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Kinetics of Vitamin E Regeneration by Water-Soluble Antioxidants in Micellar Dispersions

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The kinetics of reactions between water-soluble antioxidants (glutathione, uric acid, and cysteine) and vitamin E radicals in aqueous Triton X-100 micellar dispersions were studied by using a spectrophotometer, and the second-order rate constants for these reactions were compared to those for the corresponding reactions between vitamin C (ascorbic acid) and vitamin E radicals. These reactions are regarded as a model for regeneration of vitamin E by the water-soluble antioxidants in human blood, and the relative contributions of each antioxidant to the total regeneration in blood are discussed.

The ability of water-soluble and lipid-soluble antioxidants to protect cell membranes is a topic of current interest,[1] and several water-soluble compounds are known to function as antioxidants (AH's). Vitamin C (ascorbic acid, AsA), glutathione (GSH), uric acid (UA), and cysteine (Cys) (Fig. 1), for example, all suppress free-radical-mediated chain oxidation of lipids in cell membranes.[2] AsA is more reactive than GSH and Cys,[3] but in a biological tissue where the concentration of GSH or Cys is larger than that of AsA, free-radical-mediated chain oxidation may be inhibited more by GSH or Cys than by AsA, because the degree to which an AH contributes to the total antioxidant action in a tissue depends on the product of its reactivity and its concentration in that tissue. Furthermore, Terao and Niki[4] have reported that GSH, UA, and Cys suppress some kinds of radical-induced tissue damage that are not suppressed by AsA. Accordingly, the radical-scavenging and antioxidant functions of GSH, UA, and Cys deserve more attention and should be examined in detail. Of particular interest are the reactions by which vitamin E (tocopherol, TocH) is regenerated by these AH's.

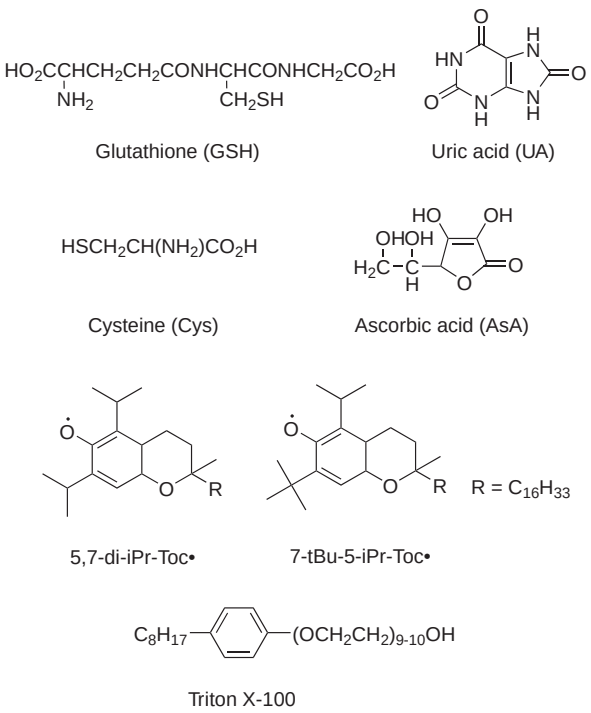

Fig. 1. Structures of molecules used in the present work.

TocH is localized in cell membranes and functions as an antioxidant protecting unsaturated lipids (LH's) from peroxidation (Fig. 2).[2,5–7] The antioxidant actions of TocH have been ascribed to the oxidation of its phenolic hydroxy group, producing a tocopheroxyl radical (Toc•). Toc•, formed in the membranes, reacts with AH's, such as AsA, GSH, UA, and Cys, at the interface between the membrane and the water phase to regenerate TocH (Fig. 2).

$$\text{Toc}\bullet + \text{AH} \xrightarrow{k_r} \text{TocH} + \text{A}\bullet \quad (1)$$

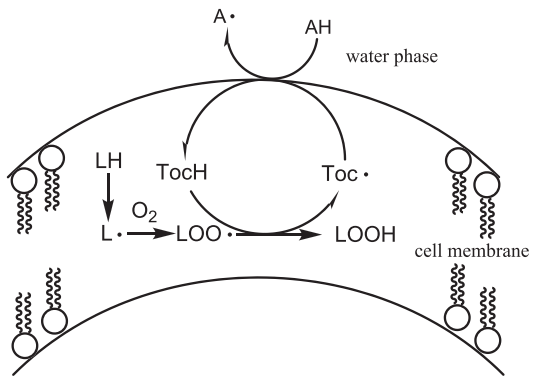

Fig. 2. Scheme of lipid peroxyl radical (LOO•) production and some of the antioxidant and regeneration reactions of TocH in cell membranes. A lipid radical (L•) formed from an unsaturated lipid (LH) reacts with oxygen to produce LOO•. The LOO• and TocH react to produce the hydroperoxide (LOOH) and Toc•, which reacts with AH at the interface between the membrane and the water phase to regenerate TocH (reaction 1).

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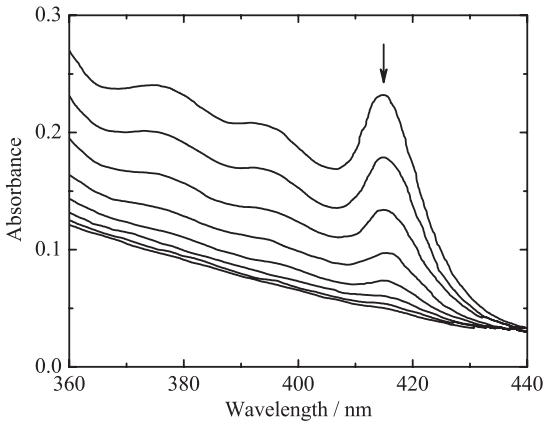

Fig. 3. An example of the absorption-spectrum change during the reaction between GSH and 5,7-di-*i*Pr-Toc•. The arrow indicates the decrease of the Toc• peak.

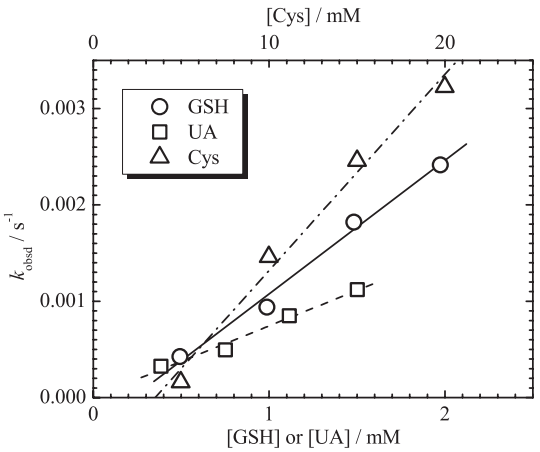

Fig. 4. Dependence of k_{obsd} on [AH] in the reaction of AH with 5,7-di-*i*Pr-Toc• under condition 1 in Table 1. The k_{obsd} value was obtained by monitoring the decrease of the Toc• peak at 415 nm.

The relative importance of each of these AH's, that is, the contribution of each to the total antioxidant capacity of biological tissues, is unknown. Furthermore, although reaction 1 in determining the kinetics of TocH-inhibited autoxidation and the efficiency of this inhibition is important and the activities of GSH, UA, and Cys have been discussed,[1,3,10,11] there have been only a few measurements of the absolute second-order rate constants for the reactions between these AH's and Toc•'s.[10]

We therefore determined by using a spectrophotometer the absolute values of the second-order rate constants (k_r's) for the reactions between these three AH's and two Toc•'s, 5,7-diisopropyltocopheroxyl (5,7-di-*i*Pr-Toc•) and 7-*t*-butyl-5-isopropyltocopheroxyl (7-*t*Bu-5-*i*Pr-Toc•) (Fig. 1), in aqueous Triton X-100 micellar dispersion. The relative contribution of each AH to the total regeneration action in human blood is discussed here.

Experimental

Commercial GSH, UA, Cys, and Triton X-100 were used as received. 5,7-di-*i*Pr-Toc• and 7-*t*Bu-5-*i*Pr-Toc• were used in this work because typical tocopheroxyl radicals like α-Toc• are unstable.[12] 5,7-diisopropyltocopherol and 7-*t*-butyl-5-isopropyltocopherol were synthesized according to the method reported in a previous paper,[13] and the corresponding Toc•-containing micellar dispersions were prepared according to the method reported in a previous paper.[14] The buffer solution was prepared using distilled water treated with a Millipore Q system, and its pH was adjusted to 7.0 using KH_2PO_4–Na_2HPO_4 buffer.

The kinetic data was obtained on a JASCO spectrophotometer Model UVIDEC-660 by mixing equal volumes of a solution of an AH and a dispersion of a Toc•. The reaction was studied under pseudo-first-order conditions, and the observed rate constant (k_{obsd}) was evaluated in the usual way using a standard least-squares analysis. All the measurements were performed at 25.0 ± 0.5 °C.

Table 1. k_r and Ratio of k_r to k_r^{AsA} (k_r/k_r^{AsA})

AH	Toc•	k_r/M^{-1} s^{-1} (1 M = 1 mol dm^{-3})	k_r/k_r^{AsA}
Condition 1[a]			
GSH	5,7-di-*i*Pr-Toc•	1.4	1.2×10^{-3}
UA	5,7-di-*i*Pr-Toc•	7.4×10^{-1}	6.2×10^{-4}
Cys	5,7-di-*i*Pr-Toc•	2.0×10^{-1}	1.7×10^{-4}
AsA	5,7-di-*i*Pr-Toc•	1.2×10^{3}	1.0
Condition 2[b]			
GSH	5,7-di-*i*Pr-Toc•	1.7	6.8×10^{-4}
UA	5,7-di-*i*Pr-Toc•	1.1	4.4×10^{-4}
Cys	5,7-di-*i*Pr-Toc•	1.1×10^{-1}	4.4×10^{-5}
AsA	5,7-di-*i*Pr-Toc•	2.49×10^{3} [c]	1.0
Condition 3[d]			
GSH	5,7-di-*i*Pr-Toc•	1.4	5.0×10^{-4}
UA	5,7-di-*i*Pr-Toc•	1.3	4.6×10^{-4}
AsA	5,7-di-*i*Pr-Toc•	2.8×10^{3}	1.0
GSH	7-*t*Bu-5-*i*Pr-Toc•	2.1×10^{-1}	6.6×10^{-4}
UA	7-*t*Bu-5-*i*Pr-Toc•	1.9×10^{-1}	5.9×10^{-4}
Cys	7-*t*Bu-5-*i*Pr-Toc•	1.8×10^{-2}	5.6×10^{-5}
AsA	7-*t*Bu-5-*i*Pr-Toc•	3.2×10^{2} [e]	1.0
Condition 4[f]			
GSH	5,7-di-*i*Pr-Toc•	1.5	—
GSH	7-*t*Bu-5-*i*Pr-Toc•	1.9×10^{-1}	—
Cys	7-*t*Bu-5-*i*Pr-Toc•	2.2×10^{-2}	—
Ref. 10[g]			
GSH	α-Toc•	2.5×10	—

a) AH: 0.5 wt % micellar dispersion in 0.02 M buffer. Toc•: 0.5 wt % micellar dispersion in 0.02 M buffer. b) AH: 5.0 wt % micellar dispersion in 0.02 M buffer. Toc•: 5.0 wt % micellar dispersion in 0.02 M buffer. c) Ref. 21. d) AH: in 0.1 M buffer solution. Toc•: 10 wt % micellar dispersion in 0.1 M buffer solution. e) Ref. 14. f) AH: 5.0 wt % micellar dispersion in 0.1 M buffer. Toc•: 5.0 wt % micellar dispersion in 0.1 M buffer. g) Reaction studied with nanosecond laser flash photolysis.

Results and Discussion

5,7-di-*i*Pr-Toc• and 7-*t*Bu-5-*i*Pr-Toc• in aqueous Triton X-100 micellar dispersions at pH 7.0 were comparatively stable in the absence of AH and showed absorption peaks at 375,

Table 2. [AH] in Human Blood (Erythrocytes, Plasma, and Whole Blood), k_r[AH], and k_r[AH]/k_r^{AsA}[AsA][a)]

AH	Erythrocytes			Plasma			Whole blood		
	[AH] /μM	k_r[AH] /s^{-1}	k_r[AH] /k_r^{AsA}[AsA]	[AH] /μM	k_r[AH] /s^{-1}	k_r[AH] /k_r^{AsA}[AsA]	[AH] /μM	k_r[AH] /s^{-1}	k_r[AH] /k_r^{AsA}[AsA]
GSH	1700 ± 150[b)]	2.4×10^{-3}	0.1	6.27 ± 1.18[c),d)]	8.8×10^{-6}	1.5×10^{-4}	2100[e)]	2.9×10^{-3}	5.4×10^{-2}
UA	—[f)]	—	—	160–450[e),g)]	2.3×10^{-4}	3.8×10^{-3}	60–180[h)]	8.9×10^{-5}	1.6×10^{-3}
Cys	5.79 ± 0.214[c),d)]	1.2×10^{-6}	5.0×10^{-5}	231 ± 15.2[c),d)]	4.6×10^{-5}	7.7×10^{-4}	—	—	—
AsA	20 ± 10[b)]	2.4×10^{-2}	1.0	50 ± 20[d),e)]	6.0×10^{-2}	1.0	42.3–48.4[d),i)]	5.4×10^{-2}	1.0

a) The k_r value obtained under condition 1 in Table 1 is used here. b) Ref. 17. c) Ref. 16. d) Normal adult. e) Ref. 8. f) Ratio of [UA] in erythrocytes to [UA] in plasma is $58.7 \pm 1.0\%$ (Ref. 20). g) Males. h) Ref. 18. i) Ref. 19.

395, and 415 nm. These peaks disappeared when excess AH's were added to the micellar dispersions, showing that the corresponding reactions can be regarded as a model for the regeneration of TocH by AH's in human blood. An example of the interaction between 5,7-di-*i*Pr-Toc• and GSH is shown in Fig. 3, and the pseudo-first-order rate constant k_{obsd} could be estimated from the exponential decay of the peak absorbance at 415 nm. Since the natural decay of Toc• is very slow, the k_{obsd} for Toc• bleaching by an AH is given by

$$k_{obsd} = k_0 + k_r[AH], \quad (2)$$

where k_0 and k_r respectively denote the natural-decay rate-constant of Toc•[14] and the second-order rate constant of reaction 1. These rate parameters can be evaluated by plotting k_{obsd} against [AH] (Fig. 4). The k_r values for GSH, UA , and Cys are listed in Table 1 together with the k_r values for AsA (k_r^{AsA}) that were obtained under the same experimental conditions.

The k_r values decreased in the order AsA > GSH > UA > Cys, whereas Tsuchiya et al.[3] have reported that the k_{obsd} value of Cys is greater than that of GSH. The k_r values for 5,7-di-*i*Pr-Toc• are larger than those for 7-*t*Bu-5-*i*Pr-Toc• (see the results listed under condition 3 or 4 in Table 1) because the steric hindrance of the *t*-butyl group of 7-*t*Bu-5-*i*Pr-Toc• impedes the approach of AH to the O• and thus reduces the k_r values. The k_r values for α-Toc•,[10,15] in which the steric hindrance is even less than that in 5,7-di-*i*Pr-Toc•, are even larger than those for 5,7-di-*i*Pr-Toc•. The k_r value for GSH scarcely changes with changes in the concentrations of buffer and micelle (conditions 1, 2, and 4). The k_r value is much the same whether the AH reacting with a micellar dispersion of Toc• is initially in a buffer solution without micelles (condition 3) or is itself initially in a micellar dispersion (condition 4).

The relative importance of reaction 1 for an AH in human blood depends on the product of the reactivity (k_r) and the concentration ([AH]). In Table 2 are listed the [AH] in erythrocytes (red cells), plasma, and whole blood,[8,16–19] the product of k_r and [AH], and the ratio of k_r[AH] to k_r^{AsA}[AsA]. Since the product of k_r^{AsA} and the [AsA] in human blood is larger than those for GSH, UA, and Cys, AsA should play a major role in the regeneration of TocH.

More than 99% of the GSH in whole blood is localized in erythrocytes,[16] but AsA is distributed more uniformly,[8,17,19] so the [GSH] in erythrocytes is 85 times the [AsA] there (Table 2). As a result, although the k_r for GSH is 1/860 of k_r^{AsA} under condition 1 (Table 1), in erythrocytes k_r[GSH] would be about 1/10 of k_r^{AsA}[AsA]. Accordingly, GSH would play a not insignificant role in the regeneration of TocH in erythrocytes.

Although UA, like AsA, is uniformly distributed in human blood,[8,18,20] the k_r value for UA is much less than k_r^{AsA}. Thus, UA should not play an important role in the regeneration of TocH in human blood.

In whole blood, 97% of the Cys is in the plasma,[16] where the [Cys] is 4.6 times the [AsA]. Since the k_r value for Cys, however, is only 1/6000 of k_r^{AsA} under condition 1 (Table 1), in plasma, k_r[Cys] would be about 1/1300 of k_r^{AsA}[AsA], which means that Cys contributes relatively little to the regeneration of TocH in plasma.

In conclusion, the kinetics of the reactions between the AH's (GSH, UA, and Cys) and Toc• in aqueous Triton X-100 micellar dispersions was studied by using a spectrophotometer, and the k_r values for these AH's was compared to those for AsA. The reactivity of AsA in the regeneration of TocH is much higher than that of GSH, UA, or Cys. In erythrocytes, however, where the [GSH] is much larger than the [AsA], GSH may compete with AsA in the regeneration of TocH.

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