CHEMISTRY LETTERS, pp. 1573-1576, 1986.

Antioxidant Activity of Vitamin E in Liposomal Membranes

Etsuo NIKI,* Mareyuki TAKAHASHI, and Erika KOMURO
Department of Reaction Chemistry, Faculty of Engineering,
The University of Tokyo, Hongo, Tokyo 113

Antioxidant activity of vitamin E in liposomal membranes was compared with that in homogeneous solution. It was found that vitamin E could move in the liposomal membranes rapidly but that the antioxidant efficiency in the membranes was considerably lower than that in homogeneous solution.

Vitamin E (tocopherols) is now accepted to function as a lipophilic chain-breaking antioxidant in biological systems and protect the aerobic organisms from oxygen toxicity.[1] Fundamental chemistry of the inhibition of oxidation by vitamin E in homogeneous solution has been studied extensively and it is now fairly well understood.[2,3] On the other hand, the detailed mechanism of inhibition of oxidation by vitamin E in the membranes is not satisfactorily elucidated. In the present study, we have tried to measure the antioxidant activity of vitamin E in liposomal membranes, a good model of biological membranes, to compare with that in homogeneous solution.

One of the interesting questions frequently asked on the role of vitamin E is why small amount of vitamin E can suppress the oxidation of lipids in biological membranes where the mobility or the lateral diffusion of vitamin E must be restricted. The phytyl side chain of vitamin E enhances the incorporation and retainment in the membranes,[4] but at the same time it should decrease the mobility in the membranes. We have found recently that α-tocopherol reacts with β-, γ-, and δ-tocopheryloxyl radicals rapidly in the homogeneous solution to regenerate β-, γ-, and δ-tocopherols.[5] We have studied in this work whether such an interaction of tocopherol and tocopheryloxyl radical can occur in the liposomal membranes.

The liposomal membranes were prepared from soybean phosphatidylcholine (PC) and, in some cases, dimirystoyl PC (14:0 PC) and cholesterol. Commercial soybean PC and methyl linoleate were purified by alumina and silica-gel columns before use, while 14:0 PC and cholesterol were used as received. Natural d-α- and d-δ-tocopherols were kindly supplied from Eisai Co. Ltd. 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2'-azobisisobutyronitrile (AIBN) were used as water-soluble and lipid-soluble radical initiators, respectively, in order to generate the initiating free radicals at a known and measurable rate. The multilamellar liposomal membranes were prepared as described previously[4] by making a thin film of PC containing lipid-soluble additives on the glass wall followed by shaking with 0.1 N-NaCl aqueous solution. The unilamellar liposomal membranes were prepared by ultrasonic irradiation for 10 min at 4 °C.[4]

α-Tocopherol and δ-tocopherol were incorporated into liposomes of soybean PC separately and these liposomes were mixed and subjected to oxidation initiated with AAPH. The concentrations of tocopherols were followed by HPLC as reported previously.[5] As shown in Fig. 1A, α-tocopherol and δ-tocopherol decayed at a similar rate. On the other hand, when α- and δ-tocopherols were incorporated into the same liposomal membranes, α-tocopherol disappeared predominantly and δ-tocopherol remained almost unchanged and began to disappear after substantially all of α-tocopherol was depleted. (Fig. 1B) The oxidation was suppressed but lipids were rapidly oxidized after both tocopherols disappeared. The similar results were obtained when β- and γ-tocopherols were used insead of δ-tocopherol. Furthermore, substantially same results were observed when the oxidation was carried out in the liposomal membranes prepared from soybean PC, 14:0 PC, and cholesterol (4:4:2 by weight). α-Tocopherol is more reactive toward peroxyl radicals than β-, γ-, and δ-tocopherols[3] but the above results can not be interpreted solely by the different activities of tocopherols and they suggest that α-tocopherol and δ-tocopheryloxyl radical incorporated into different liposomal membranes do not interact with each other but that they readily interact within the same liposomal membranes.

It was found that ascorbate reduced α-tocopheryloxyl radical to regenerate α-tocopherol both in homogeneous solution and in liposomal system.[2,6-8] It was found in this study that when α-tocopherol and 5,6-ascorbyl dipalmitate were incorporated into the same liposomal mambranes, they suppressed the oxidation but α-tocopherol was not consumed appreciably at first and then it began to disappear after ascorbic acid ester was exhausted. The above results strongly indicate that vitamin E and its radical move in the liposomal membranes rapidly and that the life time of vitamin E radical is long enough to interact with another vitamin E and ascorbic acid ester before the radical reacts with other peroxyl radical to give stable product.

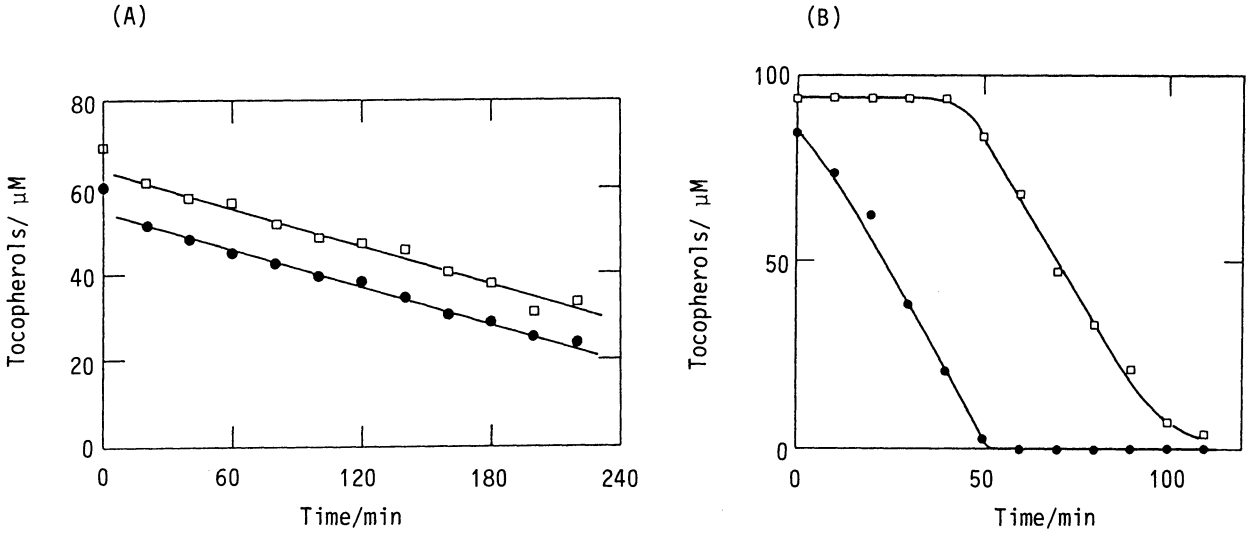

Fig. 1 Rate of disappearance of α-tocopherol (●) and δ-tocopherol (□) during the oxidation of soybean PC liposomes in 0.1 M NaCl aqueous dispersions at 37°C initiated with AAPH.
(A) : Tocopherols were incorporated into different multilamellar liposomes.
(B) : Tocopherols were incorporated into the same liposomes, which were sonicated for 10 min.

The antioxidant activity of vitamin E in the liposomal membranes was compared with that in the homogeneous solution. The lipid peroxyl radicals ($LO_2\cdot$) either abstract hydrogen atom from lipid (LH) intermolecularly to propagate chain oxidation (reaction 1) or they are scavenged by vitamin E (IH) (reaction 2).

$$LO_2\cdot + LH \xrightarrow{k_p} LOOH + L\cdot \quad (1)$$

$$LO_2\cdot + IH \xrightarrow{k_{inh}} LOOH + I\cdot \quad (2)$$

The relative efficiency of antioxidant, a, is determined by the ratio of the rate of reaction 2 (R_2) to that of reaction 1 (R_1).

$$a = \frac{R_2}{R_1} = \frac{k_{inh}[LO_2\cdot][IH]}{k_p[LO_2\cdot][LH]} = \frac{k_{inh}[IH]}{k_p[LH]} \quad (3)$$

The length of induction period, t_{inh}, and the rate of oxidation during the induction period, R_{inh}, are given by Eqs. 4 and 5, respectively, where n is the stoichiometric number of peroxyl radicals trapped by each antioxidant and R_i is the rate of chain initiation.[2)]

$$t_{inh} = n[IH]/R_i \quad (4)$$

$$R_{inh} = k_p[LH]R_i/nk_{inh}[IH] \quad (5)$$

Therefore, the ratio of the rate constants k_{inh}/k_p can be calculated from Eq. 6.[2)]

$$\frac{k_{inh}}{k_p} = \frac{[LH]}{R_{inh}t_{inh}} \quad (6)$$

The example of the oxidation of soybean PC liposomes inhibited by α-tocopherol is shown in Fig. 2. A clear induction period was observed. The pertinent results of the oxidations both in homogeneous solution and in aqueous dispersions are shown in Table 1. Table 1 shows that the ratio k_{inh}/k_p for α-tocopherol in liposomal membranes is much smaller than that in homogeneous solution. For example, when the ratio of antioxidant to lipid [IH]/[LH] is 100, which is close to physiological condition, the ratio of inhibition to oxidation is R_2/R_1 = 50 and 0.5 for homogeneous solution and liposomal membranes, respectively.

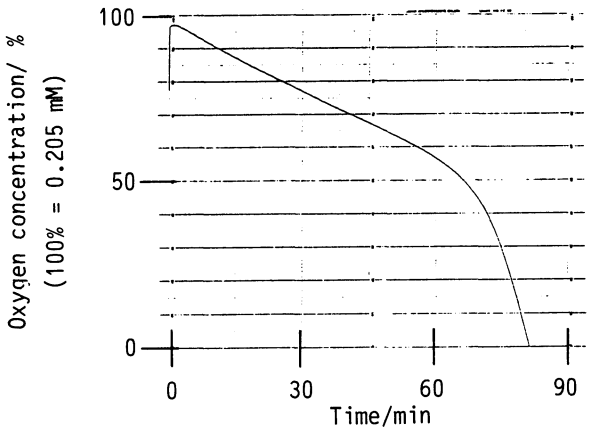

Fig. 2. Rate of oxygen uptake in the oxidation of 5.15 mM unilamellar soybean PC liposomes in 0.1 M NaCl aqueous dispersion initiated with 10.1 mM AAPH at 37 °C in the presence of 52.2 μM α-tocopherol.

Table 1. Antioxidant activities of vitamin E in the oxidation of methyl linoleate in homogeneous solution and of soybean PC liposomes in aqueous dispersions at 37 °C

(1) Methyl linoleate in t-butyl alcohol, [LH] = 0.604 M

[AIBN] / mM	[IH] / μM	t_{inh} / s	$10^8 R_{inh}$ / Ms^{-1}	$10^8 R_p$ a) / Ms^{-1}	R_{inh}/R_p	$10^3(R_2/R_1)$	$10^{-3}(k_{inh}/k_p)$
3.99	0.272	980	14.0	19.8	0.707	1.98	4.40
3.99	0.815	1680	5.05	18.3	0.276	9.61	7.12
4.03	0.815	2140	7.25	19.1	0.380	5.25	3.89
3.99	0.997	2260	5.78	25.6	0.226	7.63	4.62
3.99	1.50	3040	3.37	18.6	0.181	14.6	5.90
3.99	1.71	3560	3.37	17.8	0.189	14.3	5.03

(2) Soybean PC liposomes in 0.1 N-NaCl aqueous dispersions, [LH] = 1.16 M b)

[AAPH] / mM	[IH] / mM	t_{inh} / s	$10^6 R_{inh}$ / Ms^{-1}	$10^6 R_p$ / Ms^{-1}	R_{inh}/R_p	R_2/R_1	k_{inh}/k_p
1.85	2.39	4280	2.90	9.11	0.318	0.193	93.5
8.73	12.3	4120	3.54	29.4	0.120	0.843	78.5
15.0	18.5	4280	7.14	32.9	0.217	0.605	38.0
15.4	18.5	3920	8.56	52.0	0.165	0.551	34.6
15.7	24.0	4460	5.46	36.3	0.150	0.986	47.6

a) R_p : Rate of oxidation after induction period. b) Concentration expressed for lipid region except that for AAPH in total aqueous dispersion.

This lower k_{inh}/k_p, that is, the lower efficiency of inhibition of oxidation by α-tocopherol in liposomal membranes than in homogeneous solution may be attributed to lower mobility of α-tocopherol and, more importantly, to higher chance of propagation in the tightly packed structure of liposomal membranes. Apparently, the mobilities of both vitamin E and lipids are reduced in the membrane, but further study is necessary for the quantitative discussion. However, it may be noteworthy that the antioxidant activity is determined not just by the mobility of vitamin E alone but the competition between reactions 1 and 2.

The above results suggest that α-tocopherol can move around in the liposomal membranes rapidly but that the efficiency of inhibition of oxidation in the membranes is considerably lower than that in homogeneous solution.

References

1) "Vitamin E," ed by L. J. Machlin, Marcel Dekker, New York (1980).

2) E. Niki, T. Saito, A. Kawakami, and Y. Kamiya, J. Biol. Chem., 259, 4177 (1984).

3) G. W. Burton, T. Doba, E. J. Gabe, L. Hughes, F. L. Lee, L. Prasad, and K. U. Ingold, J. Am. Chem. Soc., 107, 7053 (1985).

4) E. Niki, A. Kawakami, M. Saito, Y. Yamamoto, J. Tsuchiya, and Y. Kamiya, J. Biol. Chem., 260, 2191 (1985).

5) E. Niki, J. Tsuchiya, Y. Yoshikawa, Y. Yamamoto, and Y. Kamiya, Bull. Chem. Soc. Jpn., 59, 497 (1986).

6) J. E. Packer, T. F. Slater, and R. L. Willson, Nature, 278, 737 (1979).

7) E. Niki, A. Kawakami, Y. Yamamoto, and Y. Kamiya, Bull. Chem. Soc. Jpn., 58, 1971 (1985).

8) T. Doba, G. W. Burton, and K. U. Ingold, Biochim. Biophys. Acta, 835, 298 (1985).

(Received June 21, 1986)