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RADIATION CHEMISTRY

Effect of Histidine-Containing Dipeptides on the Free-Radical Fragmentation of Biologically Active Phospho Derivatives of Glycerol

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Abstract—The effects of carnosine, anserine, and glycyl-histidine on the free-radical fragmentation of glycero-1-phosphate and dimiristoyl phosphatidylglycerol induced by γ-radiation or Fe^{2+}(Cu^{2+})-containing systems have been studied. The histidine-containing dipeptides (HCDs) exert a radioprotector effect on the fragmentation; this effect depends on the concentration of O_2 and reaches a maximum in deaerated systems (by a factor of ~1.7). The HCDs can be anti- or prooxidants under the conditions of the Fe^{2+}(Cu^{2+})-mediated generation of HO• radicals. Carnosine exhibits protector properties in a dose-dependent manner regardless of the inductor. Carnosine more effectively inhibits Cu^{2+}-induced fragmentation (by a factor of 2–2.5) than the Fe^{2+}-mediated process (by a factor of 1.4).

Keywords: glycerophospholipids, free-radical fragmentation, dipeptides, carnosine

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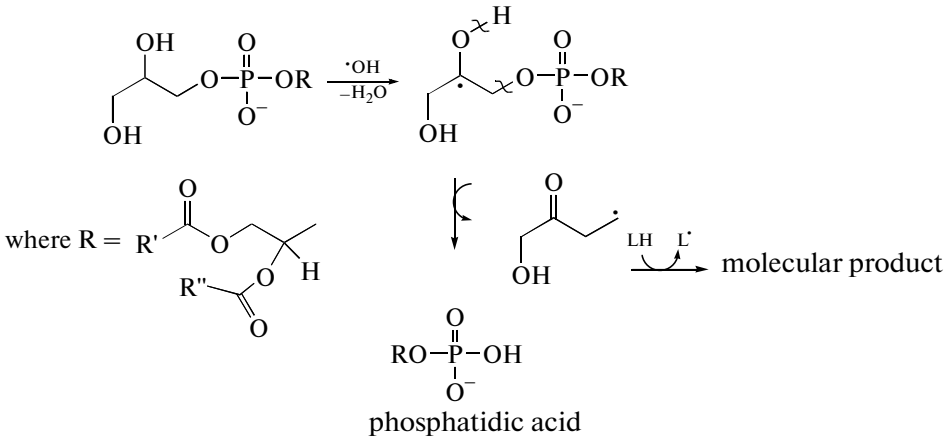
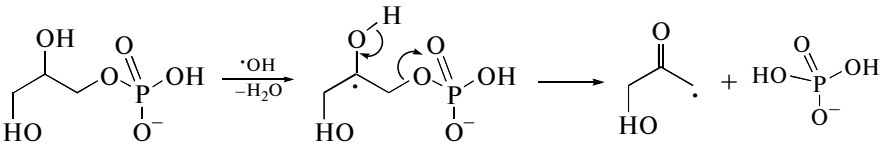
It is well known that a number of endogenous proteins and peptides, including those containing histidine, in biosystems are involved in the development and regulation of oxidative stress caused by both internal (autooxidation and electron transfer in mitochondria and microsomes) and external (ionizing and UV radiation, ultrasound, xenobiotics, etc.) factors [1, 2]. Only the reactions of the peroxide oxidation of lipids (POL) are considered in the studies of the effect of histidine-containing dipeptides (HCDs), in particular, carnosine and its analogs, on the free-radical transformations of membrane glycerophospholipids [2, 3]. The POL occurs in the hydrophobic part of a bilayer membrane by a free-radical mechanism through the stage of the formation of the peroxyl radicals of lipids [1]. However, α-hydroxyl-containing carbon-centered radicals with an unpaired electron in the β-position to the phosphoester bond are formed upon the interaction of HO• radicals with the hydrophilic fragment of glycerophospholipids phosphatidylglycerol, cardiolipin, phosphatidyl-inositol, and lysophospholipids) [4]. These radicals undergo fragmentation with phosphoester bond cleavage to result in the degradation of a lipid molecule with the formation of phosphatidic acid, a secondary messenger in biosystems, as shown in the reaction scheme below with the use of phosphatidylglycerol as an example:



Free-radical fragmentation affects the rate of the development of POL process in the lipophilic layer of a phospholipid membrane [4, 5]. The influence of hydrophilic HCDs on the occurrence of homolytic processes in the polar part of glycerophospholipids is not understood.

The aim of this work was to study the effects of histidine-containing dipeptides on the fragmentation of dimiristoyl phosphatidylglycerol and glycero-1-phosphate induced by γ-radiation or the $Fe^{2+}(Cu^{2+})$–H_2O_2–ascorbate systems. Glycero-1-phosphate (GP) is a structural fragment of glycerophospholipids, and it can be used as a model substance. Furthermore, GP is an important cell component, which participates not only in the synthesis of lipids but also in some metabolic processes.

In a study of the radiation-initiated transformations of GP, it was found [6, 7] that the cleavage of a phosphoester bond in its molecule predominantly occurs due to the fragmentation reaction of the primary radicals $H_2C(OH)–C^{\cdot}(OH)–H_2C–OP(O)O_2H^-$):



In this work, we studied the following HCDs: carnosine, anserine, and glycyl-histidine. Carnosine (β-alanyl-L-histidine) occurs in large amounts in human skeletal muscles (2–20 mM) and the olfactory bulbs of the cerebrum (0,3–5 mM) [2]. Anserine (β-alanyl-1-methyl-L-histidine) was found in the muscles of vertebrates (for example, to 40 mM in the muscles of birds [2]). Glycyl-histidine is a metabolite in the body.

It seems important to study the free-radical processes of biomolecules under conditions similar to those under which active particles are formed in biosystems. Therefore, in this work, we used both physical (ionizing radiation) and chemical (redox systems) initiators of free-radical processes.

EXPERIMENTAL

Glycero-1-phosphate disodium salt, dimiristoyl phosphatidic acid (DMPA), dimiristoyl phosphatidylglycerol (DMPG), β-alanine, L-histidine, L-carnosine, L-anserine, and glycyl-L-histidine from Sigma-Aldrich (Germany) were used in this study.

The solutions of GP with or without additives in hermetically sealed ampoules were irradiated on an MRKh-γ-25M unit with a ^{60}Co radiation source. The dose rate of this unit was (0.22 ± 0.01) Gy/s. For the preparation of deaerated samples or samples saturated with oxygen, the aqueous solutions of GP were placed in glass ampoules and blown with argon (99.9 %) for 60 min or with oxygen (99.9 %) for 30 min; then, the ampoules were sealed.

The chemical initiation of free-radical processes was performed with the aid of the $Fe^{2+}(Cu^{2+})$–H_2O_2–ascorbate systems. To the solutions of GP, $FeSO_4 \cdot 7H_2O$ or $CuSO_4 \cdot 5H_2O$, H_2O_2, and ascorbic acid were added; the final concentrations of the additives are specified in the figure captions. Then, the samples were thoroughly stirred and thermostatically controlled at a temperature of 37°C. The test substances were introduced into the substrate solution before the addition of redox system components.

The free-radical fragmentation of GP was evaluated from the accumulation of inorganic phosphate in the solutions. The phosphate anion was determined by photocolorimetry in accordance with a modified published procedure [9]. The fragmentation of DMPG in liposomes was evaluated based on the accumulation of DMPA. The preparation of multilamellar liposomes and the HPTLC analysis of lipids were carried out in accordance with previously published procedures [5]. The concentration of phospholipids after their HPTLC separation was determined based on the inorganic phosphorus content of the molecules as described previously [5].

RESULTS AND DISCUSSION

Under the action of γ-radiation on the deaerated aqueous solutions of GP, the phosphate anion is formed with a radiation-chemical yield of 3.58 ± 0.30 molecule/100 eV. The introduction of histidine-containing dipeptides in a concentration of 0.01 mol/L into a solution of GP decreased the yield of $H_2PO_4^-$ to a value 2.25 ± 0.25, 2.01 ± 0.15, or 1.91 ± 0.21 for Gly-His, Car, or Ans, respectively (Fig. 1). Carnosine inhibited the radiation-initiated fragmentation of GP in a concentration-dependent manner. With increasing the concentration of a dipeptide, its radioprotector effect increased; this was accompanied by a decrease in the radiation-chemical yield of the phosphate anion in the system according to the data given in the table. The chosen concentrations of HCDs were determined by their concentrations in biosystems.

Figure 2 shows the dependences of the accumulation of the phosphate anion on absorbed dose in the γ-radiolysis of the aqueous solutions of GP saturated

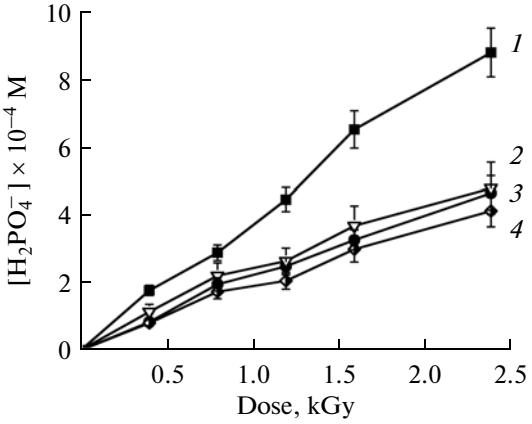


Fig. 1. Dose dependence of the buildup of $H_2PO_4^-$ on the γ-irradiation of a deaerated 100 mM aqueous solution of GP: (*1*) without additives and in the presence of (*2*) 10 mM Gly-His, (*3*) 10 mM Car, and (*4*) 10 mM Ans.

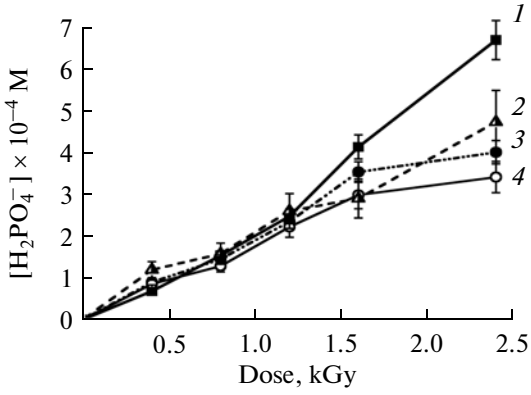


Fig. 2. Dose dependence of the buildup of $H_2PO_4^-$ during the γ-irradiation of a 100 mM aqueous solution of GP saturated with oxygen: (*1*) without additives and in the presence of (*2*) 10 mM Gly-His, (*3*) 10 mM Car, and (*4*) 10 mM Ans.

with oxygen. The radiation-chemical yield of $H_2PO_4^-$ in the system without additives was 1.94 ± 0.14 molecule/100 eV, and it was 1.88 ± 0.14, 1.78 ± 0.20, or 1.92 ± 0.16 in the presence of Car, Ans, and Gly-His, respectively. The phosphate anion was accumulated in approximately equal quantities in reference samples and samples with HCD additives in a dose range of (0–1.25) kGy. However, starting with a dose of 1.25 kGy, when the concentration of oxygen in the systems decreased as a result of the occurrence of different radiolytic processes, the level of $H_2PO_4^-$ decreased in the presence of dipeptides.

According to the experimental results, HCDs exert a protective action on the radiation-initiated fragmentation of GP in the deaerated solutions. In the presence of HCDs, the radiation-chemical yield of the phosphate anion, a molecular product of fragmentation, decreased, on the average, by a factor of ~1.7.

Hydroxyl radicals make the main contribution to the initiation of the fragmentation of GP and hydroxyl-containing lipids [5–8]. The radioprotective action of HCDs on the fragmentation of GP can be explained by their ability to effectively react with active particles and, thus, to prevent their interaction with target molecules. All of the HCDs effectively react with the radicals $HO^\bullet$, but they cannot be the effective scavengers of H_2O_2 and $O_2^{\bullet-}$, particles because they weakly interact with them [2, 10]. The rate constants of the reactions of Ans and Car with the radicals $HO^\bullet$ are 5.2×10^9 and 4.0×10^9 $M^{-1} s^{-1}$, respectively [10]. Gly-His also effectively scavenges $HO^\bullet$ particles, and its activity in this reaction is higher than that of Car [11]. The histidine residue in HCD molecules facilitates the formation of the radical intermediates of dipeptides with the radicals $HO^\bullet$; in this case, either a radical with the radical center on an imidazole ring is formed or the histidine residue stabilizes a radical with the radical center in the side chain [11, 12].

In the oxygen-containing systems, the radiation-chemical yields of the phosphate anion in the presence of Car, Ans, and Gly-His were similar and not substantially different from that in a reference sample. In an excess of oxygen, radiolytic processes that are more complex occurred in the systems. The primary carbon-centered radicals $H_2C(OH)–C^\bullet(OH)–H_2C–OP(O)O_2H^-$) interact with O_2 molecules with the formation of peroxyl radicals (the rate constant of the interaction of hydroxyalkyl radicals with O_2 is about 10^9 $M^{-1} s^{-1}$ [1]). The oxidation of primary GP radicals is a competing process with respect to their fragmentation reaction with the cleavage of two β-bonds. This leads to the fact that the yield of the phosphate anion upon the γ-irradiation of GP in the oxygen-containing systems without additives decreased, as compared with that in the deaerated solutions. At the initial stages of irradiation in the oxygen-containing systems, both HCDs at the stage of process initiation and molecular oxygen at the stage of process development can make a contribution to the inhibition of GP fragmentation.

Radiation-chemical yield of $H_2PO_4^-$ upon the γ-irradiation of a deaerated 100 mM aqueous solution of GP in the presence of carnosine

c(carnosine), mM	G ($H_2PO_4^-$), molecule/100 eV
0	3.57 ± 0.24
0.5	3.18 ± 0.20
1	2.75 ± 0.11
5	2.39 ± 0.10
10	1.97 ± 0.07
20	1.91 ± 0.10

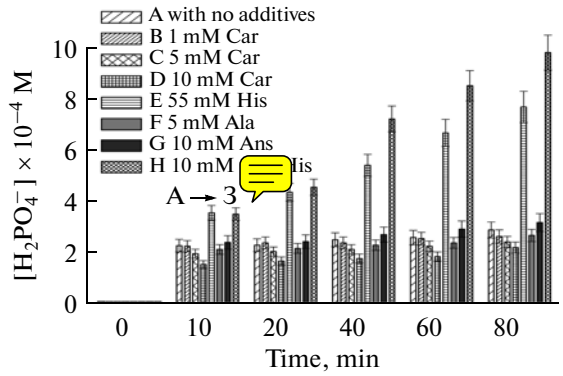


Fig. 3. Effects of carnosine, anserine, glycyl-histidine, histidine, and β-alanine on the dephosphorylation of GP in a 100 mM aqueous solution incubated with Fe^{2+}–H_2O_2–ascorbate (0.5/10/1 mM) at 37°C.

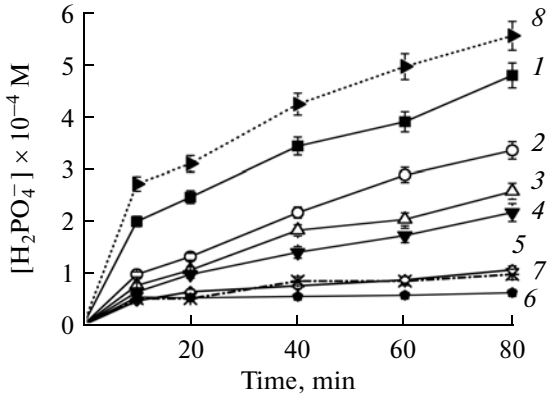


Fig. 4. Accumulation of $H_2PO_4^-$ in a 100 mM [illegible]ous solution of GP, incubated at 37°C with $CuSO_4$–H_2O_2–ascorbate (0.5/10/0.5 mM): (*1*) without additives and in the presence of (*2*) 1 mM Car, (*3*) 5 mM Car, (*4*) 10 mM Car, (*5*) 10 mM Ans, (*6*) 10 mM Gly-His, (*7*) 5 mM His, and (*8*) 5 mM Ala.

Consequently, upon the "burning" of O_2 in the systems, the protective action of HCDs manifested itself to a larger degree.

It is universally recognized that transition metal ions in biosystems catalyze hydroperoxide decomposition to give hydroxyl radicals in the Fenton or Haber–Weiss reaction [1]. In a study of the effects of HCDs on the fragmentation of GP under the conditions of the Fe^{2+}(Cu^{2+})-mediated generation of HO$^\bullet$ radicals, it was found that the dipeptides can exert both anti- and prooxidant effects.

The HCDs influence the Fe^{2+}-mediated fragmentation of GP in different ways (Fig. 3). Car exerts an inhibiting effect as its amount in the system is increased; the level of the phosphate anion decreased by a factor of 1.4 at a dipeptide concentration of 10 mM. Gly-His (10 mM) increased the destruction of GP; in the presence of it, the concentration of $H_2PO_4^-$ increased by a factor of 3. Ans (10 mM) did not exert a substantial effect on the dephosphorylation of GP.

All of the HCDs are good scavengers of HO$^\bullet$ radicals, and difference in their action on the Fe^{2+}-mediated fragmentation can be explained by the interaction of dipeptides with iron ions. On the one hand, HCDs in a complex with the Fe^{2+} ions can strengthen (a prooxidant effect) or weaken (an antioxidant effect) the catalytic activity of the metal. On the other hand, HCDs can reduce Fe^{3+} ions to facilitate their return to the catalytic cycle. In general, the complexes of Car and its analogs with iron(II) and iron(III) ions are still not clearly understood, and data on the possibility of their formation are contradictory [13]. According to published data [10], Car does not chelate Fe^{2+} ions to decrease their prooxidant activity. It is likely that the mechanism of the action of HCDs on Fe^{2+}-mediated fragmentation is caused by a balance of their radical-acceptor and complex-forming properties, which calls for further investigation.

The results obtained in the work on the influence of free amino acids on the Fe^{2+}-mediated fragmentation of GP indicate an important role of the composition and the presence of a peptide bond in the antioxidant action of HCDs. β-Alanine exerts a weaker protective effect on the process, as compared with that of Car; histidine considerably accelerates the fragmentation of GP (Fig. 3). It is most likely that the histidine residue is responsible for the prooxidant properties of Gly-His.

In the case of the Cu^{2+}-mediated fragmentation of GP, the protective action of all of the test HCDs was established (Fig. 4). In this case, the antioxidant properties of HCDs can be caused by both their interaction with the radicals HO$^\bullet$ [40] and the chelation of Cu^{2+} ions [40, 46]; this, probably, decreases the catalytic activity of copper in reactions with hydroperoxide. The difference in the action of HCDs can be caused by the structure and properties of their complexes with the Cu^{2+} ions.

Regarding the effect of free amino acids on the Cu^{2+}-mediated fragmentation of GP, β-alanine activates this process, whereas histidine exerts a significant protective effect. In the case of the Cu^{2+}-mediated process, it is likely that the protector properties of HCDs mainly depend on the histidine residue.

A comparison between data on the effect of carnosine on the metal-mediated fragmentation of GP suggests that the dipeptide was more effective in the case of the Cu^{2+}-induced process; the level of fragmentation products decreased by a factor of 2.5, whereas it decreased by a factor of only 1.4 in the case of Fe^{2+} ions. The antioxidant action of the dipeptide on the Cu^{2+}-mediated fragmentation of GP manifested itself in entire test range of concentrations (1–10 mM) or starting with a concentration of 5 mM in the case of induction by Fe^{2+}-containing systems. The experi-

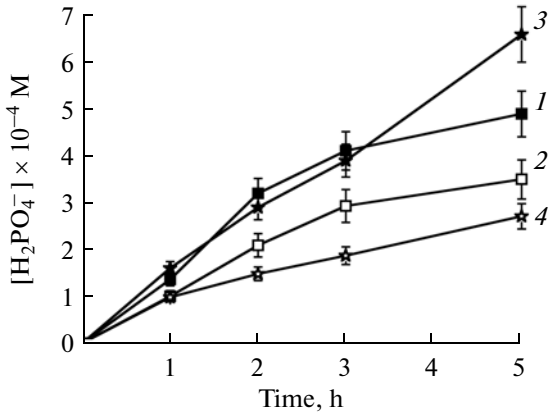


Fig. 5. Accumulation of DMPA in DMPG liposomes (20 mM) incubated at 37°C with (curves *1*, *2*) Fe^{2+}–H_2O_2 (1/10 mM) or (curves *3*, *4*) $CuSO_4$–H_2Ol_2–ascorbate (0.5/10/0.5 mM): (*1, 3*) without additives and (*2, 4*) in the presence of 20 mM Car.

mental results are consistent with published data [16], in accordance with which Car inhibited the copper-catalyzed oxidation of ascorbic acid, but it was ineffective in the case of the Fe^{2+}-mediated process.

The action of Car on the fragmentation of DMPG in model membranes also depends on the conditions of the generation of $HO^{\bullet}$ radicals in the systems. In phospholipid membranes, the dipeptide decreased the level of DMPA, a molecular product of fragmentation, by a factor of ~1.4 in the case of the Fe^{2+}-induced fragmentation or by a factor of >2 in the Cu^{2+}-mediated process (Fig. 5).

The membrane-protective action of Car was related to its ability to regulate POL in the nonpolar part of a membrane [2]; its hydrophobic analogs were more effective than the hydrophilic dipeptide itself [17]. The experimental results indicate that Car can also control processes mediated by reactive oxygen species in the hydrophilic layer of a membrane.

CONCLUSIONS

The free-radical fragmentation of the biologically active phospho derivatives of glycerol (glycerophosphates and phosphatidylglycerols) leads to the destruction of molecules with the cleavage of phosphoester bonds. The occurrence of this process in the polar part of a biomembrane will lead to a change in its properties and functions. In a study of the role of water-soluble peptides in the regulation of oxidative stress, attention is focused on peroxide oxidation in the hydrophobic layer of a membrane. In this work, we established that the histidine-containing dipeptides exert an anti- or prooxidant effect on the free-radical fragmentation of the phospho derivatives of glycerol depending on the method of process initiation. Carnosine exhibits protector properties regardless of the form of an inductor. Carnosine possesses membrane-protective action in vitro to decrease the degree of the free-radical destruction of phosphatidylglycerol in the polar part of a membrane. The experimental results make it possible to understand the mechanisms of the antioxidant action of bioactive peptides and to develop effective remedies for the protection of biosystems from the action of disturbing factors.

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