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Oxidation of Lipids. XII. Inhibition of Oxidation of Soybean Phosphatidylcholine and Methyl Linoleate in Aqueous Dispersions by Uric Acid

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The activity of uric acid as a chain-breaking antioxidant was studied in the oxidations of methyl linoleate micelles and soybean phosphatidylcholine liposomes in aqueous dispersions at 37°C. Uric acid was found to act as a radical scavenger. Uric acid located in an aqueous phase could trap the radicals in an aqueous phase, but it could not scavenge radicals within the lipid region of micelles and liposomal membranes. The kinetics of the oxidations of lipids in aqueous dispersions inhibited by uric acid was discussed.

Toxicity caused by oxygen and its active species has received much attention recently in connection with its pathological effects and aging.[1-11] Aerobic organisms have an array of protective mechanisms both for preventing the formation of active oxygen species and lipid peroxidation and for repairing oxidative damage. Enzymes such as superoxide dismutase and glutathione peroxidase play an important role in the protective systems. Chain-breaking antioxidants are also important and it has been reported[12] that vitamin E is the most potent and probably the only lipid soluble, chain-breaking antioxidant in erythrocyte membranes. Water soluble ascorbic acid also acts as an antioxidant.[13-17]

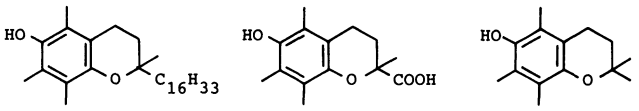
Uric acid may be another water soluble antioxidant. In 1963, Matsushita[18] found that uric acid was as effective antioxidant as α-tocopherol in the oxidation of linoleic acid in water at 37°C. Kellogg and Fridovich[19] observed that urate inhibited hemolysis of erythrocytes caused by xanthine oxidase–acetaldehyde system. Recently, Ames[20] suggested that uric acid may play a role in protecting man from oxygen radical toxicity. More recently, on the other hand, Kittridge and Willson[21] have shown that urate does not always show a protective effect against free radicals. In the course of our study on the oxidation of lipids and its inhibition, we have measured the activity of uric acid as a chain-breaking antioxidant. This paper shows convincingly that uric acid can scavenge peroxyl radicals.

Experimental

Materials. Commercial soybean phosphatidylcholine (PC) purchased from Daigo Chemicals Co. (Osaka) was purified by alumina and silica-gel columns. The purified PC gave only one spot on thin-layer chromatography and no conjugated diene was observed before oxidation. The composition of fatty acids in PC measured by gas-liquid chromatography after hydrolysis and esterification with HCl–methanol was as follows: palmitic acid 12.1%, stearic acid 1.6, oleic acid 9.6, linoleic acid 69.6, and linolenic acid 7.1 mol%. Methyl linoleate was obtained from Sigma Chemical Co. (St. Louis) and Tokyo Kasei Kogyo Co. (Tokyo) and purified by silica-gel column.

2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) were used as water-soluble and oil-soluble radical initiators, respectively, in order to generate initiating free radicals at a constant rate outside or within liposomes or micelles as requested. Both AAPH and AMVN were obtained from Wako Chemicals (Tokyo) and used as received. The rate of chain initiation was determined by the conventional method[22-24] from the induction period observed by the addition of a chain breaking antioxidant.

The rate of radical generation from AAPH in an aqueous phase was measured using a water soluble vitamin E model, 2-carboxy-2,5,7,8-tetramethyl-6-chromanol (CTMC), as a radical scavenger, whereas the rate of chain initiation in the lipid phase was measured using 2,6-di-*t*-butyl-4-methylphenol incorporated into lipid region of micelles or liposomes as a chain-breaking antioxidant.



α-tocopherol CTMC PMC

Uric acid, hypoxanthine, xanthine, purine, L-histidine, indole, and Triton X-100 were of the highest grade available commercially and used without further purification. *d*-α-Tocopherol and its models, CTMC and 2,2,5,7,8-pentamethyl-6-chromanol (PMC), were kindly supplied from Eisai Co. (Tokyo). 2,6-Di-*t*-butyl-4-methylphenol was recrystallized from ethanol.

Pure water was usually used for aqueous dispersion. In some experiments, the effect of pH of the medium was studied. Table 1 shows the buffer system used in this study.

Procedures. The liposome was prepared as follows. PC and oil soluble additives were dissolved in chloroform and the solution was taken into a small flask. The solvent was removed to obtain a thin film. An appropriate amount of 0.1 M NaCl (M=mol dm^{-3}) aqueous solution containing, when necessary, water-soluble additives was added and the film was slowly peeled off by shaking to obtain a white, milky liposome solution. An aliquot of the liposome solution was taken into a reaction vessel and immersed in a water bath which was carefully thermostated at the desired temperature.

The micelle was prepared as follows. Oil soluble additives were dissolved in benzene and the solution was taken into a 30 ml Pyrex glass ampoule. The ampoule was connected to

Table 1. Preparation of Buffer Solutions

pH	Buffer system
3.0	0.02 M Na_2HPO_4 4.11 ml+0.1 M Citric acid 15.89 ml
5.0	0.02 M Na_2HPO_4 10.30 ml+0.1 M Citric acid 9.70 ml
7.0	0.02 M Na_2HPO_4 16.47 ml+0.1 M Citric acid 3.53 ml
9.0	(0.2 M H_3BO_3+2 M KCl) 63 ml+0.2 M Na_2CO_3 37 ml+H_2O 100 ml

Table 2. Inhibition of Oxidation of Methyl Linoleate (LH) Micelle by Uric Acid (UA) in 10 mM Triton X-100 Aqueous Dispersions under Air at 37°C

[LH][a]	[AAPH]	[UA]	R_i(aq)[b]	t_{inh}[c]	R_{inh}	R_i(lipid)[d]	R_p	$(kcl)_p$[e]	$r^{2,f}$	k_{inh}/k_6
mM	mM	μM	nM/s	s	μM/s	nM/s	μM/s			
116	19.1	0	24.8	0		15.5	1.83	118		
114	18.5	10.5	24.1	1125	n.d.[g]	15.0	1.48	99		
111	17.8	25.5	23.1	2025	0.134	14.5	1.58	109	0.0036	12.1×10^5
108	17.7	37.3	23.0	3225	0.184	14.4	1.43	99	0.0081	3.4×10^5
106	17.3	48.7	22.5	3945	0.120	14.0	0.905	65	0.0081	2.7×10^5
88.0	19.1	205	24.8	8700	0.055	15.5	0.883	57	0.0009	4.8×10^5

a) Concentration of methyl linoleate in total aqueous dispersions. The concentration of neat methyl linoleate is 3.02 M. b) Rate of free radical generation in aqueous phase, $R_i(aq)=1.30\times10^{-6}[AAPH]$. c) Induction period. d) Rate of initial lipid radical production in micelles in the absence of uric acid; $R_i(lipid)=8.12\times10^{-7}[AAPH]$. e) Kinetic chain length in the absence of uric acid or after the induction period. f) $r^2=k_6[LH]/(k_{inh}[IH]+(1+\alpha)k_6[LH])$=(rate of reaction 6)/(rate of reactions 4+5+6), see text. g) n.d.=not determined.

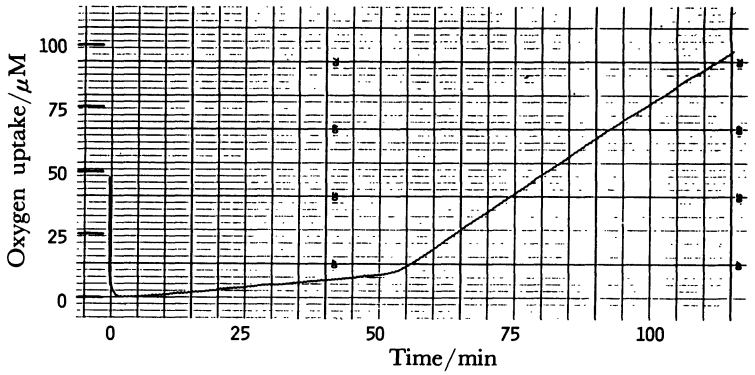


Fig. 1. Rate of oxygen uptake during the oxidation of 0.108 M methyl linoleate in 0.01 M Triton X-100 aqueous dispersion at 37°C under air in the presence of 17.7 mM AAPH and 37.3 μM uric acid. Oxygen uptake was followed by pressure transducer.

a water aspirator and benzene was removed at room temperature. Appropriate amount of methyl linoleate was added and oil-soluble materials were dissolved and then 0.01 M aqueous Triton X-100 containing, when necessary, water-soluble additives was added into the ampoule and shaken vigorously on Vortex mixer for two minutes.

The oxidation was carried out under air at 37°C. The reaction mixture was agitated with a magnetic stirrer. The rate of oxygen uptake was measured in an automatic recording gas absorption apparatus with either a pressure transducer or oxygen electrode and the rate of consumption of α-tocopherol was followed at 294 nm with high-performance liquid chromatography (HPLC) as reported previously.[15]

Results and Discussion

Oxidation of Methyl Linoleate Micelles in Aqueous Dispersion and Its Inhibition by Uric Acid. Methyl linoleate was oxidized in aqueous dispersions using Triton X-100 as a surfactant. In the absence of radical initiator, the rate of oxidation was quite slow, but the addition of AAPH induced the oxidation and methyl linoleate was oxidized at a constant rate without any noticeable induction period. When uric acid was added to the aqueous phase, the oxidation was suppressed quite markedly and after some induction period the rate of oxidation increased as shown in Fig. 1. The rate of oxidation after the induction period was as large as that in the absence of uric acid.

The pertinent results are shown in Table 2. The induction period was proportional to the uric acid concentration. The quantitative evaluation of the data in Table 2 will be discussed later in the text.

Figure 2 shows the results of oxidation of methyl linoleate micelles in aqueous dispersions of different

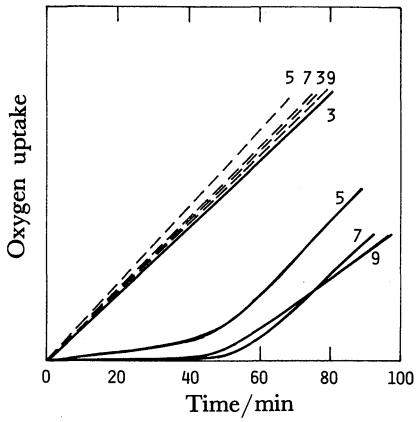


Fig. 2. Effect of pH of the medium on the inhibition of oxidation of 0.11 M methyl linoleate by 51 μM uric acid in 0.01 M Triton X-100 aqueous dispersion at 37°C under air initiated with 18 mM AAPH.
——: with uric acid, ----: without uric acid.

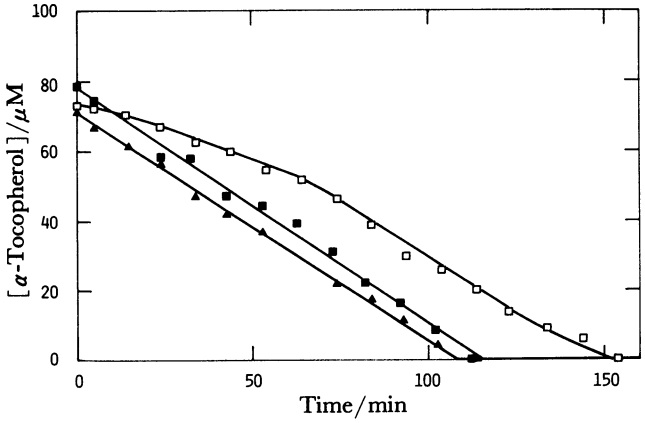


Fig. 3. Rate of disappearance of α-tocopherol during the oxidation of methyl linoleate micelle in 0.010 M Triton X-100 aqueous dispersion at 37°C under air.
■: [α-tocopherol]=78.7 μM; [uric acid]=none; [AMVN]=13.0 mM, ▲: [α-tocopherol]=71.3 μM; [uric acid]=49.9 μM; [AMVN]=10.9 mM, □: [α-tocopherol]=73.0 μM; [uric acid]=38.3 μM; [AAPH]=17.6 mM.

pH in the presence and absence of uric acid. It shows that pH of the medium has little effect in the absence of uric acid but that the antioxidant activity of uric acid depends on pH and it is not effective as an antioxidant at pH 3.

Although uric acid was an effective antioxidant, hypoxanthine, xanthine, purine, and L-histidine which have similar structures as uric acid did not suppress the oxidation of methyl linoleate in aqueous dispersions initiated with AAPH. Xanthine was not effective as an antioxidant over the pH range between 3 and 9. Indole did not suppress the oxidation of methyl linoleate in benzene solution initiated with AMVN.

Similarly, AMVN located in methyl linoleate micelles in Triton X-100 aqueous dispersions induced the oxidation. AMVN generates initiating free radicals

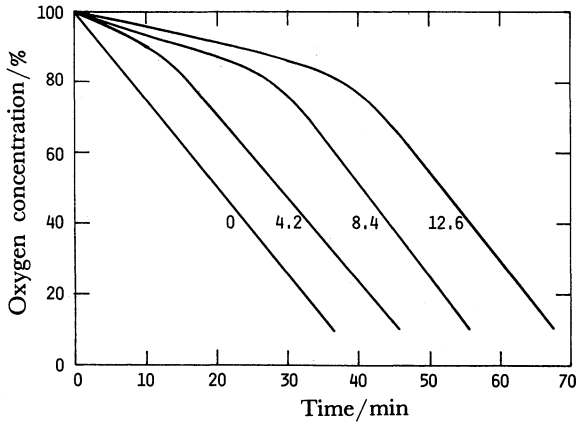


Fig. 4. Inhibition of oxidation of 1.95 mM soybean PC liposome by uric acid in 0.1 M NaCl aqueous dispersion at 37°C under air initiated with 10 mM AAPH. Rate of oxygen uptake was followed by oxygen electrode. Number show the concentration of uric acid in μM.

in the lipid region of micelles. Interestingly, however, uric acid could not suppress the oxidation when it was initiated with AMVN, suggesting that uric acid located in an aqueous phase can not scavenge radicals that are present in the lipid region of micelles, as observed for ascorbic acid.[15,16]

Figure 3 shows the rate of decay of α-tocopherol in the presence and absence of uric acid during the oxidation of methyl linoleate micelles in aqueous dispersions initiated with AAPH or AMVN. In the absence of uric acid, α-tocopherol disappeared linearly with time. In the presence of uric acid, the rate of decay of α-tocopherol was suppressed when the oxidation was initiated with AAPH, whereas uric acid had little effect on the rate of α-tocopherol consumption when the oxidation was initiated with AMVN.

Oxidation of Soybean PC Liposomes and Its Inhibition by Uric Acid. The results are summarized in Table 3 and in Fig. 4. Substantially the same results were obtained as in the oxidation of methyl linoleate micelles in aqueous dispersions. Both AAPH and AMVN induced the oxidation of soybean PC liposomes. Uric acid suppressed the oxidation initiated with AAPH, but it could not suppress the oxidation efficiently when it was induced by AMVN. The induction period was proportional to the concentration of uric acid.

Quantitative Evaluation of Antioxidant Activity of Uric Acid. The oxidation of lipids initiated with AAPH (A-N=N-A) proceeds by the following mechanism.[15-17,25,26]

Initiation:

$$\text{A-N=N-A} \xrightarrow{k_d} [\text{A}\cdot\ \text{N}_2\ \cdot\text{A}] \longrightarrow (1-e)\text{A-A} \quad (1)$$

$$\searrow\ 2e\text{A}\cdot \quad (2)$$

$$\text{A}\cdot + \text{O}_2 \xrightarrow{k_3} \text{AO}_2\cdot \quad (3)$$

Table 3. Inhibition of Oxidation of Soybean PC Liposomes by Uric Acid under Air at 37°C[a)]

[PC][b)]	[AAPH][c)]	[UA]	t_{inh}	R_{inh}[d)]	R_p[e)]	$(kcl)_p$[f)]	r^2[g)]	k_{inh}/k_6
mM	mM	μM	s	nM/s	nM/s			
1.95	10.0	0	0		87.4	29		
1.95	10.0	4.21	820	44.5	87.8	29	0.0441	1.0×10^4
1.64	10.0	7.87	1610	22.2	61.5	20	0.0081	2.5×10^4
1.95	10.0	8.42	1660	29.6	94.1	31	0.1000	2.3×10^4
1.95	10.0	12.6	2350	23.1	91.9	30	0.0049	3.1×10^4

a) Concentrations are for total aqueous dispersions. b) Concentration of neat soybean PC is 1.16 M. c) $R_i(aq)=1.30\times10^{-6}$[AAPH]$=1.30\times10^{-8}$ M/s. $R_i(lipid)=3.06\times10^{-9}$ M/s. d) Rate of oxidation during the induction period. e) Rate of oxidation after induction period. f) Kinetic chain length after induction period. g) Same as footnote f in Table 2.

$$AO_2\cdot + IH \xrightarrow{k_{inh}} \text{stable products} \quad (4)$$

$$AO_2\cdot + AO_2\cdot \xrightarrow{k_5} \text{stable products} \quad (5)$$

$$AO_2\cdot + LH \xrightarrow{k_6} AOOH + L\cdot \quad (6)$$

Propagation:

$$L\cdot + O_2 \xrightarrow{k_7} LO_2\cdot \quad (7)$$

$$LO_2\cdot + LH \xrightarrow{k_p} LOOH + L\cdot \quad (8)$$

Termination:

$$2LO_2\cdot \xrightarrow{k_t} \text{non-radical products} \quad (9)$$

The water soluble AAPH generates geminate radicals by its thermal decomposition, a portion of which recombine within the solvent cage (reaction 1) but rest of them escape from the solvent cage and become free radicals (reaction 2). They rapidly interact with oxygen to give peroxyl radicals (reaction 3). The peroxyl radicals are either scavenged by an antioxidant (IH) that is present in the aqueous phase (reaction 4), interact with another peroxyl radical to give stable products (reaction 5), or attack micelles or liposomes and abstract active, doubly-allylic hydrogens from lipids (LH) (reaction 6) to initiate free radical chain oxidation (reactions 7 and 8). As shown above, the uric acid in the aqueous phase can not scavenge radicals in the lipid region and therefore the chains are terminated by bimolecular interactions of lipid peroxyl radicals (reaction 9). Thus the oxidation proceeds by a free radical chain mechanism and one initiating radical can produce many lipid peroxides.

When the rate of chain initiation is constant, that is, when the new chains are started at a constant rate, the steady state treatment can be applied for the reaction sequence 1 to 9.

The rate of free radical generation in an aqueous phase from AAPH, $R_i(aq)$, is given by

$$R_i(aq) = 2ek_d[AAPH] = n[CTMC]/t_{inh} \quad (10)$$

where e is the efficiency of free radical production and k_d is the rate constant for the unimolecular decomposition of AAPH. The rate of radical generation in an aqueous phase can be determined from the induction period (t_{inh}) produced by CTMC. The stoichiometric number (n) of peroxyl radicals trapped by each CTMC was assumed to be 2.[15,25,27]

On the other hand, the rate of chain initiation, that is, the rate of initial radical generation in the lipid phase, $R_i(lipid)$, was determined from the induction period produced by 2,6-di-*t*-butyl-4-methylphenol incorporated into lipid phase of micelles or liposomes.[22,23,24] The constant n was taken as 2.[15,22,23] If all the free radicals from AAPH produced in an aqueous phase attack micelles or liposomes, then $R_i(aq)=R_i(micelle)=R_i(liposome)$.

$$R_i(lipid) = n[IH(lipid)]/t_{inh} \quad (11)$$

The steady state treatment for the above reactions 1 to 9 gives Eqs. 12 to 14,

$$[AO_2\cdot] = \frac{R_i(aq)}{k_{inh}[IH] + (1+\alpha)k_6[LH]} \quad (12)$$

$$[LO_2\cdot] = \left(\frac{k_6[AO_2\cdot][LH]}{2k_t}\right)^{1/2} \quad (13)$$

$$= \left\{\frac{k_6[LH]}{2k_t}\cdot\frac{R_i(aq)}{k_{inh}[IH] + (1+\alpha)k_6[LH]}\right\}^{1/2} \quad (14)$$

where

$$\alpha = 2k_5[AO_2\cdot]^2/k_6[AO_2\cdot][LH] \quad (15)$$

The rate of oxidation during the induction period (R_{inh}) is given by Eqs. 16 to 18,

$$R_{inh} = k_3[A\cdot][O_2] + k_7[L\cdot][O_2] \quad (16)$$

$$= R_i(aq) + k_6[AO_2\cdot][LH] + k_p[LO_2\cdot][LH] \quad (17)$$

$$= R_i(aq) + r^2R_i(aq) + rk_p[LH]\left(\frac{R_i}{2k_t}\right)^{1/2} \quad (18)$$

where

$$r = \left(\frac{k_6[LH]}{k_{inh}[IH] + (1+\alpha)k_6[LH]}\right)^{1/2} \quad (19)$$

and k_{inh}, k_p, and k_t are the rate constants for scavenging by uric acid of peroxyl radicals derived from AAPH, propagation reaction 8, and termination reaction 9, respectively.

In the absence of an antioxidant or after the induction period, where [IH]=0, $r=(1/(1+\alpha))^{1/2}$, and the rate of oxidation (R_p) is given by Eq. 20.

$$R_p = R_i(aq) + \frac{1}{1+\alpha}R_i(aq) + k_p[LH]\left(\frac{R_i(aq)}{2(1+\alpha)k_t}\right)^{1/2} \quad (20)$$

Equations 18 and 20 gives Eq. 21

$$r = \left\{\frac{R_{inh}}{R_i(aq)} + \left(\frac{(1+\alpha)^{1/2}R_q}{2R_i(aq)}\right)^2 - 1\right\}^{1/2} - \frac{(1+\alpha)^{1/2}R_q}{2R_i(aq)} \quad (21)$$

where

$$R_q = R_p - R_i(aq) - \frac{1}{1+\alpha}R_i(aq) \quad (22)$$

The constant α is the ratio of the rate of reaction 5 to that of reaction 6; that is, the ratio of $AO_2\cdot$ radicals that interact with each other to those that attack lipids. The value $1/(1+\alpha)$ gives the ratio of free $AO_2\cdot$ radicals generated in the aqueous phase that attack lipids in micelles or liposomes in the absence of an antioxidant in the aqueous phase (Eq. 23), and this can be obtained from R_i(aq) and the rate of chain initiation in the lipid phase, R_i(lipid), obtained from Eq. 11. On the other hand, the values of r^2 show the fraction of free $AO_2\cdot$ radicals generated in an aqueous phase that attack lipid in micelles or liposomes instead of being scavenged by uric acid and reacting with another $AO_2\cdot$ radicals.

Since R_i(aq), R_i(lipid), R_{inh}, and R_p can be measured experimentally, and α is calculated from Eq. 24, r can be calculated from Eqs. 21 and 22. Equation 19 gives Eq. 25 and, if k_6 is known, k_{inh} can be obtained.

$$\frac{1}{1+\alpha} = \frac{k_6[AO_2\cdot][LH]}{2k_5[AO_2\cdot]^2 + k_6[AO_2\cdot][LH]} \quad (23)$$

$$= \frac{R_i(lipid)}{R_i(aq)} \quad (24)$$

$$\frac{k_{inh}}{k_6} = \left(\frac{1}{r^2} - 1 - \alpha\right)\frac{[LH]}{[IH]} \quad (25)$$

Figure 5 shows the plot of induction period observed in the presence of uric acid and CTMC. The induction period was proportional to the concentration of antioxidant and inversely proportional to that of

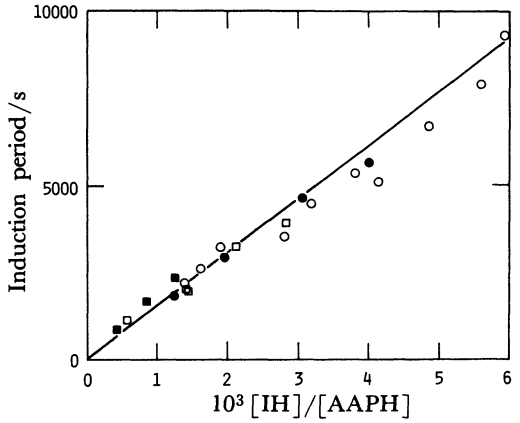


Fig. 5. Plot of induction period as a function of [antioxidant]/[AAPH] observed in the oxidations of methyl linoleate micelles and soybean phosphatidylcholine liposomes in aqueous dispersions initiated with AAPH and inhibited by uric acid and 2-carboxy-2,5,7,8-tetramethyl-6-chromanol (CTMC) at 37°C.

	Uric acid	CTMC
Micelles	□	○
Liposomes	■	●

AAPH as expected from Eq. 10. It is noteworthy that the same induction periods were observed in micelle and liposome systems. Furthermore, CTMC and uric acid gave substantially the same induction period, suggesting that both of them have same n values. Since the value of n for CTMC may reasonably be assumed to be 2,[15,25,27] the n value for uric acid must be also 2. The rate of free radical generation in an aqueous phase was obtained from Fig. 5 as

$$R_i(aq) = 1.30 \times 10^{-6}[AAPH] \quad (s^{-1}) \quad (26)$$

In Tables 2 and 3 are shown the rates of oxidation of methyl linoleate micelles and soybean phosphatidylcholine liposomes during and after the induction period. The values of r^2 calculated from Eq. 21 are also included in Tables 2 and 3. They show that substantially all of the peroxyl radicals derived from AAPH are scavenged by uric acid before they attack lipids in micelles and liposomes. The ratio of rate constants, k_{inh}/k_6, calculated for micelle and liposome systems from Eq. 25 is also included in Tables 2 and 3 respectively. Since r values are obtained from Eq. 21 as a small difference between large numbers, these values may contain considerable error, but the average values of k_{inh}/k_6 are obtained as 5.8×10^5 and 2.2×10^4 for micelle and liposome systems respectively.

The difference between R_i(aq), R_i(micelle), and R_i(liposome) is interesting. The rates of radical production in aqueous phase are similar in micelles

and in liposome systems as shown in Fig. 5. The ratio is R_i(aq): R_i(micelle): R_i(liposome)=1.30×10^{-6}: 8.12×10^{-7}: 3.06×10^{-7}=1:0.62:0.24, when the lipid concentrations in total aqueous dispersions are about 110 and 2 mM for micelle and liposome systems respectively. This shows that under these conditions, considerable amounts of the radicals generated in an aqueous phase are terminated and only small fraction of the free radicals attack lipids and initiate chain oxidation even in the absence of an antioxidant in the aqueous region. In other words, the structure of liposomal membranes is by itself effective in protecting from the attack of oxygen radicals.

If we consider the concentration difference, it may be said that liposomes are more readily attacked than micelles by oxygen radicals. The higher k_{inh}/k_6 for micelle systems than that for liposome systems must be also ascribed to less accessibility of peroxyl radicals to lipids in micelles than to those in liposomes. These results are consistent with the previous finding that ascorbic acid can interact, although less readily than in homogeneous solution, with peroxyl radicals in liposomes whereas it can not interact with peroxyl radicals in micelles,[28] and that ascorbic acid, cysteine, and glutathione located in aqueous phase interact faster with galvinoxyl radical located in liposomes than they do with galvinoxyl located in micelles in Triton X-100 aqueous dispersions.[29] It may be noteworthy, however, that the reactivity of lipids in micelles toward radicals in aqueous phase must depend on the surfactant.

Synergistic Inhibition with Vitamin E. Ascorbic acid inhibits the oxidation synergistically in combination with vitamin E.[14,15,28,30-40] It has been shown[14,15,28,38-40] that ascorbic acid reacts with vitamin E radical to regenerate vitamin E. It was examined whether uric acid could interact with vitamin E radical and inhibit the oxidation synergistically. That the rate of disappearance of α-tocopherol during the oxidation initiated with AAPH was suppressed by uric acid (Fig. 3) must be due to the trapping by uric acid of radicals from AAPH rather than the trapping of substrate peroxyl radicals and/or α-chromanyloxyl radical from α-tocopherol and it does not necessarily indicate the interaction of uric acid with α-chromanyloxyl radical.

It has been found[28] previously that ascorbic acid in an aqueous phase can interact with chromanyloxyl radical from 2,2,5,7,8-pentamethyl-6-chromanol (PMC) located in micelles and that ascorbic acid can contribute to the inhibition of the oxidation of methyl linoleate micelles in aqueous dispersions in the presence of PMC even though the oxidation is initiated with AMVN and ascorbic acid can not scavenge lipid peroxyl radicals in the oil region. However, uric acid did not show any synergistic activity in the similar oxidation of methyl linoleate micelles in the presence of PMC initiated with AMVN. The above results and Fig. 3 suggest either that uric acid does not interact with chromanoxyl radical at all or that its rate is not fast enough to compete with the interaction of chromanoxyl radical with peroxyl radical.

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