ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 4, pp. 470-478. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © I. N. Shtarkman, S. V. Gudkov, A. V. Chernikov, V. I. Bruskov, 2008, published in Biokhimiya, 2008, Vol. 73, No. 4, pp. 576-586.

Effect of Amino Acids on X-Ray-Induced Hydrogen Peroxide and Hydroxyl Radical Formation in Water and 8-Oxoguanine in DNA

I. N. Shtarkman[1,2], S. V. Gudkov[1], A. V. Chernikov[1], and V. I. Bruskov[1,2]*

[1]*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia; fax: (496) 733-0553; E-mail: bruskov_vi@rambler.ru*
[2]*Pushchino State University, 142290 Pushchino, Moscow Region, Russia*

Received July 3, 2007
Revision received November 29, 2007

Abstract—Generation of hydrogen peroxide and hydroxyl radicals in L-amino acid solutions in phosphate buffer, pH 7.4, under X-ray irradiation was determined by enhanced chemiluminescence in the luminol–*p*-iodophenol–peroxidase system and using the fluorescent probe coumarin-3-carboxylic acid, respectively. Amino acids are divided into three groups according to their effect on the hydrogen peroxide formation under irradiation: those decreasing yield of H_2O_2, having no effect, and increasing its yield. All studied amino acids at 1 mM concentration decrease the yield of hydroxyl radicals in solution under X-ray irradiation. However, the highest effect is observed in the order: Cys > His > Phe = Met = Trp > Tyr. At Cys, Tyr, and His concentrations close to physiological, the yield of hydroxyl radicals decreases significantly. Immunoenzyme analysis using monoclonal antibodies to 8-oxoguanine (8-oxo-7,8-dihydroguanine) was applied to study the effect of amino acids with the most pronounced antioxidant properties (Cys, Met, Tyr, Trp, Phe, His, Lys, Arg, Pro) on 8-oxoguanine formation *in vitro* under X-ray irradiation. It is shown that amino acids decrease the content of 8-oxoguanine in DNA. These amino acids within DNA-binding proteins may protect intracellular DNA against oxidative damage caused by formation of reactive oxygen species in conditions of moderate oxidative stress.

DOI: 10.1134/S0006297908040135

Key words: amino acids, X-ray irradiation, hydrogen peroxide, hydroxyl radicals, antioxidants, 8-oxoguanine in DNA

Reactive oxygen species (ROS) are formed both during normal aerobic cell metabolism and in response to environmental factors like ionizing and UV irradiation, xenobiotics, etc. [1, 2]. It has been shown recently that heating results in ROS formation in aqueous solutions [3-6]. Thus, various physical effects cause transformation of aqueous solutions into a chemically reactive medium. Biological molecules, in turn, are sensors of the processes taking place in the aquatic environment. According to present day concepts, ROS formed inside cells and in intercellular space play an ambiguous role. On one hand, they cause damage to biological structures like DNA, proteins, lipids, etc. [1, 2] during oxidative stress. On the other hand, ROS present in certain physiological concentrations carry out an important signal function, showing that this aquatic environment is suitable for maintenance of vital activity and redox-regulation of different cell functions [7].

There are data showing that in the case of the most reactive ROS, hydroxyl radicals ($OH^{\bullet}$), proteins are the main target, more sensitive than DNA and lipids [8]. It was shown by EPR spectroscopy that in protein solutions and in cells ionizing radiation stimulates formation of long-lived protein radicals (LLPR) [9-20] with half-life times up to 20 [9] and more hours [19]. LLPR are sources of ROS generation in the aquatic environment and maintain conditions suitable for continuous oxidative stress in biological systems [14]. It was also found that LLPR serve as intermediates in transfer of oxidative damage to different cell components [20] including DNA [17, 18].

All living organisms have systems of antioxidant cell protection against excessive ROS content; these systems

Abbreviations: ABTS) 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); CCA) coumarin-3-carboxylic acid; DRF) dose reduction factor; DVF) dose variation factor; EPR) electron paramagnetic resonance; LLPR) long-lived protein radicals; 7-OH-CCA) 7-hydroxy-coumarin-3-carboxylic acid; ROS) reactive oxygen species.

* To whom correspondence should be addressed.

include specialized enzymes and low molecular weight antioxidants. The ability of most natural low molecular weight antioxidants to decrease ROS content is caused by the presence in their structure of readily oxidized groups [21]. Antioxidant properties of widespread biological compounds have not been sufficiently studied. Recently, it has been found that among natural ribonucleosides, guanosine and inosine exhibit clearly defined antioxidant and X-ray-protective properties [22, 23].

Peptides play an important role in antioxidant protection against oxidative environmental factors. They are involved in maintenance of redox homeostasis as one of the factors necessary for functioning of biological systems. Such peptides include glutathione [24], carnosine [25], cytomedines and their small fragments [26], etc.

L-Amino acids comprise one of the most important classes of low molecular weight organic compounds. They are the main structural components of all natural peptides and proteins. However, their antioxidant properties and the role as components of peptides and proteins in physicochemical processes accompanying oxidative stress and its consequences are insufficiently studied.

It was found in some works on model systems that amino acids are able to reduce damaging effects caused by various oxidative entities at different levels of biological organization. Supposedly, these effects are due to the physicochemical properties of individual amino acids providing for their interaction with ROS. For example, L-citrulline and L-arginine appeared to be able to eliminate superoxide anion radical, thus normalizing the work of myocardium on exposure to oxidizing factors [27, 28]. Proline was shown to be an efficient quencher of singlet oxygen and to prevent cell death under oxidative stress [29]. The ability of histidine to intercept peroxyl radicals was revealed along with its involvement in prevention of protein carboxylation and cross linking [30]. A number of amino acids prevent 8-oxoguanine formation in DNA by protection of guanine against one-electron oxidation to the guanine cation radical [31].

The goals of this work were as follows. First, to study antioxidant properties of amino acids under conditions close to physiological in order to evaluate their possible role in intracellular redox processes. Second, to determine the ability of individual amino acids to serve as scavengers of hydroxyl radicals formed under X-ray irradiation in order to reveal the most vulnerable amino acid residues in proteins. Third, to study the possibility of using amino acids with the most pronounced antioxidant properties for DNA protection against oxidative damage.

MATERIALS AND METHODS

Materials. L-Amino acids Arg, Cys, Lys, Phe, Ser, Trp, Tyr, Val (Ajinomoto, Japan), Glu, Gln (Panreac, Spain), His, Asn (Matrix, Germany), Pro (Fluka, Germany), Gly, Ala, Hyp (hydroxyproline), Thr (Reanal, Hungary), and Met (Sigma, USA) were used without additional purification. Highly polymerized DNA from salmon sperm (ICN, USA) was used for immunoenzyme analysis. The following reagents were used for immunoenzyme analysis: anti-mouse IgG conjugate with horseradish peroxidase, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Tris, and casein (Sigma); *p*-iodophenol, coumarin-3-carboxylic acid, and 7-OH-coumarin-3-carboxylic acid (Aldrich, USA); citric acid and sodium citrate (Reakhim, Russia). All solutions were prepared in bidistilled water with specific conductance of 200 μS/m. $Na_2HPO_4{\cdot}7H_2O$, $NaH_2PO_4{\cdot}H_2O$ (Amresco, USA), sodium chloride, luminol (AppliChem, Germany), Triton X-100 (Koch-Light, Great Britain), and H_2O_2 (Khimmed, Russia) were used. Monoclonal antibodies to 8-oxoguanine (8-oxo-2′-deoxyguanosine) were obtained as described previously [32].

Irradiation. Solutions were irradiated at room temperature using an RUT-15 X-ray apparatus (Mosrentgen, Russia) with doses from 1 to 10 Gy at the dose rate 1 Gy/min (focal length 37.5 cm) and 4.5 Gy/min (focal length 19.5 cm).

Determination of hydrogen peroxide. The highly sensitive method of enhanced chemiluminescence in the luminol–*p*-iodophenol–horseradish peroxidase system was used [3, 6]. Amino acids at concentration of 1 mM in 5 mM phosphate buffer, pH 7.4, were irradiated in Eppendorf tubes. The chemiluminescence intensity was measured by a Beta-1 liquid scintillation counter (Medapparatura, Ukraine) under single photon counting conditions. The H_2O_2 solutions of known concentration were used for calibration of the measurement [3, 6].

Determination of hydroxyl radicals. Hydroxyl radicals were determined using a highly specific fluorescent probe coumarin-3-carboxylic acid (CCA). Experimental conditions have been described in detail elsewhere [5]. Amino acid solutions (1 mM) in 5 mM phosphate buffer, pH 7.4, were irradiated in glass or polypropylene flasks for liquid scintillation counter (Beckman, USA). The probe fluorescence intensity was measured on a SFM 25A spectrofluorometer (Kontron Instruments, Italy) at λ_{ex} = 400 nm and λ_{em} = 450 nm in a mirror quartz cell at room temperature. Solutions of an authentic preparation of 7-OH-coumarin-3-carboxylic acid (7-OH-CCA) of known concentration were used to calibrate the results.

Immunoenzyme analysis. The method of immunoenzyme analysis was described in detail previously [3]. DNA samples were denatured in advance in a boiling water bath for 5 min and cooled on ice for 15 min. DNA solutions (43 μl, 355 μg/ml) were applied onto the bottom of the immunoenzyme plate wells (Costar, USA). DNA was immobilized by adsorption during drying for 2 h at 80°C till complete dryness. Nonspecific adsorption sites were blocked using 300 μl of 1% casein solution in 0.15 M Tris-HCl buffer, pH 8.7, 0.15 M NaCl. The plates were incu-

bated at room temperature overnight (14-18 h). Formation of antigen complexes with 8-oxoguanine-specific antibodies was carried out in the blocking solution by incubation with stirring for 2 h at 30°C. The samples were washed (300 μl per well) with a washing solution (50 mM Tris-HCl, pH 8.7, 0.15 M NaCl, 0.1% Triton X-100). Then secondary complex of antibodies with IgG–horseradish peroxidase conjugate was formed for 2 h at 30°C in blocking solution. This complex was washed three times by the above-described method after which a chromogenic substrate (18.2 mM ABTS) and 2.6 mM hydrogen peroxide in 75 mM citrate buffer, pH 4.2, were added. After achievement of green staining, the reaction was stopped by addition of equal volume of 1.5 mM NaN_3 in 0.1 M citrate buffer, pH 4.3. The sample absorption was measured on a multiplate scanning photometer (Titertek Multiscan, Finland) at $\lambda = 405$ nm. The content of 8-oxoguanine in DNA was determined on the basis of known value of radiation–chemical yield [3].

RESULTS

Effect of amino acids on the H_2O_2 formation in solution under X-ray irradiation. Production of H_2O_2, the most stable ROS, was determined by enhanced chemiluminescence in the luminol–*p*-iodophenol–horseradish peroxidase system in 5 mM phosphate buffer, pH 7.4, in the presence of 1 mM amino acids under the dose of X-ray irradiation of 7 Gy (see table). Doses under study were chosen in the range of 1-10 Gy based on sensitivity of the methods used to register reliably the products of water radiolysis. On the other hand, the dose of 7 Gy is lethal for mammals [2], and owing to this, the possibility to decrease the consequences of the ionizing radiation effect, caused by amino acids in the concentrations under study, in principle can be used to reduce the amount of lethal outcomes caused by radiation. In all studied cases in the range of 1-10 Gy, linear dose–effect dependence was observed. The results given in the table show that the studied amino acids can be divided into three groups according to their effect on H_2O_2 production. The first group includes Ala, Arg, Gln, Glu, Gly, Hyp, Lys, Met, and Phe that do not influence the H_2O_2 formation remaining at the level of ~0.14 μM/Gy at the dose of 7 Gy. The second group includes amino acids decreasing the yield of hydrogen peroxide. Among them, there are Trp and Tyr decreasing the H_2O_2 yield by 70 and 80%, respectively, and His, Pro, and Val decreasing the H_2O_2 yield by 36, 30, and 27%, respectively. The third group consists of two amino acids, Ser and Thr, incorporating a hydroxyl group. These amino acids increase the yield of hydrogen peroxide by 90 and 45%, respectively. Figure 1 shows graphs of the H_2O_2 yield dependences on concentrations of some amino acids from the three different groups at the dose of X-ray irradiation of 7 Gy. The effect of amino acids is significant at ~0.1 mM concentrations corresponding to their physiological levels in mammals. At 0.1 mM concentration the decrease of the hydrogen peroxide yield changes in the order: Tyr > Trp = Pro > His (Fig. 1).

Effect of 1 mM amino acids on hydrogen peroxide formation in 5 mM phosphate buffer, pH 7.4, induced by X-ray radiation dose of 7 Gy (dose rate 1 Gy/min)

Substance	Hydrogen peroxide concentration, μM	DVF
Control	1.06 ± 0.09	–
Group 1		
Ala	0.82 ± 0.18	0.8
Arg	1.09 ± 0.19	1.0
Gln	1.05 ± 0.11	1.0
Glu	0.92 ± 0.08	0.9
Gly	1.12 ± 0.17	1.0
Hyp	0.93 ± 0.07	0.9
Lys	1.04 ± 0.04	1.0
Met	1.08 ± 0.16	1.0
Phe	1.14 ± 0.02	1.1
Group 2		
His	0.68 ± 0.21*	0.6
Pro	0.74 ± 0.09*	0.7
Trp	0.33 ± 0.11*	0.3
Tyr	0.22 ± 0.12*	0.2
Val	0.77 ± 0.09*	0.7
Group 3		
Ser	2.02 ± 0.29*	1.9
Thr	1.45 ± 0.11*	1.4

Note: DVF, dose variation factor.
* The difference is significant compared to control ($p < 0.05$).

Effect of amino acids on formation of OH radicals in solution under X-ray irradiation. Production of the most reactive variety of ROS, OH radicals, was detected using CCA, a convenient fluorescent probe for determination of formation of hydroxyl radicals. Linear dependence of the hydroxyl radical yields on the irradiation dose is observed at doses of 1-10 Gy in 5 mM phosphate buffer, pH 7.4, in the presence of 1 mM amino acids. Some examples of such dependences are shown in Fig. 2. Data for all studied amino acids at the dose of 7 Gy are shown in Fig. 3. Radiation-chemical yield of 7-OH-CCA at this dose in the absence of amino acids was 11.3 nM/Gy. Upon addition to the buffer solution, all studied amino acids decreased the yield of 7-OH-CCA and therefore of

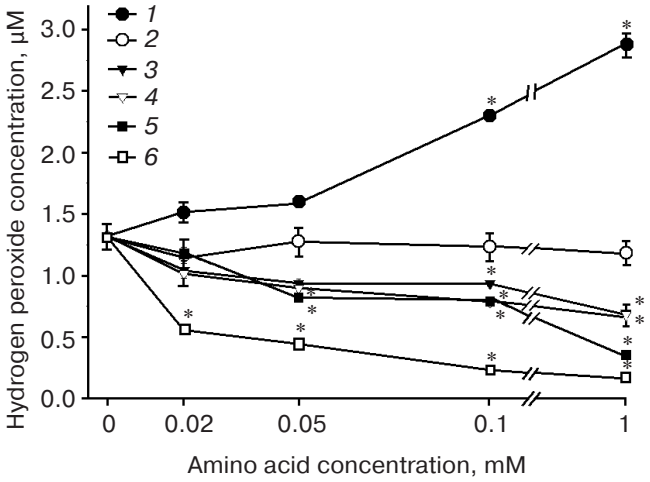

Fig. 1. Dependence of hydrogen peroxide yield on the amino acid concentration in 5 mM phosphate buffer, pH 7.4, under X-ray radiation dose of 7 Gy (dose rate 1 Gy/min): *1*) Ser; *2*) Gly; *3*) His; *4*) Pro; *5*) Trp; *6*) Tyr. Mean values and standard errors are shown (n = 2-5). * The difference is significant compared to control without amino acids ($p < 0.05$).

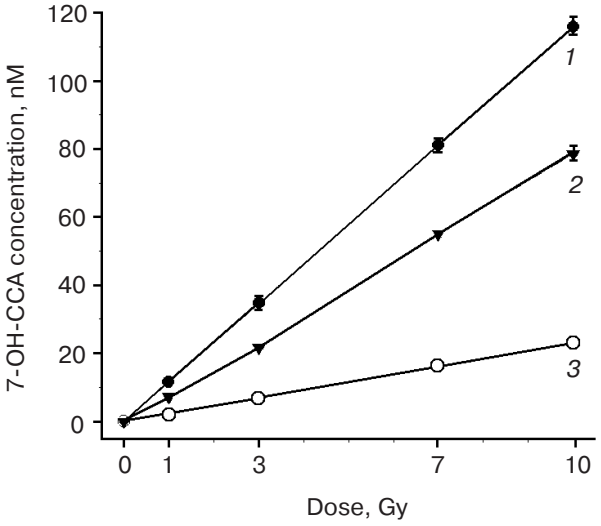

Fig. 2. Dependence of hydroxyl radical yield on the dose of X-ray radiation (dose rate 1 Gy/min) for some amino acids at a concentration of 1 mM in 5 mM phosphate buffer, pH 7.4: *1*) control; *2*) Val; *3*) Cys. Mean values and standard errors are shown (n = 3). * The difference is significant compared to control ($p < 0.05$).

OH radicals. All amino acids can be divided into two groups according to the strength of their effect. The first group (Fig. 3) includes amino acids decreasing the yield of fluorescent product by 70-80% in the order Cys > His > Phe = Met = Trp > Tyr. For them dose reduction factor (DRF) associated with formation of hydroxyl radicals was 5.5, 4.7, 4.3, and 3.1, respectively. Amino acids of the second group decrease the yield of fluorescent product by 5-30%. In this group amino acids are arranged in the following order on the basis of their increasing effect: Gly < Pro < Glu < Hyp < Thr < Ser < Lys < Arg < Gln < Val. DRF corresponding to formation of hydroxyl radicals for these amino acids was from 1.05 to 1.45 (Fig. 3). Figure 4 shows dependences of the hydroxyl radical yields on amino acid concentrations at the irradiation dose of 7 Gy. It is seen that at physiological concentrations of amino acids (~0.1 mM) there is a significant decrease of the hydroxyl radical yields by 2.1, 1.9, and 1.7 times for Cys, Tyr, and His, respectively. Increasing the amino acid concentration to 1 mM results in almost fivefold decrease of OH˙ formation, and this effect appears to be close to that for six amino acids—Cys, Tyr, His, Trp, Phe, and Met. In this work, we have studied the effect of amino acids on generation of OH radicals at pH 7.4, corresponding to physiological value of mammalian blood serum. In the case of pH change near this value, only histidine with pK = 6.0 is able to influence this process. Figure 5 shows the effect of the solution pH in the range from 5.5 to 8.0 on the yield of hydroxyl radicals for this amino acid under X-ray irradiation. In this case, increasing pH is accompanied by 1.5-fold decrease of OH˙ formation. The effect of changing the pH of phosphate buffer alone in the same interval under the same dose of irradiation caused about 5% of the level observed for histidine.

Protective effect of amino acids against X-ray-induced formation of 8-oxoguanine in DNA. Immunoenzyme analysis was used to study the effect of 1 mM amino acids (the most active scavengers of ROS induced

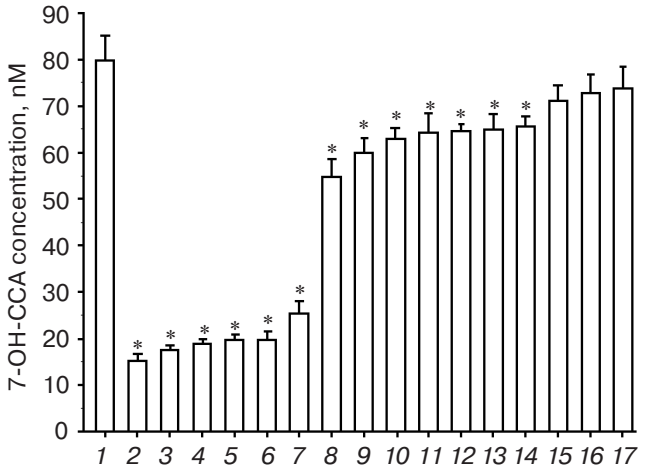

Fig. 3. Effect of 1 mM amino acids on generation of hydroxyl radicals in 5 mM phosphate buffer, pH 7.4, under X-ray irradiation in the dose of 7 Gy (dose rate 1 Gy/min): *1*) control; *2-7*) the first group of amino acids with pronounced antioxidant properties (Cys (*2*), His (*3*), Phe (*4*), Met (*5*), Trp (*6*), Tyr (*7*)); *8-17*) the second group (Val (*8*), Arg (*9*), Ser (*10*), Lys (*11*), Thr (*12*), Glu (*13*), Hyp (*14*), Pro (*15*), Gly (*16*), Ala (*17*)). Mean values and standard errors are shown (n = 3). * The difference is significant compared to control ($p < 0.05$).

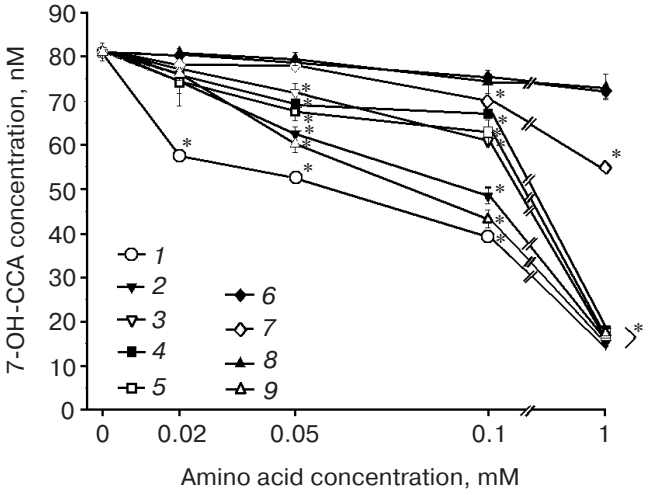

Fig. 4. Dependence of hydroxyl radical yield on amino acid concentration in 5 mM phosphate buffer, pH 7.4, under X-ray irradiation at the dose of 7 Gy (dose rate 1 Gy/min): *1*) Cys; *2*) His; *3*) Phe; *4*) Met; *5*) Trp; *6*) Pro; *7*) Val; *8*) Gly; *9*) Tyr. Mean values and standard errors are shown ($n = 3$). * The difference is significant compared to control ($p < 0.05$).

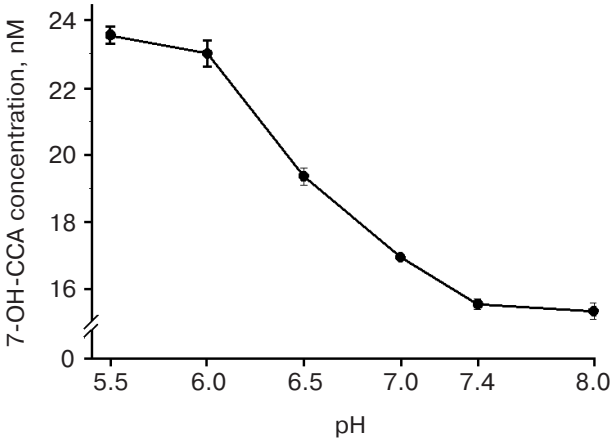

Fig. 5. Effect of 1 mM histidine on the yield of hydroxyl radicals in 5 mM phosphate buffer at different pH values under X-ray irradiation at a dose of 7 Gy (dose rate 1 Gy/min). Mean values and standard errors are shown ($n = 2$).

by X-ray irradiation) on formation of 8-oxoguanine (the key biomarker of DNA damage by reactive oxygen species) in DNA solution in 5 mM phosphate buffer, pH 7.4, under X-ray irradiation with the dose of 10 Gy. The results are shown in Fig. 6. Under these conditions of irradiation in the absence of amino acids, 7.8 bases of 8-oxoguanine are formed in DNA solution per 10^5 guanines. All studied amino acids except Gly and Ser decreased the yield of 8-oxoguanine as follows: Arg by 56%, Phe and His by 80%, Met and Tyr by 90%, and Cys by more than 90%. Serine appeared to somewhat increase the level of 8-oxoguanine formation in DNA, but this increase was within the limits of experimental error. Protective action of these amino acids against X-ray-induced oxidative damage to DNA is described by DRF of 1.6 for Pro, 2.0 for Arg, 2.2 for Lys, 4.4 for His, 6.0 for Phe, 10.8 for Trp, 15.6 for Tyr, and 15.9 for Met.

Figure 7 shows the dependence of 8-oxoguanine formation on tryptophan, tyrosine, methionine, histidine, and arginine concentrations. A significant protective

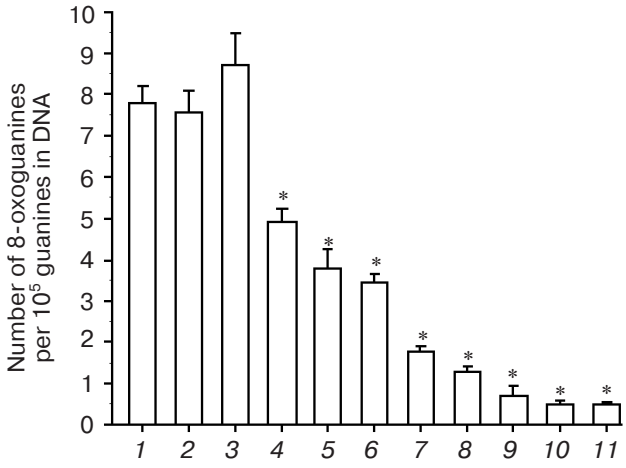

Fig. 6. Effect of 1 mM amino acids on 8-oxoguanine formation in solution of DNA from salmon sperm in 5 mM phosphate buffer, pH 7.4, under X-ray irradiation at the dose of 10 Gy (dose rate 4.5 Gy/min): *1*) control; *2*) Gly; *3*) Ser; *4*) Pro; *5*) Arg; *6*) Lys; *7*) His; *8*) Phe; *9*) Trp; *10*) Tyr; *11*) Met. * The difference is significant compared to control ($p < 0.05$).

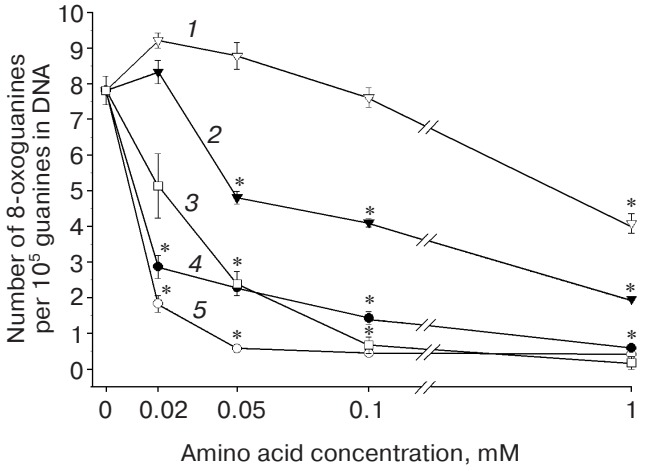

Fig. 7. Concentration dependence of the effect of some amino acids on 8-oxoguanine formation in solution of DNA from salmon sperm in 5 mM phosphate buffer, pH 7.4, under X-ray irradiation at the dose of 10 Gy (dose rate 4.5 Gy/min): *1*) Arg; *2*) His; *3*) Tyr; *4*) Met; *5*) Trp. Mean values and standard errors are shown ($n = 3$). * The difference is significant compared to control ($p < 0.05$).

effect for Trp, Met, and Tyr is revealed already at amino acid concentration of 20 μM. In the case of His there is no protective effect, whereas for Arg concentrations of 20 and 50 μM result in 25 and 20% increase in 8-oxoguanine formation compared with irradiated control DNA. At these Arg and DNA concentrations, the amino acid ratios to guanine in DNA are close to each other. At amino acid concentrations of 0.1 mM, close to physiological, the yield of oxidized guanines for His, Met, Tyr, and Trp significantly decreases.

DISCUSSION

It is known that ROS are formed in aqueous solutions under ionizing radiation as a result of radiolysis of water [2]. The longest lived variety of ROS is hydrogen peroxide, able both to damage different cell structures [1] by generation of hydroxyl radicals, using multivalent metal ions in the Fenton reaction, and to play signaling and regulatory roles associated with activation of damage repair or apoptosis processes [7]. Hydroxyl radicals are the most reactive kind of ROS and lead to oxidative damage to biological macromolecules like proteins, DNA, etc.

Our data show that free amino acids are able to influence production of hydrogen peroxide and OH radicals in aqueous solutions in response to X-ray irradiation. To interpret these data, we should take into account interactions of the amino acid side groups with different reaction products such as hydroxyl radical, superoxide anion-radical ($O_2^{\cdot-}$), hydrated electron (e_{aq}^-), the atomic hydrogen radical ($H^{\cdot}$), hydroperoxide radical ($HO_2^{\cdot}$), and hydrogen peroxide generated by ionizing radiation.

The process of water radiolysis with account for generation of the short-lived radical products and long-lived molecular forms can be presented by the following formulas [2, 33]:

$$2H_2O = 2OH^{\cdot} + H^{\cdot} + H^+ + e_{aq}^-,$$

$$2H^{\cdot} = H_2;\ OH^{\cdot} + H^{\cdot} = H_2O,$$

$$2OH^{\cdot} = H_2O_2,$$

$$OH^{\cdot} + e_{aq}^- = OH^-,$$

$$H^+ + e_{aq}^- = H^{\cdot}.$$

The total process accounting for formation of hydrogen peroxide is as follows:

$$H_2O \rightarrow OH^{\cdot} + H^{\cdot} + H^+ + e_{aq}^- + H_2 + H_2O_2.$$

In the presence of air oxygen dissolved in water, as under our experimental conditions, an additional generation of hydroperoxide radical takes place:

$$H^{\cdot} + O_2 = HO_2^{\cdot},$$

$$e_{aq}^- + O_2 = O_2^{\cdot-},$$

$$O_2^{\cdot-} + H^+ = HO_2^{\cdot}.$$

Dismutation of hydroperoxide radicals results in formation of hydrogen peroxide and oxygen:

$$2HO_2^{\cdot} = H_2O_2 + O_2.$$

Thus, these processes result in formation of OH radicals as well as of H_2O_2 as the product of recombination of these radicals and dismutation of hydroperoxide radicals, whereas these processes are influenced by the presence of hydrated electron, hydrogen atom radical, and hydroperoxide radical. In principle, radiation can cause both the decrease and increase of H_2O_2 concentration in the presence of amino acids, depending on their ability to react with $OH^{\cdot}$, $H^{\cdot}$, $HO_2^{\cdot}$, and e_{aq}^-.

According to published data the α-carbon atom, present in all amino acids, is able to react with $OH^{\cdot}$; in this case the hydrogen atom at the α-carbon is abstracted and an amino acid radical is formed [34]. Our results, showing that all amino acids decrease the yield of hydroxyl radicals under X-ray irradiation, are in agreement with these data. Besides, side chains of amino acid residues are also able to react with $OH^{\cdot}$, which are responsible for different effects of individual amino acids on formation of OH radicals.

Side chains of amino acid can be divided into two groups on the basis of their ability to react with OH radical. The first group consists of six amino acids (Cys, His, Phe, Met, Trp, Tyr) that most actively react with OH radical and significantly decrease its production, whereas the other group interacts more weakly with this radical (Fig. 3).

According to data in the literature, radiosensitivity of amino acids determined by the rate constants of reaction with OH radical decreases in the order: Tyr, Trp, Phe, His, Arg [35]. Our results show that the group of amino acids decreasing formation of OH radicals by 70-80% in response to X-ray irradiation includes amino acids whose protective properties change in the following order: Cys > His > Phe = Met = Trp > Tyr. It is assumed that the most sensitive targets for the irradiation-induced oxygen radicals are aromatic amino acids tryptophan and tyrosine [34]. Our data on the effect of Trp and Tyr on formation of OH radicals and generation of H_2O_2 are in line with this. These amino acids of the group of most efficient scavengers of OH radicals cause the lowest production of hydrogen peroxide, perhaps due to the decreased recombination of such radicals. Histidine, that even more efficiently decreased the yield of OH radicals in response to X-ray irradiation, less intensively influenced the yield of H_2O_2. In the case of Met and Phe, no H_2O_2 production

was observed. Such effect of these amino acids can be explained by the compensatory decrease of hydrated electron formation or by increased formation of hydroperoxide radicals in the presence of these substances. Indeed, methionine in general does not form peroxides and phenylalanine produces only insignificant amounts [36].

Rate constants of the reactions of hydrated electron with amino acids show that the most reactive are cysteine and histidine, less active ones being tryptophan, tyrosine, arginine, asparagine, and phenylalanine, whereas methionine exhibits even lower reactivity, and the other amino acids react relatively weakly with it [37].

It should be noted that histidine can differently influence the ROS formation in solution depending on pH, because in the pH range from 7.0 to 8.0 the histidine acceptor activity towards hydrated electron significantly decreases [33].

In BSA, all amino acids are susceptible to modification caused both by hydroxyl radicals and their combined action with superoxide anion radicals, and the most sensitive are tryptophan, tyrosine, histidine, and cysteine [38]. In this case, structural alterations in the protein significantly increase its susceptibility to proteolysis.

Among other most frequent sites of amino acid oxidation in proteins are His, Met, Pro, Arg, and Lys [34]. Histidine and methionine belong to the group of amino acids most efficiently intercepting hydroxyl radicals, whereas the three other amino acids did not exhibit such properties (Fig. 3). Though Pro noticeably decreased formation of H_2O_2, Arg, Lys, and Met did not produce such effect, although all these amino acids decreased generation of OH radicals. It can be assumed that in the case of Arg, Lys, Met, and Pro oxidation of these amino acids partially follows a different pathway not connected with the action of hydroxyl radicals, via superoxide anion radicals and peroxides. In fact, interaction with superoxide anion radicals is characteristic of arginine [27, 28, 33] and lysine [39], while proline quite efficiently forms peroxides [36]. The ability of all amino acids to form peroxides in response to OH radicals induced by γ-radiation was studied earlier. In this case, amino acids valine, proline, leucine, isoleucine, lysine, and glutamic acid most efficiently formed peroxides, whereas tryptophan, glutamine, arginine, and alanine exhibited intermediate efficiency and the other amino acids were only slightly efficient [36].

The increase in H_2O_2 production upon irradiation of serine and threonine is not accompanied by increased generation of OH radicals. A possible factor responsible for increased yield of H_2O_2 could be the electron acceptor activity of these amino acids, because in this case the increased production of H_2O_2 should take place [7, 33]. However, no such electron acceptor activity was detected for serine [37]. It should be taken into account that hydrogen peroxide is released at one of the stages of amino acid oxidation stimulated by ionizing radiation in the presence of oxygen upon splitting off of hydroperoxide [34], which can be the cause of its increased content in this case. Thus, under X-ray irradiation serine and threonine, unlike other amino acids, exhibit prooxidant properties. The mechanism of increased yield of H_2O_2 upon serine and threonine irradiation is still unclear and requires further investigation.

It is known that arginine scavenges superoxide anion-radical by direct chemical interaction with it [27, 28]. Earlier the ability of lysine, glutamate and, to a lesser extent, of histidine for superoxide scavenging activity was revealed [39]. It is supposed that individual amino acids can play an important role in antioxidant protection of biological structures during oxidative stress [40]. It has been shown recently that proline is an efficient antioxidant and apoptosis inhibitor for the pathogenic fungus *Colletotrichum trifolii* [29]. The knowledge of antioxidant properties of amino acids can be helpful both in understanding the mechanisms of functioning of presently known biologically active peptides [26] and in the design of new perspective compounds of this class.

It is known that guanine in DNA has the lowest oxidation–reduction potential among natural bases [41]; therefore, as an electron donor it is able to donate its own electron to different acceptors forming the guanine radical cation. The guanine radical cation or "hole" then migrates in jumps along the DNA chain from one guanine to another until its oxidation with formation of 8-oxoguanine occurs [42, 43]. It was shown using the pulse radiolysis that the single-electron-oxidized radicals of dGMP can be efficiently repaired chemically by aromatic amino acids tyrosine and tryptophan and peptide Arg-Tyr by means of the electron transport reaction [44].

It was shown previously that oligolysines have a radioprotective effect on plasmid DNA by lowering the yield of single-stranded breaks caused by γ-irradiation [45]. Milligan et al. [31, 46] studied γ-irradiation of plasmid DNA and by change of its supercoiling in the presence of some oxidizable amino acids obtained indirect data indicating that Trp, Tyr, and Met react with guanine radical cation and thus cause efficient chemical repair due to prevention of oxidative damage to DNA. These results agree with our data for Trp, Tyr, and Met, directly detecting lowering the yield of 8-oxoguanine formed during oxidation of guanine radical cation. However, according to Milligan et al. histidine is inactive, whereas in our studies it exhibited protective properties. These discrepancies may be due in part to different extent of histidine ionization at different pH values of phosphate buffer (7.4 in our work and 7.0 in work [31]). In fact, as pH increases from 7.0 to 8.0, acceptor activity of histidine relative to hydrated electron significantly decreases [33], which makes lower the probability of the guanidine radical cation formation. Moreover, protective properties for Pro, Arg, and Phe, which were not studied before, were also revealed in this work. It is supposed that some amino acids within DNA-binding proteins may protect DNA against oxidative dam-

age. It was found that under γ-irradiation tyrosine-containing cationic peptides complexed with DNA are able to prevent oxidative damage of the latter [47]. These data suggest that tyrosine residues in DNA-binding proteins may be involved in DNA protection against oxidative damage caused by ionizing radiation.

However, it should be noted that in model investigations on the interaction of histone H4 N-terminal region with DNA under conditions of high concentrations of H_2O_2 and Cu^{2+} ions the amount of different oxidative damage in DNA, including formation of 8-oxoguanine, increases [48]. In the presence of oxygen, hydroperoxides formed in histones in response to γ-irradiation cause guanine-specific damage in DNA in the presence of Cu^{+}. It was shown that hydroperoxides of histone H1 induce formation of 8-oxoguanine in DNA [49]. Thus, under strong oxidative stress conditions, oxygen and overloading with multivalent metal ions, histones more likely stimulate an increase in the amount of DNA damage rather than being protective. Besides, it was found that the long-lived protein radicals is source of prolonged formation of ROS and intermediates in oxidative stress development in biological systems [15]. They were shown to be capable of radical damage transfer to DNA [17, 18]. In chromosomes, DNA-binding proteins protect it against damage induced by radiation, which is associated both with DNA compaction and with the reaction of protein molecules with the products of water radiolysis. However, interception of free DNA radicals by protein and the reverse process are possible [17, 18], which may induce additional damage to DNA.

Thus, it was found that a number of amino acids in an *in vitro* system exhibit antioxidant properties. It may be assumed that in the concentrations exceeding physiological ones they will exhibit radioprotective properties in mammals. The data suggest that some amino acids may be involved in formation of intracellular oxidation–reduction balance, and their side groups within chromatin proteins are evidently able to protect DNA against damage caused by ROS formation under conditions of a moderate oxidative stress. However, it remains possible that under certain conditions proteins may stimulate additional oxidative damage to DNA. Further investigations are necessary to answer this question. It is also reasonable to study possible bioantioxidant properties of free amino acids, i.e. the enhancement of antioxidant activity in the case of their additional introduction into animals [50].

This work was supported in part by the Russian Foundation for Basic Research (grant 07-04-00406-a).

REFERENCES

1. Men'shchikova, E. B., Lankin, V. Z., Zenkov, N. K., Bondar', I. A., Krugovykh, N. F., and Trufakin, V. A. (2006) *Oxidative Stress. Prooxidants and Antioxidants* [in Russian], Slovo, Moscow.
2. Kudryashov, Yu. B. (2004) *Radiation Biophysics (Ionizing Radiations)*, [in Russian], Fizmatlit, Moscow.
3. Bruskov, V. I., Malakhova, L. V., Masalimov, Z. K., and Chernikov, A. V. (2002) *Nucleic Acids Res.*, **30**, 1354-1363.
4. Bruskov, V. I., Masalimov, Z. K., and Chernikov, A. V. (2002) *Dokl. RAN*, **384**, 821-824.
5. Chernikov, A. V., and Bruskov, V. I. (2002) *Biofizika*, **47**, 773-781.
6. Bruskov, V. I., Chernikov, A. V., Gudkov, S. V., and Masalimov, Z. K. (2003) *Biofizika*, **48**, 1022-11029.
7. Oktyabrskii, O. N., and Smirnova, G. V. (2007) *Biochemistry (Moscow)*, **72**, 132-145.
8. Du, J., and Gebicki, M. (2004) *Int. J. Biochem. Cell Biol.*, **36**, 2334-2343.
9. Koyama, S., Kodama, S., Suzuki, K., Matsumoto, T., Miyazaki, T., and Watanabe, M. (1998) *Mutat. Res.*, **421**, 45-54.
10. Kumagai, J., Nakama, M., Miyazaki, T., Ise, T., Kodama, S., and Watanabe, M. (2002) *Radiat. Phys. Chem.*, **64**, 293-297.
11. Miyazaki, T., Morikawa, A., Kumagai, J., Ikehata, M., Koana, T., and Kikuchi, S. (2002) *Radiat. Phys. Chem.*, **65**, 151-157.
12. Kumagai, J., Masui, K., Itagaki, Y., Shiotani, M., Kodama, S., Watanabe, M., and Miyazaki, T. (2003) *Radiat. Res.*, **161**, 95-102.
13. Kumagai, J., Kawaura, T., Miyazaki, T., Prost, M., Prost, E., Watanabe, M., and Quentin-Leclercq, J. (2003) *Radiat. Phys. Chem.*, **66**, 17-25.
14. Ostdal, H., Davies, M. J., and Andersen, H. J. (2002) *Free Rad. Biol. Med.*, **33**, 201-209.
15. Kumagai, J., Katon, H., Miyazaki, T., Hidema, J., and Kumagai, T. (1999) *J. Radiat. Res.*, **40**, 303-310.
16. Pietraforte, D., and Minetti, M. (1997) *Biochem. J.*, **325**, 675-684.
17. Luxford, C., Morin, B., Dean, R. T., and Davies, M. J. (1999) *Biochem. J.*, **344**, 125-134.
18. Gudkov, S. V., Shtarkman, I. N., Chernikov, A. V., Usacheva, A. M., and Bruskov, V. I. (2007) *Dokl. RAN*, **413**, 261-263.
19. Yoshimura, T., Matsuno, K., Miyazaki, T., Suzuki, K., and Watanabe, M. (1993) *Radiat. Res.*, **136**, 361-365.
20. Nauser, T., Koppenol, W. H., and Gebicki, J. M. (2005) *Biochem. J.*, **392**, 693-701.
21. Aruoma, O. I. (2003) *Mut. Res.*, **523**, 9-20.
22. Gudkov, S. V., Shtarkman, I. N., Smirnova, V. S., Chernikov, A. V., and Bruskov, V. I. (2006) *Dokl. RAN*, **407**, 115-118.
23. Gudkov, S. V., Shtarkman, I. N., Smirnova, V. S., Chernikov, A. V., and Bruskov, V. I. (2006) *Radiat. Res.*, **165**, 538-545.
24. Meister, A., and Anderson, M. E. (1983) *Ann. Rev. Biochem.*, **52**, 711-760.
25. Boldyrev, A. A. (1998) *Carnosine* [in Russian], Moscow State University Publishing House, Moscow.
26. Khavinson, V. Kh., Kvetnoy, I. M., and Ashmarin, I. P. (2002) *Uspekhi Sovrem. Biol.*, **122**, 190-203.
27. Lass, A., Suessenbacher, A., Olkart, G. W., Mayer, B., and Brunner, F. (2002) *Mol. Pharmacol.*, **61**, 1081-1088.

28. Hayashi, T., Juilet, P. A. R., Matsui-Hirai, H., Miyazaki, A., Fukatsu, A., Funami, J., Iguchi, A., and Ignarro, L. J. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 13681-13686.
29. Chen, C., and Dickman, M. B. (2005) *Proc. Natl. Acad. Sci. USA*, **102**, 3459-3464.
30. Decker, E. A., Livisay, S. A., and Zhou, S. (2000) *Biochemistry (Moscow)*, **65**, 766-770.
31. Milligan, J. R., Aguilera, J. A., Ly, A., Tran, N. Q., Hoang, O., and Ward, J. F. (2003) *Nucleic Acids Res.*, **31**, 6258-6263.
32. Bruskov, V. I., Gaziev, A. I., Malakhova, L. V., Mantsygin, Yu. A., and Morenkov, O. S. (1996) *Biochemistry (Moscow)*, **61**, 535-540.
33. Pikaev, A. K. (1986) *Modern Radiation Chemistry. Radiolysis of Gases and Liquids* [in Russian], Nauka, Moscow.
34. Stadtman, E. R. (1993) *Annu. Rev. Biochem.*, **62**, 797-821.
35. Dorfman, L. M., and Adams, G. E. (1973) *Natl. Stand. Ref. Data Ser.*, *US Natl. Bur. Stand.*, No. 46, 43-49.
36. Gebicki, S., and Gebicki, J. M. (1993) *Biochem. J.*, **289**, 743-749.
37. Braams, R. (1966) *Radiat. Res.*, **27**, 319-329.
38. Davies, K. J. A., Delsignore, M. E., and Lin, S. W. (1987) *J. Biol. Chem.*, **262**, 9902-9907.
39. Chistyakov, V. A., Kornienko, I. V., Kletskii, M. E., Kornienko, I. E., Lisitsin, A. S., and Novikov, V. V. (2005) *Biofizika*, **50**, 601-605.
40. Stadtman, E. R., and Berlett, B. S. (1991) *J. Biol. Chem.*, **296**, 17201-17211.
41. Jovanovic, S. V., and Simic, M. G. (1986) *J. Phys. Chem.*, **90**, 974-978.
42. Hall, D. B., Holmin, R. E., and Barton, J. K. (1996) *Nature*, **382**, 731-735.
43. Henderson, P. T., Delanay, J. C., Gu, F., Tannenbaum, S. R., and Essigmann, J. M. (2002) *Biochemistry*, **41**, 914-921.
44. Pan, J., Lin, W., Wang, W., Han, Z., Lu, C., Yao, S., Lin, N., and Zhu, D. (2001) *Biophys. Chem.*, **89**, 193-199.
45. Newton, G. L., Ly, A., Tran, N. Q., Ward, J. F., and Milligan, J. R. (2004) *Int. J. Radiat. Biol.*, **80**, 643-651.
46. Milligan, J. R., Tran, N. Q., Ly, A., and Ward, J. F. (2004) *Biochemistry*, **43**, 5102-5108.
47. Ly, A., Bullick, S., Won, J.-H., and Milligan, J. R. (2004) *Int. J. Radiat. Biol.*, **82**, 421-433.
48. Midorikawa, K., Murata, M., and Kawanishi, S. (2005) *Biochem. Biophys. Res. Commun.*, **333**, 1073-1077.
49. Furukava, A., Hiraku, Y., Oikawa, S., Luxford, C., Davies, M. J., and Kavanishi, S. (2005) *Biochem. J.*, **388**, 813-818.
50. Burlakova, E. B. (2007) *Ross. Khim. Zh.*, **51**, 3-12.