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RADIATION CHEMISTRY

Effect of Structure of Polar Head of Phospholipids on Their Fragmentation during γ-Irradiation of Model Membranes

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Abstract—The ability of phospholipids with different structures of the polar head (phosphatidylpropandiol, phosphatidylglycerol, phosphatidylinositol, and cardiolipin) to sustain radiation-induced fragmentation was studied. It was shown by thin-layer chromatography and MALDI–TOF mass spectroscopy that all test lipids entering the composition of model membranes subjected to γ-irradiation suffered fragmentation yielding phosphatidic acid. The radiation-chemical yield of phosphatidic acid increased in this phospholipid series on passing from phosphatidylpropanediol to cardiolipin.

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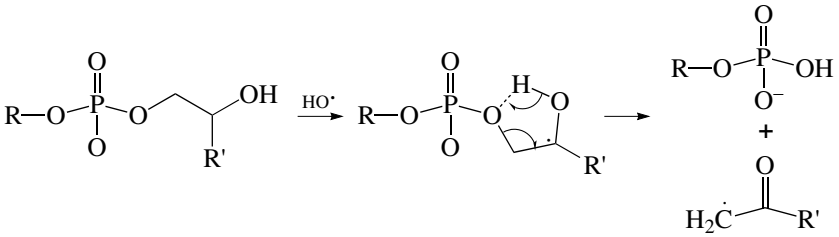
The important role of phospholipids in the functioning of biosystems makes the study of their transformations by the action of different free-radical initiators, in particular, ionizing irradiation, a matter of topical interest. It is known [1–3] that ionizing radiation can cause damage to membrane lipids via the peroxide oxidation of unsaturated fatty acid residues involved in their composition. As shown in our studies [4–10], γ-irradiation along with initiation of peroxide oxidation can also cause the destruction of the hydrophilic moiety of the phospholipid molecules by free-radical fragmentation. A necessary condition for this process is the presence of a free hydroxyl group in the β-position to the phosphoester bond in the polar head of the phospholipid molecule (Scheme 1)



Scheme 1.

where R = $-OCH_2CH(OCOC_nH_{2n-1})CH_2OCOC_2H_{2n+1}$ and R' = $-CH_2Y$ (Y = –H, –OH). From naturally occurring lipids, only lipids with polyol residues (phosphatidylglycerol, cardiolipin, and phosphatidylinositol) are potential fragmentation substrates. In this work, we studied whether these lipids and phosphatidylpropane-1,2-diol synthesized for the purpose are prone to radiation-induced fragmentation with the aim of revealing the effect of the structure of the phospholipid polar head on this reaction.

EXPERIMENTAL

Phosphatidylglycerol (PG) was prepared from egg yolk phosphatidylcholine (PC) according to the procedure described in [11]. Phosphatidylinositol (PI) from liver (Avanti Polar Lipids, USA), phosphatidic acid (PA) prepared from egg yolk phosphatidylcholine, and cardiolipin (CL) from bovine heart (Sigma–Aldrich, Germany) were also used in the study.

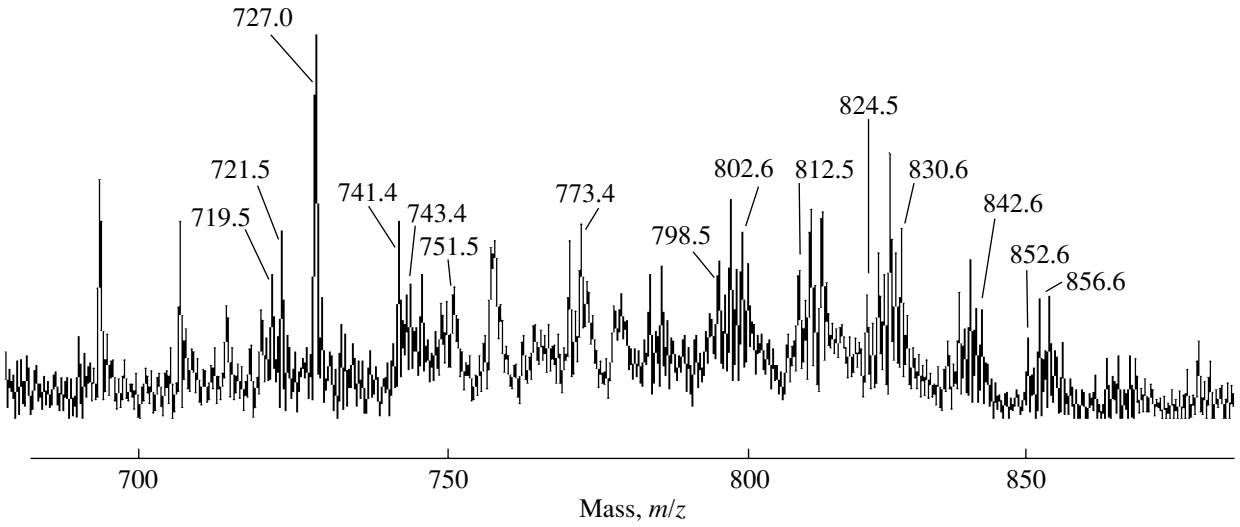


Fig. 1. MALDI–TOF mass spectrum of the organic extract of phosphatidic acid formed by γ-irradiation of cardiolipin in a liposome (radiation dose of 8.4 kGy). After irradiation, phosphatidic acid was separated from the parent cardiolipin by TLC.

1-Phosphatidylpropane-1,2-diol (PP) was obtained from egg yolk, like phosphatidylglycerol, via the transphosphatidylation reaction catalyzed by phospholipase D [11] in the presence of propane-1,2-diol as an acceptor alcohol. Phosphatidylpropane-1,2-diol was isolated by flash chromatography on silica gel with a mixture of chloroform with a methanol gradient as an eluent. Fractions containing a phospholipid with R_f = 0.6 (TLC, silica, chloroform–methanol–7 N NH_4OH) were collected, and the solvent was evaporated. The PP yield (Na^+ form) was 65 mg (43%). ^1H NMR spectrum ($CDCl_3$, 500 MHz), δ: 5.33 (m, ~3H, CH=CH), 5.24 (bs, 1H, sn-2 CH), 3.90–4.05 (m, 4H, 2 sn-2 CH_2O), 3.80 (m, 2H, CH_2CHOH), 3.41 (bs, 1H, CHOH), 2.76 (t, ~1H, $CH=CHCH_2CH=CH$), 2.30 (m, 4H, CH_2CO), 2.02 (m, ~4H, $CH=CHCH_2$), 1.58 (m, 4H, CH_2CH_2CO), 1.26 (s, ~37H, CH_2), 1.17 (d, 3H, $CHCH_3$), 0.88 (t, 6H, $2CH_3$). IR (KBr, disk), ν, cm^{-1}: 3350, 2930, 2860, 1750, 1470, 1385, 1250, 1170, 1080.

Multilamellar liposomes were prepared as described in [10]. The phospholipid concentration in liposomes was 0.02 mol l^{-1}. To remove oxygen, the samples were purged with argon for 40 min.

Liposomes were irradiated in sealed ampoules on a ^{137}Cs (Belarus State University) or ^{60}Co (the Leipzig University) γ-radiation source at a dose rate of 0.33 Gy s^{-1}.

The thin-layer chromatographic (TLC) analysis of lipids and sample preparation for the subsequent mass-spectrometric measurements were performed as described in [10]. The lipid concentration after their high-performance TLC separation was determined by measuring their phosphorus content [12].

Matrix-assisted laser desorption ionization–time of flight mass spectra were recorded on a Voyager Biospectrometry DE workstation (PerSeprive Biosystems, Framingham, MA). The spectra were obtained in the positive ion mode in all cases [10].

RESULTS AND DISCUSSION

The inclination of phospholipids to fragment into free radicals depending on the structure of their polar head was estimated from the radiation-chemical yield of PA formed upon γ-irradiation of liposomes containing naturally occurring PI and CL, as well as PG and PP synthesized from egg PC. All lipids mentioned above contain a free hydroxyl group in the β-position to the phosphoester bond and can undergo fragmentation according to Scheme 1.

A TLC analysis of the extract of irradiated liposomes showed that a new band with R_f characteristic of standard PA appeared in the chromatogram of each of irradiated lipids. To confirm the PA formation, lipids of this band were isolated by extraction and were analyzed by MALDI–TOF mass spectrometry. The mass spectrum of the extract of the band isolated from cardiolipin that predominantly contains the residues of linoleic (87%) and oleic (8%) acids in the hydrophobic moiety are shown in Fig. 1. The mass spectrum contains peaks at 719.5 and 741.4 characteristic of PA adducts containing linoleic acid residues ((PA (18 : 2, 18 : 2) + 2H + Na) and ((PA (18 : 2, 18 : 2) + H + 2Na), respectively), and peaks at 721.5 and 743.4 characteristic of PA adducts containing linoleic and oleic acid residues ((PA (18 : 1, 18 : 2) + 2H + Na) and (PA (18 : 1, 18 : 2) + H + 2Na), respectively). Other peaks in the spectrum correspond to PA with the acyl moiety altered by free-radical reactions of peroxide oxidation occurring in the hydrophobic portion of liposomes [13]. Thus, the MALDI–TOF MS data imply that the product formed by γ-irradiation of liposomes containing the test glycerophospholipids is phosphatidic acid.

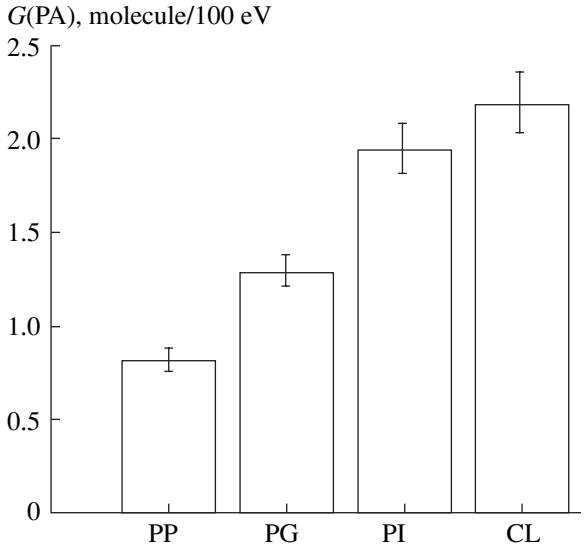
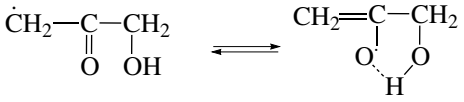


Fig. 2. Radiation-chemical yields of phosphatidic acid formed as a result of γ-irradiation of deaerated liposomes from phospholipids (0.02 mol/l) in 0.15 mol/l NaCl.

The total PA concentration was determined from the phosphorus content in a lipid according to the procedure [12] based on TLC separation. The PA radiation-chemical yields were calculated from the dose dependences for the buildup of the given product. The amount of PA in the dose range used (0–5.94 kGy) increases linearly with an increase in the absorbed radiation dose.

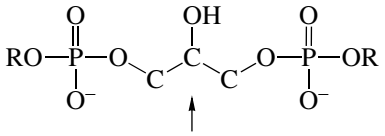
One can see from the data given in Fig. 2 that the radiation-chemical yields of PA formed in the γ-radiolysis of CL, PG, and PP differ significantly. For example, the yield of PA is higher in the radiolysis of PG than that for PP. This difference in the PA yields may be accounted for by the formation of a more stable radical intermediate via PG fragmentation. The fragmentation of PP according to scheme 1 gives acetone radical ($CH_2C(O)CH_3$) in addition to PA, and the PG fragmentation gives the hydroxyacetone radical ($CH_2C(O)CH_2OH$). The latter can be stabilized by H-bonding between the hydroxyl hydrogen atom and the carbonyl oxygen atom (Scheme 2).



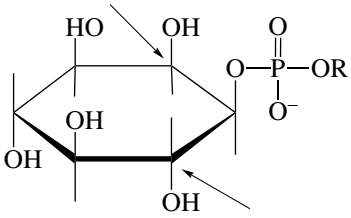
Scheme 2.

The radiation-induced transformation of CL is characterized by the highest radiation-chemical yield of PA, which is almost twice that of PA formed in the radiation-initiated transformations of PG (Fig. 2). Unlike the case of PG, the hydrophilic moiety of the CL molecule contains two phosphoester bonds β to the OH group (Scheme 3). Therefore, any fragmentation route for the radical that contains the OH group in the α-position should afford PA.

Cardiolipin



Phosphatidylinositol



Scheme 3.

where R = $–CH_2CH(OCOC_nH_{2n-1})CH_2OCOC_nH_{2n+1}$. Here, the arrows mark the carbon atoms that give radicals causing PL fragmentation with the elimination of PA.

The naturally occurring lipid phosphatidylinositol contains in its molecule the inositol (cyclic alcohol) residue with five free OH groups, of which two are β to the phosphoester bond. The ability of this lipid to undergo free-radical fragmentation is confirmed by the presence of PA among the radiolysis products. The PA yield in the case of PI fragmentation exceeds the yields of PA formed in the PP and PG radiolysis (Fig. 2). The difference in the PA yields is accounted for by the probability of the formation of radicals of two types in the case of PI, at C_2 and C_6 atoms in the alcohol moiety (Scheme 3). These radicals undergo fragmentation with the cleavage of the phosphoester bond. As shown by ESR [14] and pulse radiolysis [15] techniques, radicals of these types fragment mainly via phosphate elimination.

Thus, the ability of hydroxyl-containing phosphoglycerides in model membranes to fragment upon their γ-irradiation substantially increases owing to the possibility of formation of either one radical having the unpaired electron on the carbon atom β to two phosphoester bonds (CL) or several such radicals with the free valence β to a phosphoester bond (PI). Note that the two phospholipids play an important role in cell functioning. For example, a decrease in the CL level in a membrane may be accompanied by changes in the mitochondria structure [16], alteration in the activity of mitochondrial enzymes in oxidative phosphorylation

[17], the liberation of CL-associated cytochrome *c*, and apoptosis initiation [18]. As a result of the enzymatic hydrolysis of PI in the cell, inositol phosphate and diacylglycerol can be formed, which are secondary messengers activating changes in the cellular biochemical status [19].

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