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ROLE OF URIC ACID, CYSTEINE, AND GLUTATHIONE AS CHAIN BREAKING ANTIOXIDANT IN AQUEOUS PHASE

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Uric acid, cysteine, and glutathione suppressed the oxidations of methyl linoleate, soybean phosphatidylcholine, and erythrocyte ghosts in aqueous dispersions initiated with a water-soluble azo initiator. It was suggested that these compounds might function as a chain breaking antioxidant *in vivo* by scavenging oxygen radicals in an aqueous phase.

Toxicity caused by oxygen and its active species has received much attention recently in connection with its pathological effects and aging.[1] Aerobic organisms have an array of protective mechanisms both for preventing the formation of active oxygen species and lipid peroxide and for repairing oxidative damage. Enzymes such as superoxide dismutase and glutathione peroxidase play an important role in the protective systems. Chain breaking antioxidant is also important and it has been accepted that vitamin E acts as an important lipid soluble antioxidant and scavenges oxygen radicals in the lipid phase.[2] In this communication, we wish to present the experimental evidence which shows that uric acid, cysteine, and glutathione can scavenge oxygen radicals and that they may function *in vivo* as a chain breaking antioxidant in an aqueous phase.

The antioxidant activities of uric acid, cysteine, and glutathione were studied in the oxidations of methyl linoleate, soybean phosphatidylcholine, and erythrocyte ghosts in aqueous dispersions. Healthy human blood was obtained from Central Blood Center, Japanese Red Cross. The erythrocyte ghosts were prepared as described previously.[3] Purification of methyl linoleate and phosphatidylcholine and preparation of micelles and liposomes were carried out as reported previously.[3,4]

2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) was used as a water-soluble radical initiator. It has been found that AAPH-induced oxidations of lipids and erythrocyte ghosts proceed by a free radical chain mechanism.[3,4] The oxidations were carried out in a glass ampoule immersed in a water bath maintained at 37 °C. The rate of oxidation was measured by following the rate of oxygen uptake with an oxygen electrode or a pressure transducer.[3,4]

Figure 1 shows the example of the rate of oxygen uptake during the oxidation of methyl linoleate micelles in 0.01 M(mol dm^{-3}) Triton X-100 aqueous dispersion in the presence of uric acid. In the absence of uric acid, the oxidation proceeded smoothly without any noticeable induction period and a constant rate of oxygen uptake was observed. However, when uric acid was added, the oxidation was suppressed markedly as shown in Fig. 1 and after the induction period the oxidation proceeded rapidly at the similar rate as that in the absence of uric acid. As observed in the oxidation of methyl linoleate micelles, no induction period was observed in the absence of uric acid in the oxidation of soybean phosphatidylcholine liposomes, but the addition of uric acid produced a distinctive induction period.

The oxidation of erythrocyte ghosts proceeded similarly in aqueous dispersions as methyl linoleate micelles and soybean phosphatidylcholine liposomes in aqueous dispersions, and it was suppressed by uric acid.

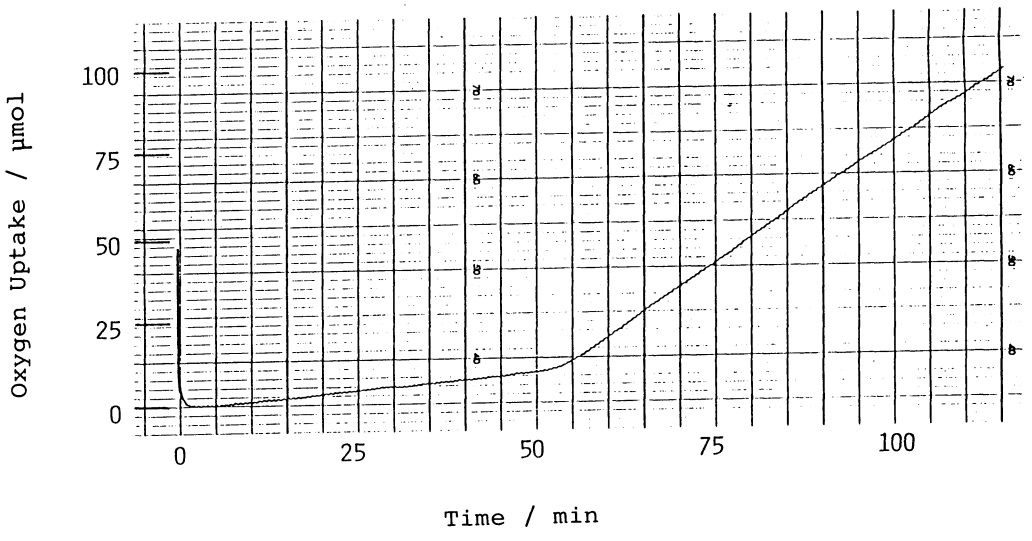

Fig. 1. Rate of oxygen uptake during the oxiation of 0.108 M methyl linoleate micelles in 0.01 M Triton X-100 aqueous dispersion at 37 °C under air in the presence of 17.7 mM AAPH and 37.7 µM uric acid. Oxygen uptake was measured with a pressure transducer.

Figure 2 shows the plot of induction period produced by uric acid in the oxidations of methyl linoleate micelles, soybean phosphatidylcholine liposomes, and erythrocyte ghosts in aqueous dispersions. As predicted from the kinetics of inhibited oxidation,[5,6] the induction period was proportional to the concentration of uric acid and inversely proportional to that of AAPH. It is noteworthy that the induction period was independent of the substrates and reaction media.

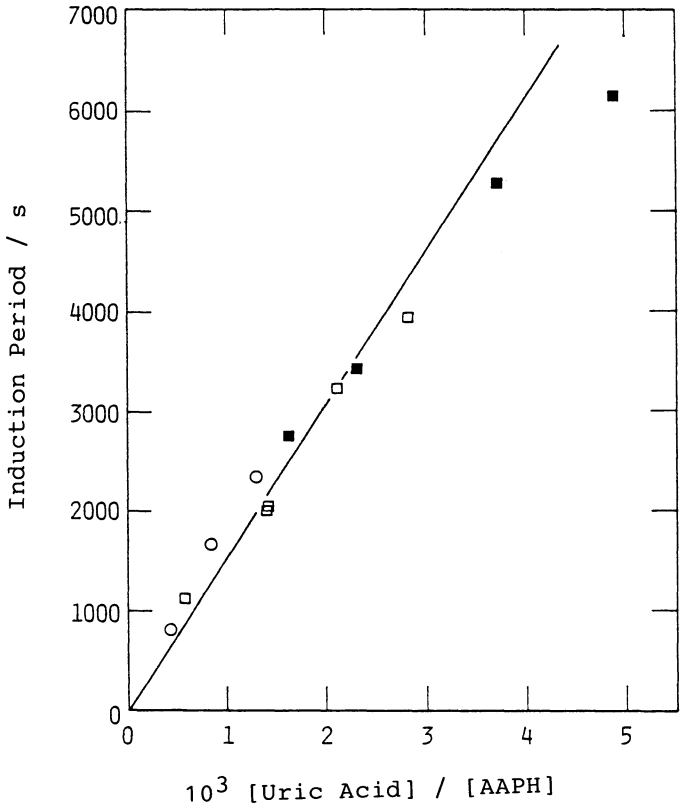

Fig. 2. Plot of induction period as a function of [uric acid]/[AAPH] in the oxidations of methyl linoleate micelles (□), soybean phosphatidylcholine liposomes (○), and erythrocyte ghosts (■) in aqueous dispersions initiated with AAPH and inhibited by uric acid at 37 °C under air.

Cysteine and glutathione also suppressed the oxidations and produced an induction period. The pertinent data are summarized in Table 1.

As described above, uric acid, cysteine, and glutathione suppressed the oxidations when they were initiated with AAPH. On the other hand, they did not suppress the oxidation efficiently when it was initiated with a lipid soluble azo initiator, 2,2'-azobis(2,4-dimethylpentanenitrile), which was incorporated into lipid phase of micelles and liposomes. These results suggest that uric acid, cysteine, and glutathione located in an aqueous phase can not scavenge radicals in the lipid phase. They must scavenge the initiating radicals generated from AAPH before these radicals attack lipid in micelles and membranes.

In biological systems, the radicals may be generated initially in both aqueous and lipid phases, and they must attack membranes to induce free radical chain oxidations. The above results suggest that uric acid, cysteine, and glutathione may contribute as a chain breaking antioxidant by scavenging oxygen radicals in an aqueous phase in conjunction with vitamin E which acts as a chain breaking antioxidant within membranes.

Table 1. Inhibition of oxidations of methyl linoleate, soybean phosphatidylcholine, and erythrocyte ghosts in aqueous dispersions by uric acid, cysteine, and gultathione at 37 °C under air

Substrate[a]		Medium[b]	[AAPH]	IH[c]	[IH]	t_{inh}[d]	R_p[e]
	mM		mM		μM	s	nMs^{-1}
18:2 LH	116	A	19.1	none	0	0	1790
18:2 LH	111	A	17.8	UA	25.5	2025	1560
18:2 LH	106	A	17.6	CSH	100	2580	1350
18:2 LH	111	A	18.1	GSH	105	2220	1120
Soy PC	1.95	B	10.0	none	0	0	80.9
Soy PC	1.95	B	10.0	UA	8.42	1660	87.6
Soy PC	5.08	B	22.1	CSH	210	3420	211
Soy PC	5.08	B	22.2	GSH	460	8400	224
Ghost	f)	C	9.96	none	0	0	38.7
Ghost	f)	C	9.96	UA	16.0	2760	40.9

a) 18:2 LH: methyl linoleate; Soy PC: soybean phosphatidylcholine; Ghost: erythrocyte ghosts. b) Reaction medium. A: micelles in 0.01 M Triton X-100 aqueous dispersions. B: liposomes in 0.1 M aqueous NaCl dispersions. C: ghost membranes in 10 mM isotonic phosphate buffer, pH 7.4. c) UA: uric acid; CSH: cysteine; GSH: glutathione. d) Induction period. e) Rate of oxygen uptake in the absence of an antioxidant or after induction period. f) [Fatty acids of membrane lipids] = 116 mg/l suspension.

References

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