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The effects of ascorbic acid on homolytic processes involving α-hydroxyl-containing carbon-centered radicals

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ABSTRACT

Effects of ascorbic acid and 5,6-*O*-isopropylidene-2,3-*O*-dimethylascorbic acid on final product formation in radiolysis of ethanol, aqueous solutions of ethanol, ethylene glycol, α-methylglycoside, maltose, α-glycerophosphate, and α-glucose phosphate were studied. It was found that ascorbic acid is able to suppress reactions involving various α-hydroxyl-containing carbon-centered radicals and depending on the experimental conditions can either oxidize or reduce α-hydroxyethyl radicals.

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The essential role played by ascorbic acid (AA) in various functions of a human organism determines the interest towards studying its properties. There are extensive discussions in the literature concerning the relationship between the high reactivity of AA towards reactive oxygen species (ROS) and its useful medicinal properties.[1,2] The latter are believed to be in many respects due to the ability of AA to either accept ROS or reduce the oxygen-centered radicals formed from α-tocopherol, enhancing thereby its antioxidant properties manifested in a living organism.[1,3] At the same time, there is virtually no information as to how AA affects other types of free-radical transformations of biologically relevant substances.

In studies performed earlier,[4–11] a concept was developed that the reactions of free-radical fragmentation play an important role in biosystem injury. These processes occur via the stage of formation of α-hydroxyl-containing carbon-centered radicals (HCR) and lead to destruction and modification of carbohydrates,[9,10] amino acids,[6] and lipids.[7,8] Quinones,[5,11] flavoniods,[12] and group B vitamins[13] were found to suppress free-radical fragmentation of hydroxyl-containing organic compounds. There is a carbonyl group conjugated with a C=C bond in the AA structure. The presence of such structural moiety in quinones and flavonoids determines in many respects their reactivity towards HCR. In order to assess the role of the named structural moiety of AA in its reactions with HCR 5,6-*O*-isopropylidene-2,3-*O*-dimethylascorbic acid (**I**) has been synthesized using the procedure described earlier.[14] In this study, interactions of AA and **I** (Fig. 1) with HCR formed in radiolysis of deaerated ethanol, as well as aqueous solutions of ethanol, ethylene glycol, α-methylglycoside, maltose, α-glycerophosphate, and α-glucose phosphate were investigated using the steady state radiolysis method.

Except for **I**, all of the compounds necessary for the study were commercially available (Sigma–Aldrich, Merck), and were used without additional purification, if not stated otherwise. Ethanol was purified with molecular sieves Wolfen Zeosorb LA, followed by rectification; ethylene glycol was distilled at 5 mm Hg. Twice-distilled water was used to prepare aqueous solutions. Phosphate buffer was used to maintain the solution pH value at 7 ± 0.05. The pH value of aqueous ethanol was adjusted to 3 ± 0.1 with diluted perchloric acid. For solutions of the organic phosphates, the pH value was adjusted to 7 ± 0.05 by adding the required amounts of perchloric acid. After dissolving the additives ($C = 10^{-3}$ mol/l)[†], the solutions were poured into ampoules, deaerated with argon, and the ampoules were sealed. The irradiation was performed using a MPX-γ-25 M unit (^{60}Co, dose rate 0.50 ± 0.0076 Gy/s, absorbed dose range 0.15–1.8 kGy). Determination of the major final products of radiation-induced transformations of ethanol and α-methylglycoside (acetaldehyde, 2,3-butanediol, and methanol) was performed using a Shimadzu GC-17AAF/APC gas chromatograph according to procedures described in Lagutin et al.[13] Radiolysis products of aqueous ethylene glycol (glycolic aldehyde, acetaldehyde) were determined in the form of adducts with 2,4-dinitriphenylhydrazine by HPLC using a Shimadzu LC-10AVP liquid chromatograph.[15] Glucose in maltose solutions was determined by HPLC according to Edime-

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† The concentration of compound **I** in aqueous ethanol and ethylene glycol solutions was 5.10^{-4}mol/l.

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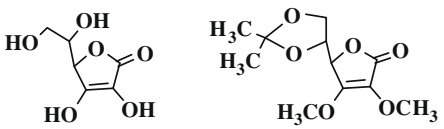
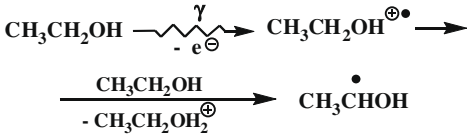


Ascorbic acid (AA) 5,6-O-isopropylidene-2,3-O-dimethylascorbic acid (I)

Figure 1. Structures of compound studied.

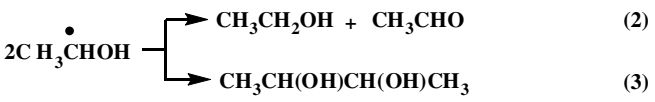
cheva et al.[9] Inorganic phosphate in the presence of organic phosphates was determined using a reagent/spectrophotometric method.[5] Concentrations of AA and **I** were measured by HPLC at 245 nm. Analysis conditions: column Nucleosil 120-5 C-18; flow rate 0.5 ml/min; eluents: for AA—aqueous phosphate buffer pH 3; for **I**—methanol/water (4:1). Radiation-chemical yields (G, mol/J) were calculated from linear portions of relationships between the products (or starting compounds) concentrations and dose absorbed.

The α-hydroxyethyl radical (HER) is the main radical product in radiolysis of ethanol, and its formation occurs according to the scheme shown below[16]:

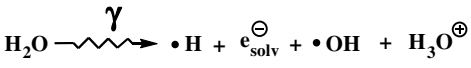


(1)

Thereafter, HER undergoes disproportionation and recombination reactions to form acetaldehyde and 2,3-butanediol, respectively:

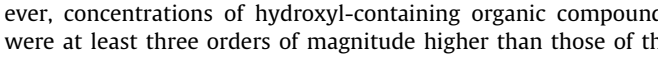


In radiolysis of diluted aqueous solutions, the free-radical processes are initiated by radical products of water radiolysis, mainly •OH and •H species, formed by the following reaction:



(4)

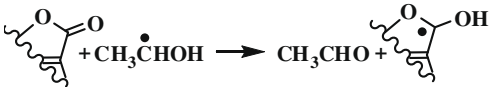
The rate constants for reactions of •OH and •H species with the hydroxyl-containing organic compounds are comparable with the respective values for AA and its derivative **I**.[17] In this study, however, concentrations of hydroxyl-containing organic compounds were at least three orders of magnitude higher than those of the additives. Therefore, any interference from interaction of the water radiolysis products with the additives may be neglected. Hence, the effects observed are exclusively due to reactions of AA and **I** with HCR formed from the initial compounds. On interaction of ethanol with •OH and •H species, HER are formed, which are then consumed in biradical reactions (2) and (3):



(5)

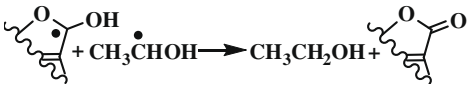
The obtained values for final product yields in radiolysis of ethanol and its aqueous solutions in the presence of AA and its derivative **I** are presented in Table 1. The compound **I** has no movable hydrogen atom in its structure, and hence it is a priori incapable of playing the role of a reducing agent in reactions with HER.

At the same time, it is able to oxidize HER due to the presence of a carbonyl group, or to add HER to the C=C double bond. The observed yields of decomposition for AA and **I** on radiolysis, when conducted in ethanol or aqueous ethanol, were substantially lower than the radiation-chemical yield of HER in radiolysis of ethanol (G = 5.5),[16] or radiation-chemical yields of the water radiolysis products formed according to (4) ($G_{\bullet OH}$ = 2.7; $G_{\bullet H}$ = 0.55).[17] These facts suggest a low probability of HER addition to C=C double bonds of the test compounds. On radiolysis of ethanol or its aqueous 1 M solution at pH 7, compound **I** decreases the 2,3-butanediol yield significantly, while increasing the yield of acetaldehyde, thereby evidencing oxidation of HER by **I** according to the following reaction:



(6)

Since the increase in acetaldehyde yield was not proportional to the decrease in yield of 2,3-butanediol, the involvement of reaction (7), leading to suppression of the 2,3-butanediol formation and regeneration of **I**, may be assumed:



(7)

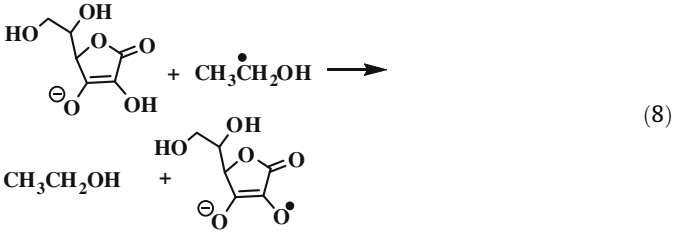
Reactions (6) and (7) may concurrently cause a significant decrease of radiation-chemical yields of 2,3-butanediol, an increase in the yields of acetaldehyde and regeneration of **I** on radiolysis performed in ethanol or its aqueous solutions. In the case of AA, the manifested effects depended to a significant extent on the form in which it was present. Judging from the changes in yields of radiolysis products (cf. Table 1), in ethanol and aqueous 1 M ethanol at pH 3, when AA is essentially undissociated[18], it behaves like **I** and, most probably, is involved in reactions of type (6) and (7). However, in aqueous 1 M ethanol at pH 7, when it is present in the form of a mono-anion, an abrupt fall in the 2,3-butanediol yield is seen, whereas no increase in the yield of acetaldehyde is observed. These facts point to the capability of AA of reducing HER under such conditions:

Table 1
Effects of AA and **I** on the yields (G) of major products formed in radiolysis of deaerated ethanol or aqueous ethanol solutions.

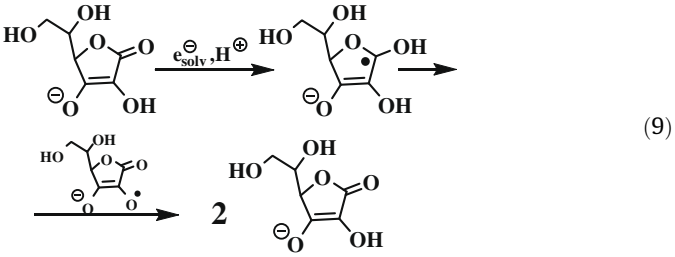
Initial system	Products	$G \times 10^7$ (mol/J)		
		Without additive	AA	**I**
Ethanol	Acetaldehyde	1.94 ± 0.17	2.37 ± 0.14	2.65 ± 0.20
	2,3-Butanediol	2.05 ± 0.09	0.55 ± 0.15	0.88 ± 0.11
	Additive decomposition		−0.48 ± 0.11	−0.99 ± 0.13
1 M Ethanol, pH 3	Acetaldehyde	1.95 ± 0.28	2.32 ± 0.14	—
	2,3-Butanediol	2.06 ± 0.19	0.19 ± 0.09	—
	Additive decomposition		−1.03 ± 0.13	—
1 M Ethanol, pH 7	Acetaldehyde	0.77 ± 0.11	0.70 ± 0.09	2.25 ± 0.19
	2,3-Butanediol	1.94 ± 0.18	0.15 ± 0.03	0.40 ± 0.08
	Additive decomposition		−1.29 ± 0.14	−1.85 ± 0.01

Table 2
Effects of AA and compound **I** on the yields (G) of major products formed in radiolysis of deaerated aqueous solutions of ethylene glycol, α-methyl glycoside, maltose, 0.1 M α-glycerophosphate, 0.1 M α-glucosophosphate at pH 7.

Initial system	Products	$G \times 10^7$ (mol/J)		
		Without additive	AA	**I**
1 M Ethylene glycol	Acetaldehyde	3.24 ± 0.19	1.64 ± 0.15	2.20 ± 0.37
3 M Ethylene glycol	Acetaldehyde	9.05 ± 1.03	3.26 ± 0.07	5.42 ± 0.09
0.1 M α-Methylglucoside	Methanol	1.72 ± 0.11	1.10 ± 0.07	–
0.1 M Maltose	Glucose	1.20 ± 0.10	0.80 ± 0.10	–
0.1 M α-Glycerophosphate	Phosphate	3.44 ± 0.12	2.48 ± 0.04	2.53 ± 0.07
0.1 M α-Glucosophosphate	Phosphate	2.63 ± 0.05	1.78 ± 0.03	2.12 ± 0.06

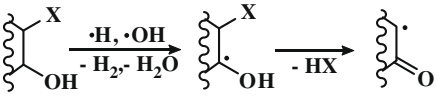

(8)

The low yields of decomposition observed for AA (cf. Table 1) suggest that its regeneration is possible:


(9)

Thus, depending on its form (undissociated or mono-anion), AA is able to act like either donor or acceptor of a hydrogen atom in reactions with HER.

Unlike HER, its β-substituted analogues, formed in radiolysis of aqueous ethylene glycol, carbohydrates or organic phosphates solutions, are able to undergo free-radical fragmentation reactions according to a general scheme shown below[5]:


(10)

X = OH, OMe, OPO_3^{2-} etc.

On radiolysis of ethylene glycol, its dehydration takes place, and this process occurs according to a chain mechanism in concentrated solutions (Table 2). Processes of a similar kind are responsible for modification of carbohydrates and transformation of ribonucleosides in deoxyribonucleosides.[19] Radiolysis of aqueous solutions of α-methylglycoside and maltose, where a rupture of the O-glycoside bond is realized, are a good models for studying destruction of polysaccharides and cerebrosides under the action of ROS.[9,10] Radiolysis of aqueous solutions of organic phosphates leads to dephosphorylation. In the case of RNA, reactions of such kind result in cleavage of phosphodiester bonds,[20] and in the case of phospholipids, the result is formation of phosphatidic acids playing the role of signaling molecules.[7] The yield values obtained for products of radiation-induced free-radical fragmentation in aqueous solutions of ethylene glycol, α-methylglycoside, maltose, α-glycerophosphate and α-glucose phosphate in the presence of AA and **I** are presented in Table 2. As follows from the obtained data, the test compounds lower the yields of products formed due to reactions of type (10) on radiolysis of aqueous solutions of the compounds under study. This fact evidences the capability of AA to interact with HCR of various structures and to act as a regulator of free-radical processes involving biologically important compounds.

Thus, the obtained data point to unique properties of ascorbic acid, which are manifested as the ability to regulate not only oxidation processes of biologically relevant substances, but also recombination and fragmentation reactions of hydroxyl-containing biomolecules, induced in biosystems by radiation or other sources of ROS.

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