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Free-radical reactions of glycerolipids and sphingolipids

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Contents

Abstract. Free-radical reactions of glycero- and sphingolipids occurring in their polar moiety (fragmentation) and in their hydrophobic residue (peroxidation) under the action of reactive oxygen species are considered. The main attention is focused on free-radical fragmentation; its mechanism and regularities are discussed. Lipid peroxidation has been shown to modify the residues of polyunsaturated fatty acids, while the free-radical fragmentation results in the cleavage of ester, O-glycosidic and amide bonds in lipid molecules to give glycerophosphatides, ceramides and fatty acid amides functioning as secondary messengers in biosystems. The bibliography includes 132 references.

I. Introduction

Glycero- and sphingolipids are important structural components of biomembranes and bioeffectors that control the intracellular reactions and intercellular interactions; their content in the membrane may vary from 25% to 80%.[1–3] A way of chemical modification of lipids is represented by free-radical transformations. These processes are initiated by reactive oxygen species (ROS), namely, $HO^{\bullet}$, $O_2^{-\bullet}$, $^1O_2$, H_2O_2, HClO, or reactive nitrogen species (RNS), namely, $^{\bullet}NO$, $^{\bullet}NO_2$, $ONOO^-$, which are formed in cells upon biochemical processes or under the action of external factors.[4–7] During the last decades, sufficient knowledge has been accumulated indicating that free radicals play an important role in the development of many diseases (immunodeficient and allergic states, neurodegenerative and autoimmune pathologies, ischemic damage, inflammatory processes, premature ageing, diabetes, cancer and so on).[7–11] Therefore, elucidation of the mechanisms of free-radical processes that may develop in the cells remains a topical task. The purpose of this review is to generalize the published data about the free-radical transformations of the most common types of lipids: glycerolipids (for example, compounds **1**) and sphingolipids **2**.†

The reactions of ROS with amphiphilic glycero- and sphingolipid molecules may involve both the hydrophobic moieties (peroxidation) and the hydrophilic moieties (free-radical fragmentation). The free-radical fragmentation results in the destruction of the lipid molecules with the cleavage of ester (amide) bonds,[12] and peroxidation modifies the lipophilic moiety as a result of oxidation of acyl residues.[13]

The lipid peroxidation (LPO) is the best known free-radical process involving these compounds; it has been studied for more than half a century and currently it is regarded as the fundamental mechanism of cell pathology. During the last decade, numerous reviews that summarize the data on the role of LPO in the development of oxidative stress and related diseases have been published.[13–22] The mechanisms of both the formation of LPO products in various biological systems[14–16,19,23–28] and the interaction of these products with proteins,[21,26,29] DNA[17,18,26] and aminophospholipids[21,26,30] are being elucidated more precisely and in more detail. The in-depth studies into the effect of these processes on the properties and functions of biomembranes,[20,21,31–33] cell signalling system and gene expression[16,20–22] are in progress. The recent build-up of the knowledge on LPO and the proper glycero- and sphingolipids has been associated with the advent of new highly sensitive methods of analysis, in particular, the methods combining chromatography (HPLC, GLC, TLC) and mass spectrometry, especially using mild ionization techniques: matrix-assisted laser desorption/ionization (MALDI MS), electrospray ionization (ESI MS) and so on.[24,34–37]

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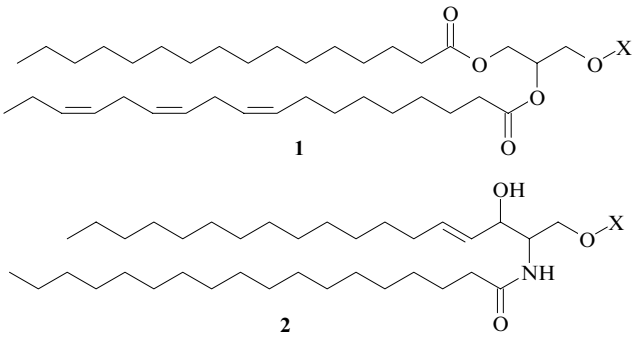
† Natural glycerolipids often contain carboxylic acid residues with 16 and 18 carbon atoms and, as a rule, one residue located in the *sn*-2 glycerol core position has double bonds. Sphingolipids **2** (sphingosine derivatives) typically contain a saturated fatty acid residue, most often, C_{18}.



X	1	2
H	1,2-diacylglycerol	*N*-acylsphingosine (ceramide)
Carbohydrate residue	glycerolglycolipid	glycosphingolipid
Phosphoric acid ester	glycerophospholipid	sphingophospholipid

Despite the diversity of reviews published on this topic, they do not consider homolytic reactions that can occur between ROS and the hydrophilic moieties of glycero- and sphingolipids exposed to the aqueous phase where the reactive species are formed. Therefore, in this review, we consider LPO only briefly, the primary attention being focused on the ROS-mediated processes that occur in the polar moiety of glycero- and sphingolipids, in particular, on free-radical fragmentation reactions.

II. Lipid peroxidation

Lipid peroxidation involves the oxidation of unsaturated fatty acid residues in lipid molecules to give hydroperoxides. In the lipid bilayer, acyl residues with two or more double bonds are oxidized, whereas monounsaturated moieties resist oxidation.[4, 20, 23] Polyunsaturated fatty acids (PUFAs) occur mainly in glycerolipids **1**, the peroxidation of which is the subject of the main bulk of publications in this area. Sphingolipids **2** contain predominantly saturated and monounsaturated fatty acid residues. In natural lipids, double bonds exist mainly in the *cis*-configuration, which is oxidized more readily than the *trans*-form.[38]

Under the action of free-radical initiators ($In^{\bullet}$), lipids are oxidized by the chain mechanism comprising the following three steps.[14–16, 21–23, 39]

1. Chain initiation by abstraction of an H atom from the CH_2 group located in a bis-allylic position, *i.e.*, between C=C double bonds in the PUFA residue of the lipid molecule (LH),

$$LH + In^{\bullet} \longrightarrow L^{\bullet} + InH. \quad (1)$$

2. Chain propagation by the reaction of the lipid radical $L^{\bullet}$ with oxygen to give conjugated peroxyl radical $LOO^{\bullet}$, which adds the H atom to be converted to lipid hydroperoxide LOOH.

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet}, \quad (2)$$

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet}. \quad (3)$$

3. Chain termination through disproportionation and recombination of the radicals $LOO^{\bullet}$, $LO^{\bullet}$ and $L^{\bullet}$ or reactions of these radicals with antioxidant (AH) molecules.

$$L^{\bullet} + LOO^{\bullet} (L^{\bullet}, LO^{\bullet}, AH) \longrightarrow \text{products}. \quad (4)$$

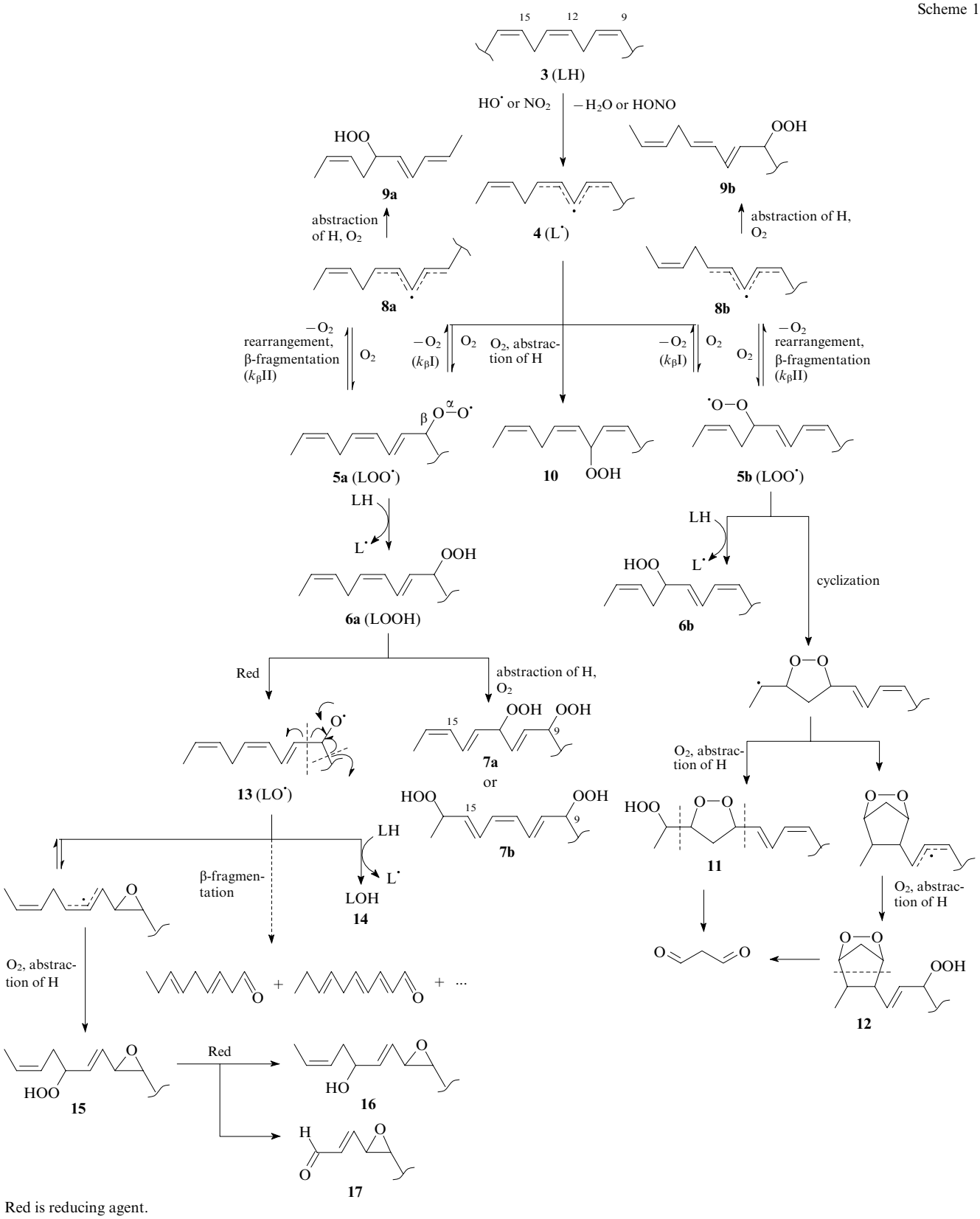
The set of hydroperoxides formed from lipids depends on the PUFA chain length, the number and the positions of double bonds and on their chemical environment. Below we consider in brief the chemistry of the LPO and give data on some secondary products the role of which in biosystems is the subject of intensive research. As an example, Scheme 1 shows free-radical oxidation of the octadeca-9*Z*,12*Z*,15*Z*-trienoic acid residue (**3**) [C(9)–C(16) fragment]. Detailed consideration of the mechanism of lipid peroxidation can be found in reviews[14–16, 21–23] and in the published sources cited therein.

Among ROS, the hydroxyl radical $HO^{\bullet}$ is considered to be the most reactive LPO initiator [see reaction (1)].[21, 23] (The involvement of RNS in the lipid oxidation process is addressed below.) Note that despite the numerous publications devoted to LPO in various systems, it is not entirely clear how the hydrogen atom is split off from a bis-allylic CH_2 group by hydrophilic $HO^{\bullet}$. According to EPR and pulse radiolysis data, hydroxyl radicals interact mainly with the polar part of the lipid bilayer.[40] However, later studies of glycerophospholipid liposomes using lipophilic spin traps in combination with NMR and EPR spectroscopy indicate that some of the $HO^{\bullet}$ radicals may penetrate behind the glycerol ester groups and reach the upper part of the hydrophobic layer.[41] Probably, the initiation step is affected by the state of packing and the composition of the hydrophilic layer.[40–42] According to an opinion,[43, 44] the $HO^{\bullet}$ radical non-selectively eliminates any H atom from the PUFA residue to give an alkyl radical, and then the free valence migrates to the energetically favourable allylic position as a result of secondary intramolecular processes.

The superoxide radical anion ($O_2^{-\bullet}$) cannot penetrate through the membrane polar layer and initiate LPO, unlike the conjugate acid $HO_2^{\bullet}$ ($pK_a = 4.8$).[21] However, a study of the reactivity of $O_2^{-\bullet}$ in neutral liposomes has shown[45, 46] that ~16% of the species can diffuse inside the lipid bilayer to a moderate depth. The singlet oxygen oxidizes lipids by a non-radical mechanism; it adds to the double bonds in the PUFA residue to give hydroperoxides.[6, 22]

The chain propagation step of LPO is more complex than reaction equations (2) and (3): the O_2 molecule adds to pentadienyl radical **4** and the H atom migrates from the substrate to peroxyl radicals **5a**,**b**. The initially formed radical **5a** or **5b** with *trans*,*cis*-conjugated system of double bonds adds a hydrogen atom, being converted to *trans*,*cis*-hydroperoxide **6a** or **6b**. Monohydroperoxides like **6a** can be further oxidized to 9,12- (**7a**) and 9,16-dihydroperoxides (**7b**). The formation of various monohydroperoxides is caused by the fact that peroxyl radicals can participate in β-fragmentation (reversible addition of oxygen to the organic radical) and cyclization as a result of intramolecular rearrangements. Upon β-fragmentation, radicals **5a**,**b** generate either the starting *cis*,*cis*-pentadienyl radical **4** [the rate constant for this reaction (k_{β}I) is 70 s^{-1}] or the rearranged (due to rotation about the C–C bond) *trans*,*trans*-pentadienyl radicals **8a**,**b** (k_{β}II = 625 s^{-1}). The latter are converted to *trans*,*trans*-hydroperoxides **9a**,**b**.[23, 39, 47]

Scheme 1



Red is reducing agent.

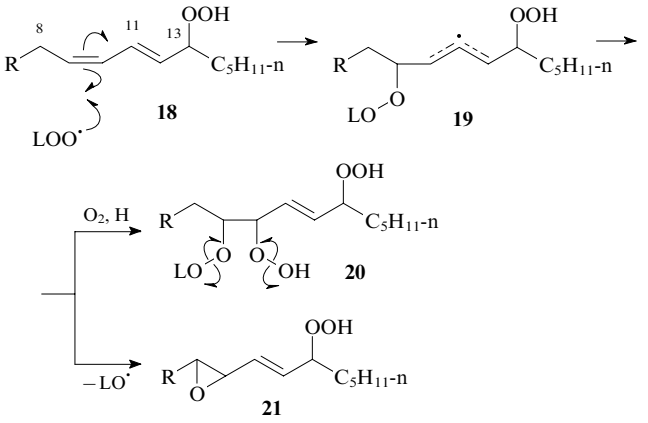
Apart from hydroperoxides **6a**,**b** and **9a**,**b** containing conjugated double bonds, radical **4** can be converted to bis-allylic hydroperoxide **10**. However, these hydroperoxides can be generated only if the system contains effective hydrogen donors (for example, α-tocopherol), as the β-fragmentation of the preceding bis-allylic peroxyl radical is very fast ($k_\beta = 1.9 \times 10^6$ s^{-1}).[23] When peroxyl radicals of type **5b** have a double bond in the β,γ-positions relative to the carbon atom bearing the peroxy group, they cyclize to give various mono- and bicyclic peroxides, for example, hydro-

peroxycycloperoxide **11** and hydroperoxy biscycloendoperoxide **12**.[23, 39]

The transformations of lipid hydroperoxides afford secondary LPO products having functional substituents, hydroxy and keto groups and epoxide rings. Indeed, the reduction of hydroperoxides (*e.g.*, compound **6a**, see Scheme 1) with transition metal ions (Fe^{2+}, Cu^{+}) gives rise to the alkoxyl radicals $LO^{\bullet}$ (**13**).[11, 13, 14, 20] Radicals **13** are able to detach H atom to give hydroxy derivatives of lipids **14** or to transform to epoxyhydroperoxides **15**, which can be reduced to yield epoxy-hydroxy (**16**) and epoxy-oxo compounds (**17**).[13] Also, radicals **13** undergo β-fragmentation with rupture of the α,β-C−C bond (relative to the radical centre) in the PUFA residue, giving rise to various low-molecular-mass compounds.[13] These may include unsaturated aldehydes (nona-3,6-dienal, deca-2,4,7-triental, *etc.*) and products of their subsequent destruction upon the retro-aldol reaction. Generally, β-fragmentation of the alkoxyl radicals furnishes alcohols, ketones, aldehydes and glycerolipids with short-chain acyl residues containing various functional groups in the carbon chain.[13–16, 21–23, 26, 28, 48]

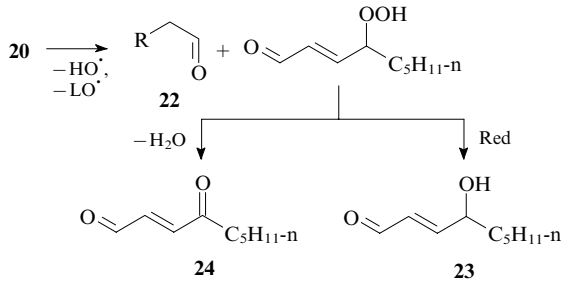
According to several publications,[25, 49, 50] in the chain propagation step, the $LOO^{\bullet}$ radicals can add to double bond of the phospholipid hydroperoxide (*e,g.*, compound **18**) producing unstable cross-linked dimers **19** (Scheme 2).

Scheme 2



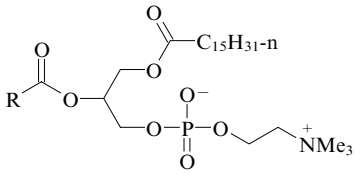
R is the glycerophospholipid residue.

Within the classical LPO paradigm, dimer formation through intermolecular cross-linking is possible mainly in the chain termination step upon interaction of two radicals. After destruction, both dimers **19** and the products of their subsequent oxidation **20** become sources of the free radicals $LO^{\bullet}$. This gives epoxy derivatives **21** and carbonyl compounds: aldehydes **22**, (*E*)-4-hydroxynon-2,3-enal (**23**) and (*E*)-4-oxonon-2,3-enal (**24**).[25, 49, 50]



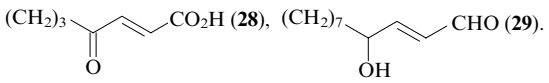
Phospholipid hydroperoxides function in biosystems as signalling molecules; they are involved in the regulation of cell proliferation and programmed cell death, and the hydroxy derivatives formed upon their reduction exhibit proinflammatory properties and affect cell differentiation.[14, 16, 22]

The oxidized glycerophospholipids with short acyl groups at the C(2) atom of the glycerol core have biological activity and can activate neutrophils and monocytes.[16, 19, 28, 33, 48, 51] Examples of these compounds are 1-hexadecanoyl-2-(5-oxopentanoyl)- (**25**), 1-hexadecanoyl-2-(4-carboxybutanoyl)- (**26**), 1-hexadecanoyl-2-(9-oxononanoyl)- (**27**), 1-hexadecanoyl-2-(7-carboxy-5-oxohept-6*E*-enoyl)- (**28**), 1-hexadecanoyl-2-(9-hydroxy-12-oxododec-10*E*-enoyl)-*sn*-glycero-3-phosphocholine (**29**).



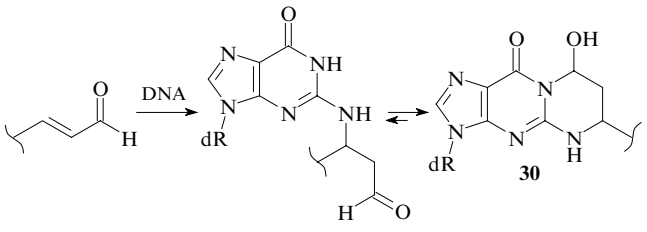
25–**29**

R = $(CH_2)_3CHO$ (**25**), $(CH_2)_3CO_2H$ (**26**), $(CH_2)_7CHO$ (**27**),



Oxidized lipids and lipid moieties form non-covalent complexes or covalent adducts with cell components comprising nucleophilic functional groups and thus modify proteins,[14, 16, 17, 21, 26, 28, 29, 51–53] DNA[14, 16, 17, 18, 26, 28, 54] and aminophospholipids (phosphatidylethanolamine, phosphatidylserine).[14, 21, 26, 30, 28] Polymerization of the oxidized lipids and proteins affords ceroid (age-related) pigments and lipofuscine.[13]

The major aldehydes formed as secondary LPO products include (*E*)-4-hydroxynon-2,3-enal (4-HNE, **23**), (*E*)-4-oxonon-2,3-enal (**24**), prop-2-enal, 4-hydroxyhex-2-enal and malondialdehyde (propane-1,3-dial, MDA). α,β-Unsaturated aldehydes are highly reactive. They form stable Michael adducts with protein amino acids upon the addition of the S atom of cysteine sulfhydryl group, the N atom of lysine amino group (this is also typical of aminophospholipids) or the imidazole nitrogen atom of histidine to the C=C double bond. They also give Schiff's bases upon condensation of the carbonyl group with primary amino groups (see below).[16, 26, 52, 53] These aldehydes modify the nitrogenous bases of DNA. For example, they react with guanine residues to give six-membered exocyclic 1,N^2-γ-hydroxypropano-dG adducts **30**, which produce cross-links in the DNA molecule.[17, 18, 52, 54]

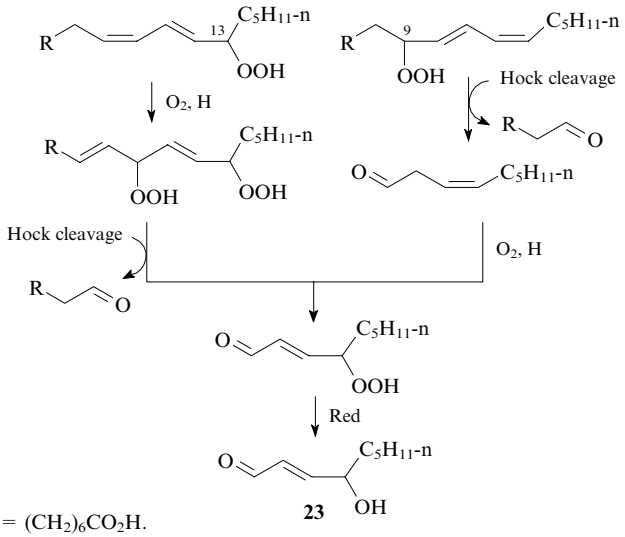


dR is 2′-deoxyribose residue in the polynucleotide chain.

The biochemistry of the aldehyde LPO products was surveyed in detail.[18,26] Here the processes involving two best studied aldehydes is considered: 4-HNE [17,18,21,22,25,49,52,53] and MDA.[18,21,22,26]

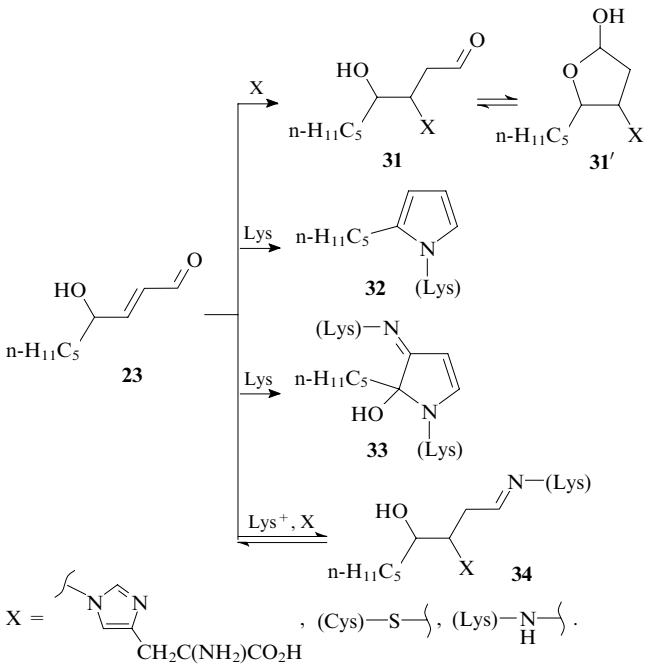
The hydroperoxides of ω-6 PUFA residues in the phospholipid molecules may serve as sources of 4-HNE.[52,53] Scheme 3 shows the transformations of 13-hydroperoxyoctadeca-9,11-dienoic and 9-hydroperoxyoctadeca-10,12-dienoic acids, resulting in the formation of aldehyde **23**.

Scheme 3



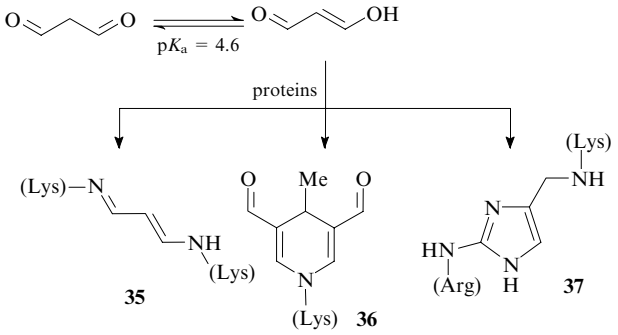
A proposed [25,49] mechanism of the formation of compound **23** includes the step of formation of dimer **19** from the hydroperoxides through cross-linking of the peroxyl radical with lipid hydroperoxide followed by its decomposition as shown in Scheme 2.

Compound **23** modifies proteins by giving Michael adducts **31** with cysteine, histidine and lysine residues; the adducts cyclize to give hemiacetals **31′**. Alternatively, addition to the double bond with condensation at the carbonyl group gives adducts **32**–**34** [notation N—(Lys) means that the nitrogen atom is a part of the lysine residue].[26,52]

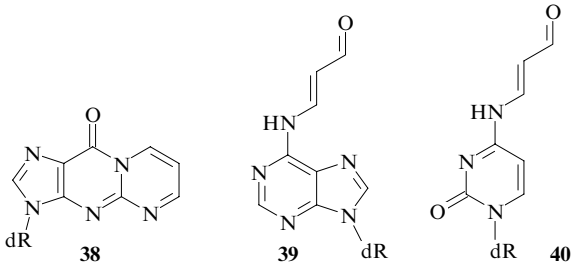


The DNA modification by aldehyde **23** consists of the formation of exocyclic five-membered (ethene-based) or six-membered (propane-based, *e.g.*, compounds **30**) adducts with the guanine, adenine and cytosine residues.[18,51,52] In biosystems, 4-HNE behaves as a signalling molecule and affects biochemical processes in the cell.[21,52,53]

Malondialdehyde can be formed upon transformations of cyclic peroxides (see Scheme 1) or other cyclic products of more extensive oxidation of acyl residues with three or more double bonds, although the mechanism of its formation is not entirely clear.[13,26] Malondialdehyde adds to proteins to give fluorescent cross-linked adducts: the reaction with two Lys residues yields *N*,*N*′-disubstituted 1-amino-3-iminopropene (**35**); the condensation of the Lys residue with three MDA molecules affords *N*-Lys-4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde (**36**), and the imidazole adduct Arg-MDA-Lys (**37**) is formed upon the reaction with arginine and lysine.[26]

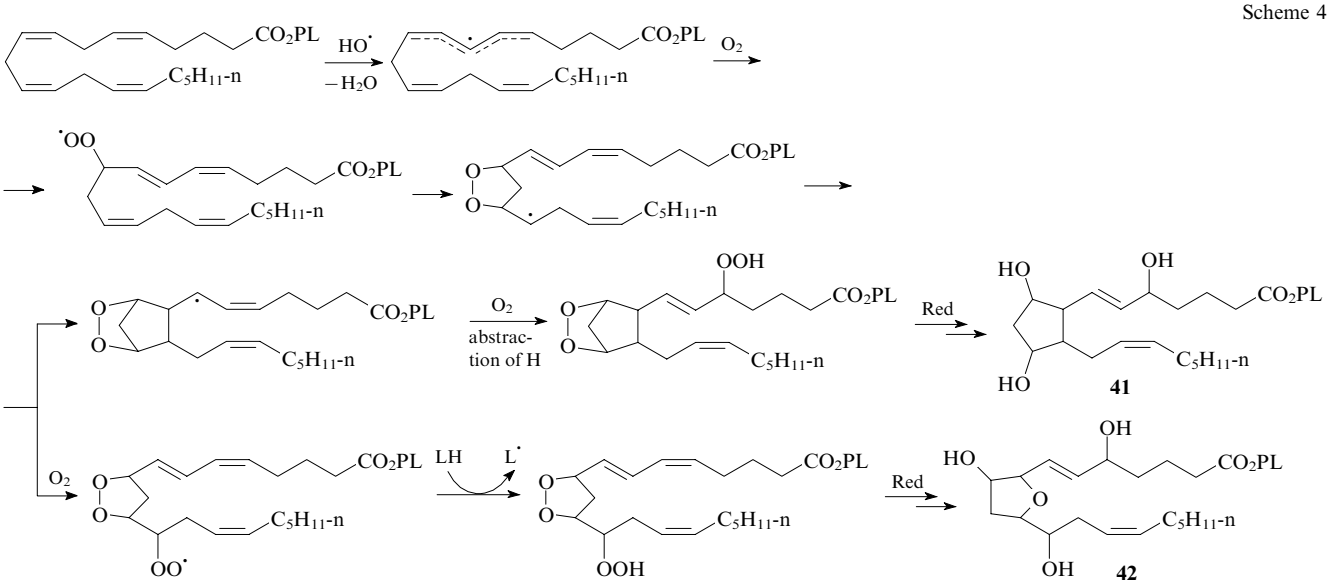


At physiological pH values, MDA reacts with the nitrogenous bases of DNA: the interaction with the N(1) and N(2) atoms of deoxyguanosine produces a new nucleoside, pyrimido[1,2-α]purin-10(3*H*)-one (**38**), while the addition to exocyclic amino groups of deoxyadenosine or deoxycytidine affords N^6-(3-oxopropenyl)deoxyadenosine (**39**) or N^4-(3-oxopropenyl)deoxycytidine (**40**), respectively.[18,26,52,54]



Isoprostanes and neuroprostanes, as well as isofurans and neurofurans, are important biologically active secondary products of LPO.[15,23] Isoprostanes structurally resemble natural prostaglandins but do not possess their spatial isomerism. They are initially formed in phospholipids *in situ* in the esterified form and are released by means of phospholipases. Similarly to prostaglandins, depending on the structure of the cyclopentane fragment, isoprostanes are classified into subgroups (F, H, E, D), which include large numbers of regioisomers.[15]

Scheme 4



PL is a glycerophospholipid residue.

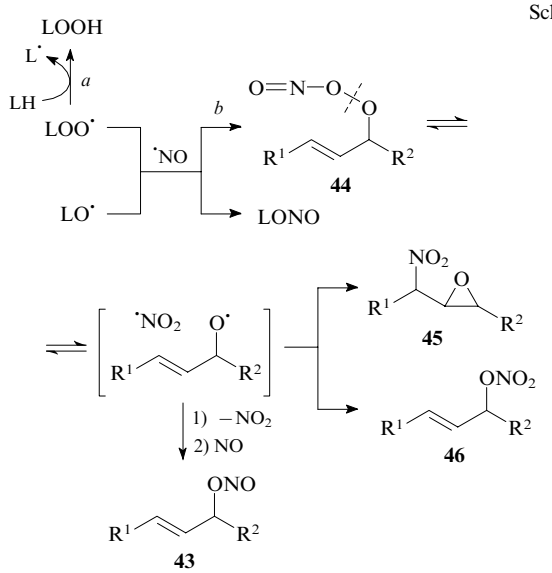
F_2-Isoprostanes **41** and isofurans **42** are formed from phospholipids containing eicosatetraenoic (20 : 4, ω-6) acid (Scheme 4).

Eicosapentaenoic (20 : 5, ω-3) and docosahexaenoic (22 : 6, ω-3) acids serve as the sources of F_3-isoprostanes and F_4-neuroprostanes, respectively. The formation mechanism, the properties and the methods of analysis of these LPO products were comprehensively covered in reviews.[15, 23, 24]

In the last decades, the role of active nitrogen species in the development of LPO and modification of membrane lipids has been intensively studied; the results were reported in detail.[27, 55, 56] Nitrric oxide is not a strong oxidant and cannot directly initiate LPO by abstracting the bis-allylic H atom from the PUFA. However, owing to high lipophilicity, it readily diffuses to the bilayer membrane [56] where it reacts with the radicals L•, LOO• and LO•, which are formed in the chain propagation step, and this inhibits the oxidation.[55] These transformations involving •NO yield non-radical nitrogen-containing glycerophospholipid derivatives: nitrites **43**, nitrosoperoxides **44**, nitroepoxides **45** and nitrates **46** (Schemes 5 and 6 show only the carbon atoms that are involved in the reactions and the substituents R^1 and R^2 designate the corresponding lipid moieties).[27]

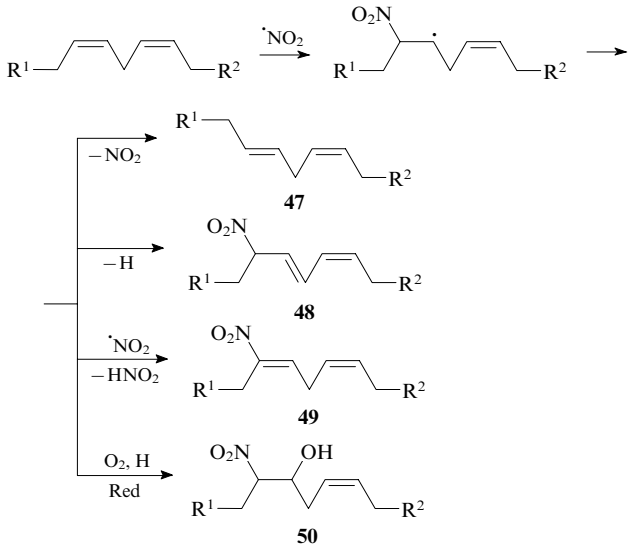
On the other hand, •NO reacts with the superoxide radical anion, thus generating peroxynitrite (ONOO•), the protonated form of which (ONOOH, $pK_a = 6.8$) undergoes homolytic cleavage to give HO• and $•NO_2$.[6, 57] Nitrogen dioxide, like the hydroxyl radical, can initiate LPO (see Scheme 1). The $•NO_2$ radical can also interact with the double bond in the PUFA residue by catalyzing the *cis*,*trans*-isomerization (see the formation of compound **47**

Scheme 5



(*a*): $k_v = (30-172)$ litre mol^{-1} s^{-1} (Ref. 27 and 39);
(*b*): $k = 3 \times 10^9$ litre mol^{-1} s^{-1} (Ref. 27).

Scheme 6



in Scheme 6) of the *cis*,*cis*-double bonds. In addition, reactions with this radical afford nitro derivatives of lipids,[27, 56] specifically, nitroallylic (**48**), nitroalkene (**49**) and nitrohydroxyl (**50**) derivatives.

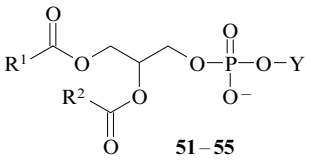
Usually, the lipid oxidation in biosystems is catalyzed by enzymes,[13, 16, 58] namely, lipoxygenases and cyclooxygenases, and involves the intermediate formation of lipid radicals. The mechanisms of these processes were described in detail in a review.[58] Lipoxygenases catalyze the oxidation of PUFAs to hydroperoxides that serve as sources of leukotrienes and lipoxins. Cyclooxygenases catalyze the addition of oxygen to PUFAs to give endoperoxides, intermediates in the syntheses of prostaglandins, thromboxanes and prostacyclins.[58] Unlike the LPO induced by ROS, reactions involving enzymes are regio- and stereospecific.[58, 59]

Thus, peroxidation, which is a multistep process, involves transformations of the PUFA residues in the lipid molecules; the ester bonds are not cleaved and the hydrophilic part remains unchanged.

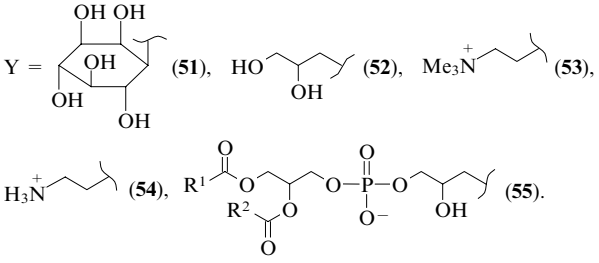
III. Free-radical fragmentation of glycerophospholipids

1. General features

Glycerophospholipids: phosphatidylinositol (PI, **51**), phosphatidylglycerol (PG, **52**), phosphatidylcholine (PC, **53**), phosphatidylethanolamine (PE, **54**), diphosphatidylglycerol [cardiolipin (CL), **55**], differ by the alcohol residues in the polar moieties.‡



51–55

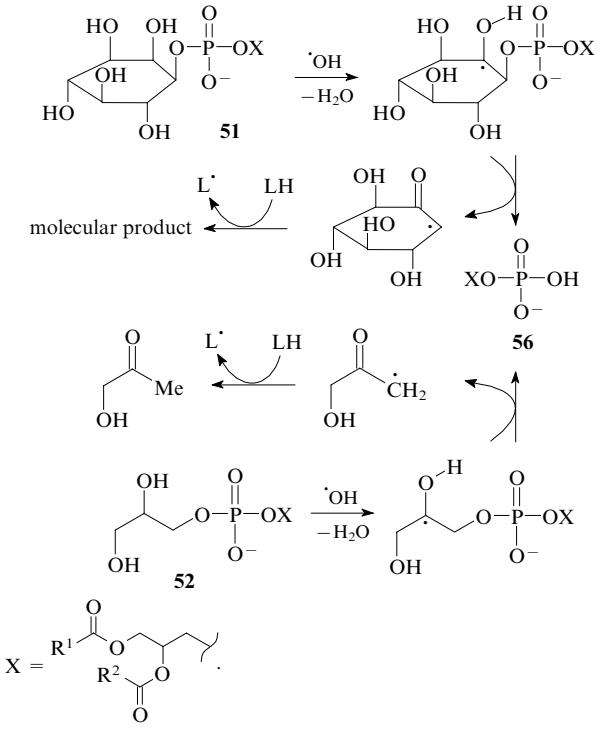


A steady-state radiolysis study of the free-radical transformations of glycerophospholipids showed that lipids **51** and **52** containing polyol residues in the hydrophilic part are destroyed to give phosphatidic acids (PA, **56**) (Scheme 7).[12, 60–66]

The cleavage of the phosphoester bond in lipid molecules **51** and **52** and, as a consequence, the formation of product **56** is a result of free-radical fragmentation taking

‡ Hereinafter, unless stated otherwise, R^1 and R^2 are hydrocarbon residues of the lipid; usually R^1 is a saturated hydrocarbon chain and R^2 is an unsaturated hydrocarbon chain.

Scheme 7



place in the polar moiety. The essence of the process is as follows. The HO$^\bullet$ radicals formed upon water radiolysis [12]

$$H_2O \longrightarrow HO^\bullet + H + e_{aq},$$

react with the hydrophilic part of the glycerophospholipids to give α-hydroxyl-containing carbon-centred radicals with the unpaired electron in the β-position to the $-OCH_2-C^\bullet(OH)-CH_2O-$ phosphoester bond. These radicals decay with cleavage of two bonds in the β-positions relative to the radical centre to give a molecular product and a radical intermediate.[12, 60–66] The formation of PA is one of the key processes of radiolysis of the studied lipids, as its radiation chemical yield (G) is ~50%–70% of the yield of HO$^\bullet$ radicals ($G_{OH} = 2.8 \times 10^{-7}$ mol J^{-1}).[12, 61–66]

Phosphatidylcholine (**53**) differs from lipids **51** or **52** by the presence of the aminoalcohol (choline) residue in the hydrophilic part and the absence of hydroxyl groups. Compound **53** was more stable against free-radical destruction with cleavage of the ester bonds than PG or PI.[12, 61] It was shown by steady-state radiolysis that PC is not destroyed to give diacylglycerol or free fatty acids. The radiation-induced transformations of PG did not result in the accumulation of these compounds either. The difference of free-radical transformations of PC from the reactions of PG was the fact that no phosphatidic acid was detected among the products of γ-radiolysis of aqueous suspensions of PC. The action on the PC liposomes of redox systems [$Fe^{2+}(Cu^{2+})-H_2O_2$ or $Fe^{2+}(Cu^{2+})-H_2O_2-$ascorbate] capable of generating the HO$^\bullet$ radicals [67] also did not give PA, which was accumulated upon the transformations of phosphatidylglycerols under identical conditions.[68] Stu-

dies of free-radical reactions of phosphatidylethanolamine (**54**) by high-performance thin layer chromatography (HPTLC) and MALDI MS showed that compound **54**, like lipid **53**, is stable against destruction with cleavage of ester bonds.

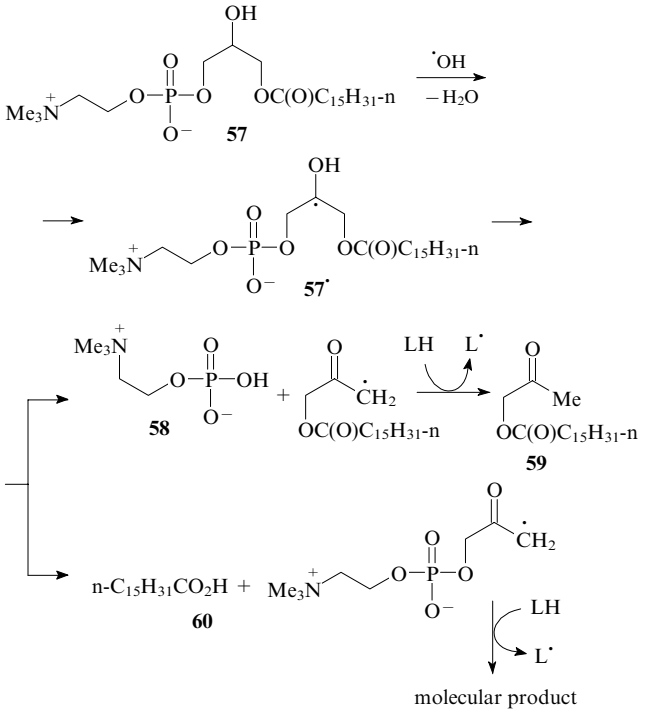
The effect of the structure of the polar moiety of glycerophospholipids on their tendency for fragmentation on treatment with free-radical process initiators on the model membranes can be readily traced by comparing the data obtained for 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (1,2-dipalmitoylphosphatidylcholine, DPPC) and 1-hexadecanoyl-*sn*-glycero-3-phosphocholine (lyso-PC, **57**).[12, 61, 69]

Thus the reactions of HO$^{\bullet}$ with the polar moiety of lyso-PC (Scheme 8) afford α-hydroxyl-containing carbon-centred radicals **57**$^{\bullet}$.

Their fragmentation with cleavage of two β-bonds results, depending on the decomposition route, in phosphocholine (**58**) and a radical intermediate that is reduced to 1-palmitoyloxypropan-2-one (**59**) and in hexadecanoic acid (**60**). The radicals $OCH_2-C^{\bullet}(OH)-CH_2OC(O)C_{15}H_{31}$-n (**57**$^{\bullet}$) are more prone to decomposition with cleavage of the phosphoester rather than ester bond, as the yield of phosphocholine (**58**) is three times higher than the yield of acid **60**. The rate constant (k_v) for elimination of compound **58** from radicals **57**$^{\bullet}$ generated selectively is 3.5×10^6 s^{-1}, while that for compound **60** is $k_v = 3.8 \times 10^5$ s^{-1} (Ref. 70). These results are consistent with the data obtained in the study of free-radical transformations of compounds containing fragments of the polar moiety of glycerophospholipids. For glycero-1-phosphate, it was shown by EPR and pulse radiolysis that the radicals $HOCH_2-C^{\bullet}(OH)-CH_2OPO_3^{2-}$ are mainly fragmented with elimination of inorganic phosphate, the rate constant for this process exceeding 10^6 s^{-1} (Refs 71 and 72).

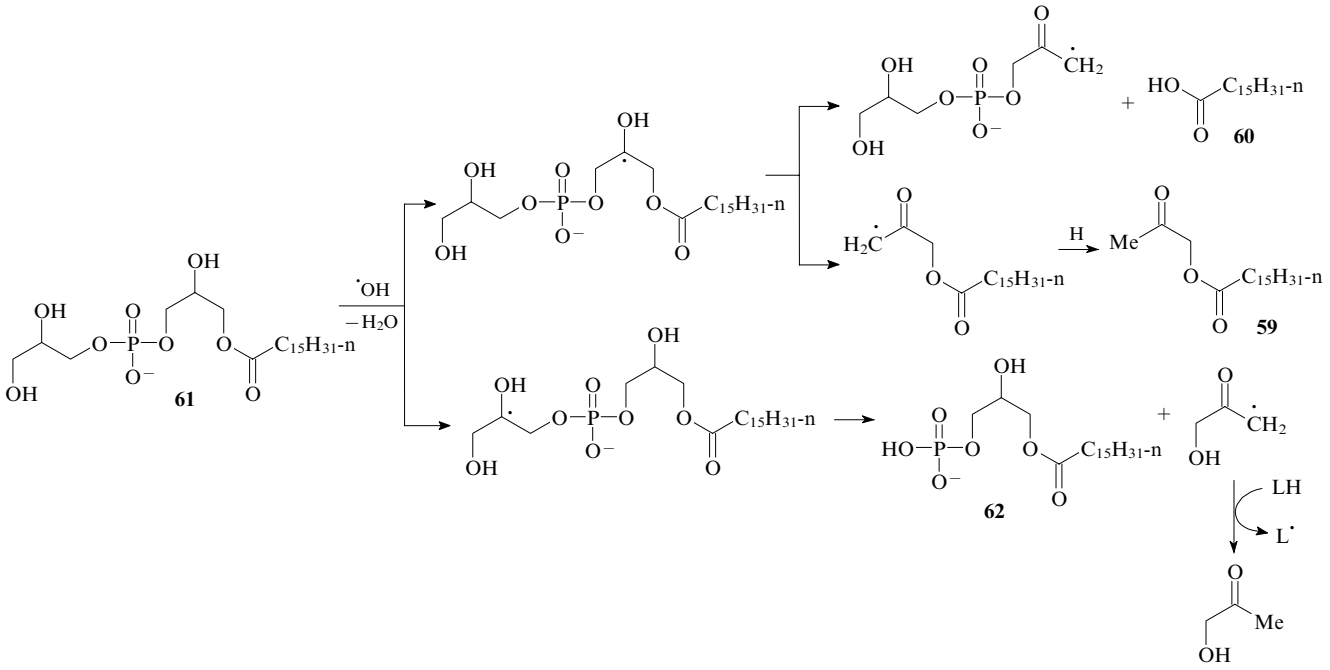
The free-radical transformations of DPPC did not give compounds **58** or **60**. This indicates that the presence of free OH group in the β-position to the (phospho)ester bond in the glycerophospholipid molecule determines its susceptibility for free-radical fragmentation.

Scheme 8



This conclusion is confirmed by the results of steady-state radiolysis studies of free-radical transformations of 1,2-dihexadecanoyl-*sn*-glycero-3-phospho-(1′-*sn*-glycerol) (dipalmitoylphosphatidylglycerol, DPPG) and 1-hexadecanoyl-*sn*-glycero-3-phospho-(1′-*sn*-glycerol) (lyso-PG, **61**) carried out by the research groups of the author of this review. Dipalmitoylphosphatidylglycerol has one free hydroxyl group in the hydrophilic moiety and can form one type of α-hydroxyl-containing carbon-centred

Scheme 9



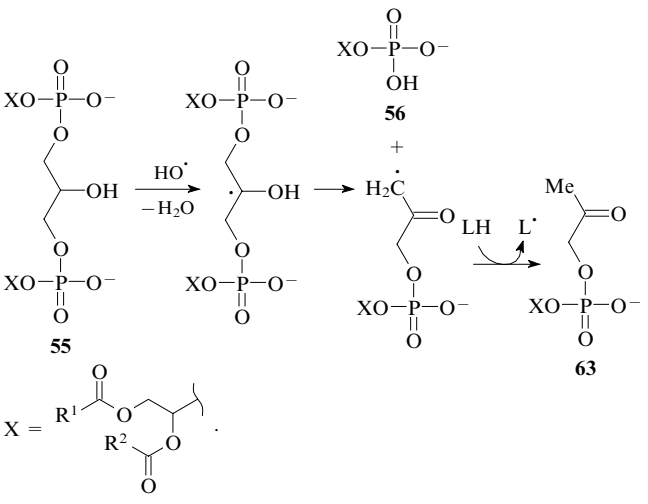
$HOCH_2-C^{\bullet}(OH)-CH_2OP(O)(O^-)OX$ radicals, which decompose to give acid **56** and a radical intermediate, the latter being reduced to 1-hydroxypropan-2-one (see Scheme 7).[12,61] The generation of radicals of this type is also typical of lyso-PG. This compound, like DPPG, decomposes to give phosphatidic acid [in this case, this is 1-hexadecanoyl-*sn*-glycero-3-phosphate, lyso-PA (**62**)] and 1-hydroxypropan-2-one (Scheme 9).

The products of radiolysis of lipid **61** were also found to contain 1-palmitoyloxypropan-2-one (**59**) and hexadecanoic acid (**60**); these compounds are not formed upon the action of free-radical initiators on DPPG liposomes. Owing to the replacement of the acyl residue in the lyso-PG molecule by the HO group at C(2) of the glycerol core, the formation of α-hydroxyl-containing carbon-centred radicals of another type, $CH_2O-C^{\bullet}(OH)-CH_2OC(O)C_{15}H_{31}$-n, becomes possible for compound **61** (unlike DPPG); decomposition of these radicals finally gives rise to compounds **59** and **60**.

Cardiolipin (**55**) is also a glycerophospholipid containing a free OH group in the polar moiety. This unique lipid with the dimeric structure accounts for ~25% of all lipids of the mitochondrial membrane.[73] The mitochondria are the main source of ROS in the cell.[5,8,74] Among the mitochondrial components, CL is the lipid that is most sensitive to the attack by ROS because it is localized on the inner membrane near the sites where they are generated. The content of CL sharply decreases upon the development of ROS-mediated oxidative stress.[75,76]

It was shown[64,66,77–79] that the hydrophilic part of the CL is subjected to free-radical fragmentation. This results in the destruction accompanied by cleavage of the phosphoester bond to give a molecular product, phosphatidic acid (**56**), and a radical intermediate, which is reduced to phosphatidylhydroxyacetone (PHA, **63**) (Scheme 10). The latter is not a native lipid and can serve as the marker of the process.

Scheme 10

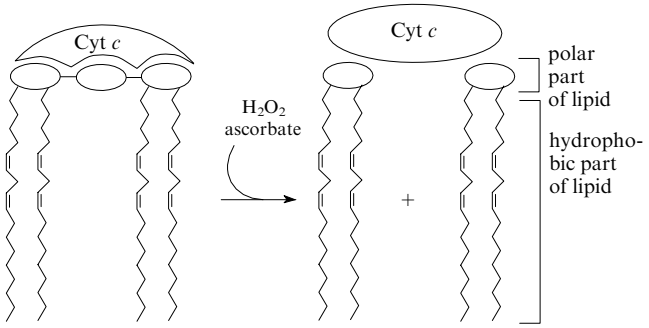


Product **63** is accumulated in a much lower amount (~2 times) than acid **56**.[77] This indicates that the preceding radical intermediate can participate not only in atom abstraction reactions but also in other competing processes.

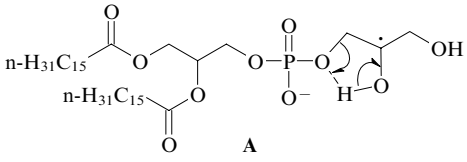
In model membranes, conditions for Fe^{2+}/Cu^{2+}-mediated generation of HO• radicals resulted in a decrease in the cardiolipin level correlated with accumulation of compounds **56** and **63**.[68,78,79] Studies by HPTLC and MALDI MS and their combination demonstrated that phosphatidic acid whose acyl moiety is identical, according to the fragmentation mechanism, to the substituent X in CL is accumulated in isolated mitochondria subjected to oxidative stress[79,80] and in mitochondria of the genetically modified Atp7b$^{\neq}$ mice.[81] The latter serve as a model for investigating the molecular mechanisms of Wilson's disease and show ~12–18 times higher copper accumulation in liver than the control species.[82]

Consumption of CL and increase in the concentration of phosphatidic acid and phosphatidylhydroxyacetone were observed in a model phospholipid membrane with incorporated ferricytochrome *c* in the presence of H_2O_2 and ascorbic acid.[83] Elimination of CL and simultaneous liberation of cytochrome *c* from mitochondria to the cytosol occurs in response to programmed cell death-inducing factors, in particular, ROS.[84–86] The fact that cytochrome *c* (Cyt *c*) mediates the free-radical fragmentation in the polar part of the CL may be significant for determination of the mechanism of disturbance of CL interaction with cytochrome *c* in the development of programmed cell death (Scheme 11).

Scheme 11



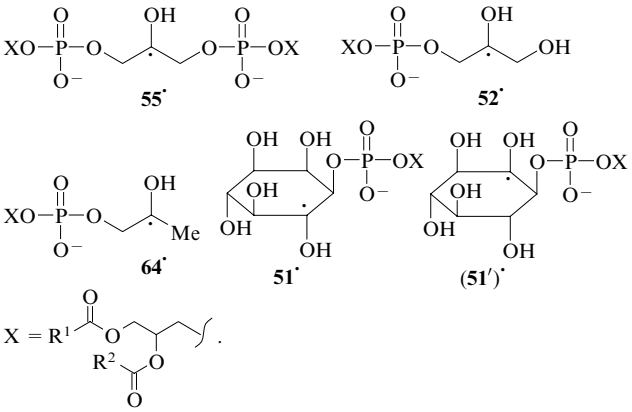
There are two viewpoints on the fragmentation mechanism of α-hydroxyl carbon-centred radicals: it is considered as either heterolytic[87] or homolytic.[88] Davies and Gilbert[87] believe that the $CH(Y)C^{\bullet}(OH)$ radicals decompose through the formation of a radical cation, and the presence of a proton at the oxygen atom in the α-position relative to the radical centre is not necessary. Meanwhile,[70,88] fragmentation of the $CH(Y)C^{\bullet}(OH)$ radicals occurs by a concerted mechanism, through a cyclic transition state, with simultaneous cleavage of two bonds that are vicinal relative to the radical centre. The radical fragmentation of glycerophospholipids containing a free HO group in the β-position to the (phospho)ester bond was explained in terms of the concerted mechanism of the reaction.[12,60–66] From this standpoint, the lipid molecule has to contain a mobile H atom in the functional group, which enables the cyclic transition state **A** and promotes fragmentation of radicals having an unpaired electron on the β-C atom relative to the (phospho)ester bond.



It is noteworthy that in neutral medium CL can form bicyclic structures stabilized by hydrogen bonds between the hydroxyl and phosphate groups in the polar part.[89] Cardiolipin containing an OH group has two acid dissociation constants: $pK_1 = 2.8$ and $pK_2 > 8$ (the initial value of 7.5 shifts to 9.5 during the titration). Meanwhile, CL devoid of the OH group has two close pK values ($pK_1 = 1.8$, $pK_2 = 4.0$).[89] This special feature of CL may facilitate its free-radical fragmentation by the concerted mechanism.

In a comparative study of the radiation-induced fragmentation of CL, PI, PG and phosphatidylpropane-1,2-diol (PP), the highest radiation chemical yield of phosphatidic acid (**56**) was observed for CL.[66] This was twice as high as the yield of the acid upon the radiolysis of PG or PP. The yield of the acid in the case of PI was similar to that of CL.

The hydrophilic part of cardiolipin contains two phosphoester bonds in the β-position relative to the OH group and, hence, the fragmentation of the resulting α-hydroxyl-containing carbon-centred radical **55**$^{\bullet}$ [unlike the transformations of PG (**52**$^{\bullet}$) or PP (**64**$^{\bullet}$) radicals] should result in the formation of PA with higher probability. The polar part of phosphatidylinositol contains the cyclic alcohol, inositol, residue, which has five free OH groups, two of them being located in the β-position to the phosphoester bond.



An increase in the yield of PA in irradiated PI liposomes compared with PG or PP is attributable to the fact that in the case of PI, there is a probability of formation of two types of radicals, namely, at C(2) (**51**$^{\bullet}$) and C(6) [(**51**′)$^{\bullet}$] in the alcohol residue; the radicals can be fragmented with cleavage of the phosphoester bond. As shown by EPR[71] and pulse radiolysis,[72] radicals of this type are mainly fragmented with elimination of phosphate.

The free-radical fragmentation results in not only destruction of glycerophospholipid molecules, which are involved in the regulation of signalling processes in biosystems, apart from playing a structure-forming role.[90,91] The fragmentation affords also phosphatidic acid, which is an important secondary messenger involved in the regulation of cell proliferation and differentiation and programmed cell death.[92] In addition, the radical intermediates formed during fragmentation can initiate further free-radical processes.

2. Effect of chemical factors on the free-radical fragmentation of phosphatidylglycerols

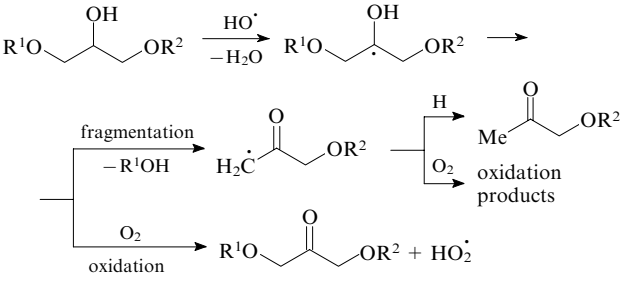
a. Calcium cations

The Ca^{2+} ions play an important role in the regulation of membrane processes.[93] Their effect on the free-radical fragmentation was studied in various model membranes, comprising phosphatidylglycerol.[62] The introduction of calcium cations into multilamellar or monolamellar PG liposomes or into monolamellar liposomes of an equimolar mixture of PG and PC leads to a 1.5–2-fold decrease in the radiation chemical yield of PG fragmentation products depending on the concentration of ions in the system.[62] Most likely, calcium cations do not change the liposome structure or size but interact with the lipid molecule. A distinctive feature of metal ions is the ability to form coordination bonds with oxygen atoms of not only negatively charged but also electrically neutral compounds.[93] The formation of a Ca^{2+} complex with the electronegative phosphate group of PG evidently results in charge neutralization and electron density redistribution in the cyclic transition state of α-hydroxyl-containing carbon-centred radical **A**, and, as a consequence, the concerted cleavage of two β-bonds becomes less favourable.

b. Molecular oxygen

Lipid peroxidation depends on the presence of molecular oxygen in the system. The amount of hydroperoxides resulting from the radiation-induced LPO in the phospholipid membrane decreases with a decrease in the oxygen concentration.[94] A change in the oxygen concentration also influences the free-radical fragmentation of glycerophospholipids but the pattern of this influence is different. Oxygen saturation of aqueous dispersions of 1,2-ditetradecanoyl-*sn*-glycero-3-phospho(1′-*sn*-glycerol) and 1′,3′-bis(1,2-ditetradecanoyl-*sn*-glycero-3-phospho)-*sn*-glycerol leads to a twofold decrease in the yield of products of the radiation-induced fragmentation of this lipids as compared with that in deaerated media.[63,77] α-Hydroxyl-containing carbon-centred radicals formed in the polar part of these lipids interact with oxygen, and this process competes with the fragmentation (Scheme 12). It was shown[88] that the oxidation of α-hydroxyalkyl radicals derived from α-diols, which competes with fragmentation, to the corresponding radicals of carbonyl compounds proceeds *via* the addition of O_2 molecule followed by radical destruction through a five-membered transition state.

Scheme 12



The oxygen saturation of aqueous suspensions of glycerophospholipids decreases the yield of the fragmentation products but does not suppress this process completely. This indicates that the destruction of the $R^1OCH_2C^{\bullet}(OH).CH_2OR^2$ radicals formed in the polar part with cleavage of the phosphoester bond occurs in aqueous solutions at a substantial rate even in the presence of oxygen.§

§ Carbon-centred radicals are known to react[94] with O_2 with a rate constant of $\sim 10^9$ litre mol^{-1} s^{-1}.

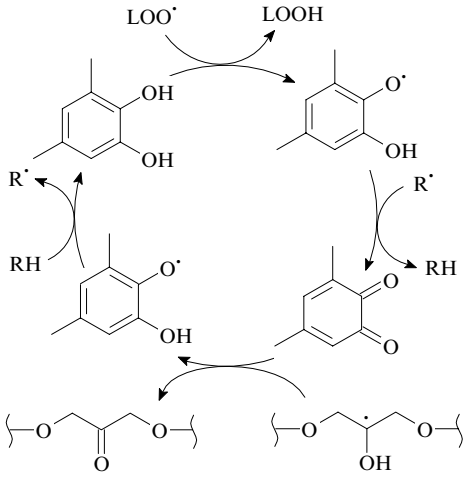
The inhibiting action of the molecular oxygen on the free-radical fragmentation is less pronounced for glycerophospholipids containing PUFA residues. Apparently, this is a consequence of the competition between the fragmentation and the peroxidation, which consumes oxygen.[63]

c. Diphenol and quinone derivatives

Studies of the effect of diphenol and quinone derivatives that mimic various structural moieties of natural antioxidants (tocopherols, flavonoids, ubiquinones, vitamin K) on the free-radical fragmentation of glycerophospholipids and compounds modelling them have demonstrated [95,96] that quinones are more effective inhibitors of the process than catechols. The yield of the products of the radiation-induced fragmentation of lipids decreased 1.8–2 fold in the presence of quinone derivatives.[95]

Carbonyl compounds are known to oxidize α-hydroxyalkyl radicals.[88] It was shown by pulse radiolysis that the $RC^{\bullet}HOH$ radical derived from some biologically important compounds (alcohols, deoxyriboses, thymine, cytosine, *etc.*) are oxidized with *p*-benzoquinone to give the corresponding carbonyl compound and the semiquinone radical with a high rate constant ($k_v \sim 10^9$ litre mol^{-1} s^{-1}).[97] The $R^1OCH_2C^{\bullet}(OH)CH_2OR^2$ radicals formed in the polar part of glycerophospholipids can be oxidized by quinones, this reaction competing with fragmentation accompanied by cleavage of two β-bonds.[95] Diphenol and aminophenol derivatives efficiently inhibit LPO by reducing $LOO^{\bullet}$ to hydroperoxides LOOH.[98–101] These facts matter for determining the ways of control of free-radical processes both in polar and in non-polar parts of the lipid bilayer. Diphenols and aminophenols can function as antioxidants and inhibit the LPO; they are thus oxidized to quinones or quinone-imines, which efficiently inhibit fragmentation in the polar part of lipids (Scheme 13).

Scheme 13

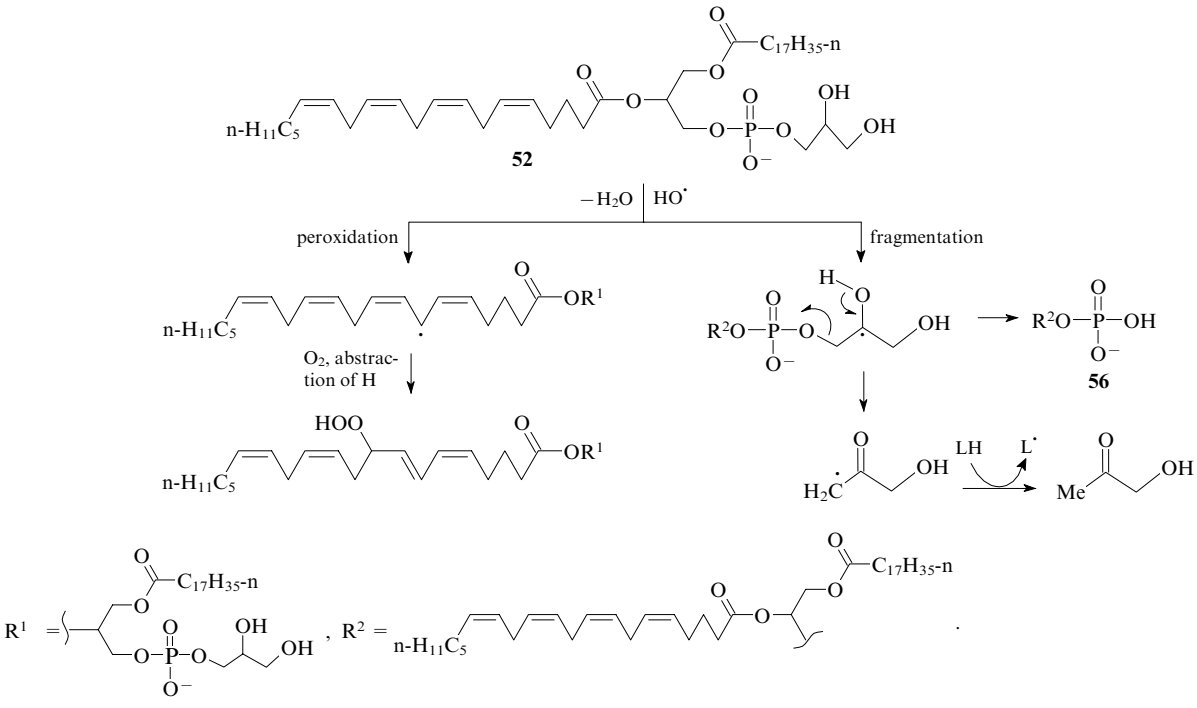


3. Oxidation and fragmentation processes in the model membrane incorporating hydroxyl-containing glycerophospholipids

On treatment of model membranes incorporating hydroxyl-containing phospholipids with ROS, peroxidation takes place in the hydrophobic layer and free-radical fragmentation occurs in the hydrophilic layer.[63,65,102] Phosphatidylglycerol (**52**), which contains PUFA residues, can undergo both the oxidation and the fragmentation (Scheme 14).[63,65]

Phosphatidic acid (**56**) thus formed has both saturated and unsaturated acyl residues; however, it remains obscure whether the oxidized PG is fragmented or PA is oxidized.[65] The amounts of LPO and fragmentation products formed upon γ-irradiation of hydroxyl-containing lipids are approximately equal.[63] In deaerated media, the concentration of the primary products of free-radical fragmentation is

Scheme 14



several times higher compared with that of the primary LPO products. In other words, as the O_2 concentration considerably decreases, fragmentation starts to predominate in the system. This circumstance may be significant for biosystems under hypoxic conditions.[63, 64]

The fragmentation taking place in the polar part of the lipid bilayer affects the peroxidation in the lipophilic layer of the model membrane upon γ-irradiation.[102–104] The oxidation of PG is accompanied by accumulation of 1.5–1.8 times larger amounts of products than the oxidation of PC for identical acyl compositions of the lipids.[63] The irradiation of PG liposomes in the presence of Ca^{2+} ions, which inhibit the fragmentation,[62] halves the amounts of both fragmentation and LPO products. The products formed in the free-radical fragmentation can change the properties of the lipid bilayer and thus influence the processes that occur in the hydrophobic part. The accumulation of the LPO products in the γ-irradiated PC liposomes containing phosphatidic acid, the molecular fragmentation product, is 1.7–2 times greater than that in liposomes of only PC.[63, 102, 105]

When various glycerophospholipids were introduced in PC liposomes, the more pronounced activating effect on the radiation-induced LPO was made by the phospholipids able to undergo the fragmentation, which is manifested as a significant (more than twofold) increase in the concentration of the oxidation products.[102, 103] It is known [106] that the hydroperoxides formed upon LPO activate phospholipase A_2, which acts on the membrane lipids to increase the amount of lysophospholipids. As has been discussed above, lysophospholipids undergo free-radical fragmentation, and this fragmentation occurring in the lipid bilayer may in turn affect the peroxidation.[104]

IV. Free-radical fragmentation of sphingolipids

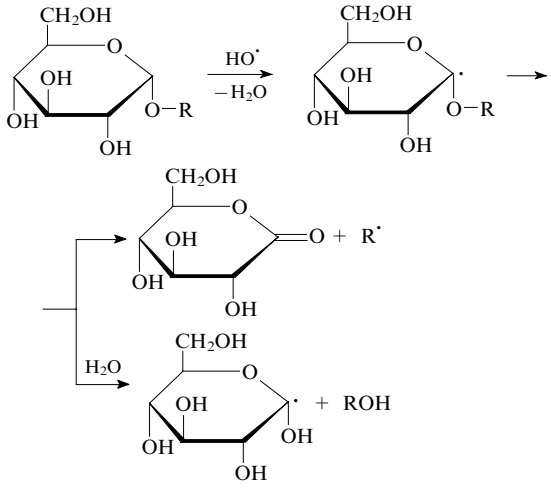
The sphingolipid family includes sphingomyelin, ceramides and glycosphingolipids (galactosyl- and glucosyl-ceramides, -sulfatides, -globosides and -gangliosides). In the polar part, sphingolipids contain OH groups in the β-position to the O-glycosidic or amide bond and participate in the free-radical fragmentation. It was shown [64, 68, 78, 107–109] that *N*-acyl-1-(β-galactopyranosyl)sphing-4-enynes (galactosylceramides, GalCer) and *N*-acyl-1-(β-glucosyl)sphing-4-enynes (glucosylceramides) as parts of mixed micelles are destroyed with O-glycosidic bond cleavage to give ceramides during Fe^{2+}-mediated formation of the $HO^{\bullet}$ radicals or on exposure to ionizing radiation. The radiation chemical yield of ceramides amounts to >70% of the decomposition yield of the initial GalCer and decreases as an $HO^{\bullet}$ acceptor is added to the micellar solution being irradiated. This attests to considerable contribution of hydroxyl radicals to the radiation-induced cleavage of the O-glycosidic bond in the starting glycolipids.[109]

According to von Sonntag,[110] the $HO^{\bullet}$ radicals react with carbohydrates with abstraction of the carbon-bonded hydrogen atom with the rate constant $k_v > 10^9$ litre $mol^{-1}\ s^{-1}$, the reactivity of the H atom being more than an order of magnitude lower and that of the hydrated electron being even lower ($k < 5 \times 10^6$ litre $mol^{-1}\ s^{-1}$). The reaction of $HO^{\bullet}$ with the carbohydrate moiety of glycosphingolipids may afford carbon-centred radicals of various type. The mechanism of cleavage of the O-glycosidic bond has been extensively investigated in the study of radiation-induced destruction of carbohydrates.[111–114] The cleavage of the O-glycosidic bond in carbohydrates involves the radicals that are formed upon abstraction of hydrogen from the C(1) and C(2) atoms of the glucopyranose ring.[111–114] The reactions of the radicals that have the unpaired electron at C(5) [C(5)-radicals] with pyranose ring opening can also cleave the O-glycosidic bond; however, the probability of these reactions is low.[111, 113] The C(1)-radicals can be either destroyed by a monomolecular mechanism through β-cleavage or hydrolyzed (Scheme 15).

The fragmentation of C(2)-radicals is accompanied by cleavage of two β-bonds (Scheme 16).[114]

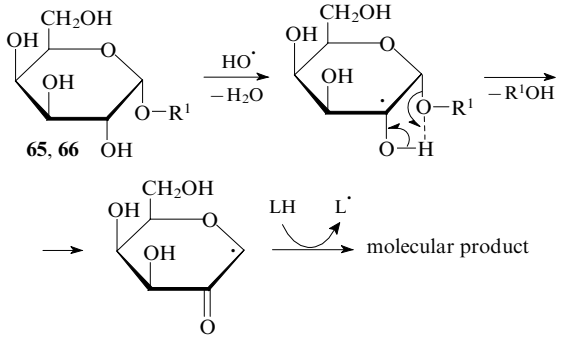
As noted above, the radiolysis of GalCer results in high radiation chemical yield of ceramide (~70% relative to the yield of $HO^{\bullet}$ radicals).[109] The results on the radiation-induced destruction of GalCer (**65**) (see Scheme 16) with cleavage of the O-glycosidic bond were interpreted taking into account the fact that a considerable contribution to this

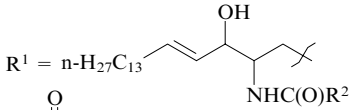
Scheme 15



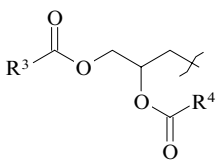
ROH is sphingolipid (ceramide).

Scheme 16





(**65**),



(**66**); R^2, R^3 and R^4 are hydrocarbon residues.

process is made by fragmentation of the C(2)-radicals.[109] As regards the C(1)-radicals, their contribution may be due to hydrolysis, while β-cleavage does not directly give ceramide.

Data on the radiation-induced destruction of carbohydrates attest to an important role of fragmentation of C(2)-radicals in the overall cleavage of the O-glycosidic bond.[112, 114] The destruction of glycosides sharply decreases (~5–10-fold) upon esterification; the replacement of the hydroxy group at C(2) by an ester group does not promote the formation of the necessary cyclic transition state of C(2)-radicals (see Scheme 16), which sharply decreases the probability of their destruction.[114] Note that the same trend is followed in the radiolysis of fully esterified glycerides, which are more stable against destruction with cleavage of the ester bonds than glycerol monoesters.[12, 61]

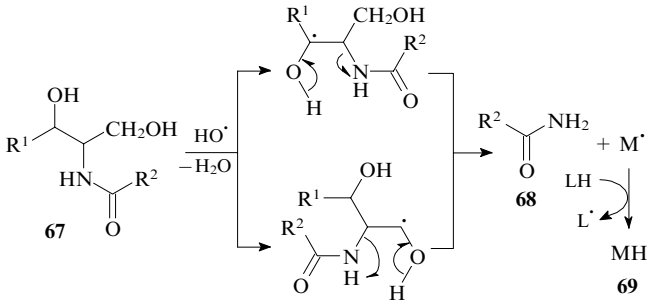
The destruction of GalCer (**65**) with cleavage of the O-glycosidic bond can also be induced by the action of the dopamine–Fe^{2+} system,[78, 108] which is able to generate the $HO^{\bullet}$ radicals.[115–117] Dopamine, a catecholamine neurotransmitter, can function as a neurotoxin and participate in triggering of neurodegenerative processes.[115–117] The transformation of GalCer to ceramide in the presence of dopamine can be one of the steps of the dopamine neurotoxicity mechanism.

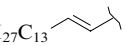
A study of free-radical transformations of 1,2-di-*O*-acyl-3-*O*-(β-D-galactopyranosyl)-*sn*-glycerol (**66**) demonstrated that this lipid also undergoes destruction with cleavage of the *O*-glycosidic bond in its polar part, and this gives rise to 1,2-diacyl-*sn*-glycerol (see Scheme 16).[64]

The ability of galactosphingolipids, which account for 20% of myelin lipids,[118] to decompose *via* free-radical fragmentation of their polar part can have serious consequences for functioning of biosystems. The deficiency of GalCer in myelin is not counterbalanced by other lipids.[119, 120] The free-radical fragmentation not only leads to destruction of galactosphingolipid but also gives rise to a new lipid, ceramide. In biosystems, ceramides regulate cell differentiation, proliferation and death by activating signalling cascades.[121–123] Reactive oxygen species, ionizing and ultraviolet radiation stimulate the production of ceramide, thus mediating the programmed cell death.[123–125]

Most of the relevant literature focuses on the enzymatic route of ceramide production, namely, the production from sphingomyelin (SM) induced by sphingomyelinases.[125] However, generally the mechanism of ceramide formation during the oxidative stress development is open to question. According to Grether-Beck *et al.*,[126] the formation of ceramide on exposure of SM liposomes to UV radiation occurs without participation of enzymes; the mechanism of sphingomyelinase activation by ionizing radiation is also obscure.[125] The ceramide accumulation in brain in early stages of Alzheimer's disease is accompanied by a decrease in the amount of galactosphingolipids (by 92%), whereas the level of sphingomyelin remains invariable, and the acyl composition of ceramide corresponds to galactolipids rather than to SM.[127, 128] Presumably, free-radical fragmentation of glycosphingolipids makes a contribution to the formation of ceramide in biosystems.

The routes of ROS-mediated formation of ceramides as signalling molecules are under extensive research; however, ceramides may be involved in free-radical reactions as substrates. It was established by MALDI MS that ROS sources (ionizing radiation or the $FeSO_4–H_2O_2$–ascorbate system) induce destruction of the ceramide (*E*)-*N*-acylsphing-4-enyne (**67**) with amide bond cleavage.[129] The key stage of the process is decomposition of α-hydroxyl-containing carbon-centred radicals with cleavage of two β-bonds. Upon free-radical fragmentation of the polar part, ceramide **67** is converted to give fatty acid amide (**68**) and a compound with the gross formula $C_{18}H_{34}O_2$ (**69**) containing carbonyl and hydroxyl groups. Depending on the fragmentation route, compound **69** may represent 1-hydroxyoctadec-4-en-3-one or 3-hydroxyoctadec-4-enal.[129]



R^1 = n-$H_{27}C_{13}$  , R^2 = n-C_nH_{2n+1},

M = $R^1C(O)–\dot{C}H–CH_2OH$ or $R^1CH(OH)–\dot{C}H–CHO$.

Ceramides account for more than 40% of the stratum corneum lipids, their level decreasing during skin diseases that appear as a result of the formation of free radicals in the skin.[130–132] The susceptibility of ceramides for destruction *via* free-radical fragmentation may be responsible for the damage of the epidermis integrity.

The destruction with amide bond cleavage *via* free-radical fragmentation is also typical of sphingomyelin,[12] which accounts for about 10% of brain lipids.

V. Conclusion

The research into free-radical transformations of glycero- and sphingolipids initiated by reactive oxygen and nitrogen species is being intensively developed. This is due to the biological role of these compounds and participation of ROS in the development of pathological processes. Homolytic reactions occur in both hydrophobic (peroxidation) and hydrophilic parts (free-radical fragmentation) of lipids. Peroxidation mainly affects the membrane glycerolipids that contain polyunsaturated fatty acid residues and results in modification of their hydrophobic parts. Glycero- and sphingolipids containing OH group in the β-position to the ester bond, O-glycosidic bond or amide bond undergo free-radical fragmentation. This process leads not only to the destruction of the lipid molecule accompanied by cleavage of the ester, O-glycosidic or amide bond but also to the formation of compounds functioning as secondary messengers in biosystems, in particular, phosphatidic acid, ceramides, diacylglycerols and fatty acid amides.

A basic difference was revealed for the free-radical fragmentation and LPO depending on the oxygen concentration in the system: in deaerated media, the concentration of fragmentation products increases several-fold compared to the concentrartion of the oxidation products. Quinone derivatives efficiently inhibit the fragmentation, and their reduced forms inhibit peroxidation. In model phospholipid membranes, fragmentation affects the peroxidation by changing its rate.

The peroxidation of lipids has been intensively studied for decades, and its mechanism as a whole and its role in biosystems are better understood than the role of free-radical fragmentation. Therefore, it is important to continue the investigation of free-radical transformations of glycero- and sphingolipids taking into account the fact that homolytic reactions can occur both in lipophilic and hydrophilic parts of their molecules. These works would favour more in-depth understanding of the role of lipids in the mechanisms of development of the oxidative stress in biosystems and the development of effective chemical pharmaceutical means for reducing its adverse consequences.

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References

1. R B Gennis *Biomembranes: Molecular Structure and Function (Springer Advanced Texts in Chemistry)* (New York: Springer, 1989)
2. C N Serhan *Prostaglandins Other Lipid Mediat.* **77** 4 (2005)
3. A D Postle *J. Lipid Sci. Technol.* **111** 2 (2009)
4. R Kohen, A Nyska *Toxicol. Pathol.* **30** 620 (2002)
5. A Yu Andreev, Yu E Kushnareva, A A Starkov *Biokhimiya* **70** 246 (2005) [a]
6. W A Pryor, K N Houk, C S Foote, J M Fukuto, L J Ignarro, G L Squadrito, K J A Davies *Am. J. Physiol., Regul. Integr. Comp. Physiol.* **291** R491 (2006)
7. W Dröge *Physiol. Rev.* **82** 47 (2002)
8. M Ott, V Gogvadze, S Orrenius, B Zhivotovsky *Apoptosis* **12** 913 (2007)
9. E Cadenas, K J A Davies *Free Radical Biol. Med.* **29** 222 (2000)
10. M Goetz, A Luch *Cancer Lett.* **266** 73 (2008)
11. M Valko, H Morris, M T D Cronin *Curr. Med. Chem.* **12** 1161 (2005)
12. M A Kisel, O I Shadyro, I L Yurkova *Khim. Vys. Energ.* **31** 99 (1997) [b]
13. G Spiteller *Exp. Gerontol.* **36** 1425 (2001)
14. G Spiteller *Free Radical Biol. Med.* **41** 362 (2006)
15. L J Roberts II, G L Milne *J. Lipid Res.* **50** (Suppl.) S219 (2009)
16. G O Fruhwirth, A Loidl, A Hermetter *Biochim. Biophys. Acta* **1772** 718 (2007)
17. I G Minko, I D Kozekov, T M Harris, C J Rizzo, R S Lloyd, M P Stone *Chem. Res. Toxicol.* **22** 759 (2009)
18. U Nair, H Bartsch, J Nair *Free Radical Biol. Med.* **43** 1109 (2007)
19. J A Berliner, N Leitinger, S Tsimikas *J. Lipid Res.* **50** (Suppl.) S207 (2009)
20. A Catala *Int. J. Biochem. Cell Biol.* **38** 1482 (2006)
21. A Catala *Chem. Phys. Lipids* **157** 1 (2009)
22. E Niki *Free Radical Biol. Med.* **47** 469 (2009)
23. H Yin, N A Porter *Antioxid. Redox Sign.* **7** 170 (2005)
24. S S Davies *Eur. J. Lipid Sci. Technol.* **111** 64 (2009)
25. W Liu, N A Porter, C Schneider, A R Brash, H Yin *Free Radical Biol. Med.* **50** 166 (2011)
26. F Gueraud, M Atalay, N Bresgen, A Cipak, P M Eckl, L Huc, I Jouanin, W Siems, K Uchida *Free Radical Res.* **44** (Spec. Issue) 1098 (2010)
27. H Rubbo, A Trostchansky, V B O'Donell *Arch. Biochem. Biophys.* **484** 167 (2009)
28. E A Podrez, E Poliakov, Z Shen, R Zhang, Y Deng, M Sun, P J Finton, L Shan, B Gugiu, P L Fox, H F Hoff, R G Salomon, S L Hazen *J. Biol. Chem.* **277** 38503 (2002)
29. P A Grimsrud, H Xie, T J Griffin, D A Bernlohr *J. Biol. Chem.* **283** 21837 (2008)
30. C B Sullivan, E Matafonova, L J Roberts II, V Amarnath, S S Davies *J. Lipid Res.* **51** 999 (2010)
31. G Stark *J. Membr. Biol.* **205** 1 (2005)
32. J Wong-ekkabut, Z Xu, W Triampo, I-M Tang, D P Tieleman, L Monticelli *Biophys. J.* **93** 4225 (2007)
33. M E Greenberg, X-M Li, B G Gugiu, X Gu, J Qin, R G Salomon, S L Hazen *J. Biol. Chem.* **283** 2385 (2008)
34. M Laguerre, J Lecomte, P Villeneuve *Prog. Lipid Res.* **46** 244 (2007)
35. M R M Domingues, A Reis, P Domingues *Chem. Phys. Lipids* **156** 1 (2008)
36. H Y Yin, B E Cox, W Liu, N A Porter, J D Morrow, G L Milne *J. Mass Spectrom.* **44** 672 (2009)
37. A Kuksis, J P Suomela, M Tarvainen, H Kallio *Mol. Biotechnol.* **42** 224 (2009)
38. R M Sargis, P V Subbaiah *Biochemistry* **42** 11533 (2003)
39. L Xu, T A Davis, N A Porter *J. Am. Chem. Sos.* **131** 13037 (2009)
40. D J W Barber, J K Thomas *Radiat. Res.* **74** 51 (1978)
41. M Gamliel, M Afri, A A Frimer *Free Radical Biol. Med.* **44** 1394 (2008)
42. E Schnitzer, I Pinchuk, D Lichtenberg *Eur. Biophys. J.* **36** 499 (2006)
43. I I Ivanov *Mol. Biol.* **18** 512 (1984) [c]
44. K Hasegawa, L K Patterson *Photochem. Photobiol.* **28** 817 (1978)
45. M Afri, H E Gottlieb, A A Frimer *Free Radical Biol. Med.* **32** 605 (2002)
46. S Shachan-Tov, M Afri, A A Frimer *Free Radical Biol. Med.* **49** 1516 (2010)
47. K A Tallman, C L Rector, N A Porter *J. Am. Chem. Soc.* **131** 5635 (2009)
48. Z Cheng, Y Li *Chem. Rev.* **107** 748 (2007)
49. C Schneider, N A Porter, A R Brash *J. Biol. Chem.* **283** 15539 (2008)
50. M Morita, M Tokita *Lipids* **43** 589 (2008)
51. B G Gugiu, K Mouillesseaux, V Duong, T Herzog, A Hekimian, L Koroniak, T M Vondriska, A D Watson *J. Lipid Res.* **49** 510 (2008)
52. K Uchida *J. Clin. Biochem. Nutr.* **36** 1 (2005)
53. Y Yang, R Sharma, A Sharma, S Awasthi, Y C Awasthi *Acta Biochem. Polon.* **50** 319 (2003)
54. I A Blair *J. Biol. Chem.* **283** 15545 (2008)
55. A C Nicolescu, J N Reynolds, L R C Barclay, G R J Thatcher *Chem. Res. Toxicol.* **17** 185 (2004)
56. P Pacher, J S Beckman, L Liaudet *Physiol. Rev.* **87** 315 (2007)
57. V L Lobachev, E S Rudakov *Russ. Chem. Rev.* **75** 375 (2006) [*Usp. Khim.* **75** 422 (2006)]
58. C Schneider, D A Pratt, N A Porter, A R Brash *Chem. Biol.* **14** 473 (2007)
59. A R Brash *J. Biol. Chem.* **274** 23679 (1999)
60. A A Akhrem, M A Kisel, O I Shadyro, I L Yurkova *Dokl. Akad. Nauk* **330** 716 (1993) [d]
61. I P Edimecheva, M A Kisel, O I Shadyro, A P Vlasov, I L Yurkova *Int. J. Radiat. Biol.* **71** 555 (1997)
62. O I Shadyro, I L Yurkova, M A Kisel *Khim. Vys. Energ.* **34** 120 (2000) [b]
63. O I Shadyro, I L Yurkova, M A Kisel *Int. J. Radiat. Biol.* **78** 211 (2002)

64. O Shadyro, I Yurkova, M Kisel, O Brede, J Arnhold *Free Radical Biol. Med.* **36** 1612 (2004)
65. I Yurkova, O Shadyro, M Kisel, O Brede, J Arnhold *Chem. Phys. Lipids* **132** 235 (2004)
66. I L Yurkova, M A Kisel, J Arnhold, O I Shadyro *Khim. Vys. Energ.* **40** 345 (2006) [b]
67. S Goldstein, D Meyerstein, G Czapski *Free Radical Biol. Med.* **15** 435 (1993)
68. I Yurkova, M Kisel, J Arnhold, O Shadyro *Chem. Phys. Lipids* **137** 29 (2005)
69. A A Akhrem, I P Edimecheva, M A Kisel, O I Shadyro, I L Yurkova *Dokl. NAN Belarusi* **39** 49 (1995)
70. S N Müller, R Batra, M Senn, B Giese, M A Kisel, O I Shadyro *J. Am. Chem. Soc.* **119** 2795 (1997)
71. A Samuni, P Neta *J. Phys. Chem.* **77** 2425 (1973)
72. M N Schuchmann, M L Scholes, H Zegota, C von Sonntag *Int. J. Radiat. Biol.* **68** 121 (1995)
73. M Schlame, D Rua, M L Greenberg *Prog. Lipid Res.* **39** 257 (2000)
74. J F Turrens *J. Physiol.* **552** 335 (2003)
75. M K Shigenaga, T M Hagen, B N Ames *Proc. Natl. Acad. Sci. USA* **91** 10771 (1994)
76. A J Chicco, G C Sparagna *Am. J. Physiol., Cell Physiol.* **292** C33 (2007)
77. O Shadyro, I Yurkova, M Kisel, O Brede, J Arnhold *Int. J. Radiat. Biol.* **80** 239 (2004)
78. I Yurkova, M Kisel, J Arnhold, O Shadyro *Cent. Eur. J. Chem.* **5** 970 (2007)
79. I L Yurkova, F Stuckert, M A Kisel, O I Shadyro, J Arnhold, D Huster *Arch. Biochem. Biophys.* **480** 17 (2008)
80. I L Yurkova, O I Shadyro, M A Kisel, J Arnhold, D Huster *Dokl. NAN Belarusi* **53** 63 (2009)
81. I L Yurkova, J Arnhold, G Fitzl, D Huster *Chem. Phys. Lipids* **164** 393 (2011)
82. D Huster, M J Finegold, C T Morgan, J L Burkhead, R Nixon, S M Vanderwerf, C T Gilliam, S Lutsenko *Am. J. Pathol.* **168** 423 (2006)
83. I Yurkova, D Huster, J Arnhold *Chem. Phys. Lipids* **158** 16 (2009)
84. A Atlante, P Calissano, A Bobba, A Azzariti, E Marra, S Passarella *J. Biol. Chem.* **275** 37159 (2000)
85. G Petrosillo, F M Ruggiero, M Pistolese, G Paradies *FEBS Lett.* **509** 435 (2001)
86. G Petrosillo, F M Ruggiero, G Paradies *FASEB J.* **17** 2202 (2003)
87. M J Davies, B G Gilbert, in *Advances in Detailed Reaction Mechanisms. Radical, Single Electron Transfers and Concerted Reactions* (Ed. J M Coxon) (Greenwich, CT: JAI Press, 1991) Vol. 1, p. 35
88. E P Petryaev, O I Shadyro *Radiatsionnaya Khimiya Bifunktsional'nykh Organicheskikh Soedinenii* (Radiation Chemistry of Bifunctional Organic Compounds) (Minsk: Belarusian State University, 1986)
89. M Kates, J-Y Syz, D Gosser, T H Haines *Lipids* **28** 877 (1993)
90. G Di Paolo, P De Camilli *Nature (London)* **443** 651 (2006)
91. W H Moolenaar *Ann. NY Acad. Sci.* **905** 1 (2000)
92. X Wang, S P Devaiah, W Zhang, R Welti *Prog. Lipid Res.* **45** 250 (2006)
93. D Papahadjopoulos, S Nir, N Düzgünes *J. Bioenerg. Biomembr.* **22** 157 (1990)
94. G Stark *Biochim. Biophys. Acta* **1071** 103 (1991)
95. O I Shadyro, G K Glushonok, T G Glushonok, I P Edimecheva, A G Moroz, A A Sosnovskaya, I L Yurkowa, G I Polozov *Free Radical Res.* **36** 859 (2002)
96. T V Pototskaya, O I Shadyro, I L Yurkova *Khim. Vys. Energ.* **38** 4 (2004) [b]
97. M Simic, E Hayon *Biochem. Biophys. Res. Commun.* **50** 364 (1973)
98. E J Lien, S Ren, H-H Bui, R Wang *Free Radical Biol. Med.* **26** 285 (1999)
99. V I Lodyato, I L Yurkova, V L Sorokin, O I Shadyro, V I Dolgopalets, M A Kisel *Bioorg. Med. Chem. Lett.* **13** 1179 (2003)
100. V I Lodyato, I L Yurkova, V L Sorokin, O I Shadyro, V I Dolgopalets, M A Kisel *Bioorg. Med. Chem. Lett.* **14** 4253 (2004)
101. E B Burlakova, N G Khrapova *Russ. Chem. Rev.* **54** 907 (1985) [*Usp. Khim.* **54** 1540 (1985)]
102. M A Kisel, O I Shadyro, I L Yurkova *Curr. Top. Biophys.* **24** 41 (2000)
103. V Yu Davydov, M A Kisel, O I Shadyro, I L Yurkova *Radiats. Biol. Radioekol.* **40** 382 (2000)
104. M A Kisel, O I Shadyro, I L Yurkova *Radiats. Biol. Radioekol.* **41** 20 (2001)
105. I L Yurkova, O I Shadyro, M A Kisel *Radiats. Biol. Radioekol.* **44** 142 (2004)
106. T Hashizume, H Yamaguchi, A Kawamoto, A Tamura, T Sato, T Fujii *Arch. Biochem. Biophys.* **289** 47 (1991)
107. I Yurkova, M Kisel, J Arnhold, O Shadyro *Chem. Phys. Lipids* **134** 41 (2005)
108. I L Yurkova, M A Kisel, J Arnhold, O I Shadyro *Dokl. NAN Belarusi* **51** 77 (2007)
109. I L Yurkova, J Arnhold *Khim. Vys. Energ.* **42** 303 (2008) [b]
110. C von Sonntag *Adv. Carbohydr. Chem. Biochem.* **37** 7 (1980)
111. N K Kochetkov, L I Kudryashov, M A Chlenov *Radiatsionnaya Khimiya Uglevodov* (Radiation Chemistry of Carbohydrates) (Moscow: Nauka, 1978)
112. B C Gilbert, D M King, C B Thomas *Carbohydr. Res.* **125** 217 (1984)
113. C von Sonntag, H-P Schuchmann, in *Radiation Chemistry — Present Status and Future Trends (Studies in Physical and Theoretical Chemistry)* (Eds C D Jonah, B S M Rao) (Amsterdam: Elsevier, 2001) Vol. 87, p. 481
114. I P Edimecheva, R M Kisel, O I Shadyro, K Kazem, H Murase, T Kagiya *J. Radiat. Res.* **46** 319 (2005)
115. D G Graham, S M Tiffany, W R Bell Jr, W F Gutknecht *Mol. Pharmacol.* **14** 644 (1978)
116. C Velez-Pardo, M J Del Rio, G Ebinger, G Vauquelin *Gen. Pharmacol. Vasc. Syst.* **31** 19 (1998)
117. A Hald, J Lotharius *Exp. Neurol.* **193** 279 (2005)
118. N Baumann, D Pham-Dinh *Physiol. Rev.* **81** 871 (2001)
119. K Tadano-Aritomi, K Hikita, H Fujimoto, K Suzuki, K Motegi, I Ishizuka *J. Lipid Res.* **41** 1237 (2000)
120. A Bosio, E Binczek, W Stoffel *Proc. Natl. Acad. Sci. USA* **93** 13280 (1996)
121. E V Dyatlovitskaya *Bioorg. Khim.* **26** 12 (2000) [e]
122. W J van Blitterswijk, A H van der Luit, R J Veldman, M Verheij, J Borst *Biochem. J.* **369** 199 (2003)
123. A Morales, H Lee, F M Goni, R Kolesnik, J C Fernandez-Checa *Apoptosis* **12** 923 (2007)
124. D Martin, M Salinas, N Fujita, T Tsuruo, A Cuadrado *J. Biol. Chem.* **277** 42943 (2002)

125. N Andrieu-Abadie, V Gouaze, R Salvayre, T Levade *Free Radical Biol. Med.* **31** 717 (2001)
126. S Grether-Beck, G Bonizzi, H Schmitt-Brenden, I Felsner, A Timmer, H Sies, J P Johnson, J Piette, J Krutmann *EMBO J.* **19** 5793 (2000)
127. X Han, D M Holtzman, D W McKeel Jr, J Kelley, J C Morris *J. Neurochem.* **82** 809 (2002)
128. X Han *Curr. Alzheimer Res.* **2** 65 (2005)
129. I L Yurkova, J Arnhold *Khim. Vys. Energ.* **43** 320 (2009) [b]
130. G Vielhaber, S Pfeiffer, L Brade, B Lindner, T Goldmann, E Vollmer, U Hintze, K-P Wittern, R Wepf *J. Invest. Dermatol.* **117** 1126 (2001)
131. I B Afanas'ev *Curr. Drug Metab.* **11** 409 (2010)
132. D R Bickers, M Athar *J. Invest. Dermatol.* **126** 2565 (2006)

[a] — *Biochemistry (Moscow) (Engl. Transl.)*
[b] — *High Energ. Chem. (Engl. Transl.)*
[c] — *Mol. Biol. (Engl. Transl.)*
[d] — *Dokl. Chem. (Engl. Transl.)*
[e] — *Russ. J. Bioorg. Chem. (Engl. Transl.)*