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Kinetic study on the prooxidative effect of vitamin C on the autoxidation of glycerol trioleate in micelles

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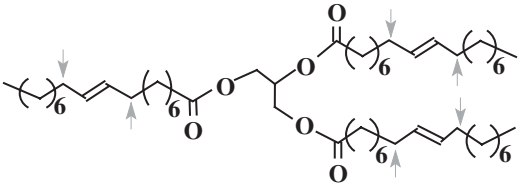
ABSTRACT: Vitamin C (L-ascorbic acid) protects human health by scavenging toxic free radicals and other reactive oxygen species formed in cell metabolism. The surplus supplementation of vitamin C, however, may be harmful to health because the level of 8-oxoguanine and 8-oxoadenine in lymphocyte DNA varies remarkably. In the process of the kinetic investigation on the 2,2′-azobis(2-amidinopropane dihydrochloride) (AAPH)-induced autoxidation of glycerol trioleate (GtH) in the micelles of cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton X-100, the addition of vitamin C accelerates the autoxidation of GtH even in the absence of the free radical initiator, AAPH. The initiating rate, R_i, of vitamin C (VC)-induced autoxidation of GtH is related to the micelle charge, i.e. $R_i = 14.4 \times 10^{-6}$ [VC] s^{-1} in SDS (anionic micelle), $R_i = 1961 \times 10^{-6}$ [VC] s^{-1} in Triton X-100 (neutral micelle) and R_i is a maximum in CTAB (cationic micelle) when the vitamin C concentration is $\sim 300\,\mu M$. Thus, vitamin C can initiate autoxidation of GtH in micelles, especially in the neutral one. Moreover, the attempt to explore whether α-tocopherol (TocH) could rectify vitamin C-induced autoxidation of GtH leads us to find that the rate constant of TocH reacting with the anionic radical of vitamin C ($VC^{\cdot -}$), k_{-inh}, is $\sim 10^3 M^{-1} s^{-1}$, which is less than that of the α-tocopherol radical ($Toc^{\cdot}$) with vitamin C ($k_{inh} = \sim 10^5 M^{-1} s^{-1}$). Thus, the equilibrium constant of the reaction $Toc^{\cdot} + VC^{-} \rightleftharpoons TocH + VC^{\cdot -}$ is prone strongly to the regeneration of $Toc^{\cdot}$ by vitamin C rather than the reverse reaction. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: vitamin C; α-tocopherol; glycerol trioleate; micelle; prooxidant; autoxidation

INTRODUCTION

Vitamin C (L-ascorbic acid) is an important antioxidant that efficiently scavenges toxic free radicals and other reactive oxygen species (ROS) formed in cell metabolism. It also acts as a co-substrate of many essential dioxygenases, regenerating enzyme, and plays a role in gene expression.[1] In particular, esterifying the 6-hydroxyl by fatty acid to enhance its lipophilicity[2] makes it a more effective antioxidant than its parent against free-radical-induced peroxidation of human low-density lipoprotein (LDL)[3] and erythrocytes.[4] In addition, it can play a mutually protective role with α-tocopherol (TocH) in the reaction $Toc^{\cdot} + VC \rightarrow TocH + VC^{\cdot -}$,[5] for which the rate constant is $k_{inh} \sim 10^5 M^{-1} s^{-1}$.[6] However, the result of the present study reveals that surplus supplementation of vitamin C to healthy people leads to levels of 8-oxoguanine and 8-oxoadenine in their lymphocyte DNA that vary remarkably, implying that vitamin C could exhibit a prooxidant property *in vivo*.[7] Hence, whether the prooxidative behavior of vitamin C can be validated in a chemical reaction system and what the kinetic circumstances are in the prooxidative process of vitamin C are major investigations in this work.

Linoleic acid is generally the substrate in the kinetic research of a free-radical-related reaction because a biallyl position in its carbon chain is very susceptible to free-radical-induced peroxidation, and cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton X-100 are always applied to mimic the cationic, anionic and neutral media, respectively.[8] However, in this work glycerol trioleate (GtH) is chosen to be the substrate in order to mimic erythrocyte membrane. Moreover, the major unsaturated fatty acid in human erythrocyte membrane is oleic acid, and the structure of GtH is similar to phosphoglyceride.[9,10]



Glycerol trioleate (GtH)

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Furthermore, it is possible for the six allyl positions in GtH (as the arrows show) to be attacked by free radicals generated from the decomposition of a water-soluble azo compound, 2,2′-azobis(2-amidinopropane dihydrochloride) (AAPH) (R—N═N—R, where $R = C(CH_3)_2C(=NH)NH_2$), at 37 °C, for which the free radical generation rate, R_g, can be calculated by Eqn (1).[11]

$$R_g = 1.3 \times 10^{-6}[\text{AAPH}]\text{s}^{-1} \quad (1)$$

Thus, presented here is a series of kinetic data indicating that vitamin C accelerates the autoxidation of GtH in the micelles of SDS, CTAB and Triton X-100, respectively.

EXPERIMENTAL

Glycerol trioleate, vitamin C, SDS and CTAB were purchased from Sino-western Chemical Ltd (Beijing, China) and AAPH, TocH and Triton X-100 were from Aldrich, used as received. The AAPH was dissolved in phosphate-buffered saline (PBS, which contains 5 mM Na_2HPO_4 and 5 mM NaH_2PO_4, pH7.0). The SDS, CTAB and Triton X-100 were dissolved in PBS to reach concentrations of 100, 20 and 20 mM, respectively, and agitated ultrasonically to form micelles. Glycerol trioleate was dispersed in various micelle–PBS solutions ultrasonically and kept at ambient temperature under an atmosphere of oxygen. α-Tocopherol was also dispersed into the corresponding micelle–PBS solution just before the experiment.

The process of AAPH-induced peroxidation of GtH was followed *in situ* by SP-3 oxygen uptake apparatus equipped with an oxygen electrode that was sensitive to oxygen concentrations as low as 10^{-8} M (Shanghai Institute of Phytobiology, Chinese Academy of Sciences). Figure 1 displays a typical oxygen uptake chart, with every determination repeated at least three times and an experimental error within 10%. Statistical analysis of the relationship between the concentration of vitamin C and the initiating rate, R_i, was performed by one-way ANOVA using Origin 6.0 professional software.

RESULTS AND DISCUSSION

Deduction of kinetic equations based on oxygen uptake determination in the process of AAPH-induced peroxidation

It has been proved that the free radical ($R^{\cdot}$)-induced peroxidation of polyunsaturated fatty acids (LH) in micelles follows the same rate law as that in homogeneous solutions,[12] which can be represented by the following procedure:

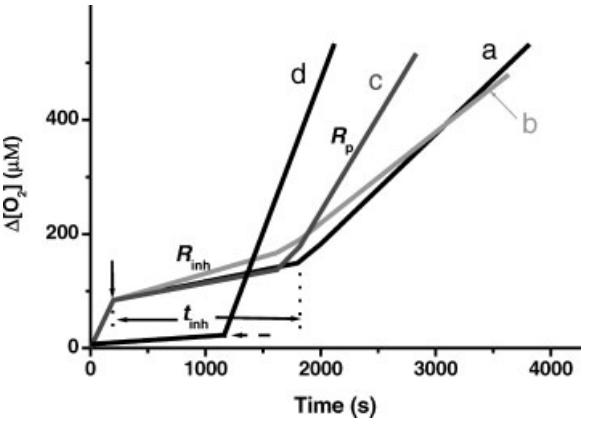


Figure 1. Oxygen uptake curves recorded during the AAPH-induced autoxidation of glycerol trioleate (GtH) in the micelles of SDS (a), CTAB (b) and Triton X-100 (c). Vertical arrow indicates the addition of α-tocopherol in concentrations of 5.08 μM (a), 20.0 μM (b) and 15.2 μM (c). [AAPH] = 20 mM; [GtH] ~ 8.00 mM; micelle concentrations are [SDS] = 100 mM, [CATB] = 20 mM and [Triton X-100] ~ 20 mM. Line d shows that the addition of vitamin C to GtH at the horizontal arrow makes the oxygen uptake rate increase rapidly, indicating that vitamin C initiates the autoxidation of GtH

Initiation:

$$\text{R} - \text{N} = \text{N} - \text{R} \xrightarrow{k_d} 2e\text{R}^{\cdot} + \text{N}_2 + (1-e)\text{R} - \text{R} \quad (2)$$

$$\text{R}^{\cdot} + \text{O}_2 \xrightarrow{fast} \text{ROO}^{\cdot} \quad (3)$$

$$\text{ROO}^{\cdot} + \text{LH} \rightarrow \text{ROOH} + \text{L}^{\cdot} \quad (4)$$

Propagation:

$$\text{L}^{\cdot} + \text{O}_2 \xrightarrow{fast} \text{LOO}^{\cdot} \quad (5)$$

$$\text{LOO}^{\cdot} + \text{LH} \xrightarrow{k_p} \text{LOOH} + \text{L}^{\cdot} \quad (6)$$

Termination:

$$\text{LOO}^{\cdot} + \text{LOO}^{\cdot} \xrightarrow{2k_t} \text{non-radical product (NRP)} \quad (7)$$

where k_d, k_p and k_t are rate constants for decomposition of the initiator, chain propagation and termination, respectively; and e designates the fraction of the initiator that is effective in initiating the peroxidation due to the cage effect of the solvent. Based on the steady-state kinetic treatment, the rate of oxygen uptake in the period of propagation, R_p, can be expressed as Eqn (8).[13,14]

$$R_p = -\text{d}[\text{O}_2]/\text{d}t = (k_p/(2k_t)^{0.5})R_i^{0.5}[\text{LH}] \quad (8)$$

where $k_p/(2k_t)^{0.5}$ is referred to as the *oxidizability* of the substrate, representing the susceptibility of LH to undergo peroxidation. When an antioxidant (AH) such as TocH is added to the above reaction system, it traps the peroxyl radical ($LOO^{\cdot}$) to generate an antioxidant radical ($A^{\cdot}$), as Eqn (9) shows. If $A^{\cdot}$ is so stable that it couples rapidly with $LOO^{\cdot}$ to form a non-radical product LOOA, as Eqn (10) shows, the peroxidation would be inhibited efficiently.

$$LOO^{\cdot} + AH \xrightarrow{k_{inh}} LOOH + A^{\cdot} \quad (9)$$

$$LOO^{\cdot} + A^{\cdot} \xrightarrow{fast} LOOA \quad (10)$$

According to the steady-state hypothesis, the rate of $LOO^{\cdot}$ formation is equal to that of $LOO^{\cdot}$ trapped by AH in the inhibitive period, thus the rate during the inhibition period, R_{inh}, is given by

$$R_{inh} = k_{inh}\, n\, [AH][LOO^{\cdot}] \quad (11)$$

where the stoichiometric factor n designates the number of $LOO^{\cdot}$ radicals trapped by every AH molecule, and can be expressed as

$$n = (R_i\, t_{inh})/[AH] \quad (12)$$

For example, the n for AH (which is TocH) is 2, meaning that one molecule of TocH traps two free radicals.[15,16] Thus, the initiating rate, R_i, can be obtained in accordance with Eqn (12) by measuring the inhibition period, t_{inh}. Meanwhile, the application of steady-state treatment to Eqns (9–12) leads R_{inh} to be expressed as Eqn (13).[17]

$$R_{inh} = -d[O_2]/dt = (k_p R_i[LH])/(nk_{inh}[AH]) \quad (13)$$

Moreover, the kinetic chain length *kcl*, which defines the cycle number of chain propagation, is given by Eqn (14) in the period of inhibition (kcl_{inh}) and propagation (kcl_p).

$$kcl_{inh} = R_p/R_i \quad \text{and} \quad kcl_p = R_p/R_i \quad (14)$$

Therefore, according to the kinetic detail, the benefit of oxygen uptake measurement for a free radical reaction could be revealed simply by determination of t_{inh}, R_{inh} and R_p.[18]

Kinetic data of AAPH-induced autoxidation of GtH in various micelles

Lines a, b and c in Fig. 1 are typical oxygen uptake curves of AAPH-induced autoxidation of GtH in SDS, CTAB and Triton X-100, respectively. Each line is divided into three parts: the rapid oxygen consumption in the first period (0–200 s) shows that AAPH initiates the autoxidation of GtH; then the oxygen-exhausting rate slows down due to the addition of TocH at the point of the vertical arrow, with the slope of the oxygen uptake curve in this period given by R_{inh}; finally, the oxygen-exhausting rate increases, with the slope of the oxygen uptake curve in this period given by R_p, indicating that the AAPH-induced autoxidation of GtH continues after depletion of TocH. The time within the TocH-adding point and the turning point is the inhibition period, t_{inh}. After measurement of R_{inh}, R_p and t_{inh} in various micelles, the above-mentioned kinetic data are calculated and collected in Table 1.

The radical-generating rate of AAPH, R_g, is 2.6×10^{-8} M s^{-1} in accordance with Eqn (1), and 20 mM AAPH is utilized. But R_i in Table 1 is less than R_g because the *phase-transfer efficiency*

$$\varepsilon = R_i/R_g \quad (15)$$

is < 1.[16] It can be calculated by Eqn (15) that in CTAB $\varepsilon \sim 1$, in Triton X-100 $\varepsilon = 0.77$ and in SDS $\varepsilon = 0.23$. This result implies that not all the free radicals derived from the decomposition of AAPH are able to transfer through the micelle to initiate the autoxidation of GtH. In particular, the anionic micelles of SDS decrease the transfer efficiency of free radical from the aqueous phase to micelles. This maybe because the cationic radicals from AAPH, $.OOC(CH_3)_2C(=NH)NH_3^+$, are adsorbed by the anionic surface of SDS micelles, thus hindering their embedding into the intra-micelle of SDS. Triton X-100 supplies a non-charged microenvironment for the cationic radicals to transfer into the intra-micelle. Thus, the 0.77 value of ε in Triton X-100 indicates the diffuse efficiency of radicals themselves without the influence of micelle charge. In contrast, the ~ 1 value of ε in CTAB indicates that all the radicals derived from AAPH transfer into the CTAB micelles to initiate autoxidation of GtH.

The *oxidizability*, $k_p/(2k_t)^{0.5}$, of GtH in the different micelles can be calculated using Eqn (8) after the corresponding R_i value has been obtained. As can be seen in Table 1, the smallest value of $k_p/(2k_t)^{0.5}$ in CTAB (0.0945) means that it is more difficult for GtH to undergo peroxidation than in the other two micelles. Meanwhile, the kcl_{inh} and kcl_p values, either in CTAB or in Triton X-100, are smaller than in SDS, revealing that the free-radical chain is much shorter in these two micelles than in SDS.

Autoxidation of GtH induced by vitamin C

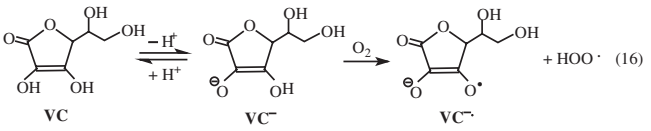
Line d in Fig. 1 shows that the addition of vitamin C at the vertical arrow accelerates the oxygen uptake remarkably,

Table 1. Inhibition of AAPH-induced autoxidation of glycerol trioleate (GtH) by α-tocopherol (TocH) in various micelles

Micelle	[GtH] (mM)	[TocH] (μM)	t_{inh} (s)	R_{inh} ($\times 10^{-8}$ M s^{-1})	R_p ($\times 10^{-8}$ M s^{-1})	R_i[a] ($\times 10^{-8}$ s^{-1})	k_{inh}[b] ($\times 10^3$ M^{-1} s^{-1})	$k_p/(2k_t)^{0.5}$[c] (M$^{-0.5}$ s$^{-0.5}$)	kcl_{inh}[d]	kcl_p[d]
SDS	7.78	5.08	1700 ± 140	4.66 ± 0.23	19.3 ± 1.3	0.598	9.81	0.321	7.8	32.3
CTAB	8.30	20.0	1518 ± 136	5.85 ± 0.26	16.9 ± 1.1	2.64	9.34	0.0945	2.2	6.4
Triton X-100	8.36	15.2	1520 ± 114	3.74 ± 0.16	37.2 ± 2.6	2.00	14.7	0.265	1.9	18.6

[a] R_i is calculated using Eqn (12).
[b] k_{inh} is calculated using Eqn (13), in which k_p is taken as 100 M^{-1}s^{-1}.[17]
[c] $k_p/(2k_t)^{0.5}$ is calculated using Eqn (8).
[d] kcl_{inh} and kcl_p are calculated using Eqn (14).

indicating that it is the addition of vitamin C that accelerates the autoxidation of GtH in these micelles. In other words, vitamin C replaces the AAPH to be an initiator to induce autoxidation of GtH. This interesting phenomenon gives us direct evidence for vitamin C as a prooxidant in the chemical reaction system. But how the vitamin C initiates the autoxidation of GtH is worth investigating and Scheme 1 may be a reasonable deduction. Firstly, vitamin C as an acid ($pK_a = 4.17$) ionizes to form its anion, VC^-, which can be oxidized easily by oxygen in the experimental system, as Eqn (16) shows.[19]



(16)

Then $VC^{-\cdot}$, as can be seen in Scheme 1, acts as the initiating radical to abstract the allyl hydrogen atom in GtH, and HOO· can also oxidize GtH to form Gt·, which combines with oxygen to form the peroxyl radical GtOO·, as Eqn (17) shows.

$$GtH + VC^{-\cdot}(HOO^{\cdot}) \xrightarrow{O_2} GtOO^{\cdot} + VC^{-}(H_2O_2) \quad (17)$$

Then the free radical propagates by GtOO·, as in Eqn (18).

$$GtOO^{\cdot} + GtH \rightarrow Gt^{\cdot} + GtOOH \quad (18)$$

Thus, the vitamin C-induced autoxidation of GtH could be regarded to proceed along with the self-oxidation of vitamin C by the oxygen dissolved in the solution.

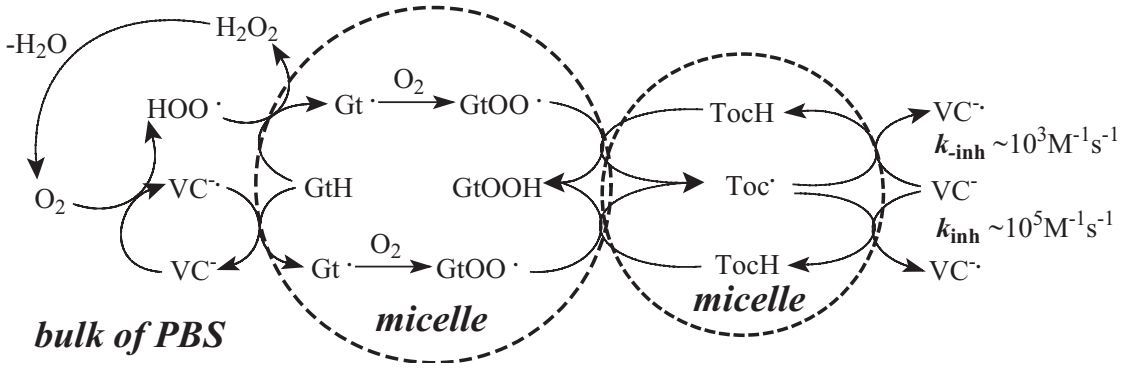
The initiating rate R_i of vitamin C-induced autoxidation of GtH is important for revealing its prooxidant property in detail. Now that the *oxidizabilities*, $k_p/(2k_t)^{0.5}$, in different micelles have been obtained (see Table 1), the R_i of vitamin C can be obtained via Eqn (8) by determination of the corresponding R_p. Furthermore, the kcl_p in the presence of vitamin C can be obtained via Eqn (14). These kinetic data are collected in Table 2, and the relationship between R_i and vitamin C concentration is illustrated graphically in Fig. 2.

Figure 2 shows that R_i increases directly with vitamin C concentration in SDS, and especially in Triton X-100. The quantitative relationship can be expressed by Eqns (19) and (20).

$$\text{In SDS:} \quad R_i = 14.4 \times 10^{-6}[VC]s^{-1} (P < 0.005) \quad (19)$$

$$\text{In Triton X} - 100: \quad R_i = 1961 \times 10^{-6}[VC]s^{-1} (P < 0.001) \quad (20)$$

The coefficients in Eqns (19) and (20) (14.4×10^{-6} and 1961×10^{-6}) are ~11 and ~1500 times larger,



Scheme 1. Proposed vitamin C-induced autoxidation of glycerol trioleate (GtH)

Table 2. Prooxidative effect of vitamin C on the autoxidation of glycerol trioleate (GtH) in various micelles

Micelle	[GtH] (mM)	[VC] (μM)	R_p ($\times 10^{-8}$ M s^{-1})	R_i ($\times 10^{-8}$ s^{-1})	kcl_p
SDS	7.89	497	22.5 ± 0.57	0.791	28.5
	8.03	402	16.7 ± 0.82	0.419	39.9
	8.03	296	13.8 ± 0.82	0.287	48.1
	7.67	201	9.76 ± 0.40	0.157	62.2
	7.67	106	8.66 ± 0.23	0.124	69.8
	8.29	10.6	3.31 ± 0.21	0.0155	213
CTAB	7.92	497	8.14 ± 0.78	1.18	6.9
	8.09	402	10.7 ± 0.17	1.95	5.5
	8.09	296	13.1 ± 0.18	2.93	4.5
	7.80	201	12.1 ± 0.51	2.67	4.5
	7.95	106	7.87 ± 0.32	1.10	7.2
	7.95	10.6	3.93 ± 0.22	0.273	14.4
Triton X-100	8.36	200	142 ± 6.8	41.1	3.5
	8.36	105	87.1 ± 5.2	15.4	5.6
	8.36	52.6	54.6 ± 1.9	6.07	9.0
	8.43	5.25	30.7 ± 1.8	1.89	16.3
	8.36	1.58	17.5 ± 0.81	0.625	28.0
	8.36	0.53	8.37 ± 0.16	0.143	58.7

respectively, than that of AAPH (1.3×10^{-6}; see Eqn (1)), indicating that vitamin C functions as a prooxidant to induce the autoxidation of GtH even more efficiently than AAPH, especially in Triton X-100 micelles. This is due to the micelle charge having a much weaker influence on $VC^{-\cdot}$ in neutral micelles. However, SDS with its anionic surface could hinder the transfer of $VC^{-\cdot}$ through its surface. On the other hand, R_i in CTAB increases directly to become a maximum ([VC] $\sim$300 μM) and then decreases with increase in vitamin C concentration. Nevertheless, the R_i of vitamin C in CTAB is still higher than that of AAPH and the fact that the micelle charge affects R_i so remarkably is in agreement with a previous report.[6]

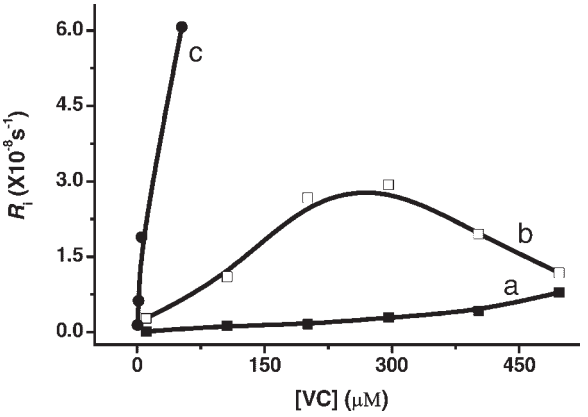


Figure 2. Relationship between the concentration of vitamin C and the initiation rate, R_i, in the micelles of SDS (a), CTAB (b) and Triton X-100 (c)

Attempt to inhibit vitamin C-induced autoxidation of GtH by addition of TocH

The synergistic antioxidative process between TocH and vitamin C (see right-hand side of Scheme 1) can be expressed as Eqn (21).[6]

$$Toc^{\cdot} + VC \rightarrow TocH + VC^{\cdot -} \qquad (21)$$

Whether the converse reaction (right-hand side of Scheme 1), as Eqn (22) shows, could take place is another attractive problem in our research.

Table 3. The effect of α-tocopherol (TocH) on vitamin C-induced autoxidation of glycerol trioleate (GtH) in various micelles

Micelle	[VC] (μM)	[GtH] (mM)	[TocH] (μM)	R_{inh} ($\times 10^{-8}$ M s^{-1})	k_{-inh} ($\times 10^{3}$ M^{-1} s^{-1})
SDS	106	8.59	127	5.95 ± 0.20	0.0679
	($R_i = 1.24 \times 10^{-9}$ s^{-1})	8.59	102	6.34 ± 0.35	0.0825
		8.59	76.2	5.54 ± 0.25	0.126
		8.37	50.8	6.48 ± 0.34	0.157
		8.37	25.4	6.28 ± 0.12	0.324
CTAB	106	8.35	126	8.97 ± 0.54	0.404
	($R_i = 11.0 \times 10^{-9}$ s^{-1})	8.10	100	8.35 ± 0.30	0.530
		8.10	76.2	8.06 ± 0.15	0.723
		8.52	50.1	8.26 ± 0.21	1.13
		8.46	26.1	7.76 ± 0.33	2.29
		8.46	6.00	8.57 ± 0.56	8.99
Triton X-100	52.6	7.94	152	15.3 ± 0.28	1.04
	($R_i = 60.7 \times 10^{-9}$ s^{-1})	8.65	122	16.9 ± 0.59	1.28
		8.65	91.3	16.7 ± 0.69	1.73
		8.85	60.9	20.7 ± 0.88	2.13
		7.94	30.4	23.4 ± 0.78	3.39
		8.85	9.13	38.4 ± 0.66	7.66

$$\mathrm{Toc}^{\cdot} + \mathrm{VC}^{-} \underset{k_{-inh}}{\overset{k_{inh}}{\rightleftharpoons}} \mathrm{TocH} + \mathrm{VC}^{\cdot -} \qquad (22)$$

Hence, TocH dispersed in the corresponding micelle is added to the vitamin C induced autoxidation of GtH for detecting R_{inh}, and k_{-inh} is calculated via Eqn (13) and collected in Table 3.

As can be found in Table 3, R_{inh} varies slightly in the presence of various concentrations of TocH, indicating that the addition of TocH cannot abate the prooxidant effect of vitamin C on the autoxidation of GtH. The reason why the reverse reaction of Eqn (22) cannot be observed significantly should be explained by calculation of the equilibrium constant of Eqn (22), K_{eq}, as Eqn (23) shows.

$$K_{eq} = k_{inh}/k_{-inh} \qquad (23)$$

The magnitudes of k_{inh} ($\sim 10^5$ M^{-1} s^{-1})[6] and k_{-inh} ($\sim 10^3$ M^{-1} s^{-1}) lead to K_{eq} $\sim$100. This result reveals that the equilibrium of Eqn (22) is prone to the regeneration of Toc$^{\cdot}$ by vitamin C rather than the reverse reaction. Moreover, the relationships between k_{-inh} and [TocH]/[VC] are illustrated in Fig. 3.

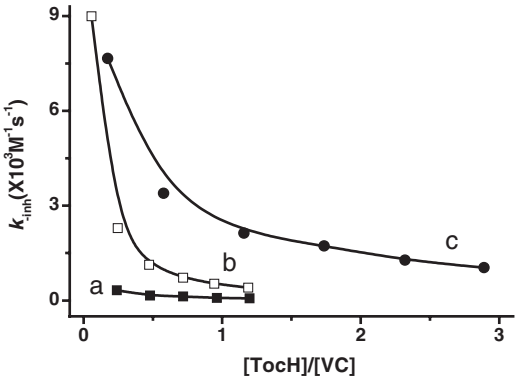


Figure 3. Relationship between the inhibition rate constant, k_{inh}, and the concentration ratio between α-tocopherol and vitamin C in the micelles of SDS (a), CTAB (b) and Triton X-100 (c)

Interestingly, along with the increase in the concentration of TocH, k_{-inh} decreases remarkably in three kinds of micelles, particularly in CTAB and Triton X-100. This phenomenon must be related also to the distribution status of the TocH and GtH, the diffusive velocity of VC$^{\cdot -}$ in different micelles and the interaction of VC$^{\cdot -}$ with TocH. As shown on the right-hand side of Scheme 1, vitamin C plays the antioxidant role by repairing Toc$^{\cdot}$ in the micelles, and the left-hand side of Scheme 1 shows that vitamin C can initiate autoxidation of GtH by its self-oxidation.

CONCLUSION

In summary, the self-oxidation of vitamin C by oxygen in solution could be the reason why vitamin C can initiate autoxidation of GtH in different micelles. The initiating rate (R_i) of vitamin C is larger than that of AAPH, especially in Triton X-100, and in CTAB micelle it tends towards a maximum with an increase of vitamin C concentration. Moreover, TocH cannot rectify the prooxidant property of vitamin C because the equilibrium of the reaction, $\mathrm{Toc}^{\cdot} + \mathrm{VC} \rightleftharpoons \mathrm{TocH} + \mathrm{VC}^{\cdot -}$ is prone to the forward rather than the backward reaction. The result obtained here is direct evidence for the prooxidant property of vitamin C in a chemical reaction system.

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