EFFECTIVENESS OF LIPID PEROXIDATION INHIBITION IN BIOMEMBRANES BY ANTIOXIDANTS WITH OR WITHOUT A HYDROCARBON TAIL

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UDC 615.272.4.014.425.015.4:[612.397.2:547.915.39

KEY WORDS: lipid peroxidation; biomembranes; structure of antioxidants

Bioantioxidants such as α-tocopherol (TP), ubiquinones, etc., are basically similar in their structure and contain an aromatic fragment with a hydroxy group and a hydrocarbon tail (HT). The antioxidant function of these compounds is concentrated essentially in the aromatic part of the molecule [3, 9]. In homogeneous systems (organic solvents) it has been shown in the case of TP that the effectiveness of interaction of the chromane ring with peroxide radicals (during autooxidation of ethylbenzene) is independent of the presence of the phytyl tail or the magnitude of the K_7 constant (the reaction of interaction with peroxide radicals) is virtuallly the same for TP and its derivatives containing shortened HT or not possessing the phytyl radical [1]. However, in orderly and essentially microheterogeneous systems and, in particular, in biomembranes in which the effectiveness of interaction of the reagents is largely determined by their orientation and mutual accessibility, the action of antioxidants containing or not containing HT may differ sharply.

The aim of this investigation was to compare the effectiveness of lipid peroxidation (LPO) in microsomal and mitochondrial membranes of rat liver by TP and 2,2,5,7,8-pentamethyl-6-hydroxychromane (PMC) – a derivative without the phytyl tail, and also by the synthetic antioxidant 4-methyl-2,6-di-tert-butylphenol (ionol) and its phospholipid derivative (Fig. 1).

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-200 g were used. The microsomal and mitochondrial fractions of rat liver were isolated by differential centrifugation [6]. Protein was determined by the biuret method, using bovine serum albumin as the standard. LPO was induced in medium containing 1 mg/ml of protein, 100 mM phosphate buffer, pH 7.4, 0.5 mM NADPH (NADH, ascorbate) or 5 mM tert-butyl hydroperoxide, 10 μM $FeSO_4$ (+0.3 mM ADP for LPO in mitochondrial membranes). Incubation was carried out at 37°C with constant mixing. Accumulation of LPO products was determined from the quantity of malonic dialdehyde (MDA) formed [7]. TP and PMC were introduced into the membranes by adding alcoholic solutions of these compounds in the necessary concentrations (the final concentration of alcohol did not exceed 0.5%). Incorporation of TI and PMC was carried out in the course of incubation for 12 h at 4°C. The measurements were made on a Perkin-Elmer 552 spectrophotometer (USA). TP and ionol were obtained from Serva (West Germany), NADPH, NADH, and ascorbate from Reanal (Hungary), and the PMC was generously provided by I. K. Sarycheva (Institute of Fine Chemical Technology, Moscow) and the phospholipid derivative of ionol by Dr. Chem. Sci. D. A. Predvoditelev, to whom the authors are grateful.

EXPERIMENTAL RESULTS

In the experiments of series I the effectiveness of the inhibitory action of TP and PMC was compared in microsomal and mitochondrial membranes during LPO induced by $O_2^{\cdot}$-generating systems (Fe + ascorbate, Fe + NADPH) or by organic peroxides (tert-butyl hydroperoxide).

In the absence of inhibitors, accumulation of LPO products was found to take place most rapidly in the case of induction by the Fe + ascorbate system, rather more slowly by

Institute of Physiology, Bulgarian Academy of Sciences, Sofia. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 419-421, October, 1986. Original article submitted November 13, 1985.

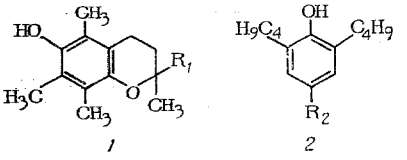

Fig. 1

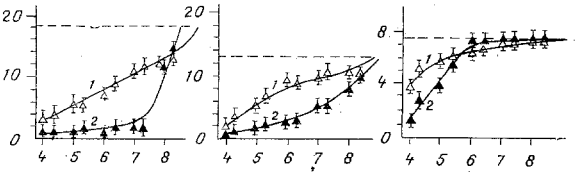

Fig. 2

Fig. 1. Structural formulas of antioxidants: 1) $R_1 = C_{14}H_{27}$ (TP), R_1 = H (PMC); 2) R_2 = H (ionol), $R_2 = (CH_2)_2PO_4(C_3H_5(C_{15}H_{31}COO)_2)_2$ (phospholipid derivative of ionol).

Fig. 2. Action of TP (1) and PMC (2) and accumulation of LPO products in rat liver microsomal membranes as a function of concentration. Abscissa, negative logarithm of antioxidant concentration; ordinate, MDA concentration (in nmoles/mg protein). Incubation time 10 min. Broken line indicates quantity of oxidation product in absence of inhibitor.

TABLE 1. 50% Inhibition Constants of TP and PMC for LPO Reactions in Rat Liver Microsomal and Mitochondrial Membranes

System of LPO induction	Microsomes		Mitochondria	
	TP	PMC	TP	PMC
Fe + ascorbate	$5{,}9\cdot10^{-7}$M	$1\cdot10^{-8}$M	$9\cdot10^{-7}$M	$5\cdot10^{-8}$M
Fe + NADPH	$6{,}3\cdot10^{-6}$M	$1{,}2\cdot10^{-8}$M	—	—
Fe + tert-butyl hydroperoxide	$1\cdot10^{-4}$M	$1\cdot10^{-5}$M	$9\cdot10^{-5}$M	$2\cdot10^{-6}$M

Legend. Incubation mixture for induction of LPO in mitochondrial membranes contained Fe + ADP.

the Fe + NADPH system, and much more slowly by the Fe + tert-butyl hydroperoxide system (Fig. 2). Both TP and PMC had a marked inhibitory action in LPO in all three induction systems; PMC was much more effective in this case than TP. When α-tocopheryl acetate, which lacks the OH-group essential for the antioxidant effect, was used virtually no inhibition of LPO was observed [8]. General data on the effectiveness of inhibition of LPO by TP and PMC are given in Table 1, where values of the 50% inhibition constant of LPO in mitochondrial and microsomal membranes are given. It will be clear from Table 1 that PMC is a more effective inhibitor of LPO in microsomal and mitochondrial membranes than TP, whose 50% inhibition constant is one or two orders of magnitude higher than those for PMC. Under these circumstances TP and PMC inhibited LPO in $O_2^{\cdot}$-dependent systems of LPO induction more effectively than in a system of Fe + tert-butyl hydroperoxide. It can be tentatively suggested that PMC, which possesses higher mobility in the membranes [5], can interact more effectively with lipid radicals and active forms of oxygen than TP, whose chromane fragment is located in the hydrophobic region of the membrane, close to the surface of the phase boundary [2]. If these views are correct, it will be evident that other antioxidants containing HT ought to give a weaker effect on LPO than their analogs which have no HT, limiting the mobility of the molecules in the lipid bilayer of the membrane.

To test this hypothesis, in the next series of experiments the effectiveness of inhibition of LPO in microsomal membranes by one of the most widely used synthetic antioxidants, namely ionol, was compared with that of its analog (Fig. 1), in which ionol was added to a phospholipid fragment containing an HT of 16 carbon atoms. It was shown that the phospholipid derivative of ionol also is a much weaker inhibitor of LPO than ionol itself; inhibition of LPO by ionol is 2-2.5 times more effective than by its derivative in the case of inhibition of LPO by Fe + ascorbate, Fe + NADPH, and Fe + NADH systems (Table 2).

It can accordingly be concluded that the presence of an HT in the molecule of antioxidants is responsible for their being less effective as LPO inhibitors in biomembranes. Meanwhile, in homogeneous systems (in solutions) the presence of an HT does not affect the

TABLE 2. 50% Inhibition Constants of LPO Reactions by Ionol and Its Phospholipid Derivatives in Microsomal Membranes

System of LPO induction	Ionol	Phospholipid derivative of ionol
Fe + NADPH	$0.8 \cdot 10^{-6}$ M	$1 \cdot 10^{-4}$ M
Fe + NADH	$3 \cdot 10^{-6}$ M	$5 \cdot 10^{-4}$ M
Fe + ascorbate	$\cdot 10^{-6}$ M	$5 \cdot 10^{-4}$ M

antiradical activity of the compounds [1]. The question accordingly arises of the biological usefulness of the phytyl chain in the TP molecule. It must be recalled that TP is a polyfunctional stabilizer of biomembranes, capable of behaving not only as an effective trap for free radicals, but also as a quencher of singlet oxygen, and a stabilizer of the structure of unorganized polyunsaturated fatty-acid residues in the hydrophobic region of the membrane [3, 4]. In this connection it must be noted that PMC and ionol not only are not structural stabilizers of biomembranes but, on the contrary, they themselves have a disorganizing, destructive action, causing a sharp increase in their permeability for ions [5]. It can therefore be concluded that the presence of an HT is a biologically useful compromise, resulting both in sufficiently high antioxidant activity of these compounds and the possibility of structural stabilization of biomembranes.

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