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Effects of various vitamins and coenzymes Q on reactions involving α-hydroxyl-containing radicals

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Abstract
Effects of vitamins B, C, E, K and P, as well as coenzymes Q, on formation of final products of radiation-induced free-radical transformations of ethanol, ethylene glycol, α-methylglycoside and glucose in aqueous solutions were studied. Based on the obtained results, it can be concluded that there are substances among vitamins and coenzymes that effectively interact with α-hydroxyl-containing radicals. In the presence of these substances, recombination reactions of α-hydroxyalkyl radicals and fragmentation of α-hydroxy-β-substituted organic radicals are suppressed. It has been established that the observed effects are due to the ability of the vitamins and coenzymes under study to either oxidize α-hydroxyl-containing radicals yielding the respective carbonyl compounds or reduce them into the initial molecules.

Keywords: *Vitamins, coenzyme, radical, oxidation, fragmentation*

Introduction

It is generally known that the free-radical oxidation processes induced by reactive oxygen species (ROS) in biologically relevant substances cause damage to biosystems [1]. One of the means to protect the latter from undesirable consequences of these processes is based on using natural and/or synthetic substances of phenolic type, which are termed antioxidants. Many of such compounds display marked pharmacological properties and are used for prevention and treatment of various diseases [2].

Our studies resulted in germination and development of the concept that ROS can injure biologically relevant substances by inducing both oxidation reactions and fragmentation processes [3–9]. The fragmentation processes involve carbon-centered radicals, and the key stage of these reactions is decomposition of intermediates of type —C˙(OH)—CH(X)—, which occurs via simultaneous rupture of two β-bonds [3]. Reactions of such type occur quite commonly in the course homolytic transformations of carbohydrates due to the presence of hydroxyl groups in molecules of these substances [10,11]. Realization of fragmentation reactions in glycerophospholipids [5–9] and cerebrosides [6] results in destruction of the starting molecules to form phosphatidic acid and ceramide, respectively, which play an important role in regulation of cell proliferation and apoptosis. Free-radical transformation of nucleosides in the corresponding deoxyribonucleosides occurs according to a similar fragmentation mechanism [12]. The aforesaid illustrates a wide prevalence of fragmentation processes, which makes it

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necessary to develop some means of regulating their probability to occur.

Derivatives of quinones [13] and quinonimines [14], as well as compounds capable of forming quinoid structures in the course of homolytic transformations [15], were found to exert a noticeable influence on directivity of processes involving α-hydroxyl-containing organic radicals while blocking their recombination and fragmentation reactions. Because many vitamins and coenzymes belong to this type of substances, this study attempts to evaluate their ability to regulate free-radical processes involving carbon-centered α-hydroxyl-containing organic radicals. For this purpose, effects of vitamins B, C, E, K and P, as well as coenzymes Q, on product formation resulting from radiation-induced free-radical transformations of deaerated ethanol, as well as deaerated aqueous solutions of ethylene glycol, methyl-α,D-glucopyranoside and maltose were studied.

Materials and methods

Structural formulas of compounds used in this study are depicted below (Figure 1). Vitamins, coenzymes and methyl-α,D-glucopyranoside from Sigma were used without further purification. Ethanol (96% vol.) was purified by sorption on ceolites Wolfen Zeosorb LA with subsequent twice-repeated distillation using a rectification column of 3-m length. Ethylene glycol was purified by twice-repeated distillation. Purity of all substances used was controlled by chromatography.

Before preparing solutions of compounds under study in ethanol, the latter was blown through with argon of high purity (99.9%) for 50 min. All further

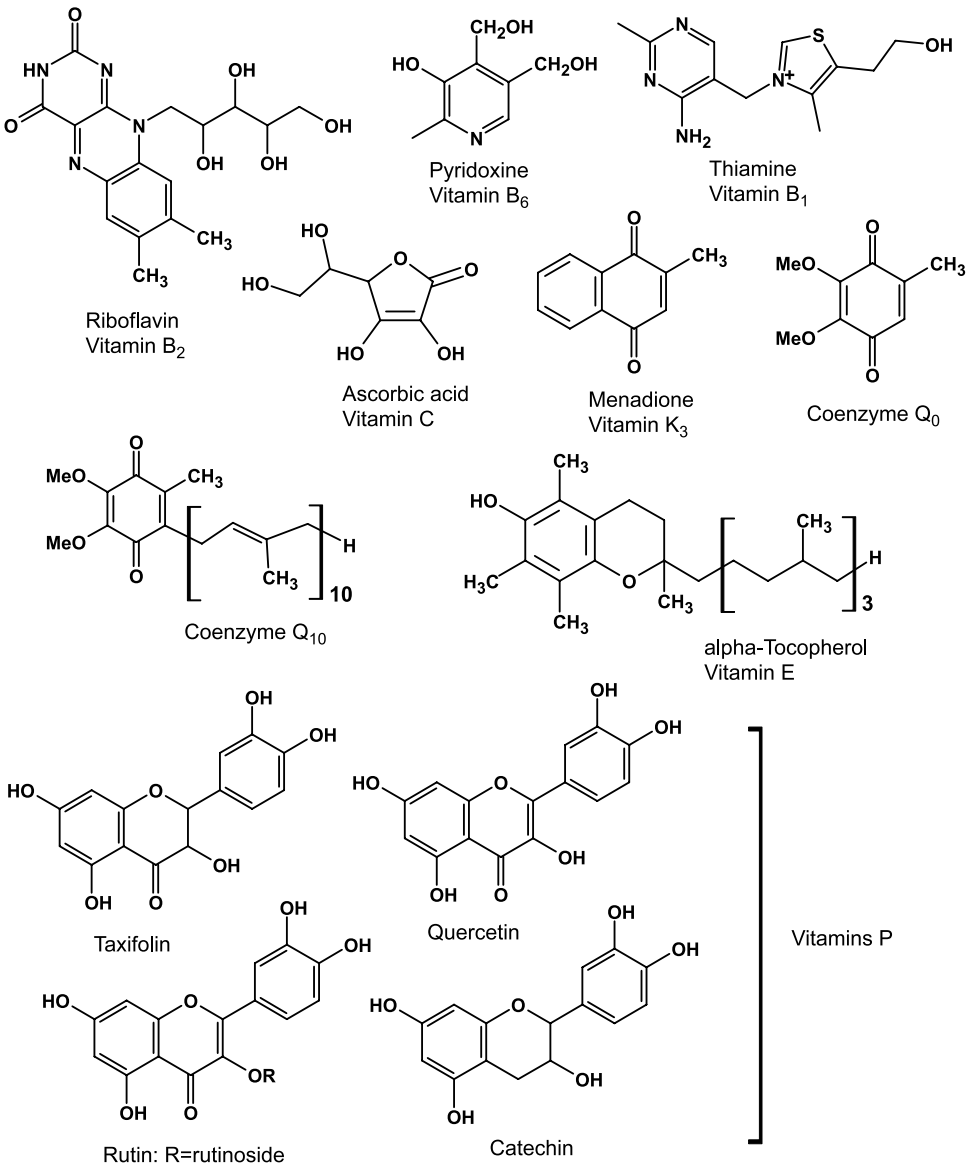


Figure 1. Structures of compounds used in the study.

procedures, up to sealing of the filled ampoules, were performed under argon atmosphere.

Solutions of ethylene glycol (3 M), methyl-α, D-glucopyranoside (0.1 M) and maltose (0.1 M) were prepared using twice-distilled water. Freshly prepared solutions were used for each series of experiments. Weighed amounts of vitamins and coenzymes were introduced into the prepared solutions. Oxygen was removed from the solutions using 5 repeated freeze–vacuum–thaw cycles, after which the ampoules were sealed under vacuum.

Free-radical processes in the model systems were initiated by γ-radiation from a ^{137}Cs source. Irradiation of samples contained in sealed ampoules was performed with a dose rate of 0.28 ± 0.01 Gy/s, and the absorbed dose range was 0.2–3.5 kGy.

Concentrations of acetaldehyde (AA), 2,3-butanediol (BD), glycolic aldehyde and methanol in the substrate solutions, were determined by gas chromatography using a Shimadzu GC-17AAF/APC instrument equipped with a quartz capillary column RTX-Wax ($l = 30$ m, ID = 0.32 mm, df = 0.5 mm). Evaporator temperature: 250°C; detector temperature: 220°C; carrier gas: nitrogen, flow rate 30 cm/s; flame ionization detector.

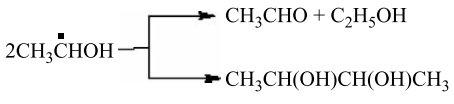
Concentration of glucose on radiolysis of maltose solutions was determined by HPLC using a Shimadzu instrument equipped with a Nucleosil Carbohydrate EC 250/4 column. Analysis conditions: flow rate—1 ml/min; mobile phase—acetonitrile/water 80:20 v/v; loop 20 μl, refractomertic detector (RID, Aux. rang. 1; Response 5).

All the data presented were obtained by averaging results of at least 3 series of experiments. Radiation-chemical formation yields were determined from relationships between accumulation of the compounds and dose absorbed.

Results

Interaction of compounds under study with α-hydroxyethyl radicals

Reactivity of a number of vitamins towards $CH_3C^{\cdot}HOH$ species was evaluated according to their influence on formation of molecular products of ethanol radiolysis.



It is well known [16] that the main products of ethanol radiolysis are AA and BD formed as a result of disproportionation and recombination of α-hydroxyethyl radicals.

By determining the yields of AA and BD on radiolysis of ethanol in the presence of various additives, one can evaluate the probability of reactions of the additives with $CH_3C^{\cdot}HOH$ radicals and the reaction pathways. It follows from the data obtained (see Table I) that vitamin K_3, as well as coenzymes Q_0 and Q_{10}, drastically suppressed BD yields, which was accompanied by a significant increase of AA formation yields.

Table I. Effects of vitamins (1×10^{-3} M) and coenzymes (1×10^{-3} M) on product yields (G) resulting from recombination ($CH_3CH(OH)CH(OH)CH_3$) and oxidation (CH_3CHO) of α-hydroxyethyl radicals in radiolysis of ethanol.

	$G \times 10^7$ mol/J	
Additives	CH_3CHO	$CH_3CH(OH)CH(OH)CH_3$
No additive	1.70 ± 0.15	1.49 ± 0.13
Ascorbic acid	2.69 ± 0.05	0.31 ± 0.03
α-Tocopherol	3.30 ± 0.32	0.20 ± 0.01
Pyridoxine (B_6)	3.17 ± 0.26	0.12 ± 0.01
Riboflavin (B_2)*	2.98 ± 0.29	0.32 ± 0.03
Thiamine (B_1)	0.36 ± 0.06	0.09 ± 0.01
Quercitin	2.66 ± 0.17	0.28 ± 0.02
Rutin	2.98 ± 0.15	0.21 ± 0.02
Catechin	2.49 ± 0.15	0.99 ± 0.06
Taxifolin	1.95 ± 0.07	0.98 ± 0.05
Menadione (K_3)	4.94 ± 0.16	0.04 ± 0.006
Coenzyme Q_0	4.35 ± 0.30	0.03 ± 0.005
Coenzyme Q_{10}	5.24 ± 0.18	0.05 ± 0.01

* Concentration of B_2 was 1×10^{-4} M.

B group vitamins, α-tocopherol and vitamin C also modified the product ratio of ethanol radiolysis in favour of AA. Of flavonoids (P group vitamins) studied, quercitin and rutin were the most effective in suppressing BD yields, evidencing their higher reactivity towards $CH_3C^{\cdot}HOH$ radicals as compared with that of catechin or taxifolin.

Interaction of compounds under study with α-diol radicals

To investigate interaction of the compounds under study with α-diol radicals, influence of the former on radiolysis of 3 M aqueous ethylene glycol solutions was studied. Predominant process observed in homolytic transformations of α-diols is free-radical dehydration of the starting compounds [17]. In the case of ethylene glycol radiolysis, the main product is AA, and its formation in a 3 M ethylene glycol solution occurs according to a chain mechanism. This is evidenced by radiation-chemical yields of CH_3CHO (see Table II), which are significantly higher than those of the initiator ($G_{OH} = 2.8$). The values of CH_3CHO yields obtained on ethylene glycol radiolysis agree with those reported in the literature [17], and the product formation occurs according to the following scheme:

$$HOCH_2CH_2OH \xrightarrow[-H_2O]{OH} HOCH_2C^{\cdot}HOH \xrightarrow[-H_2O]{}$$

$$C^{\cdot}H_2CHO \xrightarrow[-HOCH_2C^{\cdot}HOH]{HOCH_2CH_2OH} CH_3CHO$$

Table II. Effects of vitamins (1×10^{-3} M) and coenzymes (1×10^{-3} M) on product yields (G) resulting from dehydration (CH_3CHO) and oxidation ($HOCH_2CHO$) of $HOCH_2C^{\cdot}HOH$ radicals in radiolysis of aqueous 3 M solutions of ethylene glycol.

	$G \times 10^7$ mol/J	
Additives	CH_3CHO	$HOCH_2CHO$
No additive	11.54 ± 0.92	0.79 ± 0.07
Ascorbic acid	3.11 ± 0.31	1.20 ± 0.15
Thiamine (B_1)	0.44 ± 0.03	1.37 ± 0.15
Riboflavin (B_2)*	0.54 ± 0.10	4.05 ± 0.78
Pyridoxine (B_6)	1.61 ± 0.20	1.17 ± 0.25
Rutin*	4.93 ± 0.37	0.72 ± 0.07
Catechin	2.11 ± 0.12	0.72 ± 0.09
Taxifolin	4.15 ± 0.36	1.26 ± 0.14
Menadione (K_3)	1.19 ± 0.20	2.29 ± 0.23
Coenzyme Q_0	0.50 ± 0.05	4.36 ± 0.51

* Concentrations of B_2 and rutin were 1×10^{-4} M.

Glycolic aldehyde is formed on radiolysis of ethylene glycol through disproportionation of $HOCH_2C^{\cdot}HOH$ radicals [17].

As it follows from the data obtained, all compounds under study inhibited the chain process of ethylene glycol dehydration. Group B vitamins, as well as coenzyme Q_0 and vitamin K_3, suppressed this process more effectively than flavonoids and vitamin C did. Yields of glycolic aldehyde—an oxidation product of $HOCH_2C^{\cdot}HOH$ radicals—generally increased in the presence of additives, with the highest increase observed in the presence of riboflavin and coenzyme Q_0.

Effect of compounds under study on homolytic rupture of the O-glycoside bond in carbohydrates

The O-glycoside bond rupture is known to be the main process taking place when free-radical transformation initiators, such as radiation [18] or redox systems like Fenton's reagent [11,12], act upon aqueous solutions of carbohydrates. For the purpose of investigation of effects produced by a number of vitamins and coenzymes on processes of O-glycoside bond cleavage, we studied radiolysis of aqueous solutions of methyl-α,D-glucopyranoside and maltose in the presence of these substances. The yields of methanol formed on radiolysis of methyl-α,D-glucopyranoside were found to decrease in the presence of the additives under study (see Table III), the most pronounced effect being observed with coenzyme Q_0 and thiamine.

In the course of homolytic transformations of maltose, the O-glycoside bond cleavage led to formation of glucose. Its yields are shown in Table III. Of the compounds under study, vitamin B_1 and coenzyme Q_0 blocked the process of glucose formation more effectively as compared with pyridoxine and vitamin C.

Table III. Effects of vitamins (1×10^{-3} M) and coenzyme Q_0 (1×10^{-3} M) on yields (G) of CH_3OH in radiolysis of aqueous 0.1 M solutions of methyl-α,D-glucopyranoside, and on yields of glucose in radiolysis of aqueous 0.1 M solutions of maltose.

	$G \times 10^7$ mol/J	
Additives	CH_3OH	Glucose
No additive	1.71 ± 0.11	1.20 ± 0.10
Thiamine (B_1)	0.33 ± 0.03	0.10 ± 0.06
Riboflavin (B_2)*	1.18 ± 0.06	–
Pyridoxine (B_6)	1.46 ± 0.04	1.07 ± 0.09
Coenzyme Q_0	0.69 ± 0.09	0.11 ± 0.06
Menadione (K_3)	1.60 ± 0.15	–
Ascorbic acid	–	0.80 ± 0.1

* Concentrations of B_2 was 1×10^{-4} M.

Discussion

The results presented above (see Tables I–III) show that most of the substances studied modified the directivity of reactions involving various α-hydroxyl-containing organic radicals. Thus, as evidenced by the data of Table I, many of them suppressed BD yields to a significant extent by reacting with $CH_3C^{\cdot}HOH$ species, increasing thereby AA yields. Such behaviour is characteristic mainly for coenzymes Q and menadione (vitamin K_3). Earlier, the possibility of reactions was demonstrated, in which alcohol radicals were oxidized, for example, by synthetic quinone derivatives [19].

$$RC^{\cdot}HOH + Q \rightarrow RCHO + Q^{\cdot}H \qquad (1)$$

Q = quinones.

Reaction (1) occurs at a high rate ($k \sim 10^9$ l/mol·s) [19]. This indicates that the substances with quinoid structures are able to oxidize α-hydroxyalkyl radicals effectively. The effects manifested by coenzymes Q and menadione on radiolysis of ethanol in their presence (see Table I) confirm the ability of quinones to oxidize $CH_3C^{\cdot}HOH$ radicals.

Effective oxidizers of alcohol radicals are also quinonimines and their derivatives [14]. Riboflavin has a quinonimine moiety in its structure, and hence the experimentally observed changes in AA/BD yield ratio in favour of the first product (see Table I) confirm the ability of riboflavin to oxidize $CH_3C^{\cdot}HOH$ species.

As it follows from the obtained data, not only derivatives of quinones and quinonimines are capable of oxidizing alcohol radicals but also compounds containing fragments of these structural units. This relates, in the first place, to flavonoids (rutin, quercitin). The presence of a >C=O group in

the ring C of flavonoids enables the following reaction to occur:

$$Fl = O + CH_3C^{\cdot}HOH \rightarrow [Fl - OH]^{\cdot} + CH_3CHO \quad (2)$$

$$Fl = O \quad \text{is} \quad \text{rutin.}$$

On radiolysis of ethanol in the presence of flavonoids, this led to suppression of BD formation and increase in AA yields (see Table I). From comparison of the obtained data on influence of the flavonoids studied on BD yields, it follows that catechins are characterized by the lowest reactivity towards $CH_3C^{\cdot}HOH$ radicals. Since quercitin and catechin differ from one another only in structure of the ring C, the obtained results point to a key role of this structural element in reactions of flavonoids with α-hydroxyl-containing radicals.

Natural and synthetic antioxidants may also realize their functions by interacting with alcohol radicals as classical hydrogen donors [20]:

$$PhXH + CH_3C^{\cdot}HOH \rightarrow PhX^{\cdot} + CH_3CH_2OH \quad (3)$$

$$X = O, NH.$$

Realization of reaction (3) should lead to a decrease in yields of both BD and AA. We observed this, only in the case of thiamine (B_1). Structure of the latter includes an amino group attached to an aromatic ring, hence the reaction (3) can occur with this compound. Moreover, the possibility of $CH_3C^{\cdot}HOH$ addition to one of the two heterocycles of thiamine cannot be excluded, which can also provoke a decrease in yields of final products formed in the course of ethanol radiolysis.

Vitamin E and flavonoids, while decreasing BD yields on radiolysis of ethanol, increased yields of AA. This can be due to the circumstance that flavonoids and α-tocopherol, when reacting according to (3), generate radicals capable of forming chinoid structures, and this, as shown in [13–15], leads to accumulation of oxidants which react with $CH_3C^{\cdot}HOH$ species according to (1). As a result of this, suppression of formation of BD and an increase in yield of AA should be observed. The obtained data (see Table I) provide evidence for this assumption.

The compounds under study produced significant effects on transformations of diol radicals. This follows from the values of product yields formed on radiolysis of aqueous 3 M ethylene glycol solutions (see Table II). All the substances under the study blocked the chain homolytic process of ethylene glycol dehydration. While doing so, a number of these substances acted as chain-breaking acceptors oxidizing the $HOC^{\cdot}HCH_2OH$ species and increasing thereby glycolic aldehyde yields:

$$HOC^{\cdot}HCH_2OH + D \rightarrow HOCH_2CHO + D^{\cdot}H \quad (4)$$

$$D = Q_0, K_3, B_2.$$

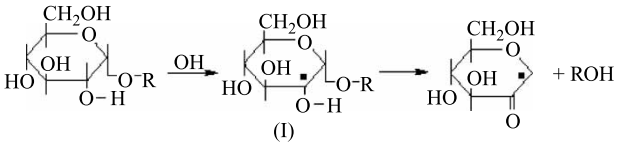
As it should be expected, mainly the substances possessing oxidative properties (coenzyme Q_0, riboflavin, menadione) reacted according to (4).

Thiamine, pyridoxine and flavonoids suppressed the formation of AA without increasing glycolic aldehyde yields to any significant extent. This point to the possibility of participation of these substances in reduction of the $HOC^{\cdot}HCH_2OH$ species to the starting molecule via a hydrogen atom transfer according to reaction (4) (chain-breaking donors):

$$HOC^{\cdot}HCH_2OH + DH_2 \rightarrow HOCH_2CH_2CHO + D^{\cdot}H$$

$$DH_2 = B_1, B_6, \text{Vit. P.}$$

It is known that the main process occurring in the course of homolytic transformations of carbohydrates [18] and glycolipids [6] is the reaction of O-glycoside bond cleavage. This process can occur according to a fragmentation mechanism similar to that of ethylene glycol dehydration:



R = CH_3, glucose residue.

(5)

In the reaction (5), a key role belongs to the stage of decomposition of a (C-2)-hydroxyl-containing radical formed from the starting molecule via rupture of two β-bonds.

As it follows from the data obtained (see Table III), thiamine and coenzyme Q_0 decreased the yields of CH_3OH and glucose substantially, and they may be regarded as effective inhibitors of reaction (5) leading to a rupture of the O-glycoside bond. Such effect may be due to either oxidation of radicals of type (I)—a behaviour characteristic of coenzyme Q_0—or their reduction to the respective starting molecules—a process possible in the presence of thiamine.

Thus, based on the obtained results, it can be concluded that among vitamins and coenzymes there are substances effectively interacting with α-hydroxyl-containing radicals. In the presence of these substances, recombination reactions of α-hydroxyl-containing radicals and fragmentation reactions of

α-hydroxy-β-substituted organic radicals are suppressed. Taking into account the fact that fragmentation reactions cause damage to many biologically important substances and lead to formation of signaling molecules, it appears to be of interest to consider in the future a possible correlation between the known pharmacological properties of vitamins and their capability of blocking free-radical fragmentation processes. This may be useful for discovering new possibilities of using vitamins for prevention and treatment of pathologic conditions, in the development of which free-radical processes play an important role.

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