybean PC (2.83 mM) multilamellar vesicles containing AMVN (1.0 mM), α-tocopherol and (A) bisphenol or (B) probucol (2.0 μM) were incubated at 37°C in air in aqueous dispersions and the formation of PC hydroperoxides and consumption of antioxidants were followed with an HPLC. Open and solid marks show the results with one of the antioxidants alone and their combination, respectively. The symbols are the same as in Figure 5.

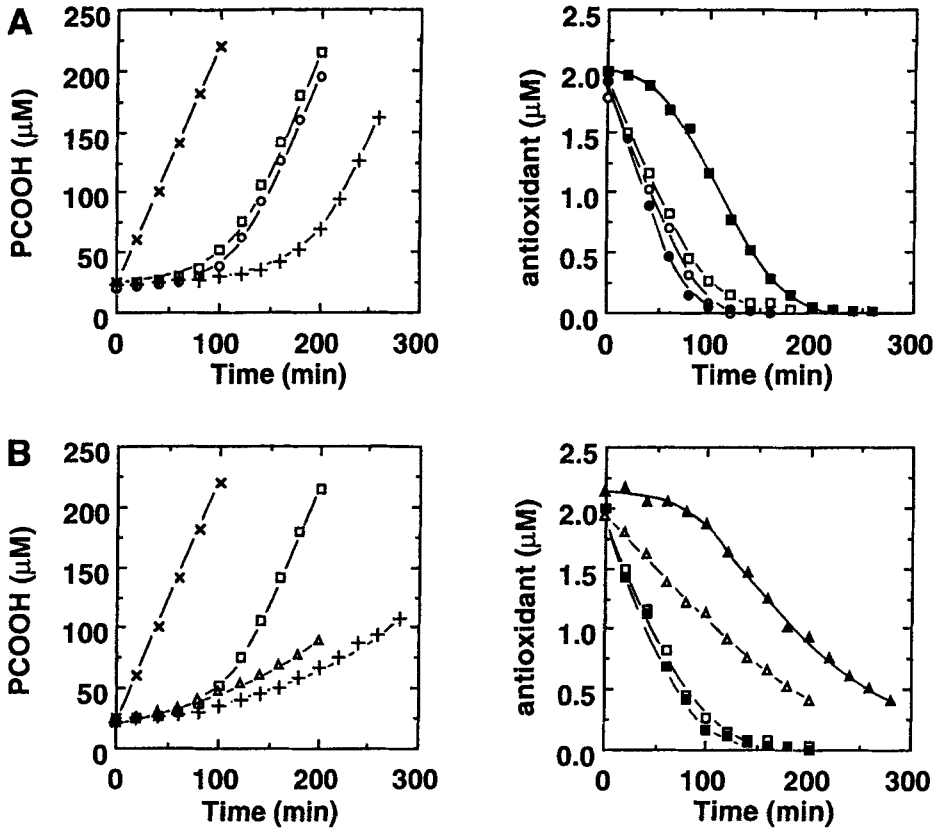


FIGURE 7 Inhibition by a combination of antioxidant of the oxidation of soybean PC (2.83 mM) unilamellar vesicles induced by AAPH (5.0 mM) at 37°C in aqueous dispersions. The conditions and marks are the same as those in Figure 6 except AAPH was used instead of AMVN.

containing AMVN, α-tocopherol was spared by bispherol as observed in soybean PC membranes. On the other hand, it was found that, when they were incorporated into dimyristoyl PC unilamellar vesicles and AAPH was used, α-tocopherol was consumed faster than bisphenol (Figure 9). These results suggest that α-tocopherol whose active phenolic group is localized at the surface of the membranes is capable of scavenging aqueous radicals, whereas bisphenol having bulky and lipophilic *tert*-butyl groups is buried in the membranes.

The interaction between ascorbate and lipophilic antioxidants is also important. It has been shown that ascorbate does not act as a potent antioxidant against lipid peroxidation taking place within the membranes but it exerts an efficient antioxidant activity in combination with α-tocopherol by reducing α-tocopheroxyl radical and regenerating α-tocopherol.[16,17] It was found in the present study that ascorbate spared bisphenol almost completely during the oxidation in methanol solution, but it did not spare bisphenol efficiently in the oxidation of soybean PC liposomes induced by AMVN (Figure 10).

Another interesting interaction was found in the combination of bisphenol and DPPD. As shown above, bisphenol is chemically less reactive toward radical than DPPD, and bisphenol exerted less potent antioxidant activity than DPPD against the oxidation of methyl linoleate in acetonitrile (Table I). However, the combination of bisphenol and DPPD exerted a synergistic effect and interestingly bisphenol spared DPPD

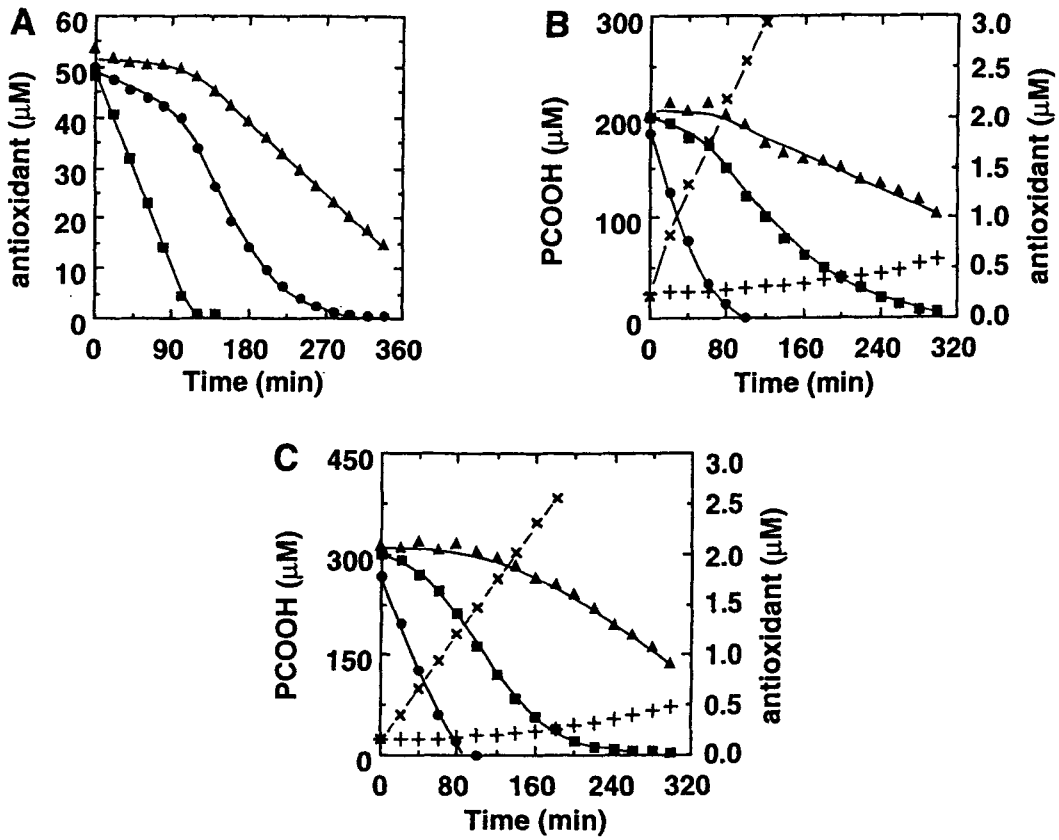


FIGURE 8 Inhibition of oxidation by a combination of bisphenol, probucol and α-tocopherol. (A) Bisphenol, probucol and α-tocopherol (50 μM each) were incubated in the presence of AMVN (5.0 mM) in acetonitrile at 37°C in air and their consumption was followed with an HPLC. Oxidation of soybean PC (2.83 mM) liposomes, (B) multilamellar vesicles containing AMVN (1.0 mM) and (C) unilamellar vesicles induced by AAPH (5.0 mM), were performed in the presence of bisphenol, probucol and α-tocopherol (2.0 μM each). ×: Without antioxidant; +: with three antioxidants; ●: bisphenol; ▲: probucol; ■: α-tocopherol.

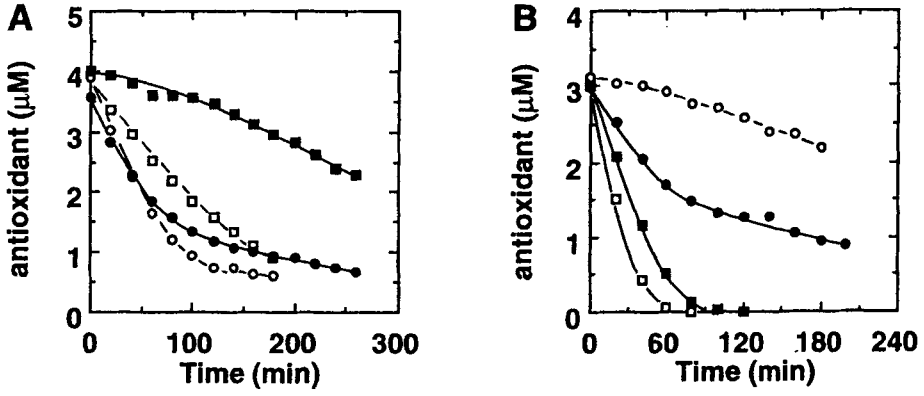


FIGURE 9 Consumption of bisphenol (○, ●) and α-tocopherol (□, ■) incorporated into dimyristoyl PC (2.83 mM) liposomal membranes induced by (A) AMVN (1.0 mM) or (B) AAPH (5.0 mM). (A): multilamellar vesicles; (B): unilamellar vesicles. Open and solid symbols show the results with either bisphenol or α-tocopherol alone and with both bisphenol and α-tocopherol, respectively.

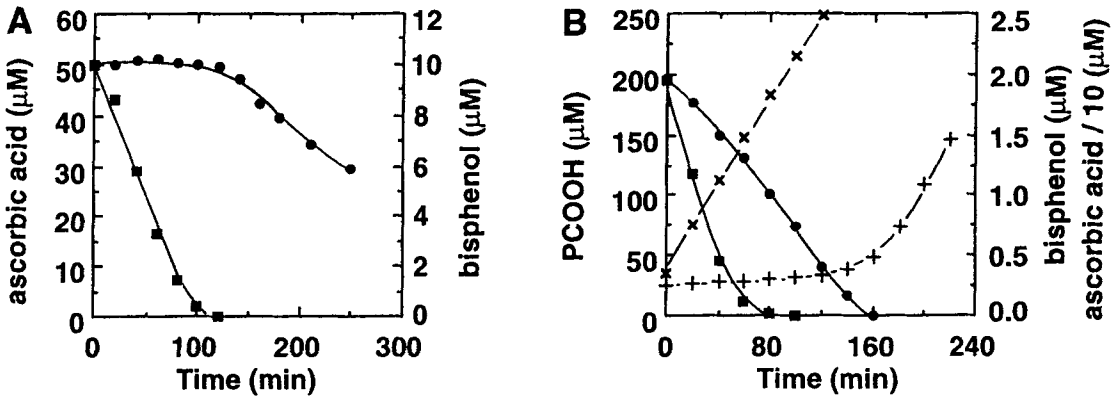


FIGURE 10 Effect of ascorbic acid on the consumption of bisphenol during the oxidation of (A) methyl linoleate in methanol solution and (B) soybean PC (2.83 mM) liposomal membranes at 37°C in air. (A) bisphenol = 10 μM; ascorbic acid = 50 μM; AMVN = 1.0 mM (B) bisphenol = 2.0 μM; ascorbic acid = 20 μM; AMVN = 0.50 mM. ●: Bisphenol; ■: ascorbic acid; ×: PCOOH with ascorbic acid alone; +: PCOOH with both bisphenol and ascorbic acid.

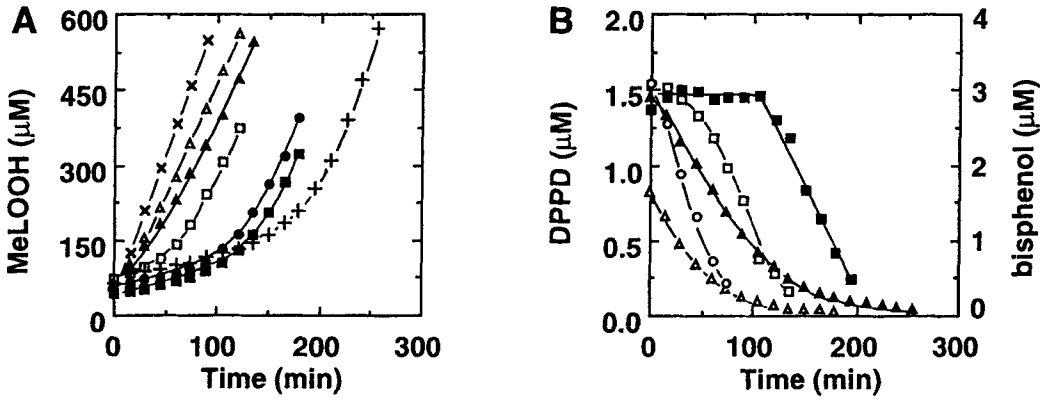


FIGURE 11 Inhibition of oxidation of methyl linoleate by bisphenol, DPPD, and their combination. Methyl linoleate (75.5 mM) was oxidized with AMVN (0.20 mM) in acetonitrile at 37°C in air in the absence and presence of either bisphenol or DPPD and their combination and (A) the formation of methyl linoleate hydroperoxide (MeLOOH) and (B) consumption of antioxidant were followed. (A) ×: Without antioxidant; □: 1.5 μM DPPD; ■: 3.0 μM DPPD; △: 1.5 μM bisphenol; ▲: 3.0 μM bisphenol; ●: 1.5 μM bisphenol + 1.5 μM DPPD; +: 3.0 μM bisphenol + 1.5 μM DPPD. (B) ▲, △: bisphenol with 1.5 μM DPPD; ○, □, ■: DPPD with 0, 1.5, 3.0 μM bisphenol, respectively.

almost completely, that is, only bisphenol was consumed at the initial stage and DPPD began to decrease only after most of bisphenol disappeared (Figure 11).

Oxidation Products from Bisphenol

The identification of the oxidation products from bisphenol is important for understanding its antioxidation mechanism. When bisphenol was reacted with galvinoxyl or DPPH in acetonitrile, diphenoquinone was formed in quantitative yield. On the other hand, diphenoquinone was not detected in the oxidation of methyl linoleate induced by AMVN in the presence of bisphenol, but two unknown products were observed in an HPLC analysis. As shown in Figure 12, the unknown product A increased concomitantly with a decrease in bisphenol and then started to decrease when bisphenol was disappeared

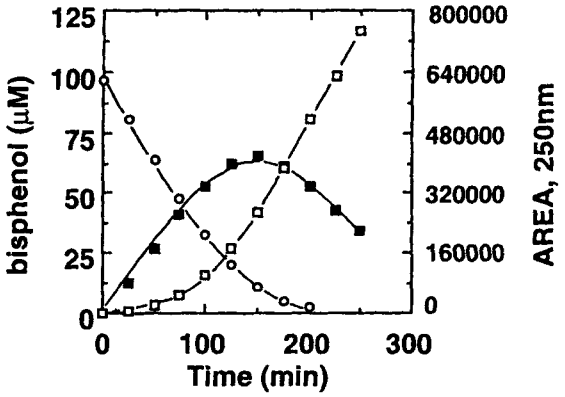


FIGURE 12 The oxidation products from bisphenol. Bisphenol (100 μM) was incubated with 5 mM AMVN in acetonitrile at 37°C under air and the reaction mixture was analyzed with an HPLC as described in Materials and Methods. ○: bisphenol; ■: unknown A; □: unknown B.

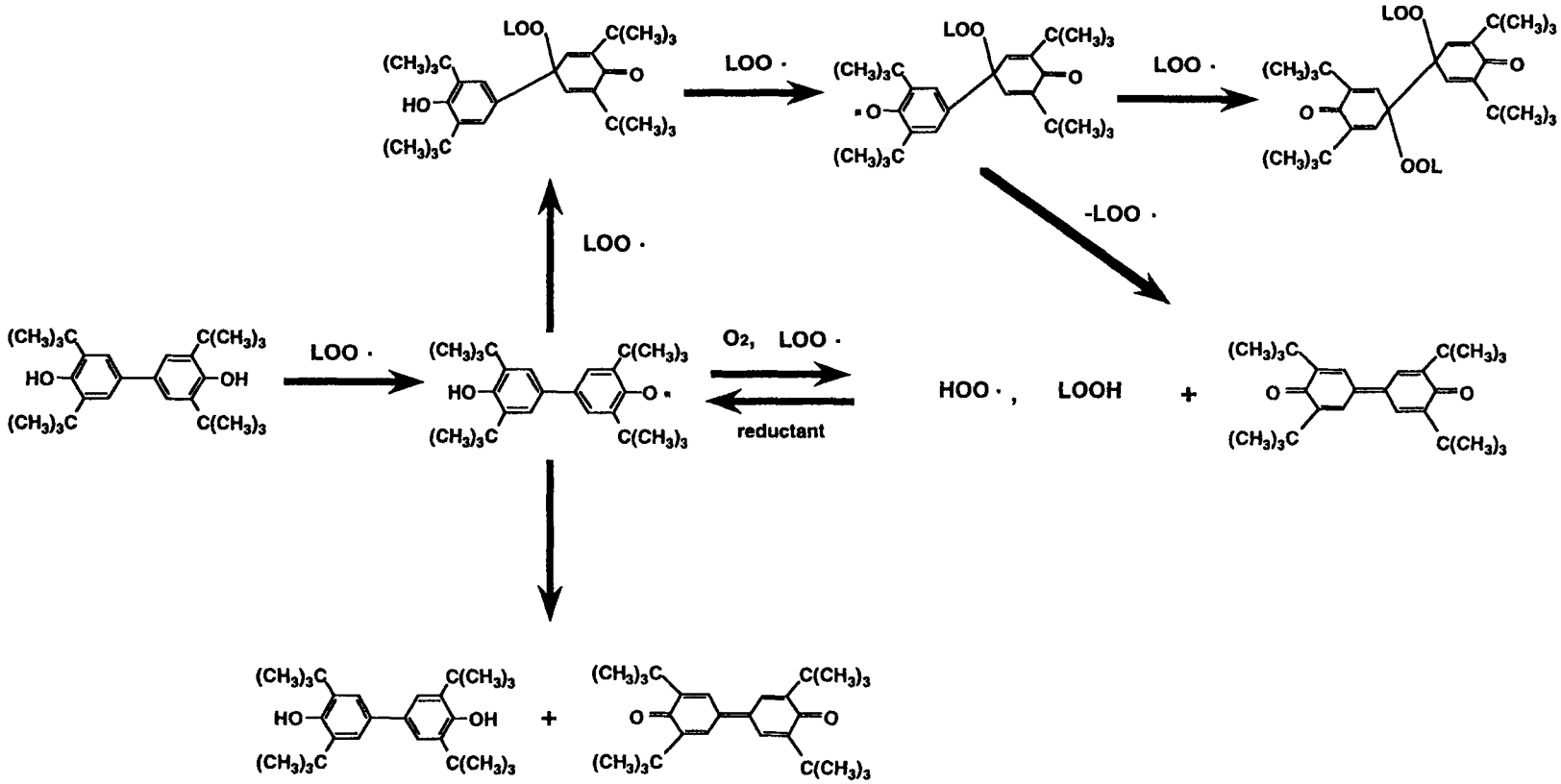
completely. Another unknown product B increased with time after some lag time. Little diphenoquinone was formed. When soybean PC, bisphenol and AMVN were incubated in methanol/*tert*-butyl alcohol solution, diphenoquinone was not formed either. On the other hand, diphenoquinone was formed almost quantitatively from bisphenol when it was oxidized by AMVN in soybean PC liposomal membranes (data not shown).

DISCUSSION

The above results show that bisphenol, a metabolite of an antiatherogenic drug probucol, is an antioxidant having a modest but higher activity than probucol and that it exerts characteristic effect in combination with α-tocopherol and DPPD. The reactivities of bisphenol toward galvinoxyl and DPPH were much smaller than those of α-tocopherol and, in accordance with this, bisphenol exerted much weaker antioxidant activity than α-tocopherol against the oxidation of methyl linoleate in organic solution, where the antioxidant potency is determined by the chemical reactivity of the antioxidant. In the membranes, bisphenol exerted similar antioxidant activity as α-tocopherol, which may be ascribed primarily to a reduced antioxidant activity of α-tocopherol in the membranes as compared with that in homogeneous solution.[18–20]

Apparently, bisphenol must scavenge radicals by donating its phenolic hydrogen to give phenoxyl radical. Although the phenoxyl radicals derived from 2,6-di-*tert*-butyl-4-methylphenol and probucol are stable and their ESR spectra can be easily observed, the ESR spectrum of the phenoxyl radical derived from bisphenol disappeared rapidly. Probably, the phenoxyl radical derived from bisphenol after hydrogen atom donation undergoes secondary reaction rapidly by donating the second phenolic hydrogen to give diphenoquinone. It was also found that, under certain conditions, the oxidation of bisphenol did not give diphenoquinone but two unknown products were observed (Figure 12). It is known that the oxidation of lipids in the presence of phenolic antioxidant such as α-tocopherol and 2,6-di-*tert*-butyl-4-methylphenol gives the peroxyl radical adduct to the para-position of the phenoxyl radical.[21] Accordingly, the mechanism of the inhibition of oxidation by bisphenol may be proposed as shown in Scheme 1. The reaction between the phenoxyl radical and peroxyl radical determines the product. The results indicate that the peroxyl radical adds to the aromatic ring to give an adduct in the homogeneous solution, whereas it abstracts another phenolic hydrogen to give diphenoquinone in the membranes. Scheme 1 shows that when bisphenol gives peroxyl radical adduct, the stoichiometric number is 2 or 4, whereas when it gives diphenoquinone, the n value is 2. As described above, the stoichiometric number was obtained experimentally as 2.2 for bisphenol in homogeneous solution where diphenoquinone was not observed. The stoichiometric number for probucol was obtained as 5.0 in the present study. A high n value has been also reported previously.[22,23]

The effect of mixing of bisphenol with other antioxidant is interesting. Bisphenol was found



SCHEME 1

to be less reactive toward radical and less potent as an antioxidant against lipid peroxidation in solution than α-tocopherol but it exerted similar antioxidant activity as α-tocopherol in the oxidation of membranes and, when they were present together, bisphenol spared α-tocopherol. It was found by ESR study that bisphenol could reduce α-tocopheroxyl radical, that is, the ESR spectrum of α-tocopheroxyl radical disappeared rapidly when reacted with bisphenol and no ESR signal was observed when the mixture of α-tocopherol and bisphenol was reacted with galvinoxyl. The phenoxyl radical of bisphenol must be capable of reducing α-tocopheroxyl radical faster than bisphenol. However, the results in the homogeneous solution (Figure 8A) and in dimyristoyl PC liposomal membranes in the presence of AAPH (Figure 9B) that only α-tocopherol was consumed predominantly and bisphenol was not capable of sparing α-tocopherol efficiently imply that the reduction of α-tocopheroxyl radical by bisphenol is not fast enough to compete with the reaction of α-tocopheroxyl radical with peroxyl radical. In other words, bisphenol is less potent than ascorbic acid as a reductant of α-tocopheroxyl radical.

What is more striking is the synergistic interaction between bisphenol and DPPD. Although bisphenol is less reactive than DPPD, bisphenol spared DPPD, acted as a synergist and suppressed the lipid peroxidation efficiently.

The phenoxyl radical derived from probucol is readily reduced by ascorbic acid[23] and it has been observed that probucol is spared by ascorbic acid during the oxidation of LDL,[24,25] although the sparing efficacy is less efficient than that for α-tocopherol by ascorbic acid. It could not be confirmed by ESR analysis whether the phenoxyl radical from bisphenol was reduced by ascorbic acid due to its instability. The results in Figure 10 show that ascorbic acid efficiently spares bisphenol in homogeneous solution, probably due to higher reactivity of ascorbic acid than bisphenol. In the oxidation of PC liposomal membranes induced by lipophilic azo compound, ascorbic acid alone did not exert efficient antioxidant activity, nor did it spare bisphenol notably. Probably, the bisphenol phenoxyl radical is not stable and it may be localized within the membrane, which makes the rate of reduction by ascorbate slow.[26]

In conclusion, bisphenol, a metabolite of probucol, may act as an effective antioxidant in combination with other antioxidants such as α-tocopherol and DPPD.

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