Peroxidation Kinetics involving the Complexes of Cytochrome *c* and Cardiolipin in the Presence of Lipid Antioxidants

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Abstract — **This paper represents a theoretical description of the kinetics, taking into account the mechanism of antiradical activity of the antioxidant. Based on the analysis of this problem, a kinetic model is formulated that includes two pathways in which the reactions characteristic of the lipid peroxidation process take place. The enzymatic pathway includes reactions involving the complex of cytochrome *c* and cardiolipin, and the non-enzymatic pathway is reactions involving the free lipid radicals. A system of differential equations describing the kinetics of the lipid peroxidation process is constructed both with and without the inhibiting effect of the antioxidant. Relying on the existing theoretical and experimental data, the considered kinetic model of the lipid peroxidation process can be used to study and compare the antiradical activity of various antioxidants, in particular vitamin E (α-tocopherol) and some of its homologues with the shortened side chains.**

Index Terms — **antioxidant, complex of cytochrome *c*–cardiolipin, free radicals, kinetics, lipid peroxidation**

I. INTRODUCTION

Owing to the fact that the complexes of the cytochrome *c* (Cyt *c*) and cardiolipins (CLs) of various types show the peroxidase activity, their properties and structures are currently being intensively studied [1-7]. Special attention of the researchers in the field of the biology and medicine attracts the problem of apoptosis of the cell (see the numerous works on this issue cited in the reviews [4, 5]). Apoptosis can be initiated by the lipid peroxidation (LPO), as it causes the pathological disturbances of the barrier properties of the lipid bilayer in the cell membranes. The fact that, namely, the oxidized CL is required for the release of the proapoptotic factors, has been confirmed experimentally [1]. The results of [1] specify a new way of launching a cell death when the reactive oxygen species (ROS) accumulate in the cell initiating a formation of the free radicals (FRs) of lipids and their oxidized forms (lipid hydroperoxides), and this was associated with the peroxidase activity of the complex of cytochrome *c* and cardiolipin (Cyt-CL) formed in the mitochondrial membranes. The main structural features of such complexes are presented in [6, 7] (see also the Refs 103 and 105 from [5]). It is important to note that Cyt *c* in the absence of CL exhibits the extremely weak peroxidase activity [6], but namely in the complex with CL its peroxidase activity grows substantially, making this complex an active participant in the membrane processes.

Before proceeding to the kinetics of the peroxidase process and the system of differential equations (DE) corresponding to it, we briefly describe the components of the Cyt-CL complex and outline some of the common properties of the antioxidants – more complete information on these issues can be found in the reviews [4, 5].

Cytochrome *c* is the smallest and the alone water-soluble protein in the cytochrome family, in which about 30 species. Cytochromes, as the components of mitochondrial membranes, are present in all eukaryotic cells (animals and plants). Cyt *c* contains in its structure a heme type *c*, localized in the internal protein cavity by means of the covalent bonds with the amino-acid residues. Due to such structure, Cyt *c*, like other cytochromes, plays the role of a catalyst for the oxidation-reduction (redox) reactions. In a living cell, Cyt *c* is usually located (loosely bound) outside the inner mitochondrial membrane, and, above all, it performs two major functions (see Refs 92-95 from [5]). Firstly, it is an one-electron carrier in the respiratory chain, since it is capable of the oxidation and reduction without the oxygen participation. Secondly, it is an activator of apoptosis, since under certain conditions it is able to detach from the membrane, passing into a solution in the intermembrane space, and then from it into the cytosol, thereby activating the apoptosis of the cell.

Phospholipid cardiolipin (diphosphatidylglycerol) in the eukaryotic cells is an important component of the internal mitochondrial membranes (for more details, see Refs 96-102 from [5]) – its fraction from their total lipid composition is about 20%, and it is found even in membranes of bacterial microorganisms. Phospholipids of this type have a dimeric structure in which two phosphatidylglycerols are connected to each other by a glycerol residue, as a "bridge". CL consists of two residues of the orthophosphoric acid H_3PO_4 (a polar head part) and four long chains of the fatty acids (a nonpolar tail part), and each of these four alkyl chains C_{18} in the majority of the animal tissues has two unsaturated bonds. Owing to the specific physical and chemical properties, the phospholipids play a very important role in the structure

and functioning of biomembranes. In the phospholipid molecules, the oxygen-containing head parts are hydrophilic, and the long massive tail parts are hydrophobic. Herewith, their head part is easily deprotonated, acquiring a negative charge. Thus, CLs belong to the complex (multicomponent) lipids – the products of their hydrolysis are the alcohols and carboxylic acids, and also, the phosphoric acid and carbohydrate. That is to say, in addition to the structural functions, the CLs also perform the metabolic functions.

In the LPO process considered here, the hydrogen peroxide (H_2O_2) performs as an activator of the Cyt-CL complex. In the organism, H_2O_2 is the most prevalent reagent from the ROS family, since, being a stable and neutral molecule it is able to penetrate almost all cellular structures. In general, the oxidation processes of the various substrates by oxygen on the cellular level those are necessary in the organism under the aerobic conditions for the normal course of metabolism occur due to ROS that are formed in the cell or come from outside [4]. The ways of using the oxidative potential of the oxygen molecule, the main sources and conditions for the appearance of ROS in the organism, and the chemical and physical properties of the various types of ROS are described in detail in the monograph [8]. In an organism, under the physiologically normal or close to normal conditions, the system of antioxidant defense (AOD) effectively protects against the destructive action of the ROS, FRs and lipid peroxides mainly through the regulation of the FR concentrations of different types and the rates of the radical and redox reactions accompanying any process on the cellular level, including the LPO process.

The model presented in this paper for the kinetics of the LPO process, caused by the peroxidase activity of the Cyt-CL complex, is formulated solely to take into account the influence of the direct-acting antioxidants, that is, antioxidants (AOs) that directly extinguish the FRs. Influence of the indirect-acting AOs, activating other components of the AOD system, this model does not imply. In the AOD system containing two main functional branchs: the enzyme and non-enzyme branchs, the direct-acting AOs are components of the non-enzyme branch, which, unlike the enzyme branch, includes mainly the low-molecular chemical compounds. The direct-acting AOs manifest themselves as the most operative means of the antiradical protection of a living cell. After the interception (scavenging) of the FRs, they pass into oxidized or stable radical forms, and then, under the action of the corresponding enzymes or other AOs, they are again transformed into the reduced forms (synergy). The manifestation of a synergy among the FRs in relation to each other (the enhancement of the antioxidant effect at the joint action and/or the conversion of each other to the reduced forms) is a very important property of the AOD system. In general, the main features and principles of interaction of the AOD system with ROS, FRs and lipid peroxides in many respects coincide for all types of the eukaryotes (in more detail the AOD system as a whole and the various types and properties of its components are discussed in the review [5]).

II. THEORETIAL APPROUCH

Turning to the description of the theoretical model of the kinetics of the peroxidase process for the Cyt-CL complexes with account of the influence of the AO, it is convenient to emphasize two ways of the LPO process – the enzymatic pathway, which is realized as a catalytic cycle, and the non-enzymatic pathway on which the reactions proceed involving the lipid FRs.

The catalytic cycle for the Cyt-CL complex will be described using the reaction scheme proposed in [4]. The graphical scheme of the reactions shown in Fig.1 describes the peroxidase activity of the Cyt-CL complex realized within the framework of its catalytic cycle. The continuation of the LPO process caused by the peroxidase activity of the Cyt-CL complex occurs on a non-enzymatic pathway involving the lipid FRs, which arise in a course of the reactions of the catalytic cycle and initiate the chain reactions of the lipid oxidation.

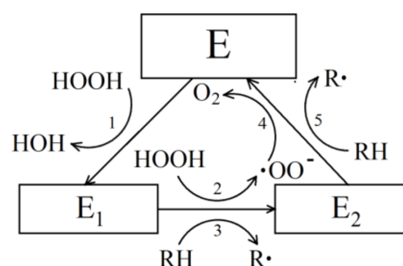

Fig.1. Scheme of the catalytic cycle for the Cyt-CL complex.

The scheme shows that the Cyt-CL complex, being initially in the non-activated state E, successively passes through three stages involving the activated states E_1 and E_2. The conformational features of the states E_1, E_2 and a description of the chemical reactions at these three stages are given in [4, 5]. Here, an attention should be paid to the important feature of the Cyt-CL complexes: the products of lipid oxidation (hydroperoxides ROOH), in addition to the lipid molecules (RH), are also the substratum on the enzymatic pathway of the POL process [9] – this makes a significant contribution to the kinetics of accumulation of the LPO products. In the experimental work [9], the kinetics of the accumulation of the POL products for the tetra-linoleoyl cardiolipid (TLCL) was studied in detail, accounting the antioxidant effect (for α-tocopherol and each of its homologues separately).

Since the formation of an excessive amount of the lipid hydroperoxides due to the reactions involving FRs proceeding on the non-enzymatic pathway is the push (impetus) for the initiation of the apoptosis process [1], with the help of the direct-acting AOs it is possible to achieve the effective control of the LPO process, mainly through its free radical stage, and hence, to gain the control over the effect of apoptosis as a whole [4, 5]. Namely, the direct-acting AOs, disrupting the radical reaction chains, inhibit the process of product formation, and are necessary as a means of the operative regulation of the peroxidase activity of the Cyt-CL complex.

The most important representative of this class of AOs, protecting the cell from the oxidative stress, which occurs at a deviation from a physiologically normal level of the

ROS concentrations involved in a cellular metabolism, is vitamin E. The results of the extensive investigations (see Refs 117-123 from [5] and the corresponding works cited in [9]), devoted to the study of the various properties of vitamin E, show that vitamin E should be considered as the main fat-soluble antioxidant. In general, two groups of chemical compounds – tocopherols and tocotrienols (4 species in each group: α-, β-, γ- and δ-) belong to vitamin E, and among them α-tocopherol is considered to be the most biologically active form of vitamin E [10].

We also note that tocopherols in the biomembranes perform a structure-forming function, through a modifying action on the phospholipid bilayers. In this connection, an attention should be paid to the ability of α-tocopherol to form the complexes with the phospholipids. The common membranotropic effect of tocopherols consists in their ability not only to intercept the lipid FRs, but also modulate the physical properties of the lipid bilayer, maintaining the necessary packing density of phospholipids, and limiting the access of oxygen to the fatty acid chains, and as well as providing other AOs in the biomembranes the conditions for performing their antioxidant function (synergy). Since at the interaction of tocopherols with the unsaturated phospholipids, which include the CLs, the membranotropic effect manifests itself in the compaction of the lipid bilayer, we suggested in [5] that the possibility of the entry of α-tocopherol into the Cyt-CL complex opens another way (the third, associated with the membranotropic effect) to regulate the peroxidase activity of the Cyt-CL complex in addition to the other two ways noted in [7].

Upon extinguishing the lipid FRs by α-tocopherol after a proton donation the α-tocopherol radical is formed which is stable and, furthermore, is also capable of interacting with the lipid FRs to produce the oxidized molecular products safe for a living cell. Let us note, another characteristic feature of α-tocopherol mentioned in the book [10]: α-tocopherol (and its radical) very actively intercepts, namely, the peroxyl radicals ROO•, and much weaker – the alkyl radicals R•. At the same time, the peroxyl radical is the most reactive radical in comparison with other lipid FRs (alkyl and alkoxyl RO•), so that the chain reaction develops mainly due to the radical ROO•. Consequently, α-tocopherol in the membranes is a very potent quencher of the chain radical reactions, protecting the membrane from the LPO. Herewith, its effectiveness as a scavenger of the FRs is enhanced by the synergy that other components of the AOD system manifest in the living cell with respect to α-tocopherol, such as, vitamin C, ubiquinone coenzyme Q_{10} and glutathione – they are able to convert α-tocopherol from the oxidized or radical form to the reduced form.

III. RESULTS AND DISCUSSION

In the given paper, the DE system (see below the expressions (1)-(4)), describing the LPO kinetics, is based on the standard scheme for the accounting of the AO action (antiradical activity), when the AO is considered only as a FR scavenger on the non-enzymatic pathway of the LPO process.

The catalytic cycle of the LPO process with the participation of the Cyt-CL complex in the presence of the AO, in particular α-tocopherol or one of its homologues with shortened tail (PMC and C_6 – see Fig.2), remains unchanged (the scheme in Fig.1), but in the FR reactions (non-enzymatic pathway), the AO acts as an additional substrate. The considered AOs (α-tocopherol, C_6 and PMC) have the following structural formulas:

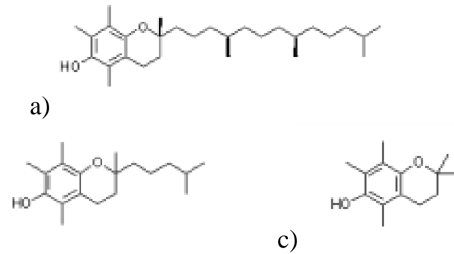

Fig.2. a) α-tocopherol (vitamin E) and its homologues with shortened tail: b) 2,5,7,8,-Tetramethyl-2(4-methylpenthyl)-6-chromanol (C_6), c) 2,2,5,7,8-Pentamethyl-6-chromanol (PMC), respectively.

Note that in our work [5], in connection with the above-mentioned third way of the regulation of the peroxidase activity of the Cyt-CL complex, the reactions involving the AO that are also possible on the enzymatic pathway of the LPO process were included in the LPO kinetics (in addition to the standard approach). So, in [5] we considered a generalized case in which the AO molecule is also the reactant in the reactions of the catalytic cycle (Fig.1). In such case, in Fig.1 and in the corresponding equations of chemical reactions, the symbol RH for the lipid molecule is formally replaced by the symbol InH for the AO molecule, and on this basis the additional terms appear in the DE system (1)-(4) in the corresponding kinetic equations – this extended DE system is given in [5]. The radical forms of PMC and C_6, as well as of α-tocopherol, are the stable radicals, and they also actively participate in the reactions with the lipid FRs, neutralizing them. It is also important that PMC and C_6, like α-tocopherol, can be easily restored with the help of vitamin C, glutathione or ubiquinone coenzyme Q_{10}.

As a result, the sought system of equations, which includes four interconnected subsystems for the enzyme, radicals, oxidation products and antioxidant, has a view:

$$\begin{cases} dE_1/dt = k_1XE - k_2XE_1 - k_3SE_1 + k_{8a}P_1E_2 \\ dE_2/dt = k_2XE_1 + k_3SE_1 - k_4R_0E_2 - k_5SE_2 + \\ + k_8P_1E - k_{8a}P_1E_2 \\ dE/dt = -k_1XE + k_4R_0E_2 + k_5SE_2 - k_8P_1E \end{cases} \quad (1)$$

$$\begin{cases} dR/dt = k_3SE_1 + k_5SE_2 - k_6YR + k_7SR_1 + \\ + k_9SR_2 - k_{11}R_0R - k_{13}I_1R - k_{16}I_2R \\ dR_0/dt = k_2XE_1 - k_4E_2R_0 - k_{11}RR_0 - k_{22}R_0 \\ dR_1/dt = k_6YR - k_7SR_1 - k_{10}R_1^2 - k_{12}P_1R_1 - \\ - k_{14}I_1R_1 - k_{17}I_2R_1 \\ dR_2/dt = k_8P_1E + k_{8a}P_1E_2 - k_9SR_2 - k_{15}I_1R_2 - \\ - k_{18}I_2R_2 \end{cases} \quad (2)$$

$$\begin{cases} dP_1/dt = k_7SR_1 - k_8EP_1 - k_{8a}E_2P_1 - k_{12}R_1P_1 + \\ + k_{14}I_1R_1 \\ dP_2/dt = k_9SR_2 + k_{15}I_1R_2 \end{cases} \quad (3)$$

$$\begin{cases} dI_1/dt = -k_{13}RI_1 - k_{14}R_1I_1 - k_{15}R_2I_1 \\ dI_2/dt = k_{13}RI_1 + k_{14}R_1I_1 + k_{15}R_2I_1 - k_{16}RI_2 - \\ -k_{17}R_1I_2 - k_{18}R_2I_2 - k_{19}I_2^2 \end{cases} \quad (4)$$

here E_1, E_2, and E, denote the concentrations of the two active forms and the inactive form of the Cyt-CL complex, respectively, and for the reagent concentrations the following notations are introduced: X≡[H_2O_2], Y≡[O_2], S≡[RH], R_0≡[$\bullet OO^-$], R≡[R•], R_1≡[ROO•], R_2≡[RO•], P_1≡[ROOH], P_2≡[ROH], I_1≡[InH], I_2≡[In•].

The DE system in the absence of the AO can be easily obtained from (1)-(4) if the variables I_1 and I_2 are set equal to zero, the reduced DE system obtained in this way makes it possible to evaluate the effectiveness of the AO influence on the lipid oxidation process by comparing the yields of the oxidation products in the presence of the AO and without it. For the numerical calculations (in the first approximation), the concentrations of the substances [H_2O_2], [O_2] and [RH] are considered to be enough high (i.e., in excess), and so, can be assume: $X \approx X_0$, $Y \approx Y_0$ and $S \approx S_0$. Also, using the matter conservation law for the catalyst, it should be taken into account that: $E_0 = E_1 + E_2 + E$. Then, using the quasi-stationary conditions for the radical forms R_0, R, R_1, R_2 the DE system (1)-(4) is reduced to a simpler and convenient form for the numerical simulation.

IV. CONCLUSIONS

The DE system (1)-(4) presented in this paper was obtained on the basis of the complete description, which was carried out in our work [5] for all chemical reactions occurring on the enzymatic and nonenzymatic pathway of the LPO process, which is caused and developing due to the peroxidase activity of the Cyt-CL complex. In [5], a detailed analysis of the various reaction channels and their contributions at a certain stage of the LPO process is given, and those reactions that can be neglected due to their insignificant contribution are discussed. The work [5] also contains a brief review of the main experimental methods used in the modern biophysical studies and a discussion of the key aspects of the functioning of the ROS and AOD systems in the organism: the main components of these systems and their properties; the possible channels of a mutual influence of the components of these systems on each other, both intrasystem and intersystem, which are essential for the optimal interaction of the ROS and AOD systems. Thus, using (1)-(4) or, in view of the foregoing, the analogous extended DE system, one can compare the antiradical (antioxidant) activity of different types of AOs. For this purpose, the numerical simulation of the kinetics of the LPO process with a participation of the Cyt-CL complexes is performed apart for each of the tested AOs, and then the effectiveness of the influence of the certain AO on the kinetics of the simulated process is determined by comparing the yields of the lipid oxidation products. Furthermore, the theoretical kinetic curves for the oxidation products can be compared with the corresponding experimental kinetic curves. The theoretical data obtained as a result of numerical simulation, their analysis and comparison with the experimental data from [9], will be published separately in the second part of our work [5].

In conclusion, we note that the DE system (1)-(4) provides the ample opportunities for modeling the kinetics of the LPO process involving the Cyt-CL complex in the presence of the antioxidant. Using (1)-(4), in addition to testing the antiradical activity of different types of antioxidants, it is also possible to find some of the unknown reaction rate constants from the known experimental kinetic curves for oxidation products, or to compare the kinetics of the POL process for the Cyt-CL complexes with the various kinds of cardiolipin molecules.

REFERENCES

[1] V. E. Kagan, V. A. Tyurin, J. Jiang, et al., "Cytochrome *c* acts as a cardiolipin oxygenase required for release of proapoptotic factors", Nat. Chem. Biol., vol. 1(4), pp. 223-232, 2005.

[2] V.E. Kagan, G. G. Borisenko, Y. Y. Tyurina, et al., "Oxidative lipidomics of apoptosis: redox catalytic interactions of cytochrome *c* with cardiolipin and phosphatidylserine", Free Radic. Biol. Med., vol. 37(12), pp. 1963-1985, 2004.

[3] V. E. Kagan, H. A. Bayir, N. A. Belikova, et al., "Cytochrome *c*/cardiolipin relations in mitochondria: a kiss of death", Free Radic. Biol. Med., vol. 46(11), pp. 1439-1453, 2009.

[4] E. V. Proskurnina, Yu. A. Vladimirov, "Free radicals as the participants of the regulatory and pathological processes", In: Fundamental Sciences – for Medicine. Biophys. Med. Technol., A. I. Grigoriev and Yu. A. Vladimirov (Eds), MAKS Press, Moscow, vol. 1, pp. 38-71, 2015. (in Russian)

[5] E. Yu. Kanarovskii, O. V. Yaltychenko and N. N. Gorinchoy, "Kinetics of the antioxidant activity of α-tocopherol and some of its homologues. Part I. Review of the problem. Theoretical model", Electronnaya Obrabotka Materialov, vol. 53(6), pp. …-…, 2017. (to be published, in Russian)

[6] L. V. Basova, I. V. Kurnikov, L. Wang, et al., "Cardiolipin switch in mitochondria: shutting off the reduction of cytochrome *c* and turning on the peroxidase activity", Biochemistry, vol. 46(11), pp. 3423-3434, 2007.

[7] V. E. Kagan, C. T. Chu, Y. Y. Tyurina, et al., "Cardiolipin asymmetry, oxidation and signaling", Chem. Phys. Lipids, vol. 179, pp. 64-69, 2014.

[8] D. A. Metelitsa, Activation of oxygen by enzyme systems. Nauka, Moscow, 1982. (in Russian)

[9] A. K. Samhann-Arias, Y. Y. Tyurina and V. E. Kagan, "Lipid antioxidants: free radical scavenging versus regulation of enzymatic lipid peroxidation", J. Clin. Biochem. Nutr., vol. 48(1), pp. 91-95, 2011.

[10] E. B. Menshchikova, V. Z. Lankin, N. V. Kandalintseva, Phenolic antioxidants in biology and medicine. Structure, properties, mechanisms of action. Saarbrucken, LAP LAMBERT Academic Publishing, 2012. (in Russian)