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Effect of some membrane lipids on radical generation in the system acetylcholine-hydroperoxide

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The influence of membrane lipids (0.001 - 0.1 mM) on the rate of β-carotene oxidation initiated by the lipophilic azoinitiator AMVN and by microaggregates formed by acetylcholine (ACh) and tert-butyl hydroperoxide (ROOH) in n-decane (37 ° C) was studied. Phosphatidylcholine, sphingomyelin and cholesterol were investigated as lipid components. In homogeneous systems, all the additives slightly decrease the consumption rate of β-carotene initiated by AMVN due to cross radical reactions. In the case of initiation by mixed microaggregates {mROOH…nACh} which generate peroxyl radicals $RO_2^{\bullet}$, a dose-dependent r etarding effect of phosphatidylcholine on the β-carotene consumption rate was observed whereas cholesterol additives caused acceleration of β-carotene oxidation. Sphingomyelin did not show any significant differences. It was found that the changes of radical initiation rate correlated with the changes of microaggregates sizes measured by dynamic light scattering.

Keywords: Acetylcholine chloride, Membrane lipids, Micellar catalysis, Cholesterol, Phosphatidylcholine

INTRODUCTION

The surfactant effect on lipid oxidation is determined by the ability of hydroperoxides (ROOH), the primary products of oxidation, to form mixed micelles-microaggregates with surfactants: mROOH + nS ⇔{mROOH…nS} [1-3]. The formation of mixed micelles that have a strong influence on the decomposition of ROOH was studied and confirmed by nuclear magnetic resonance (NMR) spectroscopy, dynamic light scattering (DLS), and tensiometry [2-4]. Depending on the chemical nature of the surfactant and oxidized substrate, catalysis of oxidation in the case of cationic surfactants (S+) [1-4], inhibition [5,6], or no effect at all [1,2] can take place. The key reaction of the catalytic mechanism of cationic surfactants (S^+) on the lipid oxidation is the accelerated decomposition of hydroperoxides into radicals in mixed micelles. With anionic and nonionic surfactants, hydroperoxides also form mixed micelles, but the radical decay is accelerated only in combination with S^+. In micelles with S^+, peroxide bond -O-O-, evidently, falls properly into a strong electric field of a double electric layer with a high voltage of ~ 10^5-10^7 V/m, which attenuates this bond and stimulates homolytic decay. Simple micellar effects on the rate of ROOH decomposition into free radicals due to the concentration of reagents in the micelle core and interface or due to the change in the polarity compared to that in bulk solution [6] do not explain the scale and selectivity of the effect inherent only to cationic surfactants [1-5]. The activation energy of the thermal decomposition of different ROOH is 90–120 kJ·mol^{-1} [7-9]; in reverse micelles {mROOH…nS$^+$}, it decreases to 40–60 kJ/mol [2, 3, 10,11]. As a result, the binary system S^+– ROOH can be applied as a lipophilic (reverse micelles) and hydrophilic (direct micelles) initiator of free radicals:

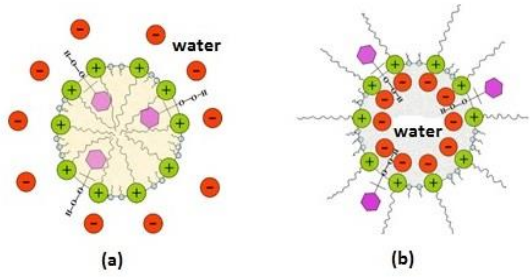

Figure 1. Direct (a) and reverse (b) micelles {mROOH…nS}

It was found that the known neurotransmitter acetylcholine chloride (ACh), forms mixed micelles with ROOH and accelerates the decomposition of ROOH into radicals and the oxidation of lipids similar to cationic surfactants [12,13]:

$$mROOH + nACh \Leftrightarrow \{mROOH…n\ ACh\} \rightarrow RO_2^{\bullet}$$

Oxidation of biomembrane lipids, such as phosphatidylcholine (PC) and cholesterol (Chol), has been recognized to be related to human diseases, such as atherosclerosis and cancer [14,15]. Along with sphingomyelin (SM), PC and Chol are constituents of rafts (“ruft-like lipids” [16]), whose role in the physiology of the cell is being intensively studied. Sphingomyelins are present in the plasma

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membranes of animal cells and are especially prominent in the brain substance and peripheral nervous system [17]. Phosphatidylcholines (lecithins), the most common molecules of cell membranes, are widely represented in the cells of various tissues; they perform both metabolic and structural functions in membranes. Cholesterol ensures the stability of cell membranes in a wide range of temperatures. Chol is necessary for the production of vitamin D, the production by adrenal glands of various steroid hormones, including cortisol, aldosterone, female sex hormones estrogen and progesterone, the male sex hormone testosterone, and according to recent data Chol plays an important role in the activity of brain synapses and the immune system, including protection from cancer.

In this work, we studied the effect of PC, Chol, and SM on the rate of β-carotene oxidation initiated by the binary system ACh and tert-butyl hydroperoxide (ROOH) in n-decane (37° C) as compared with the oxidation initiated by the lipophilic azoinitiator AMVN. β-Carotene (pro-vitamin A) is a polyunsaturated hydrocarbon of high activity in scavenging of various free radicals [18,19]. In micellar systems, hydrophobic β-carotene is localized in the organic phase and does not interact with surfactants. By this reason, β-carotene is a convenient free radical acceptor to use in the inhibitor's method for determination of the initiation rate.

EXPERIMENTAL

Acetylcholine chloride (ACh), tert-butyl hydroperoxide (ROOH), egg-phosphatidylcholine (PC) (all from Fluka), cholesterol (Chol), sphingomyelin (SM), β-carotene (A) and n-decane (all Sigma-Aldrich) were used as purchased.

The oxidation of β-carotene was carried out directly in a constant temperature-maintained quartz cell (1 cm) of an Ultrospec 1100 pro spectrophotometer at 37° C to determine the kinetics of β-carotene consumption. Its concentration was chosen in a way to intercept all the radicals escaped. ACh, dissolved in a mixture chloroform: methanol (2:1), was added to the solution of ROOH and a lipid component in n-decane. The mixture was immersed in an ultrasonic stirring bath (10 min); then 3 mL of the mixture were placed in a quartz cell, to which 9 μL of β-carotene stock solution in n-decane was added. The average size of microaggregates was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS analyzer (Malvern Instruments, United Kingdom) equipped with a laser operating at 633 nm. The particle sizes were measured in the range from 0.6 nm to 6 μm. The measurements were carried out at 25°C and a scattering angle of 173°.

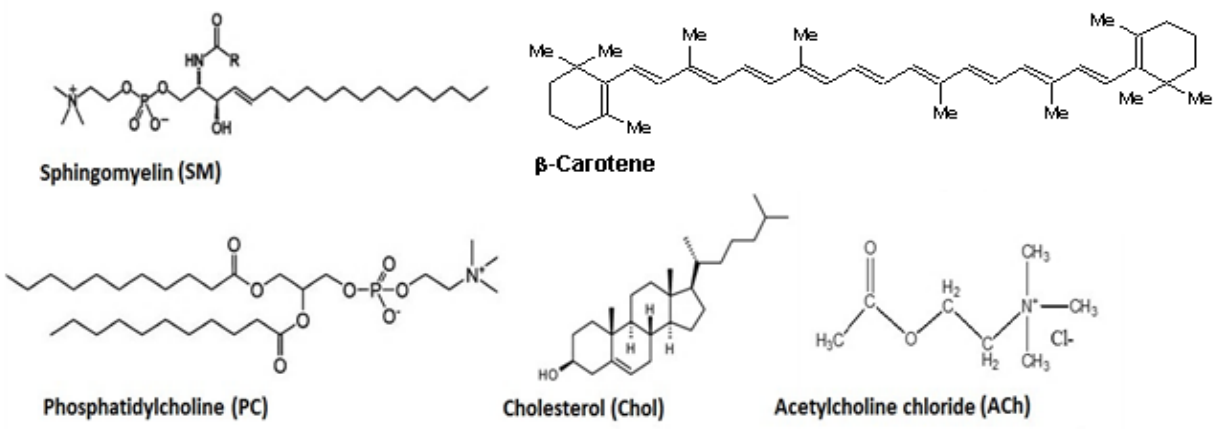

Figure 2. Structures of ACh, β-carotene and "ruft-like lipids" studied

RESULTS

There is a large number of studies on the inhibitory properties of phospholipids and cholesterol [20-24]. These compounds do not have the usual inhibitory groups to compete successfully with bulk lipids for radicals. However, SM and PC possess surface activity. Chol is known to affect the membrane structure and to contribute to the transformation of spherical direct micelles to vesicles [25]. We compared the effect of "ruft-like lipids" on β-carotene (A) consumption in homogeneous n-decane solution and in a microheterogeneous system, in which peroxyl radicals were generated in mixed micelles {mROOH…n ACh}.

Table 1. Effect of lipid additives (LH) on the rate of β-carotene (A) oxidation initiated by the azoinitiator $1.5\ 10^{-3}$ M AMVN in the presence of $1{\cdot}10^{-5}$ M lipid, [β-carotene] $=1 \cdot 10^{-5}$ M, 37ºC

Lipid additive (LH)	Without additive	Cholesterol	Phosphatidylcholine	Sphingomyelin
Rate of β-carotene consumption, $M{\cdot}s^{-1}$	$4.3{\cdot}10^{-9}$	$3.7{\cdot}10^{-9}$	$3.9{\cdot}10^{-9}$	$3.8{\cdot}10^{-9}$

Table 1 shows the influence of the additives of Chol, PC, and SM on the rate of β-carotene oxidation initiated by lipophilic AMVN in homogeneous medium. It can be seen that the additives did not lead to strong differences in rates and the decrease in the rate of β-carotene (A) consumption is probably associated with involvement of lipids in radical reactions and the increase in the rate of cross-termination (Scheme 1). In the case of radical initiation by the binary micellar system ACh-ROOH, we observed different effects of lipid additives (Table 2).

Chol additives demonstrated dose-dependent increase of β-carotene oxidation rate, whereas in the case of PC, a retardation effect was observed. Sphingomyelin demonstrated retarding influence, much weaker than PC (Table 2).

1) AMVN $\rightarrow$ $RO_2^{\bullet}$
2) A + $RO_2^{\bullet}$ $\rightarrow$ $AO_2^{\bullet}$
3) $AO_2^{\bullet}$ + $RO_2^{\bullet}$ $\rightarrow$ products
4) $AO_2^{\bullet}$ + $AO_2^{\bullet}$ $\rightarrow$ products
5) LH + $RO_2^{\bullet}$ $\rightarrow$ $LO_2^{\bullet}$ + RO_2H
6) LH + $AO_2^{\bullet}$ $\rightarrow$ $LO_2^{\bullet}$ + AO_2H
7) $LO_2^{\bullet}$ + $AO_2^{\bullet}$ $\rightarrow$ products
8) $LO_2^{\bullet}$ + $RO_2^{\bullet}$ $\rightarrow$ products

Scheme 1.

Table 2. Effect of lipid additives on the rate of β-carotene oxidation initiated by the binary system ACh-ROOH at initial concentrations: $1{\cdot}10^{-3}$ M ACh, $1{\cdot}10^{-2}$ M ROOH, $1{\cdot}10^{-5}$ M β-carotene, in n-decane, 37ºC

Concentration of additives, M	Rate of β-carotene consumption, $M{\cdot}s^{-1}$		
	Chol	SM	PC
Without additive	$1.15{\cdot}10^{-9}$		
$1{\cdot}10^{-6}$	$3.5{\cdot}10^{-9}$	$1.00{\cdot}10^{-9}$	$0.02{\cdot}10^{-9}$
$5{\cdot}10^{-6}$	$3.8{\cdot}10^{-9}$	$0.82{\cdot}10^{-9}$	$0.005{\cdot}10^{-9}$
$1{\cdot}10^{-5}$	$4.2{\cdot}10^{-9}$	$0.75{\cdot}10^{-9}$	$0.002{\cdot}10^{-9}$
$5{\cdot}10^{-5}$	$4.8{\cdot}10^{-9}$	$0.61{\cdot}10^{-9}$	$0.001{\cdot}10^{-9}$
$1{\cdot}10^{-4}$	$5.0{\cdot}10^{-9}$	$0.57{\cdot}10^{-9}$	0

It should be noted that hygroscopic acetylcholine is not soluble in organic solvents, but in combination with hydroperoxides, ACh forms relatively large mixed aggregates, with a size of ~350 nm (Fig. 3a). For comparison, a typical cationic surfactant cetyltrimethylammonium bromide (CTAB) under similar conditions forms together with tert-butyl hydroperoxide mixed reverse micelles with a size of 10 nm [13] and provides a higher rate of β-carotene consumption [2,3,5,13]. Because PC and SM are surface active substances and Chol as an essential structural component of all cell membranes, known to affect the structure of direct micelles and membranes [26,27], the influence of Chol and PC on the sizes of mixed micelles {mROOH…nACh} was studied.

In the presence of Chol, the time required to establish a stationary distribution for DLS measurement decreases, and the average size is reduced to ~300 nm (Fig. 3.b). Since size reduction is accompanied by an increase of the rate of radical generation, we can suggest that Chol promotes, to some extent, the integration of the –O-O- bond into a double electric layer, which leads to an increase in ROOH decay and radical initiation rate. PC is relatively well soluble in organic solvents to yield transparent or slightly opalescent (at [PC] > 20 mg/ml) solutions. According to the DLS data, at [PC] = 20–90 mg/ml in n-decane, reverse micelles with average hydrodynamic diameters of 6 nm are formed [28]. When PC is added to the micellar solution of {mROOH…nACh}, micelles of ~100

nm are formed along with large aggregates of 1500 nm (Fig. 3.c). Most likely, PC solubilizes the micelles {mROOH…nACh} or the individual ACh. This results in destruction of the inner structure of the {mROOH…nACh}, changing of space location of the –O-O- bond, and decrease of ROOH decay and rate of radical escape into bulk solution.

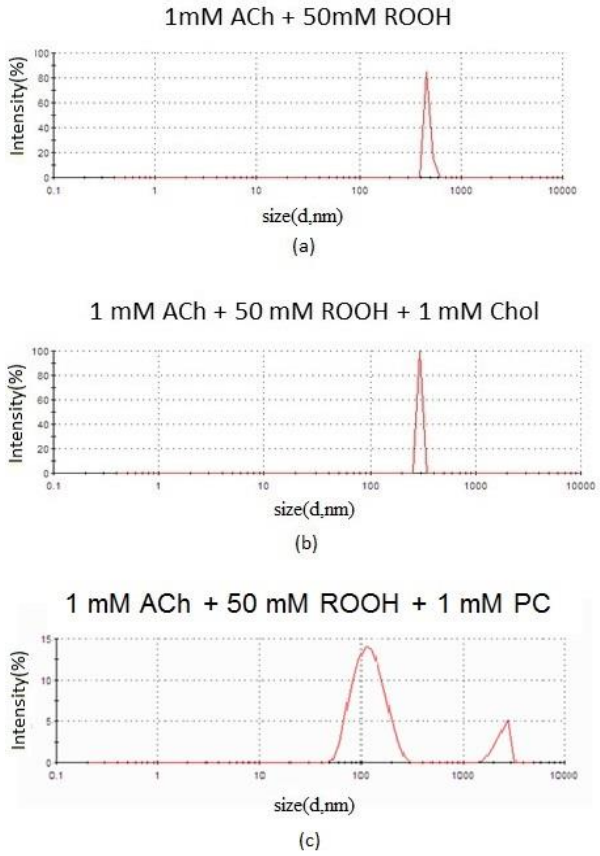

Figure 3. Effect of Chol (b) and PC (c) on the average size of microaggregates formed by the mixture of ACh with tert-butyl hydroperoxide (a) in n-decane solution.

CONCLUSIONS

Acetylcholine forms common micelles with hydroperoxides in the organic medium, which facilitates the breakdown of hydroperoxides into free radicals. The effect of the membrane lipids on the rate of free radical generation in microaggregates {mACh ... nROOH} was revealed.

Phosphatidylcholine (lecithin) solubilizes {mACh ... nROOH} in an organic medium, which results in a decrease of the radical initiation rate, and the yield of radicals into the volume is hampered. Cholesterol (Chol), on the contrary, stabilizes {mACh ... nROOH}, accompanied by an increase of the rate of radical generation.

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ЕФЕКТ НА НЯКОИ МЕМБРАННИ ЛИПИДИ ВЪРХУ ГЕНЕРИРАНЕТО НА РАДИКАЛИ В СИСТЕМАТА АЦЕТИЛХОЛИН-ХИДРОПЕРОКСИД

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(Резюме)

Влиянието на мембранни липиди (0.001 - 0.1 mM) върху скоростта на окисление на β-каротин, инициирано от липофилния азоинициатор AMVN и от микроагрегати, образувани от ацетилхолин (ACh) и трет.-бутилов хидропероксид (ROOH) е изследвано в n-декан при 37 °C. Фосфатидилхолин, сфингомиелин и холестерол са изследвани като липидни компоненти. В хомогенни системи всички добавки слабо понижават скоростта на изразходване на β-каротина, инициирано от AMVN, поради кръстосани радикалови реакции. В случая на инициране от смесени микроагрегати {mROOH…nACh}, които генерират пероксилни радикали $RO_2^{\bullet}$, се наблюдава концентрационно-зависим ефект на забавяне на консумацията на β-каротина от фосфатидилхолина, докато добавката от холестерол ускорява окислението на β-каротина. Сфингомиелинът не води до значими различия. Установено е, че промените в скоростта на радикалово инициране корелират с промените в размера на микроагрегатите, измерени чрез динамично разсейване на светлината.