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CHEMISTRY

Ways of Decreasing the Oxidative Stress Promoted by Organomercury Compounds

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The oxidative stress of an organism resulting in metabolism disorder is related to the peroxide oxidation of lipids (POL) and to changes in the structure of cell membranes [1]. Detailed studies of POL processes showed that a number of exogenous agents exist, for example, metal salts and organic radicals, which promote the formation of hydroperoxides or carbonyl compounds, which are products of their decomposition, and accumulation of these products in the cell [2]. In addition, assays carried out for the whole organism indicate that toxic organomercury compounds RHgX and R_2Hg are accumulated in cell membranes due to their lipophilic properties and accelerate POL [3, 4]. However, scarcity of the available data on the mechanism of toxic action of RHgX and R_2Hg at the molecular level do not allow one to propose a general concept for the choice of detoxifying agents. Currently, thio derivatives and complexones [5], for example, 2,3-dimercaptopropanol and calcium disodium ethylenediaminetetraacetate, are used most often as detoxifying agents against mercury poisoning. The action of these agents is based on the binding of Hg atoms to give Hg–S or Hg–O bonds; however, it does not imply the possibility of deactivation of the reactive C-centered organic radicals resulting from the homolytic cleavage of the Hg–C bond, which inevitably accompanies the involvement of RHgX and R_2Hg in biochemical redox and radical processes.

Previously, we showed [6] that RHgX and R_2Hg with various organic groups R inhibit the electron transport in respiratory chains; this results in depressed cellular respiration. The assumption that processes induced by the active organic radicals $R^{\cdot}$ contribute to the overall mechanism of the toxic action of RHgX and R_2Hg was confirmed by the observation that the toxic effect of these substances markedly decreases upon the addition of a natural inhibitor of radical reactions, namely, a group E vitamin, α-tocopherol [7].

Here, we study the action of mercury compounds on the peroxide oxidation of model substrates, structural fragments of lipids: (*Z*)-9-octadecenoic (oleic) acid ((*Z*)-$CH_3(CH_2)_6CH_2CH{=}CHCH_2(CH_2)_6COOH$) and its methyl ester, in the presence of antioxidants.

The toxic mercury compounds we studied were CH_3HgI (formed in the natural processes of biomethylation of Hg^{2+} salts), C_6H_5HgBr (used as a herbicide and a spermicide [3]), and $HgCl_2$. The concentrations of the hydroperoxides derived from oleic acid and methyl oleate and of carbonyl compounds formed upon hydroperoxide decomposition were determined by standard methods, namely, by iodometric titration and by spectrophotometry on the basis of the reaction with thiobarbituric acid in a temperature-controlled setup for 5 h at 65°C with a constant air supply [8, 9].

The figure shows the kinetic curves for the accumulation of hydroperoxides of oleic acid in the presence of mercury compounds; the curves show exponential patterns. In logarithmic coordinates, they give linear functions with correlation coefficients close to unity, which corresponds to pseudo-first-order reactions. The parameters for the oxidation of oleic acid and methyl oleate determined on the basis of accumulation of hydroperoxides and carbonyl compounds are listed in Table 1.

Our findings indicate that the concentrations of substrate oxidation products increase and the process as a whole is accelerated in the presence of mercury compounds; the protection of the carboxy group in the oleic acid by using the methyl ester has virtually no influence on the general process, indicating that the rates of radical processes and the rates of reactions of mercury compounds at the carbonyl group are incommensurable. In terms of the effect on the substrate oxidation, the mercury compounds can be arranged in the following sequence:

$$HgCl_2 > CH_3HgI > C_6H_5HgBr.$$

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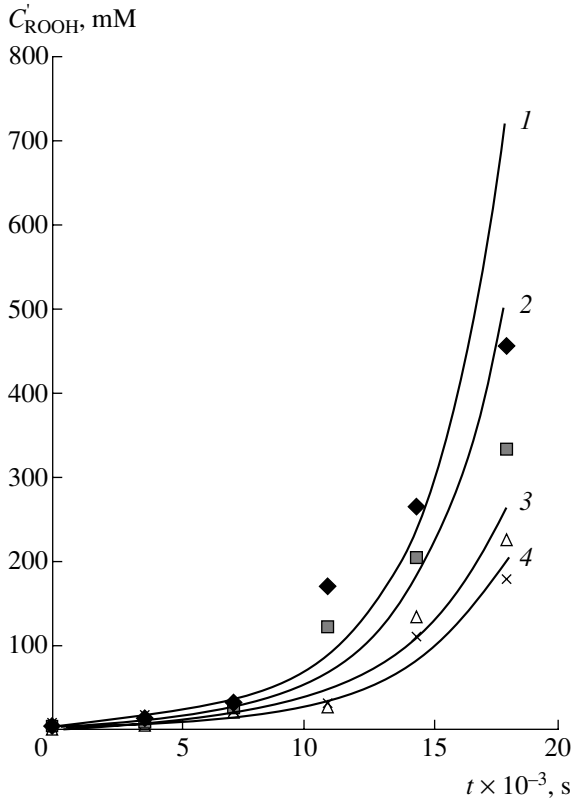
Table 1. Kinetic parameters of the autooxidation of oleic acid and methyl oleate in the presence of mercury compounds

Additives	Oleic acid		Methyl oleate	
	$k' \times 10^{-4}$, s^{-1}	$k'' \times 10^{-4}$, s^{-1}	$k' \times 10^{-4}$, s^{-1}	$k'' \times 10^{-4}$, s^{-1}
None	2.42 ± 0.08	0.69 ± 0.06	2.78 ± 0.06	0.66 ± 0.03
$HgCl_2$	3.21 ± 0.17	1.19 ± 0.08	3.54 ± 0.13	1.28 ± 0.06
CH_3HgJ	3.01 ± 0.15	1.09 ± 0.02	3.35 ± 0.12	1.16 ± 0.03
C_6H_5HgBr	2.61 ± 0.06	0.82 ± 0.01	3.34 ± 0.09	0.89 ± 0.03
	A'	A''	A'	A''
None	1	1	1	1
$HgCl_2$	2.55	2.31	3.43	3.18
CH_3HgJ	1.85	2.11	2.44	2.72
C_6H_5HgBr	1.29	1.36	1.67	1.6

Note: Here and in Table 2, k' and k'' are the initial rate constants for the overall accumulation of hydroperoxides and carbonyl compounds, respectively; A' and A'' are the changes in the concentrations of hydroperoxides and carbonyl compounds, respectively, relative to the values in the reference sample without additives ($A = [(C_i - C_{oi})/C_o] : [(C_{ir} - C_{oir})/C_{or}]$; C_i and C_{ir} are the product concentrations in the presence of additives and in the reference sample, respectively; C_{oi} and C_{or} are the initial concentrations in the presence of additives and in the reference sample, respectively).

When interpreting these results, the nature of the groups attached to mercury should be taken into account.



Kinetic curves for the accumulation of oleic acid hydroperoxides in the presence of (*1*) $HgCl_2$; (*2*) CH_3HgI; (*3*) C_6H_5HgBr; and (*4*) without additives (65°C, air bubbling, concentrations of the additives 1 mmol/l).

The results of a detailed study [10] of the autooxidation mechanism of oleic acid and methyl oleate as substrates with the general formula R'H indicate that the formation of the peroxy radical $R'OO^{\bullet}$ in the reaction of the corresponding substituted allyl radical $R'^{\bullet}$, generated at the initiation step, with dioxygen is a diffusion-controlled process; hence, the rate constant of the overall accumulation of isomeric R'OOH hydroperoxides is dominated by the abstraction of a hydrogen atom by the $R'OO^{\bullet}$ radical, whose concentration in the reaction medium is maximum. However, it is known [11] that electron-withdrawing oxygen-centered radicals (and, hence, peroxyl radicals, which belong to this group) are reactive reagents with respect to mercury compounds in the radical substitution reaction proceeding according to Scheme 1.

$$R'OO^{\bullet} + RHgX \rightarrow R'OOHgX + R^{\bullet},$$

$R'OO^{\bullet}$ are peroxyl radicals corresponding to the R'H substrates;

$$R = C_6H_5, CH_3, Cl; \quad X = Cl, Br, I.$$

Scheme 1.

When the initial concentration of the $R'OO^{\bullet}$ radicals is either comparable with or higher than the RHgX concentration, the reactive $Cl^{\bullet}$, $CH_3^{\bullet}$, and $C_6H_5^{\bullet}$ radicals, which differ in reactivity and in further transformation routes, are generated in the reaction medium. The atomic chlorine and the methyl radical are effective initiators of radical processes. However, unlike $Cl^{\bullet}$, the methyl radical is also able to enter into the competing reaction with O_2, which contributes to the total concen-

Table 2. Kinetic parameters of the autooxidation of oleic acid in the presence of mercury compounds and antioxidants

Additives	Mercury compounds			
	none	$HgCl_2$	CH_3HgI	C_6H_5HgBr
	$k' \times 10^{-4}$, s^{-1}			
None	2.42 ± 0.08	3.21 ± 0.17	3.01 ± 0.15	2.61 ± 0.06
α-Tocopherol	1.28 ± 0.05	1.86 ± 0.19	1.89 ± 0.02	1.67 ± 0.12
α-Tocopherol acetate	1.66 ± 0.12	2.13 ± 0.02	2.16 ± 0.01	1.88 ± 0.14
2,4,6-Tri-*tert*-butylphenol	0.3 ± 0.12	1.62 ± 0.01	1.12 ± 0.02	1.29 ± 0.09
2,6-Di-*tert*-butylphenol	1.73 ± 0.15	2.43 ± 0.02	2.47 ± 0.01	2.08 ± 0.14
	A'			
None	1	2.55	1.85	1.29
α-Tocopherol	0.1	0.55	0.53	0.29
α-Tocopherol acetate	0.36	0.8	0.78	0.46
2,4,6-Tri-*tert*-butylphenol	0.01	0.256	0.2	0.13
2,6-Di-*tert*-butylphenol	0.36	0.93	0.94	0.65

tration of $R'OO^{\bullet}$ and R'OOH. Both the stability and lifetime of the $C_6H_5^{\bullet}$ radical are much greater and the reaction with O_2 is unlikely; thus, the major route of transformation of this radical is also related to the initiation step, i.e., to the abstraction of a hydrogen atom from R'H. These views do not contradict the experimental dependence of the activity of mercury compounds on the nature of group R.

To confirm the role of organic radicals formed from mercury compounds, here we studied autooxidation of oleic acid and methyl oleate in the presence of RHgX and radical inhibitors: the natural antioxidant α-tocopherol and its acetate and the synthetic analogues, 2,6-di-*tert*-butylphenol and 2,4,6-tri-*tert*-butylphenol, were employed.

Table 2 presents the kinetic parameters for the accumulation of oleic acid hydroperoxides in the presence of mercury compounds and various antioxidants. It can be seen that 2,4,6-tri-*tert*-butylphenol, which forms a stable phenoxyl radical, is an effective inhibitor of the radical oxidation of oleic acid, the most pronounced effect being observed when CH_3HgI is present in the reaction mixture. These results are consistent with the data obtained in [12], dealing with determination of the rate constants for the abstraction of hydrogen from substituted phenols by peroxyl and alkyl radicals.

Thus, our findings suggest that free organic radicals formed upon the rupture of the Hg–C bond may directly participate in the molecular mechanisms of toxicity of organomercury compounds and that natural and synthetic antioxidants can be used as detoxifying agents.

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