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Non-Enzymatic Methylglyoxal Formation From glucose Metabolites and Generation of Superoxide Anion Radical During Methylglyoxal-Dependent Cross-Links Reaction

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Abstract

The paper explores the formation of a-oxoaldehydes during the interaction of glucose metabolites with hydroxyl or alkoxyl radicals. Hydroxyl radicals were generated under radiolysis of aqueous solutions, and alkoxyl radicals (*t*-BuO$^{\cdot}$) were obtained in the model system *tert*-butyl hydroperoxide/Fe^{2+}. High-performance liquid chromatography revealed that methylglyoxal was one of the organic products resulting from *t*-BuO$^{\cdot}$-induced transformations of fructose-1,6-bisphosphate under hypoxic conditions. The interaction of lysine and methylglyoxal one of the main targets of a-oxoaldehydes in proteins was also studied. As chemiluminescence and EPR spectroscopy demonstrated, this reaction generates a methylglyoxal anion radical, a cation-radical of methylglyoxal dialkylamine and a superoxide anion radical. EPR signal of methylglyoxal-derived free radicals was observed in hypoxia, whereas only the trace amounts of these free radicals were recorded in the aerated reaction medium.

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Introduction

Ionizing irradiation or metabolic disturbances in the living body can lead to hyperproduction of reactive oxygen species (ROS), which promotes the activation of free radical processes – the phenomenon which can damage some biologically significant molecules [1-3]. Oxidative stress is also an important factor in the pathogenesis of atherosclerosis and diabetes mellitus [4-7]. The elevation of the generated ROS in atherosclerosis against the background of hyperlipidemia leads to a rapid accumulation of lipohydroperoxides in blood as well as in the vascular wall [6, 8]. In the process of free radical peroxidation of the unsaturated lipids lipohydroperoxides (primary oxidation products) undergo oxidative degradation with the formation of reactive carbonyl compounds – aldehydes such as 4-hydroxynonenal and malondialdehyde (MDA) [4, 9, 10].

Substances with phosphoether bonds in their structure play an important role in biosystems. Such compounds as fructose-1,6-bisphosphate (Fru-1,6-P_2) and glyceraldehyde-3-phosphate (G-3-P) are glucose (Glc) metabolites which are formed during glycolysis. Since the interaction of oxygen-centered radicals with the monosaccharides leads to the formation of physiologically reactive carbonyl and dicarbonyl compounds [11], monosaccharide phosphates and their analogues. The latter ones being the main metabolites of glycolysis can be assumed to undergo the similar free radical transformations, which form α-oxoaldehydes.

Thus, oxidative stress, accompanied by an increased level of organic hydroperoxides, inevitably leads to a carbonyl stress, characterized by the accumulation of reactive carbonyl species. In diabetic hyperglycemia Glc gets autooxidated, which is accompanied by the formation of MDA homolog - glyoxal [10]. This process may be increased by a co-oxidation of unsaturated lipids and Glc [12-14]. The structural isomer of MDA, methylglyoxal is formed in diabetes as a result of enzymatic oxidation of triose phosphate accumulated during glycolysis [4, 10, 15].

The potential danger of accumulating dicarbonyls during carbonyl stress development in diabetes lies in the ability of aldehyde groups to react with amino groups of proteins in order to form inter- and intramolecular Schiff bases cross-links , since this process triggers the biopolymer modification and the consequent violation of their normal functioning [4, 9, 10, 15]. In particular, modifying the terminal ε-residues of L-lysine in the molecule of apoprotein B-100 of low-density lipoproteins (LDL) by dicarbonyls increases the capture of LDL particles by vascular wall cells with the help of scavenger receptors and leads to a pro-atherogenic vascular damage [16]. In addition, literature on the subject mentions about the formation of free radical intermediates during the interaction of methylglyoxal with L-alanine and albumin [17, 18]; noteworthily, the mechanism of this reaction has not been fully ascertained so far.

In the present study we investigate the possibility that the metabolites of glycolysis can create the non-enzymatic formations of methylglyoxal, as well as the possibility of ROS and other free radical products to form in the process of interaction between this high reactive dicarbonyl compound and amino acids.

Materials and Methods

The following reagents produced by Sigma-Aldrich (USA) were used in the present work: superoxide dismutase from bovine erythrocytes, fructose (Fru), fructose-1,6-bisphosphate (Fru-1,6-P_2), Glc, glucose-1-phosphate (G-1-P), glucose-6-phosphate (G-6-P), G-3-P, glyceraldehyde, methylglyoxal and glyoxal (40% water solutions), formaldehyde (37% water solution), potassium dihydrogen phosphate, *tert*-butylhydroperoxide (*t*-BuOOH), L-lysine, lucigenin, *o*-phenylenediamine (OPDA), 2,4-dinitrophenylhydrazine (DNPH), nitro blue tetrazolium (NBT), 1,1,3,3-tetraethoxypropane.

Twice-distilled water was used to prepare aqueous solutions for the compounds under investigation. Aqueous 0.1 M and 0.01 M solutions of the glucose metabolites have been saturated with argon (Ar) or oxygen (O_2) of a high purity for 30−45 minutes. The prepared samples were irradiated in a g-unit with a ^{60}Co source. The absorbed dose range, equaled to 0.13÷1.92 kGy, was determined by Fricke dosimetry using a radiation chemical yield $G(Fe^{3+}) = 16.2 \cdot 10^{-7}$mol/J [19]. In all other experiments the mixture consisted of *t*-BuOOH/Fe^{2+} with a different molar concentration of *t*-BuOOH was added to the substrate solutions. In the latter case the conversion products of the substrates

and *t*-BuOOH was determined 15 minutes after the solutions had been mixed.

Dicarbonyls (a-oxoaldehydes) were analysed employing the method of a high-performance liquid chromatography using LCMS-2020 chromatograph ("Shimadzu", Japan) after the preliminary derivatization of the samples with OPDA. Test samples were mixed with OPDA solution and have been held for 12 hours in a dark place to obtain the corresponding quinoxalins (Figure 1A). To effectively separate the quinoxalines formed from methylglyoxal and glyoxal, their gradient elution was conducted using methanol and water. The separation was carried out on a Shim-pack VP-ODS column. To identify a-oxoaldehydes, UV-detector (λ_{max} = 315 nm) and mass spectrometric detector ($[M+H]^+$ at m/z 131 for glyoxal; at m/z 145 for methylglyoxal) were used.

Carbonyls were analysed by a high-performance liquid chromatography using LCMS-2020 chromatograph ("Shimadzu", Japan) after the preliminary derivatization of the samples with DNPH (Figure 1B). Chromatographic conditions: Shim-pack VP-ODS column; a mobile phase of methanol/water; UV-detector (λ_{max} = 366 nm).

To determine the inorganic phosphate, solutions of $(NH_4)_2MoO_4$ in 1 M H_2SO_4 and 10% solution of $FeSO_4 \cdot 7H_2O$ in 7.5 mM H_2SO_4 (phosphate concentration more than $1 \cdot 10^{-5}$mol/L) or solutions of $(NH_4)_2MoO_4$, $(SbO)KC_4H_4O_6 \cdot 0.5H_2O$ in 9M H_2SO_4 and ascorbic acid (phosphate concentration less than $1 \cdot 10^{-5}$mol/L) were used. The concentration of inorganic phosphate ions was determined using a reagent-spectrophotometric method based on the formation of a yellow heteropoly acid during the reaction of orthophosphates with ammonium molybdate in an acid medium, which turns into an intensely stained blue compound (with the addition of Fe^{2+} λ_{max}=720 nm, with the addition of Sb^{2+} λ_{max}=880 nm) under the influence of reducing agents , on the SPECORD S600 spectrophotometer ("Analytik Jena", Germany).

Hydroperoxide-induced yields (Y, molecule/100 initiator molecules) were determined from the ratio between the concentration of the conversion products of Fru-1,6-P_2 and the sum of the concentrations of acetone and *tert*-butanol formed from t-BuOOH.

Radiation-chemical yields (G, 10^7 mol/J) of the formation of reaction products were determined in the linear sections of the dependence of the concentrations of substances on the absorbed dose. The results obtained in three independent experiments were used to calculate G and Y values. The error in radiation- and hydroperoxide-induced yields were estimated using the least squares method with the confidence coefficient value of 0.95.

EPR spectra were recorded at a room temperature in an E-109E spectrometer ("Varian", USA). Recording settings were as follows: microware power 20 mW, microware frequency 9.15 GHz, high frequency modulation amplitude 0.2 mT. Recording of the spectra was started 1 min after the reaction components had been mixed. The

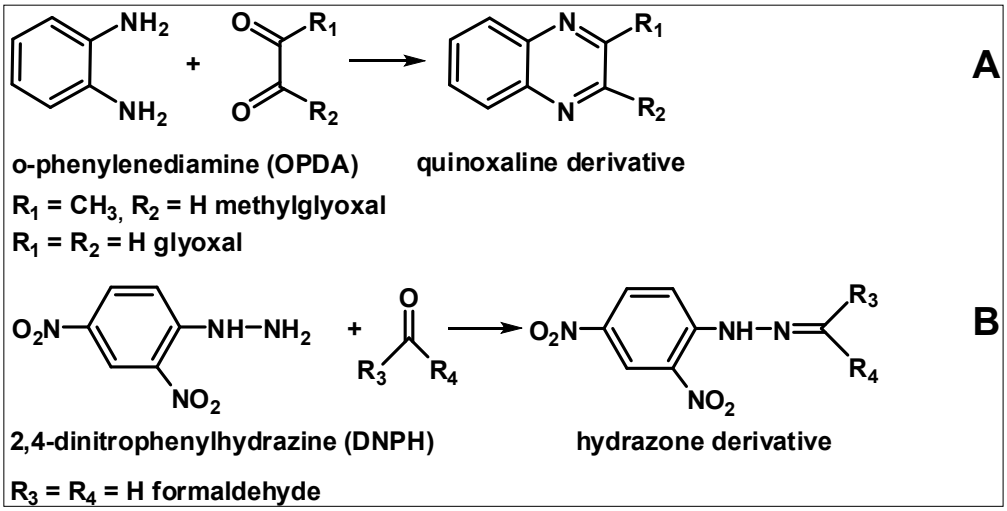

Figure 1. Derivatization of dicarbonyls and carbonyls using o-phenylenediamine and 2,4-dinitrophenylhydrazine, respectively

reaction mixture (80 µl) was introduced into PTFE Sub-Lite-Wall capillary tubing (inside diameter 0.635 mm, wall thickness 0.051 mm) ("Zeus Industrial Products", USA). The capillaries were folded four times and inserted into an EPR quartz tube, open at both ends, which allowed for an adequate gas flow (a mixture of O_2 and N_2) around the sample in EPR TE102 cavity. EPR signal of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as standard [20, 21]. EPR spectra were simulated by SimFonia software ("Bruker", Germany).

Generation of superoxide anion radical ($O_2^{\cdot-}$) was detected using two independent methods: reduction of NBT by superoxide and $O_2^{\cdot-}$ induced chemiluminescence of lucigenin. The kinetics of the accumulation of NBT reduction product, formazan, was determined by absorption at 560 nm in a Hitachi-557 spectrophotometer (Japan) at 25°C. To initiate the reaction methylglyoxal was added to the NBT-containing medium while L-lysine – to carbonate buffer (0,1M, pH 9.5). Chemiluminescence was measured by a Lum-5773 chemiluminometer (Russia) in the medium containing lucigenin (20 µM), L-lysine (0.015 M), and methylglyoxal (0.015M) in K,Na-phosphate buffer (0.1 M, pH 7.8). Measurements were performed at 37°C under the continuous stirring of the reaction medium. Statistical treatment of the data was performed using Student's *t*-criterion.

Results and Discussion

The Formation of Methylglyoxal and Other Products During the Degradation of Dlucose Metabolites Induced by Various Free Radicals

One of the main reasons behind the study of free radical processes is an extremely important and contradictory role they play in living systems. Lipid peroxidation is the most well-studied process of damaging biologically important substances, since the resultant product of this process – polyunsaturated fatty acid residues – are transformed with the formation of toxic oxidation products and oxidative degradation [1, 8-10, 22].

In our work we used a steady-state radiolysis technique to generate free radicals . It is free from the artifacts characteristic of the reagent-based methods. When exposed to radiation, water undergoes a breakdown sequence into hydroxyl radicals and other radical and molecular products (Figure 2).

Hydroxyl radicals ($^{\cdot}OH$) are highly reactive and consequently short-lived (*in vivo* their half-life is approximately 10^{-9} seconds [23]). They can also occasionally appear as byproducts of the immune response in living organisms (by macrophages and microglia) or in the mitochondria [3, 24]. Hydroxyl radicals can damage virtually all types of macromolecules: carbohydrates, nucleic acids, lipids and amino acids, which makes them very dangerous for the organism. Unlike superoxide, which can be detoxified by superoxide dismutase, hydroxyl radicals cannot be eliminated by an enzymatic reaction.

Our previous studies [25-27] proved that the fragmentation of hydroxyl-containing organic compounds procceding in parallel with (proceeding vis-à-vis) the formation of carbon-centered radicals is possible under hypoxic conditions (Figure 3).

Oxygen- and nitrogen-centered radicals formation can also trigger the destruction of the

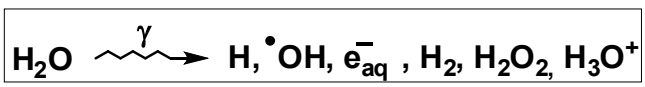

Figure 2. The main products of water radiolysis.

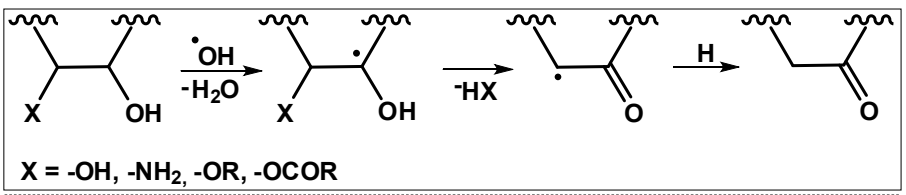

Figure 3. The mechanism of carbon-centered radicals formed in the reaction of hydroxyl radicals with organic compounds containing OH-group fragmentation [25-27].

biomolecules' carbon skeleton [11, 27, 28].

The data presented in Table 1 show the radiation-chemical yields of the major radiolysis products formed from Glc, its metabolites (Fru-1,6-P_2, G-3-P and Glc-6-P), as well as the reference compounds (Fru, Glu, Glc-1-P) in aqueous solutions.

As is well-known, the excessive formation of lipohydroperoxides, which in the Fenton-type reaction forms alkoxyl radicals ($LO^{\cdot}$), is associated with the concept of ferroptosis [22]. In the present study we used *t*-BuOOH/Fe^{2+} model system for alkoxyl radicals generation (Figure 4).

The data presented in Figure 5A show the hydroperoxide-induced yields (Y, molecule/100 initiator molecules) of the major products formed in Fru-1,6-P_2 aqueous 0.01 M deaerated solutions in the presence of *t*-BuOOH/Fe^{2+}.

As we can see from the data presented in Table 1, the main product of free radical transformations of Fru-1,6-P_2, G-3-P, Glc-1-P and Glc-6-P initiated by $^{\cdot}OH$ is an inorganic phosphate. The same can be stated for *t*-$BuO^{\cdot}$-induced transformations of Fru-1,6-P_2 (Figure 5A). Among other products of the transformations induced by hydroxyl and *tert*-butoxyl radicals dicarbonyl substances such as glyoxal and methylglyoxal (in the case of Fru derivatives and G-3-P) were found. It should be mentioned that in free radical transformations of Fru-1,6-P_2 initiated by Fe^{2+}/*t*-BuOOH the ratio between the amount of methylglyoxal and that of inorganic phosphate appeared to be by ~ 30 times higher than in the case of g-irradiation of the appropriate solutions (Figure 5A and 5B).

The resultant products obtained in the experiment can arise from the generation of carbon- and oxygen-centered radicals in the initial compounds which appeared after the interaction of the initial molecules with $^{\bullet}OH$ and *t*-$BuO^{\bullet}$. High yields of inorganic phosphate (Tables 1, Figure 5A) allow us to suggest that the mechanisms of free radical transformation of Fru-1,6-P_2 include two consecutive reactions of dephosphorylation of the initial molecule, as it is shown in Figure 6A. The formation of inorganic phosphate and methylglyoxal can also result from the sequence of reactions illustrated in Figure 6B.

In systems containing oxygen the radiation-chemical yield of inorganic phosphate from Fru-1,6-P_2 appeared to decrease by approximately 40% as compared to the argon-saturated systems (Table 1); while in oxygen-saturated systems glyoxal and methylglyoxal did not accumulate at all (Table 1). These experimental data can be account for the ability of molecular oxygen [29] to oxidize carbon-centered radicals at the α-position to hydroxyl groups, thereby

Table 1. Radiation-chemical yields (G, 10^7 mol/J) of the major radiolysis products formed in the sugars under the investigation and the aqueous solutions, their derivatives

System		G (products), 10^7mol/J			
Compound	Deaerated / oxygenated	Inorganic phosphate	Methylglyoxal	Glyoxal	Formaldehyde
G-3-P (0.003 M)	Ar	2.21±0.17	0.11±0.03	0.02±0.01	-
Fru-1,6-P_2 (0.01 M)	Ar	3.41±0.21	0.02±0.01	0.03±0.01	0.06±0.02
Fru-1,6-P_2 (0.1 M)	Ar	6.81±0.17	0.04±0.01	0.11±0.02	0.02±0.01
	O_2	2.99±0.70	n/d*	n/d	-
Fru (0.1 M)	Ar	-	0.09±0.02	0.02±0.01	0.14±0.04
	O_2	-	n/d	0	0.40±0.11
Glc (0.1 M)	Ar	-	n/d	0.04±0.01	-
Glc-1-P (0.1 M)	Ar	2.88±0.12	n/d	0.03±0.01	-
Glc-6-P (0.1 M)	Ar	1.34±0.14	n/d	0.06±0.02	-

*n/d – not detected

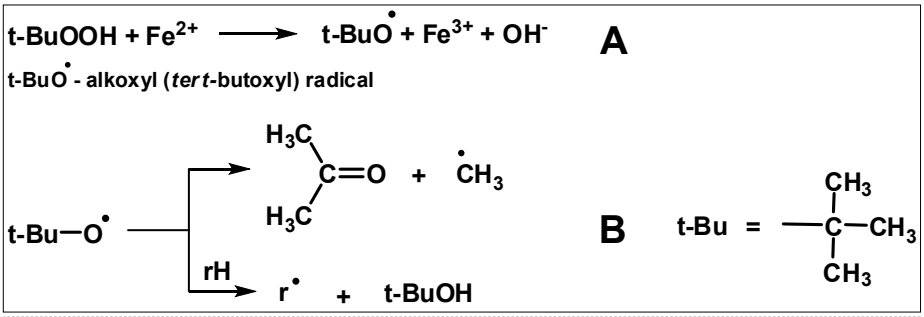

Figure 4. Generation of alkoxyl radicals (A) and the products of their further transformations (acetone and tert-butanol) (B) in a model system containing *t*-BuOOH and Fe^{2+} ions.

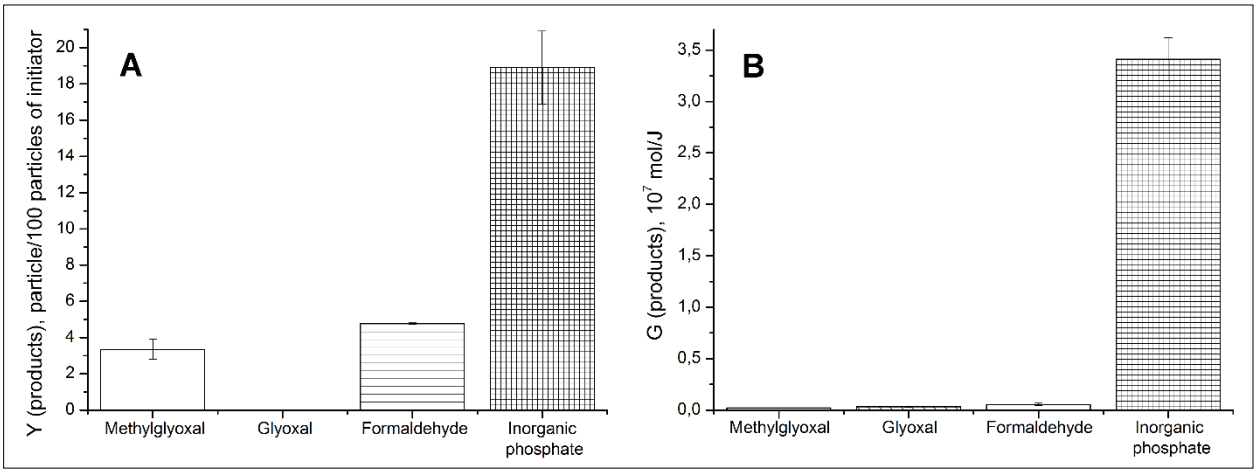

Figure 5. The ratio of the yields of Fru-1,6-P_2 transformation products in case of *tert*-butyl hydroperoxide initiation (A) *vs* radiation initiation (B) in aqueous 0.01 M deaerated solutions.

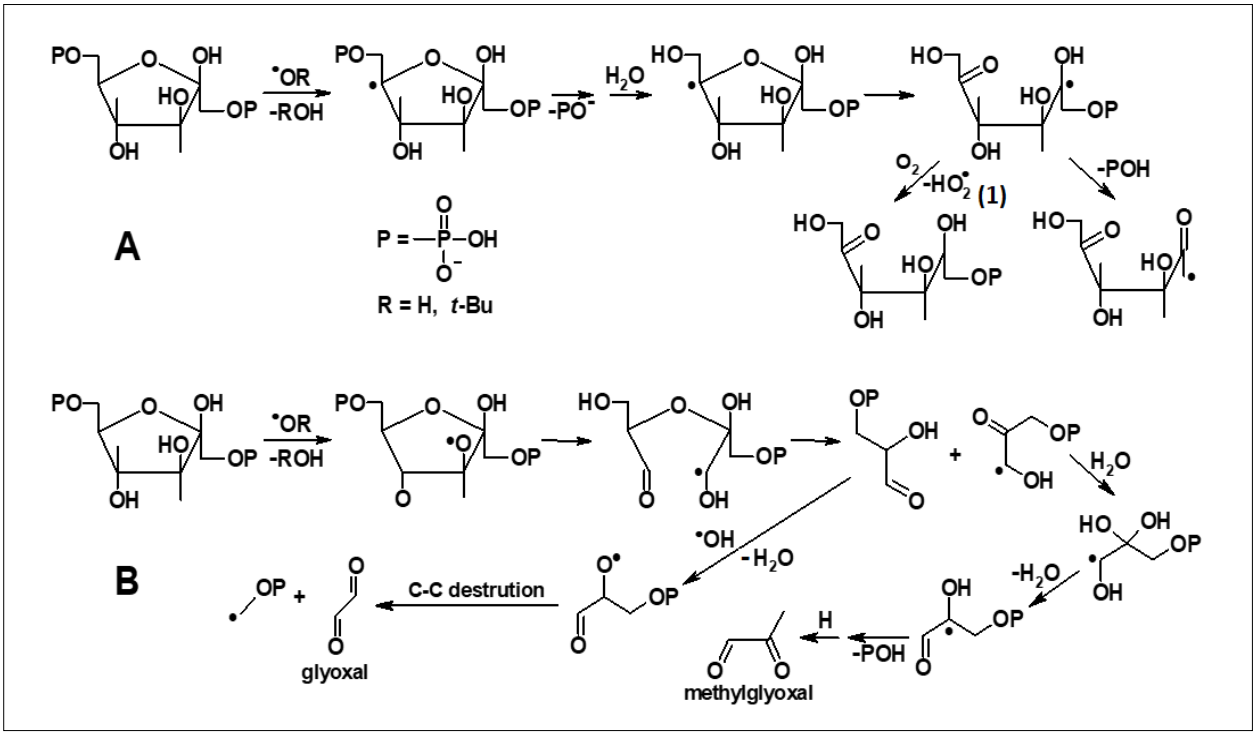

Figure 6. Proposed reaction mechanisms of Fru-1,6-P_2 transformations under the action of hydroxyl and alkoxyl radicals.

blocking their further transformations. Figure 6A shows a similar reaction 1 between oxygen and a free radical intermediate of Fru-1,6-P_2 degradation.

Formation of Superoxide and Organic Free Radicals in the Reaction of Methylglyoxal with Lysine.

According to the pertinent literature on the subject, α-oxoaldehyde such as methylglyoxal plays an important role in the non-enzymatic and enzymatic production of free radicals in biological systems [5, 17, 20, 30-32]. For example, the free radical derivatives can be formed in the reaction of methylglyoxal -induced protein glycation [18]. Since lysine elements are known to be one of the major targets for reactive carbonyl compounds in proteins [10]; we studied the interaction of this amino acid with methylglyoxal.

The application of chemiluminescence revealed that the superoxide anion radical gets formed in the mixture of methylglyoxal with L-lysine at pH 7.8 (Fig.7). Lucigenin-dependent chemiluminescence was measured in the reaction mixture that contained the following elements: K,Na-phosphate buffer (0.1 M, pH 7.8), 20 μM lucigenin, 0.015 M methylglyoxal, 0.015 M L-lysine (upper curve); the same + SOD 120 units (lower curve). In this figure the insert stands for the kinetics of formazane formation during the reaction of methylglyoxal with L-lysine. The reaction showed by the mixture ran as follows: carbonate buffer (0.1 M, pH 9.5), 10 mM methylglyoxal and 10 mM L-lysine (upper curve); the same + SOD 120 units (lower curve).

SOD under these conditions almost completely inhibits the chemiluminescence of lucigenin, which proves some dependence between this process and the presence of $O_2^{\cdot-}$ (Fig. 7, lower curve). We also assessed the superoxide anion production through the accumulation of formazan on NBT reduction in carbonate buffer, pH 9.5. (Inset, Fig. 7). The accumulation of formazan under these conditions might not depend on $O_2^{\cdot-}$, since NBT may probably be reduced by other intermediates of methylglyoxal reaction with L-lysine. Nevertheless, judging by the ability of SOD to inhibit significantly (more than 4 times) the formation of formazan under the above-mentioned conditions, we may conclude that the better part of NBT is reduced owing to the action of $O_2^{\cdot-}$ (Inset, Fig. 7).

Thus, the functioning of superoxide dismutase (SOD) effect has been revealed. Superoxide-depended

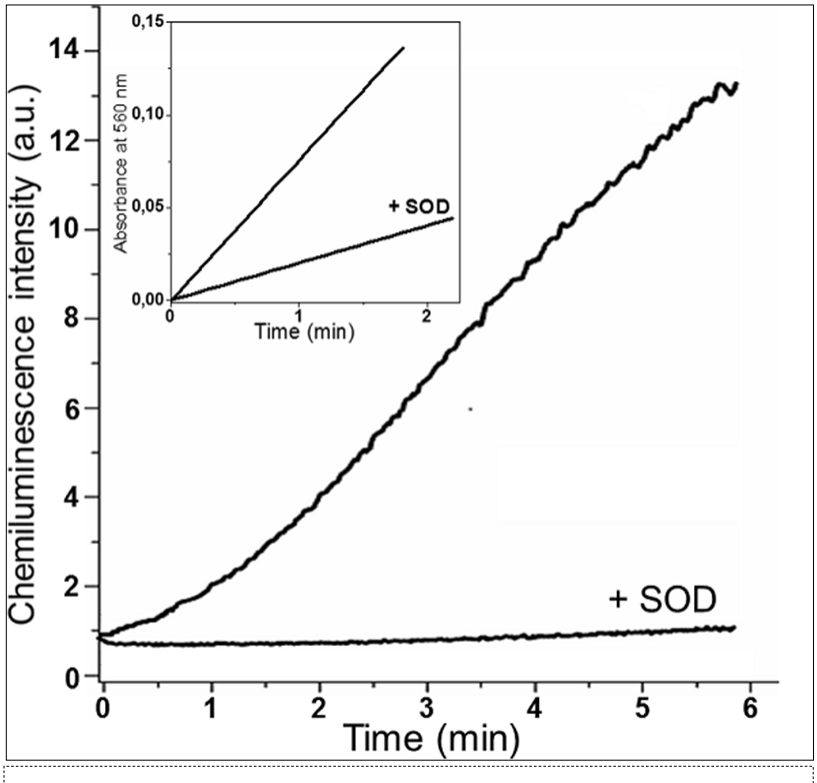

Figure 7. Production of superoxide anion radical in the reaction of methylglyoxal with L-lysine.

chemiluminescence of lucigenin in the reaction mixture contained: K,Na-phosphate buffer (0.1 M, pH 7.8), 20 μM lucigenin, 0.015 M methylglyoxal, 0.015 M L-lysine (upper curve); the same + SOD 120 units (lower curve). Inset is the kinetics of formazan formation during the reaction of methylglyoxal with L-lysine. The reaction showed by the mixture ran as follows: carbonate buffer (0.1 M, pH 9.5), 10 mM methylglyoxal and 10 mM L-lysine (upper curve); the same + SOD 120 units (lower curve).

The effect of SOD can account for the fact that this enzyme removes superoxide formed in the tested model system. Indeed, the data obtained in the experiment [17] is the evidence that $O_2^{\cdot-}$ is formed by a single-electron oxygen reduction by methylglyoxal anion radical ($MGO^{\cdot-}$) in accordance with the reaction:

$MGO^{\cdot-} + O_2 \rightarrow MGO + O_2^{\cdot-}$ .....(2).

Figure 8 shows the results of the EPR spectroscopic study of the products obtained in L-lysine reactions with methylglyoxal (Fig. 8, spectra B and E). The data presented in this figure demonstrate that free radical intermediates are formed under anaerobic conditions in the reaction between L-lysine and methylglyoxal. EPR spectra were recorded 4 min after the components had been mixed under aeration (spectrum A and D) or under nitrogen purging (spectrum B and E); several samples have been purged with nitrogen for 10 min (hypoxic conditions), which was followed by another 4 min of incubation after the switchover to aerobic conditions (spectrum C and F). In samples D-F SOD (400 units/ml) was added.

The EPR spectrum recorded in the reaction of L-lysine with methylglyoxal has a multicomponent hyperfine structure. Previously, such EPR spectrum has been recorded in the reaction mixture containing L-alanine and methylglyoxal [17]. In the present work, using C^{13}- and N^{15}-substituted and deuterated L-alanine derivatives it has been shown that the EPR spectrum is a superposition of signals of a methylglyoxal anion radical and a cation radical of Schiff base cross-linking (dialkylimine) that appears in the interaction between methylglyoxal and the amino acid. This statement allowed us to suggest that the EPR spectrum observed in our experiments can be also a superposition of $MGO^{\cdot-}$ signals and the cation-radical of methylglyoxal dialkylimine with lysine. Noteworthy as well, only trace quantities of free radical intermediates were registered under aeration of the reaction mixture (Fig. 8, spectra A and D).

In samples D-F added SOD (400 units/ml). EPR spectra were recorded 4 min after the components had been mixed under aeration (spectrum A and D) or under nitrogen purging (spectrum B and E); several samples were purged with nitrogen for 10 min (hypoxic conditions) and followed by another 4 min of incubation after the switchover to aerobic conditions (spectrum C and F). The simulation of EPR spectra of methylglyoxal anion radical (G).

The decrease in the concentration of organic free radicals recorded by EPR in aerated reaction medium is probably not associated with the inhibition of their formation. Indeed, with nitrogen purging the content of the organic free radicals, the reaction reached its maximum in 8-9 min after the reaction components had been mixed; but after gas was replaced with air the level of these free radicals in the medium dropped dramatically (Fig. 8 and 9).

Under these experimental conditions SOD most probably would reduce the rate of the intensity of EPR signal decreasing during aeration (Fig. 9). The EPR spectrum containing five components of hyperfine structure and a g-factor equal to 2.0045 were recorded during aeration of the reaction medium in the presence of SOD (Fig. 8, spectrum F).

According to the data presented in the literature, the characteristics of the EPR spectrum presented in Fig. 8 (spectrum F) correspond to the signal of *cis*-form of methylglyoxal anion radical ($MGO^{\cdot-}$) [17, 20, 33]. This fact confirms the above-mentioned assumption that free radical intermediates of L-lysine reaction with methylglyoxal are $MGO^{\cdot-}$ and the cation radical of dialkylimine. Thus, molecular oxygen seems to interact directly with the free radical derivatives of methylglyoxal and dialkylimine; the products formed in this reaction are not registered by EPR (Fig. 8 and 9). However, SOD protects the methylglyoxal anion radical under aerobic conditions, which proves the possibility of $MGO^{\cdot-}$ elimination under the effect of superoxide. Indeed, it has been found out that in aqueous media $O_2^{\cdot-}$ reduces some organic radicals [34, 35]. In particular, superoxide is an electron donor for the reduction of the protonated

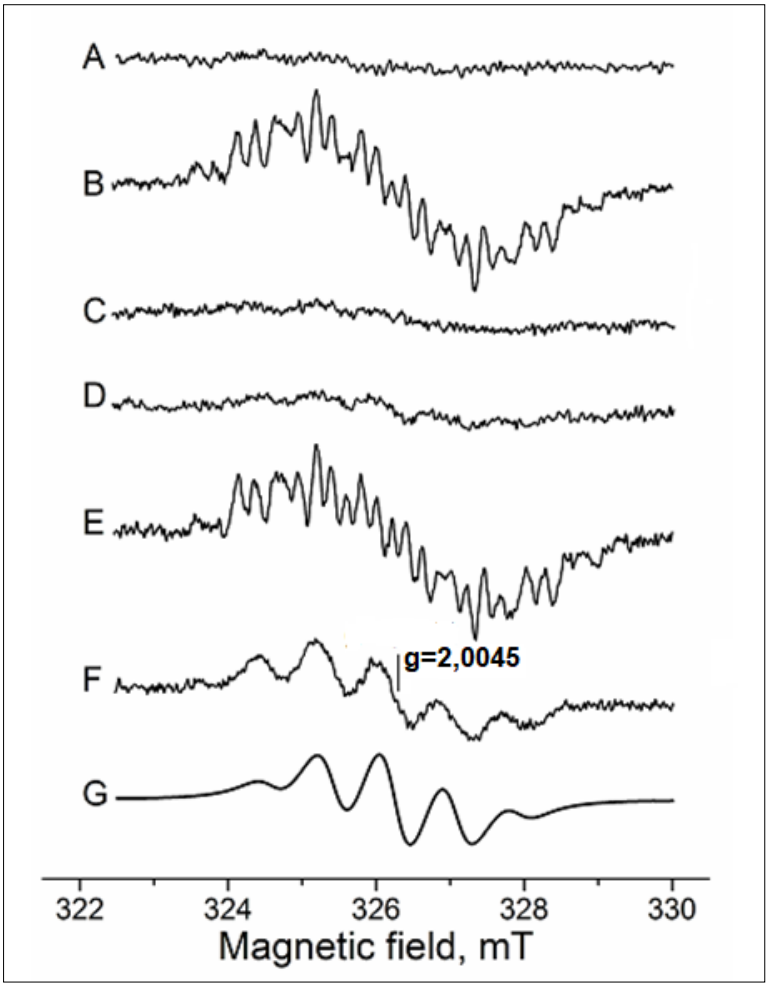

Figure 8. EPR spectra of organic free radicals formed in the reaction of a mixture containing methylglyoxal (0.16 M) and L-lysine (0.16 M) in K,Na-phosphate buffer (0.2 M, pH 7.8) (A-F). The simulation of EPR spectra of methylglyoxal anion radical (G).

nitrosobenzene anion radical [36]. By analogy, it can be suggested that $O_2^{\cdot-}$ interacts with the methylglyoxal anion radical, reducing it to non-radical products in accordance with the reaction:

$MGO^{\cdot-} + H^+ + O_2^{\cdot-} \rightarrow$ non-radical product + O_2 .....(3)

The state-of-the-arts analysis [17, 18, 30] revealed that a sequence of reactions normally result in the formation of free radicals in the interaction of amino acids with carbonyl compounds (Fig. 10). The presented scheme shows that dialkylimine is a Schiff base, which is a product of interaction of methylglyoxal carbonyl groups with two L-lysine molecules. As a result of the reaction of dialkylimine with another molecule of α-oxoaldehyde, the Schiff base cation radical and $MGO^{\cdot-}$ are formed, respectively (Fig. 10). Such a peculiarity of the chemical structure seems to reduce the ability of dialkylimines to participate in reactions of single-electron oxidation/reduction. Since SOD less effectively prevents the dissipation of the dialkylimine cation radical than that of methylglyoxal anion radical (Fig. 8 and 9), it can be suggested that a free radical derivative of dialkylimineis is mainly used in the reactions with molecular oxygen. Carbon-centered free radicals of amino acids, lipids and sugars are known to interact with molecular oxygen with the formation of peroxyl radicals, which are further transformed into non-radical products [1, 2, 9, 12, 13]. However, in contrast to the carbon-centered allyl radicals, a free electron in the dialkylimine cation radical is localized in the system of coupled double bonds between the Schiff base nitrogen atoms and methylglyoxal carbons (Fig.

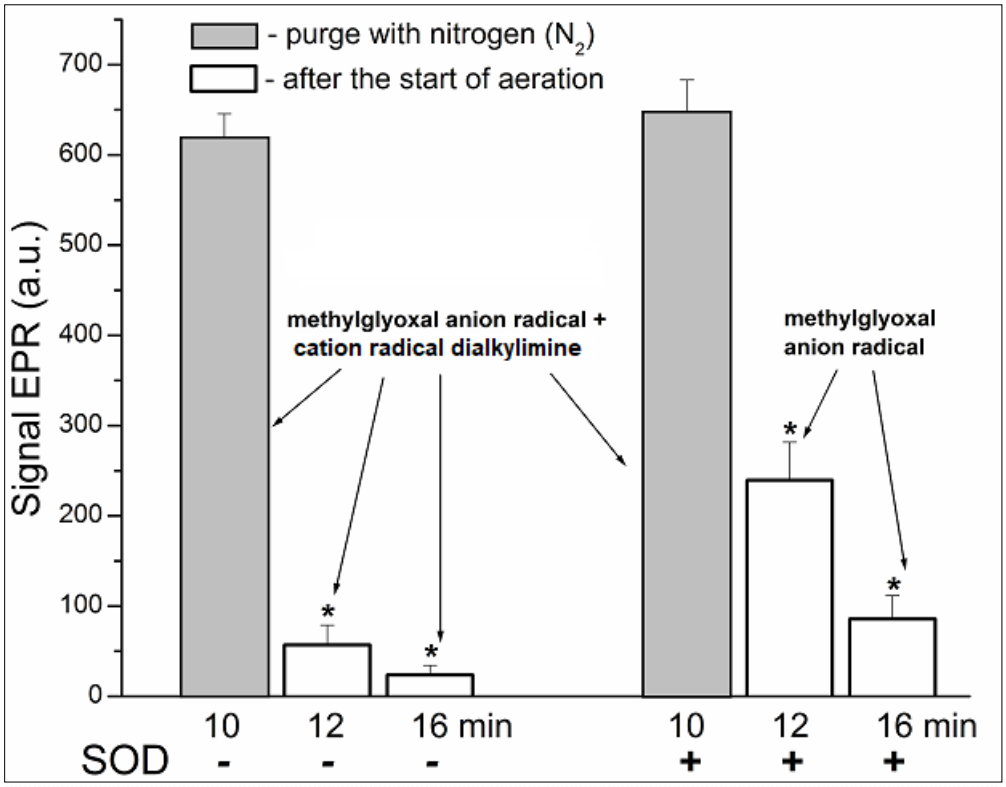

Figure 9. The effect oxygen and SOD produce on the level of organic free radicals resulting from the reaction between methylglyoxal and L-lysine. In the figure: EPR signals of these radicals recorded under nitrogen purging are put in gray; the same samples 2 and 6 min after the beginning of aeration – in white. The reaction mixture content was the same as in the legend to Fig. 3. All values are mean ± SEM. n=5; *p<0.01.

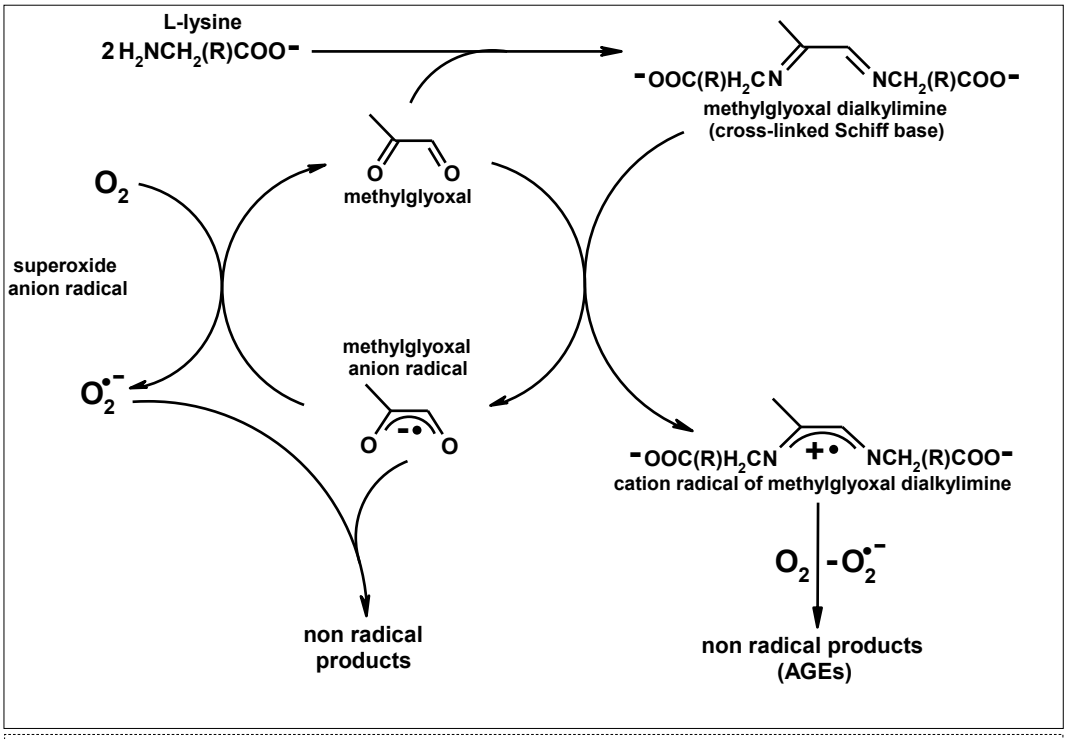

Figure 10. Proposed mechanisms for the formation of various products during the interaction of methylglyoxal with L-lysine.

10). Meanwhile, such a system of coupled double bonds is present in the molecule of paraquat (methyl viologen). The reaction of a single-electron oxygen reduction by the paraquat cation radical normally forms a paraquat dication and a superoxide anion [1, 21]. The analogous reaction between oxygen and the dialkylimine cation radical may probably occur under the conditions of our experiments.

The obtained results allow us to extend and expand the previously proposed mechanism for generating the superoxide radical during the glycation reaction of amino acids by methylglyoxal [17]. The functioning of the mechanism presupposes that the reactions presented in Fig. 10 result in the formation of $MGO^{\cdot-}$, which further reduces oxygen to $O_2^{\cdot-}$ (reaction 3). We assume that $O_2^{\cdot-}$ is not only formed in the reactions involving $MGO^{\cdot-}$ but also seems to participate in them (Fig. 10).

These assumptions explain the effect of oxygen on the kinetics of accumulation and dissipation of free radicals having emerged in the mixture of L-lysine and methylglyoxal. The paper [17] has shown that accumulation of $MGO^{\cdot-}$and Schiff base cross-linked cation radical depends on the oxygen concentration only insignificantly. According to our data, this fact may be associated with the application of L-alanine as an amino acid in the cited work and by a high pH value of the reaction medium (carbonate buffer, pH 9.5). It should be pointed out that the model system proposed in the paper (phosphate buffer, pH 7.8) is much closer to physiological conditions. Thus, it is most likely that oxidative modification of proteins and other biomolecules might be the influence a local generation of superoxide produces on the interaction between the residues of L-lysine (and probably other amino acids) and α-oxoaldehydes. This phenomenon of non-fermentative superoxide generation might be an element of autocatalytic intensification of pathophysiological action of carbonyl stress.

Glycation of biopolymers by reactive carbonyl compounds (Maillard reaction) leads to the formation of the advanced glycation end products – the biomarkers of pathophysiological processes associated with diabetes and various other metabolically grounded diseases [5, 7, 10]. Using different regulatory pathways, these products can provoke NADPH oxidase and mitochondria to form a superoxide radical [3, 24, 37]. Oxidative stress in diabetes mellitus can also be triggered by inhibition of antioxidant enzymes, which, in its turn, was caused by reactive dicarbonyl compounds [15, 38]. At the same time, as it has been noted above, the non-enzymatic reactions may get involved in the formation of $O_2^{\cdot-}$ under the carbonyl stress.

Recent studies have shown that glucose oxidation by a hydroxyl radical leads to the formation of a-oxoaldehyde as 3-deoxyglucosone [39], that agrees with the proposed mechanism of non-enzymatic synthesis of methylglyoxal. The data obtained in our study also highlight the important role the free radical reactions play in non-enzymatic transformations of sugars and other carbonyl compounds. It should be also noted that the synthesis of methylglyoxal from hexose phosphates in the presence of reactive oxygen and organic free radicals and provokes the consequent formation of $O_2^{\cdot-}$ in modifications of amino acids by methylglyoxal. Thus, under the conditions of hyperglycemia the oxidative stress can cause non-enzymatic accumulation of methylglyoxal, which, in its turn, can further stimulate oxidative stress as well. Therefore, the concepts of 'oxidative stress' and 'carbonyl stress' should be considered rather controversial, since various stages of these processes typical of these stresses not infrequently merge and converge into each other. These data may allow for proposing that molecular mechanisms provoking the diseases associated with oxidative and carbonyl stress (such as atherosclerosis and diabetes) should be more complicated than they used to be thought of and are to be studies with close attention to the interplay of reactive carbonyl compounds and ROS.

Conclusions

The obtained data should be taken into account when describing the free radicals giving rise to the formation of dicarbonyls in pathophysiological processes involving Fru-1,6-P_2 and other Glc metabolites. At the same time, the toxicity of α-oxoaldehydes can be associated with further free radical reactions. Indeed, methylglyoxal is not only a product of free radical processes, but also plays an important role in their development.

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