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Effects of B group vitamins on reactions of various α-hydroxyl-containing organic radicals

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Abstract—Effects of vitamins B_1, B_2, B_6, and pyridoxal phosphate (PPh) on final product formation in radiolysis of aqueous solutions of ethanol, ethylene glycol, α-methylglycoside, and maltose were studied. It has been found that vitamin B_2 and PPh effectively oxidize R·CHOH species, while suppressing their recombination and fragmentation reactions, thereby increasing the yields of the respective oxidation products. Vitamins B_1 and B_2 are capable of reducing alcohol radicals to the respective initial molecules, decreasing the yields of the radical transformation products.

B group vitamins possess a wide spectrum of biochemical and pharmacological properties,[1,2] making them versatile objects of numerous studies. In clinical practice, they are used in the treatment of cardiovascular and central nervous system diseases, in the genesis and development of which free-radical processes play an important role.[2,3] Increasing levels of reactive oxygen species (ROS) are known to be a cause or a consequence of the above-named and many other diseases. The deleterious effects of ROS are, in many respects, associated with inducing oxidation processes of many biologically important molecules and, first of all, hydrophobic unsaturated fatty acid residues of the cell membrane lipids.[3]

It has been shown in our studies that ROS react with polar components of a number of phospholipids and sphingolipids[4–8] causing their destruction, which is accompanied by the formation of signaling molecules. It was convincingly demonstrated that decomposition reactions of carbon-centered α-hydroxyl-containing radicals proceeding via rupture of two β-bonds play a key role in such processes. Besides lipids, good substrates for free-radical fragmentation reactions are carbohydrates,[9–11] nucleic acid components,[12] and hydroxyl-containing amino acids and their derivatives.[13] Bearing in mind the wide prevalence of fragmentation processes in homolytic transformations of bioorganic substances in aqueous solutions, the assessment of the effects of water-soluble vitamins on realization probability of such processes appears to be of significant interest. For this purpose, we investigated the effects of a number of group B vitamins on final product formation taking place during radiation-induced free-radical transformations of ethanol, ethylene glycol, α-methylglycoside, and maltose in aqueous solutions.

Free-radical processes taking place in organic substances during radiolysis of their aqueous solutions are initiated by reactive radical products of water radiolysis,[9] formed according to reaction (1):

$$H_2O \rightsquigarrow H,\ OH,\ e_{aq} \quad (1)$$

It was found that H· and ·OH radicals, while reacting with the above-named substances, mainly initiate the following processes:

(a) Aqueous ethanol solution[14]:

$$CH_3CH_2OH \xrightarrow[H_2,H_2O]{H,OH} CH_3{}^{\bullet}CHOH \quad (2)$$

$$2CH_3\dot{C}HO \longrightarrow (CH_3CHOH)_2 \ (2,3\text{-}BD) \quad (3)$$

$$2CH_3\dot{C}HO \longrightarrow CH_3CHO \ (AA) + CH_3CH_2OH \quad (4)$$

(b) Aqueous ethylene glycol solutions[15]:

$$HOCH_2CH_2OH \xrightarrow{H,\,OH} HOCH_2{}^{\bullet}CHOH \xrightarrow[-H_2O]{} {}^{\bullet}CH_2CHO \quad (5)$$

(*) $-CH_3CHO$ (AA)

(*) This reaction pathway is possible only in concentrated solutions of the substrate.

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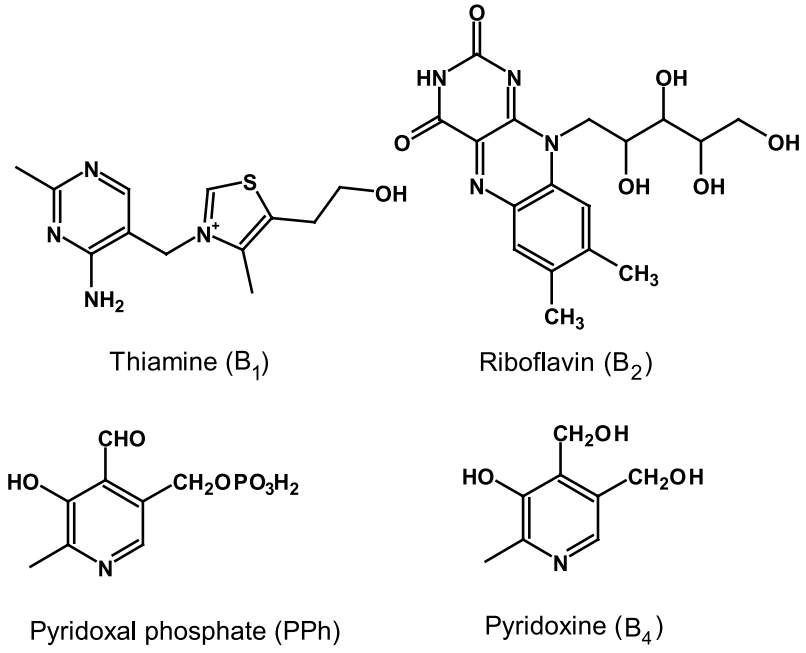
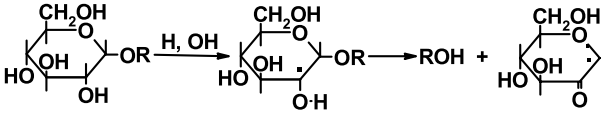


Figure 1. Structural formulas of compounds used in the study.

$$\cdot CH_2CHO + HOCH_2\cdot CHOH \rightarrow CH_3CHO + HOCH_2CHO \quad (6)$$

$$2HOCH_2\cdot CHOH \rightarrow \underset{(GA)}{HOCH_2CHO} + HOCH_2CH_2OH \quad (7)$$

(c) Aqueous glycoside solutions[9]:



(8)

R = CH_3, glucose residue.

The possibility of realization of type (8) reactions in carbohydrates being attacked by ·OH radicals of non-radiation origin was shown in studies.[11,12]

By assessing the yields of products formed in reactions (2–8) in the presence and in the absence of group B vitamins in the systems being irradiated, one can evaluate the reactivity and reaction pathways of the vitamins with various α-hydroxyl-containing radicals. With all this in mind, we bestowed efforts to create conditions, under which the possibility of the reaction of group B vitamins with radical products of water radiolysis formed according to reaction (1) would be practically excluded. This was achieved by using vitamin concentrations 3 orders of magnitude lower than the concentrations of hydroxyl-containing substances, because the reactivity of group B vitamins toward the water radiolysis products is an order of magnitude higher than those of alcohols and carbohydrates.[16]

Structural formulas of the vitamins used are shown in Figure 1.

The vitamins, methyl-α,D-glucopyranoside, and maltose, all from Sigma, were used without additional purification. Ethanol (96% vol) was purified by sorption with Wolfen Zeosorb LA zeolites, followed by twice-repeated distillation. Ethylene glycol (Merck) was purified by twice-repeated distillation. Purity of the substances used was controlled by chromatography.

Twice-distilled water was used to prepare aqueous solutions of the above-named substances. Starting solutions of ethanol, ethylene glycol, methyl-α,D-glucopyranoside, and maltose were prepared using the volumetric method. After dissolving the additives studied ($C = 10^{-3}$ mol/L),[†] ampoules with the solutions were deaerated for 40 min with argon and then sealed. Radiolysis of the starting systems was performed in a γ-unit LMB-γ-1M (^{137}Cs, dose rate 0.30 Gy/s, absorbed dose range 0.36–1.8 kGy).

Concentrations of radiolysis products, such as acetaldehyde, 2,3-butanediol, glycolic aldehyde, and methanol, were determined by 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 μm), evaporator temperature: 250 °C; carrier gas: nitrogen, 30 cm/s; flame ionization detector. Concentrations of glucose formed during radiolysis of maltose solutions were determined by HPLC using a

[†] C(riboflavin) = 10^{-4} mol/L because of its limited solubility in water.

Shimadzu instrument equipped with an EC 250/4 Nucleosil Carbohydrate column. Analysis conditions include: flow rate: 1 ml/min; mobile phase: acetonitrile/water (80/20 v/v); loop 20 μl; refractometric detector RID (Aux.-rang.1; Response 5). All the data presented were obtained by averaging the results of at least three series of experiments. The yields of product formation were determined from relationships between accumulation of the respective products and the dose absorbed.

The data on final product yields obtained after radiolysis of the systems under study are shown in Table 1.

While analyzing the data given in the table, one can ascertain that B group vitamins are reactive toward various α-hydroxyl-containing radicals, and that they are able to modify transformation pathways of the latter to a substantial extent. Thus, on radiolysis of aqueous ethanol solutions, all the B group vitamins studied decrease 2,3-butanediol yields significantly, while suppressing recombination reactions of $CH_3^{\bullet}CHOH$ species. At the same time, vitamin B_2 and PPh substantially increased the yield of acetaldehyde, an oxidation product of α-hydroxyethyl radicals. Quinones,[17] quinonimines,[18], and aldehydes[19] are known to oxidize α-hydroxyethyl radicals effectively. Since vitamin B_2 and PPh contain similar structural moieties, they also react as given below:

$$CH_3^{\bullet}CHOH + B \rightarrow CH_3CHO + {}^{\bullet}BH \quad (9)$$

$B = B_2$, PPh

The realization of reaction (9) accounts for the observed effects of suppression of the 2,3-butanediol yields and increase in acetaldehyde yields on radiolysis of aqueous ethanol solutions in the presence of vitamin B_2 and PPh.

Molecules of vitamins B_1 and B_6 contain an amino- and a hydroxyl group, respectively, and hence these substances are able to reduce organic radicals according to reaction (10), similar to phenols[20] and aromatic amines[18,21]:

$$CH_3^{\bullet}CHOH + BXH \rightarrow CH_3CH_2OH + B^{\bullet}X \quad (10)$$

X = –NH– (B_1), –O– (B_6)

Reaction (10) should lead to a decrease in yields of acetaldehyde and 2,3-butanediol. Indeed, in the presence of vitamins B_1 and B_6, a drastic drop in 2,3-butanediol yields can be noted (see Table 1). Because acetaldehyde yields decrease to a lesser extent, one can presume that interactions of $CH_3^{\bullet}CHOH$ radicals with these vitamins are not restricted to just the reaction (10). Since the structures of vitamins B_1 and B_6 contain –C=N– double bonds, these substances can, similar to quinonimines and riboflavine (B_2), partially oxidize $CH_3^{\bullet}CHOH$ radicals according to reaction (9) to form acetaldehyde.

Unlike $CH_3^{\bullet}CHOH$ species, radicals formed from ethylene glycol[15] and carbohydrates[10,11] are able to undergo fragmentation reactions, which, as noted previously, cause damage to many biologically important substances. In the case of fragmentation of ethylene glycol radicals according to reactions (5,6), acetaldehyde is formed. On changing from 1 to 3 M solutions, the process appears to switch to a chain mechanism, because acetaldehyde yields are higher than that of the initiator ($G_{OH} \sim 2.8$) in this case. Group B vitamins block the process (5), and, depending on the vitamin structure, this is realized according to different mechanisms. Thus, inhibition of acetaldehyde formation on radiolysis of ethylene glycol in the presence of vitamin B_2 and PPh is accompanied by an increase in yields of glycolic aldehyde, which points to the ability of these substances to oxidize $HO^{\bullet}CH-CH_2OH$ radicals according to a reaction of type (9). Vitamins B_1 and B_6 are more liable to block fragmentation reactions of ethylene glycol radicals by both reduction according to reaction (10) and oxidation according to reaction (9), leading to a decrease in yields of acetaldehyde and a slight increase in yields of glycolic aldehyde.

Fragmentation reaction of carbohydrate radicals (8), leading to cleavage of the O-glycoside bond, is effectively blocked by vitamin B_1 and PPh, as evidenced by the data given in the table. This fact appears to be important, because, as shown in our recent study,[5] a reaction similar to (8) taking place upon action of free-radical reaction initiators on cerebrosides leads to their destruction with the formation of ceramides involved in the regulation of apoptotic processes.

The data obtained in this study indicate that group B vitamins possess some new properties associated with their capability of regulating free-radical reactions involving carbon-centered α-hydroxyl-containing radicals. Reactions of this type can lead to destruction of biologically

Table 1. Product yields (G) obtained from radiolysis of aqueous ethanol, ethylene glycol, α-methylglycoside, and maltose solutions in the presence of group B vitamins

Initial system	Products	$G \cdot 10^7$ (mol/J)				
		Ar	B_1	B_2	B_6	PPh
Ethanol, 1 M	Acetaldehyde	0.33 ± 0.10	0.57 ± 0.07	3.56 ± 0.20	0.58 ± 0.05	3.98 ± 0.18
	2,3-Butanediol	1.68 ± 0.14	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Ethylene glycol, 1 M	Acetaldehyde	3.21 ± 0.14	0.18 ± 0.03	0.36 ± 0.05	0.32 ± 0.09	0.11 ± 0.03
	Glycolic aldehyde	1.18 ± 0.10	1.09 ± 0.15	4.34 ± 0.25	1.76 ± 0.15	5.13 ± 0.37
Ethylene glycol, 3 M	Acetaldehyde	12.4 ± 0.97	0.44 ± 0.03	0.54 ± 0.10	1.61 ± 0.20	0.30 ± 0.05
	Glycolic aldehyde	0.88 ± 0.13	1.37 ± 0.15	4.05 ± 0.78	1.71 ± 0.25	4.50 ± 0.36
α-Methylglycoside, 0.1 M	Methanol	1.71 ± 0.11	0.31 ± 0.03	1.18 ± 0.06	1.46 ± 0.04	0.43 ± 0.03
Maltose, 0.1 M	Glucose	1.20 ± 0.10	0.10 ± 0.04	—	1.07 ± 0.11	—

important substances resulting in formation of signaling molecules. The data presented above provide evidence that B group vitamins inhibit these processes effectively.

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