Interaction of Lipase with Lipid Model Systems

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Abstract: The aim of this work was to study the interaction of lipases (as an important biopolymer) with models of biomembranes based on the phospholipid and cholesterol. Lipases (triacylglycerolacyl hydrolases) are widely distributed enzymes and well-known by their hydrolytic activity. The study of the lipase interactions with lipid vesicles in aqueous dispersions is of fundamental and practical interest. The pure phosphatidylcholine from egg yolk (ePC) and cholesterol (Chol) were obtained from Sigma-Aldrich. Lipase was obtained from hog pancreas. Measurements of the current and equilibrium surface tension (ST and eST) values were carried out using a BPA-1P device and ADSA program. The particle sizes in the prepared colloidal solutions were determined by the method of dynamic light scattering. An addition of lipase led to some decrease both, of ST and eST for the samples of ePC:Chol (in the ratios from19:1 to 1:1). The mean particle diameter (MPD) and effective particle diameter (EPD) values for the samples of ePC:Chol changed drastically by lipase addition. The EPD/MPD ratios increased from 1.7 to 2.0, from 1.8 to 2.6, from 2.3 to 6.5, from 1.5 to 2.9 for the samples of ePC:Chol at the ratios of 19:1, 14:1, 9:1, 7:1, respectively by lipase concentration increase. This general tendency can be explained by strong interaction of lipase with lipid membrane that leads to the formation of the mixed particles ePC:Chol:lipase with more narrow particle size distribution as compared to the initial EPD/MPD ratio (for the ePC:Chol mixture without lipase).

Keywords: Lipase, lipids, surface tension, particle size distribution.

INTRODUCTION

In recent years, the study of the properties and applications of various lipase preparations is of high interest [1]. Lipases (triacylglycerolacyl hydrolases EC 3.1.1.3) are widely distributed enzymes [2-6] and well-known by their hydrolytic activity - the hydrolysis of fats and oils in the aqueous media [7-12]. There are numerous papers [1-12] on purification, structure-function relations and immobilization of various lipases, but the dimensions and size distributions of the lipase-containing systems are rarely described. It is important to highlight that lipase preparations are widely used in biotechnology and biomedicine, especially for therapy of the pancreas, liver and gallbladder diseases [6-13]. Recently a comparative structural and functional study of various lipases [14-16] and particular coordination between nanostructured materials and lipases for numerous applications [16-18] have been fulfilled.

The study of the effect of lipase interaction with lipid vesicles in aqueous dispersions is of fundamental and practical interest and will be a subject to discuss here. In addition to the surface properties and enzyme activity of lipases [13], it is important to study the structural features of the lipase interaction with lipid dispersions. Such dispersions from phosphatidylcholine and other "structural lipids" which are compatible with lipid bilayer (obtained by ultrasonication or by extrusion) can be considered as the model of biological membranes (biomembranes) [19]. Biomembranes are ultra-thin, highly organized systems of molecular sizes located on the surface of cells and subcellular particles [20-22]. A necessary condition for the vital processes of a cell is a combination of such properties of biological membranes as stability and lability, the ability to self-association and molecular recognition, which is ensured by their specific chemical composition [20-22]. These properties of biomembranes are mainly due to various interactions in the lipid-protein complexes, that is why lipase addition to such lipid dispersions is very important for modeling of biomembranes [20-22]. The major lipid systems (dispersions) are well-known as liposomes, which are modeling the structure and function properties of biomembranes [23, 24].

The thickness of most biomembranes ranges from 5 to 10 nm [20-22]. Conventionally, the biomembranes are consisting of the bilayer of various types of lipids (with thickness about 3.5-5.0 nm) and proteins (integral or bound to the bilayer by non-covalent bonds). The

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lipid dispersions (sometimes called liposomes) can be roughly divided onto following systems: small unilamellar vesicle (SUVs); large unilamellar vesicle or giant unilamellar vesicles (LUVs or GUVs); multilamellar vesicle (MLVs) [20-22]. The SUV's average diameter is about 50-100 nm; MLV's – varied from 100 to 500 nm, whereas LUV's or GUV's - reaching up to 1 μm or 100 μm (in some special cases) [20-22]. Of course, the particular numbers are depending on the methods of measurements, for example, the MLV's average diameters varied from 100-300 nm (by electron microscopy measurements) to 150-500 nm (by light scattering measurements) for the same phospholipid's mixtures [21, 23, 24]. In this study, the authors used photon correlation spectroscopy in order to measure so-called hydrodynamic diameter ("the particle diameter plus the double layer thickness" [25]).

The most valuable phospholipids can be obtained not only from the plasma membranes, but also - from other sources, such as egg yolk [26-28]. There are egg yolk phosphatidylcholines, phosphatidylethanolamine, phosphatidylserines and others [19]. The phosphatidylcholine (PC) is one of the most important membrane phospholipids, because PC content is about half of them and PC plays the key role in the structure stability of biological membranes [28-30]. Another important natural membrane lipid is cholesterol [19, 31-34]. Cholesterol and its derivatives play the numerous roles in the membranes and total homeostasis as well as in the metabolism in cells, brain, peripheral tissues, etc. [31-33]. It is well-known that cholesterol is essential for the regulation of the biomembrane "fluidity or rigidity" [20-22], as well as for activation of mast cell and associated with obesity and diabetes in humans and animals [19, 33-35]. Cholesterol is participating in the "durability of the vesicle" that depends on its relative content in the phospholipid layer of such membrane [20-22].

Some recent studies on the structural features of the lipid monolayers as models of the biological membranes [15-17] are especially interesting for our work because they deal with the interactions of lipase with such monolayers. For example, the mentioned study presented an interesting data concerning the interactions "of recombinant dog gastric lipase (rDGL) with model monolayers liquid–liquid phase coexistence and mimicking the outer leaflet of the milk fat globule membrane" [36]. The rDGL adsorption is mainly due to combination of the hydrophobic and electrostatic interactions and "strongly impacts on the lipid phase lateral organization" [36] that is revealed by combining some classical and modern biophysical tools such as ellipsometry, tensiometry and atomic force microscopy [36-38].

The aim of this work was to study the interaction of lipases with models of biological membranes (based on the phospholipid and cholesterol) by surface-active and light scattering measurements.

MATERIALS AND METHODS

Materials

The following compounds and reagents were used in this work: pure phosphatidylcholine from egg yolk (ePC) and synthetic cholesterol (Chol). These compounds were obtained from Sigma-Aldrich (Russian Federation): L-α-Phosphatidylcholine (Sigma-Aldrich P3556 egg yolk, Type XVI-E, lyophilized powder, ≥99% by TLC, CAS Number 8002-43-5); Cholesterol (Sigma-Aldrich C8667, Sigma Grade, ≥99% CAS Number 57-88-5).

Measurements of the current surface tension (ST) values were carried out using a BPA-1P device by the method of the maximum pressure measurements in a bubble (with a surface lifetime of 0.5 s to 10 s) [39-41].

The general principle of the device operation (based on the maximum pressure in the bubble method [39-41]) is described below [39]. The air from the compressor enters the capillary, which is lowered into the test liquid. The maximal pressure in the system is determined (Figure **1**) and used to calculate the surface tension.

Figure 1: The tensiometer BPA-1P (adapted from www.sinterface.de and publication [39, 41]).

This method of the maximal pressure measurement "in the buoyant bubble" allowed us to obtain the dependences of DST values vs. time (i.e., in the form of "tensiograms" [41]). All measurements were carried out at 20°C.

The equilibrium surface tension (eST) values were obtained by a calculation from the "ST vs. time" isotherms using the ADSA program [39-41]. The particle sizes in the obtained colloidal solutions were determined by the method of dynamic light scattering with the use of "90Plus/BI-MAS Particle Size Analyzer" device (Brookhaven Instruments Corporation, USA) [25]. Porcine pancreatic lipase (Lip) was obtained by purification from hog pancreas by well-known methods [1]. The data obtained were subjected to the statistical treatment by STATISTICA 10 (the average errors were below 1%).

RESULTS

It is important to study the interaction of lipases with the lipid dispersions based on natural phospholipids: phosphatidylcholine (from egg yolk) and cholesterol. The current and equilibrium surface tension (ST and eST) values, as well as the mean particle diameter (MPD) and effective particle diameter (EPD) values for the samples of ePC:Chol from 19:1 to 1:1 (i.e. two-component mixtures of phosphatidylcholine and cholesterol) were investigated in the presence of lipase at various concentrations.

Lipid Dispersion ePC:Chol=19:1 in the Presence of Lipase

A lipase addition (at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 a.u.) to the lipid dispersion (two-component mixture of ePC:Chol=19:1) led to the continuous ST decrease (Table **1**) as compared to the initial mixture (72.87 mN/m at 0.5 s, 72.74 mN/m at $t\rightarrow\infty$). The total decrease was from 0.85, to 1.59 mN/m by lipase addition (at maximal concentration of 1.0 a.u.) after 0.5 s and $t\rightarrow\infty$ as compared to the initial mixture, respectively (Table **1**, columns 1-5). These small changes became more pronounced (at 2.6, 4.8, 4.8, 5.0, 5.7 times) by recalculating the changes between 0.5 s and $t\rightarrow\infty$ for each of the lipase addition (at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 a.u., Table **1**, lines 2-6, respectively), as compared to the very small changes of 0.13 mN/m for the initial mixture (72.87 mN/m at 0.5 s, 72.74 mN/m at $t\rightarrow\infty$).

In general, the mean particle diameter (MPD) values decreased drastically by 11.1%, 15.8%, 23.4%, 28.5% and 41.0% by lipase addition from 0.01 to 1.0 a.u., respectively (Table **2**). The effective particle diameter (EPD) values decreased almost in the same manner by 9.6%, 17.1%, 23.8%, 29.2% and 30.3% by

Table 1: The Current and Equilibrium Surface Tension (ST and eST) Values for the Samples of ePC:Chol=19:1 as Lipid Dispersion (LD19:1) and Lipase

Samples	t=0.5 s	t=1 s	t=3 s	t=5 s	$t\rightarrow\infty$
LD19:1 + 0.01 Lip	73.01	72.49	72.39	72.33	72.67
LD19:1 + 0.05 Lip	72.92	72.64	72.52	72.44	72.30
LD19:1 + 0.1 Lip	72.69	72.64	72.39	72.33	72.07
LD19:1 + 0.5 Lip	72.68	72.61	72.38	72.28	72.03
LD19:1 + 1.0 Lip	72.02	71.99	71.77	71.65	71.27

Table 2: The Mean Particle Diameter (MPD) and Effective Particle Diameter (EPD) Values for the Samples of ePC:Chol=19:1 in the Presence of Lipase (Lip)

Samples	MPD, nm	EPD, nm	EPD/MPD
LD19:1 + 0.01 Lip	202.6	350.9	1.73
LD19:1 + 0.05 Lip	191.9	321.9	1.68
LD19:1 + 0.1 Lip	174.6	296.0	1.70
LD19:1 + 0.5 Lip	162.8	275.0	1.69
LD19:1 + 1.0 Lip	134.3	270.6	2.01

lipase addition from 0.01 to 1.0 a.u., respectively (Table **2**). The initial EPD and MPD values of the lipid mixture without lipase were about 388.2 and 227.8 nm, respectively.

Lipid Dispersion ePC:Chol=14:1 in the Presence of Lipase

The total decrease was about 0.62, 0.69, 0.59, 0.56 and 0.58 mN/m by lipase addition (at maximal concentration of 1.0 a.u.) after 0.5 s, 1 s,3 s,5 s and t→∞ for the mixture of ePC:Chol=14:1 as compared to the initial mixture, respectively (Table **3**, columns 1-5). These small changes became more pronounced (at 5.2, 3.9, 9.8, 4.7, 4.9 times) by recalculating the changes between 0.5 s and t→∞ for each of the lipase addition (at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 a.u., Table **3**, lines 1-5, respectively), as compared to the very small changes of 0.13 mN/m for the initial mixture (ePC:Chol=14:1).

In general, the mean particle diameter (MPD) values decreased drastically by 30.8%, 26.8%, 38.9% and 34.8% by lipase addition from 0.05 to 1.0 a.u., respectively (Table **4**). The effective particle diameter (EPD) values decreased almost in the same manner by 1.4%, 2.5%, 3.8% and 9.1% by lipase addition from 0.05 to 1.0 a.u., respectively (Table **4**).

Lipid Dispersion ePC:Chol=9:1 in the Presence of Lipase

In contrast to previous data (Tables **1** and **3**) the only small changes of ST values by lipase addition (at maximal concentration of 1.0 a.u.) after 0.5 s, 1 s,3 s,5 s and t→∞ found for the mixture of ePC:Chol=9:1 (Table **5**, columns 1-5). These small changes became incredibly pronounced (at 42.3, 25.4, 25.6, 19.0 and 17.3 times) by recalculating the changes between 0.5 s and t→∞ for each of the lipase addition (at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 a.u., Table **5**, lines 1-5, respectively), as compared to the very small changes of 0.16 mN/m for the initial mixture (ePC:Chol=9:1). It is interesting that the ST values after 0.5 s for the initial mixture (ePC:Chol=9:1) were a little bit lower (73.44 mN/m) than those for these mixtures with lipase (74.29 mN/m). The only exception found in the case of the extrapolated ST values at maximal time (t→∞), which was higher (73.28 mN/m) than those for this mixture with lipase (67.52-71.28 mN/m).

In general, the mean particle diameter (MPD) values decreased drastically by 34.2%, 57.7%, 62.5% and 75.8% by lipase addition from 0.05 to 1.0 a.u., respectively (Table **6**). The effective particle diameter (EPD) values changed by 3.7%, -3.6%, 12.6% and

Table 3: The Current and Equilibrium Surface Tension (ST and eST) Values for the Samples of ePC:Chol=14:1 as Lipid Dispersion (LD14:1) and Lipase (Lip)

Samples	t=0,5 s	t=1 s	t=3 s	t=5 s	t→∞
LD14:1 + 0.01 Lip	73,00	72,93	72,69	72,59	72,32
LD14:1 + 0.05 Lip	72,23	72,11	71,95	71,83	71,72
LD14:1 + 0.1 Lip	73,25	72,56	72,29	72,24	72,06
LD14:1 + 0.5 Lip	72,82	72,75	72,55	72,46	72,21
LD14:1 + 1.0 Lip	72,38	72,24	72,10	72,03	71,74

Table 4: The Mean Particle Diameter (MPD) and Effective Particle Diameter (EPD) Values for the Samples of ePC:Chol=14:1 in the Presence of Lipase (Lip)

Samples	MPD, nm	EPD, nm	EPD/MPD
LD14:1 + 0.01 Lip	212.3	395.0	1.80
LD14:1 + 0.05 Lip	146.9	389.5	2.65
LD14:1 + 0.1 Lip	155.3	385.0	2.50
LD14:1 + 0.5 Lip	129.7	379.8	2.93
LD14:1 + 1.0 Lip	138.4	359.2	2.60

Table 5: The Current and Equilibrium Surface Tension (ST and eST) Values for the Samples of ePC:Chol=9:1 as Lipid Dispersion (LD9:1) and Lipase (Lip)

Samples	t=0,5 s	t=1 s	t=3 s	t=5 s	t→∞
LD9:1 + 0.01 Lip	74.29	74.10	73.74	73.52	67.52
LD9:1 + 0.05 Lip	74.07	74.06	73.95	73.88	70.00
LD9:1 + 0.1 Lip	74.02	73.96	73.78	73.66	69.92
LD9:1 + 0.5 Lip	74.40	73.96	73.78	73.67	71.36
LD9:1 + 1.0 Lip	74.04	73.62	73.34	73.10	71.28

Table 6: The Mean Particle Diameter (MPD) and Effective Particle Diameter (EPD) Values for the Samples of ePC:Chol=9:1 in the Presence of Lipase (Lip)

Samples	MPD, nm	EPD, nm	EPD/MPD
LD9:1 + 0.01 Lip	128.6	295.7	2.30
LD9:1 + 0.05 Lip	84.6	284.8	3.37
LD9:1 + 0.1 Lip	54.4	306.2	5.63
LD9:1 + 0.5 Lip	48.2	258.5	5.36
LD9:1 + 1.0 Lip	31.1	203.8	6.51

31.1% by lipase addition from 0.05 to 1.0 a.u., respectively (Table **6**).

Lipid Dispersion ePC:Chol=7:3 in the Presence of Lipase

In contrast to previous data (Tables **1**, **3** and **5**) the small changes of ST values by lipase addition (at maximal concentration of 1.0 a.u.) after 0.5 s, 1 s,3 s,5 s and t→∞ found for the mixture of ePC:Chol=7:3 (Table **7**, columns 1-4). These small changes became more pronounced (at 1.2, 3.8, 15.0, 1.3 times) by recalculating the changes between 0.5 s and t→∞ for each of the lipase addition (at concentrations of 0.01, 0.05, 0.1 and 0.5 a.u., Table **7**, lines 1-4, respectively), as compared to the very small changes of 0.29 mN/m for the initial mixture (ePC:Chol=9:1). It is interesting that the ST values after 0.5 s for the initial mixture (ePC:Chol=7:3) were a little bit lower (73.07 mN/m) than those for these mixtures with lipase (73.29 mN/m).

In general, the mean particle diameter (MPD) values decreased drastically by 29.8%, 38.8% and 62.4% by lipase addition from 0.05 to 0.5 a.u., respectively (Table **8**). The effective particle diameter (EPD) values changed by 9.1%, 11.9% and 25.7% by lipase addition from 0.05 to 0.5 a.u., respectively (Table **8**).

Lipid Dispersion ePC:Chol=1:1 in the Presence of Lipase

The total decrease was about 0.56, 0.54, 0.49, 0.53 and 0.54 mN/m by lipase addition (at maximal concentration of 1.0 a.u.) after 0.5 s, 1 s,3 s,5 s and t→∞ for the mixture of ePC:Chol=1:1 as compared to the initial mixture, respectively (Table **9**, columns 1-5). The small changes found (at 1.2, 1.3, 1.7, 1.5, 1.2 times) by recalculating the changes between 0.5 s and t→∞ for each of the lipase addition (at concentrations of 0.01, 0.05, 0.1, 0.5 and 1.0 a.u., Table **9**, lines 1-5,

Table 7: The Current and Equilibrium Surface Tension (ST and eST) Values for the Samples of ePC:Chol=7:3 as Lipid Dispersion (LD7:3) and Lipase (Lip)

Samples	t=0,5 s	t=1 s	t=3 s	t=5 s	t→∞
LD7:3 + 0.01 Lip	73.29	73.26	73.12	73.04	72.94
LD7:3 + 0.05 Lip	73.86	73.32	73.11	72.99	72.75
LD7:3 + 0.1 Lip	73.33	73.31	73.16	73.08	72.99
LD7:3 + 0.5 Lip	73.79	71.20	71.49	73.41	73.40

Table 8: The Mean Particle Diameter (MPD) and Effective Particle Diameter (EPD) Values for the Samples of ePC:Chol=7:3 in the Presence of Lipase (Lip)

Samples	MPD, nm	EPD, nm	EPD/MPD
LD7:3 + 0.01 Lip	199.2	294.9	1.48
LD7:3 + 0.05 Lip	139.8	268.2	1.92
LD7:3 + 0.1 Lip	122.0	259.8	2.13
LD7:3 + 0.5 Lip	74.8	219.1	2.93

Table 9: The Current and Equilibrium Surface Tension (ST and eST) Values for the Samples of ePC:Chol=1:1 as Lipid Dispersion (LD1:1) and Lipase (Lip)

Samples	t=0,5 s	t=1 s	t=3 s	t=5 s	t→∞
LD1:1 + 0.01 Lip	73.20	73.14	72.90	72.78	72.54
LD1:1 + 0.05 Lip	73.07	73.00	72.71	72.56	72.38
LD1:1 + 0.1 Lip	73.26	72.81	72.62	72.50	72.30
LD1:1 + 0.5 Lip	73.02	72.91	72.66	72.55	72.21
LD1:1 + 1.0 Lip	72.64	72.60	72.41	72.25	72.00

respectively), as compared to the very small changes of 0.55 mN/m for the initial mixture (ePC:Chol=1:1).

In contrast to previous data (Tables **2**, **4**, **6** and **8**), the mean particle diameter (MPD) values increased drastically by 12.5%, 30.5%, -16.6% and 28.2% by lipase addition from 0.05 to 1.0 a.u., respectively (Table **10**). The effective particle diameter (EPD) values changed by 6.2%, 7.0%, 5.2% and 8.1% by lipase addition from 0.05 to 1.0 a.u., respectively (Table **10**).

DISCUSSION

The “maximum bubble pressure method” (i.e. BPA-method) is designed to measure surface tension at short surface life times, i.e. for non-equilibrium conditions, which may lead to quantitative differences in the obtained values as compared to the data of well-known works of other authors [36-38] using Langmuir and other methods [20-22, 36-38] for measuring the equilibrium surface tension. Based on similar considerations particle size measurements were also carried out as quickly as possible (approximately in 10 minutes). It is important to highlight that “the type of diameter obtained with photon correlation spectroscopy is the hydrodynamic diameter, which is the diameter that a sphere would have in order to diffuse at the same rate as the particle being measured” [25]. In the presented systems a relatively broad distribution of particle sizes is present. In this case, the effective particle diameter (EPD) measured is an average diameter calculated in the most simple way, i.e. “by treating the data (the correlation function) without intensity-weighted function” [25]. In contrast, the mean particle diameter (MPD) is an average diameter which is “weighted by the intensity of light scattered by each particle (the translational diffusion coefficient is intensity-weighted)” [25]. More precisely, “the effective

Table 10: The Mean Particle Diameter (MPD) and Effective Particle Diameter (EPD) Values for the Samples of ePC:Chol=1:1 in the Presence of Lipase (Lip)

Samples	MPD, nm	EPD, nm	EPD/MPD
LD1:1 + 0.01 Lip	151.5	339.0	2.24
LD1:1 + 0.05 Lip	170.4	317.9	1.87
LD1:1 + 0.1 Lip	197.7	315.2	1.59
LD1:1 + 0.5 Lip	126.3	321.5	2.55
LD1:1 + 1.0 Lip	194.2	311.6	1.60

particle diameter is calculated from the Stokes-Einstein equation (for spheres) from the experimentally determined, translational diffusion coefficient" [25]. That is why the pronounced difference in EPD and MPD values occurred: the EPD to MPD ratios increased from 1.7 to 2.0, from 1.8 to 2.6, from 2.3 to 6.5, from 1.5 to 2.9 for the samples of ePC:Chol at the ratios of 19:1, 14:1, 9:1, 7:1, respectively by lipase concentration increase from 0.05 to 1.0 a.u (Tables **2**,**4**,**6**,**8**). The initial EPD/MPD ratio (388.2 to 227.8 nm) of the lipid mixture without lipase was about 1.70.

The specified conditions with large amounts of cholesterol in the system (30-50%) led to a large scatter of data (Tables **8** and **10**). We believe that this is due to the non-equilibrium state of the system, especially with the formation of cholesterol domains on particles surface. In this case, an adsorption of lipase occurs mainly at the boundaries of the liquid-expanded layer of ePC and liquid-condensed cholesterol domains on such particle surfaces that is with agreement of the data published recently [36-38]. With lower amounts of cholesterol - 10% (Table **6**), the cholesterol domains are not the prevailing structure, and with amounts of cholesterol of 5% or less (Tables **2** and **4**), cholesterol molecules are distributed in defects of the liquid-expanded lipid layer [36-38].

Based on the literature data [20-22, 36-38] it can be concluded that the interaction of lipase occurs only with a liquid-expanded lipid layer formed mainly from PC molecules, while there is no interaction with liquid-condensed domains. The MPD and EPD values for the first three systems (Tables **2**, **4** and **6**) significantly decrease with an increase in the amount of lipase in the system what can be explained by an increase in the degree of organization of ePC molecules in the layer during the lipid-lipase interaction. It is well known that the interaction of lipase with a phospholipid particle at the initial stage occurs to a greater extent due to electrostatic interactions and only in less degree by hydrophobic or Van der Waals interactions [1, 20-22, 36-38]. The priority of electrostatic interactions is associated with the need to overcome the double electric layer of the particle, in order to open the "lid" of the enzyme and then the β-loops that close the hydrophobic region (pocket for triglyceride molecules in the active center of the enzyme) [1, 20-22].

Unlike the monolayers or milk fat droplet systems studied earlier [1, 20-22, 36-38], the systems, studied in this work, lack triglyceride molecules. Therefore, there is no hydrophobic substrate, which in the case of fat droplets could be the driving force for the further advancement of lipase into the fat particle, i.e. lipase in this case lags behind in the near-surface layer of the particle - at the level of the hydrophilic heads. Lipase, building up in the lipid layer, changes its structure and allows the formation of particles of a smaller diameter in the same way as it happens with the addition of cholesterol to the vesicles of phospholipid [20-22]. With an increase in the amount of lipase in the mixture a large number of ePC-Chol-Lipase particles of a smaller diameter (from 10% to 30% roughly) are obtained. Under equilibrium conditions such a redistribution of particle sizes would take a lot of time but since all the systems in our experiments undergo "ultrasonication" before each measurement the speed of the process increases dramatically.

CONCLUSIONS

The choice of the "maximum bubble pressure" method was dictated by the need of fast measurements in the lipid-protein systems (even at not equilibrium conditions) what is very important for preserving the native structure of biological systems especially enzymes like lipase. An addition of lipase led to small decrease both, in ST and eST for the samples of ePC:Chol (in the ratios from 19:1 to 1:1). In contrast the mean particle diameter (MPD) and effective particle diameter (EPD) values for the samples of ePC:Chol changed drastically by lipase addition. Probably, the decrease in ST, MPD and EPD occurred by rearrangements of the particles with various size distributions (in such complex colloid dispersions) in the presence of lipase. Thus, the strong interactions of lipase with the mixture of the phospholipid and cholesterol have had completely different features that we expected before the experiments. It seems an interesting a further comparative study of the inorganic and organic compounds with such models of biological membranes for both, fundamental and applied aspects.

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REFERENCES

[1] Zaitsev SYu, Savina AA, Zaitsev IS. Biochemical aspects of lipase immobilization at polysaccharides for biotechnology. Advances in Colloid and Interface Science 2019; (272): 102016. https://doi.org/10.1016/j.cis.2019.102016

[2] Javed S, Azeem F, Hussain S, Rasul I, Siddique MH, Riaz M, Afzal M, Kouser A, Nadeem H. Bacterial lipases: A review on purification and characterization. Progress in Biophysics and Molecular Biology 2018; 132: 23-34. https://doi.org/10.1016/j.pbiomolbio.2017.07.014

[3] Treichel H, de Oliveira D, Mazutti MA, Di Luccio M, Oliveira JV. A review on microbial lipases production. Food Bioprocess Technology 2010; 3: 182-96.

[4] Gupta R, Gupta N, Rathi P. Bacterial lipases: an overview of production, purification and biochemical properties. Applied Microbiology and Biotechnology 2004; 64(6): 763–81.

[5] Robles-Medina A, Gonzalez-Moreno PA, Esteban-Cerdan L, Molina-Grima E. Biocatalysis: Towards ever greener biodiesel production. Biotechnol Adv 2009; 27(4): 398-408. https://doi.org/10.1016/j.biotechadv.2008.10.008

[6] Sangeetha R. Bacterial lipases as potential industrial biocatalysts: An overview. Res. J. Microbiol. 2011; 6: 1–24. https://dx.doi.org/10.3923/jm.2011.1.24

[7] Salihu A, Alam MZ. Solvent tolerant lipases: A review. Process Biochem 2015; 50: 86–96.

[8] Jaeger K-E, Dijkstra BW, Reetz MT. Bacterial biocatalysts: Molecular Biology, Three- Dimensional Structures and Biotechnological Applications of Lipases. Annual Review of Microbiology 1999; 53(1): 315-51. https://doi.org/10.1146/annurev.micro.53.1.315

[9] Jaeger K-E, Eggert T. Lipases for biotechnology. Current Opinion in Biotechnology 2002; 13(4): 390–97. https://doi.org/10.1016/s0958-1669(02)00341-5

[10] Jaeger K-E, Reetz MT. Microbial lipases form versatile tools for biotechnology. Trends in Biotechnology 1998; 16(9): 396–403. https://doi.org/10.1016/s0167-7799(98)01195-0

[11] Andualema B, Gessesse A. Microbial Lipases and Their Industrial Applications: Review. Biotechnology 2012; 11: 100-118. https://dx.doi.org/10.3923/biotech.2012.100.118

[12] Sharma R, Chisti Y, Banerjee UC. Production, purification, characterization, and applications of lipases. Biotechnology Advances 2001; 19: 627-62. https://doi.org/10.1016/s0734-9750(01)00086-6

[13] Savina AA, Abramova OV, Garnashevich LS, Zaitsev IS, Voronina OA, Tsarkova MS, Zaitsev SYu. Study of Catalytic Activity of Lipase and Lipase-Chitosan Complexes in Dynamics. Journal of Research Updates in Polymer Science 2019; 8: 15-20.

[14] Kumar R., Goomber S, Kaur, J. Engineering lipases for temperature adaptation: Structure function correlation. Biochimica et Biophysica Acta - Proteins and Proteomics 2019; 1867: 140261. https://doi.org/10.1016/j.bbapap.2019.08.001

[15] Sankar S., Ponnuraj K. Less explored plant lipases: Modeling and molecular dynamics simulations of plant lipases in different solvents and temperatures to understand structure-function relationship. International Journal of Biological Macromolecules 2020; 164: 3546-3558. https://doi.org/10.1016/j.ijbiomac.2020.08.227

[16] Kurtovic I., Nalder TD., Cleaver H., Marshall S.N. Immobilisation of Candida rugosa lipase on a highly hydrophobic support: A stable immobilised lipase suitable for non-aqueous synthesis. Biotechnology Reports 2020; 28: e00535. https://doi.org/10.1016/j.btre.2020.e00535

[17] Bharathi D, Rajalakshmi G. Microbial lipases: An overview of screening, production and purification. Biocatalysis and Agricultural Biotechnology 2019; 22: 101368 https://doi.org/10.1016/j.bcab.2019.101368

[18] Bilala M., Iqbal HMN. Armoring bio-catalysis via structural and functional coordination between nanostructured materials and lipases for tailored applications. International Journal of Biological Macromolecules 2020; In Press. https://doi.org/10.1016/j.ijbiomac.2020.10.239

[19] Zaitsev SYu. Biological chemistry: from biologically active substances to organs and tissues of animals. Moscow: Capital Print Publishing; 2017.

[20] Zaitsev SYu. Supramolecular Nanosized Systems at the Phase Interface: Concepts and Prospects for Bio-Nanotechnologies. Moscow: LENAND; 2010.

[21] Zaitsev SYu. Membrane Structures Based on Synthetic and Natural Polymers. Moscow: Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology; 2006.

[22] Zaitsev SYu. Supramolecular Systems at the Phase Interface as Models of Biomembranes and Implications for Nanomaterials. Moscow, Donetsk: Nord Computer; 2006.

[23] Torchilin V. Nanoparticulates as Drug Carriers. New York: World Scientific Publishing Co.; 2006.

[24] Andresen TL, Jensen SS, Jorgensen K. Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release. Progress in Lipid Research 2005; 44: 68-97.

[25] BrookhavenInstruments [homepage on the Internet]. Instruction Manual for 90Plus/BI-MAS (Multi Angle Particle Sizing Option Operation Manual) Brookhaven Instruments Corporation Holtsville, NY 11742 USA 2005-2010. 73 pages. www.BrookhavenInstruments.com

[26] Chen J., Lin S., Sun N., Bao Z., Shen J., Lu X. Egg yolk phosphatidylcholine: Extraction, purification and its potential neuro-protective effect on PC12 cells. Journal of Functional Foods 2019; 56: 372-383. https://doi.org/10.1016/j.jff.2019.03.037

[27] Bao Z., Zhang P., Chen J., Gao J., Lin S., Sun, N. Egg yolk phospholipids reverse scopolamine–induced spatial memory deficits in mice by attenuating cholinergic damage. Journal of Functional Foods 2020; 69: 103948. https://doi.org/10.1016/j.jff.2020.103948

[28] Dong XF., Zhai QH., Tong JM. Dietary choline supplementation regulated lipid profiles of egg yolk, blood,and liver and improved hepatic redox status in laying hens. Poultry Science 2019; 98: 3304-3312. https://doi.org/10.3382/ps/pez139

[29] Yamamoto Y., Harada K., Kasuga S., Hosokawa M. Phospholipase A2-Mediated preparation of phosphatidylcholine containing ricinoleic acid and its anti-inflammatory effect on murine macrophage-likeRAW264.7 cells. Biocatalysis and Agricultural Biotechnology 2019; 19: 101141. https://doi.org/10.1016/j.bcab.2019.101141

[30] Janist N., Srichana P., Asawakarn T., Kijparkorn S. Effect of supplementing the laying hen diets with choline, folic acid, and vitamin B12 on production performance, egg quality, and yolk phospholipid. Livestock Science 2019; 223: 24-31 https://doi.org/10.1016/j.livsci.2019.02.019

[31] Gliozzi M., Musolino V., Bosco F., Scicchitano M., Scarano F., Nucera S., Zito MC., Ruga S., Carresi C., Macrì R., Guarnieri L., Maiuolo J., Tavernese A., Coppoletta AR., Nicita C., Mollace R., Palma E., Muscoli C., Mollace V. Cholesterol homeostasis: Researching a dialogue between the brain and peripheral tissues. Pharmacological Research 2020; 105215 In Press. https://doi.org/10.1016/j.phrs.2020.105215

[32] Oakes V., Domene C. Influence of Cholesterol and Its Stereoisomers on Members of the Serotonin Receptor Family. Journal of Molecular Biology 2019; 431: 1633-1649 https://doi.org/10.1016/j.jmb.2019.02.030

[33] Zhang X., Huang Q., Wang X., Deng Z., Li J., Yan X., Jauhiainen M., Metso J., Libby P., Liu J., Shi G.-P. Dietary cholesterol is essential to mast cell activation and associated

obesity and diabetes in mice. Biochimica et Biophysica Acta - Molecular Basis of Disease 2019; 1865: 1690-1700. https://doi.org/10.1016/j.bbadis.2019.04.006

[34] Wen X., Wu W., Fang W., Tang S., Xin H., Xie J., Zhang H. Effects of long-term heat exposure on cholesterol metabolism and immune responses in growing pigs. Livestock Science 2019; 230: 103857. https://doi.org/10.1016/j.livsci.2019.103857

[35] Lin C.-W., Huang T.-W., Peng Y.-J., Lin Y.-Y., Mersmann HJ., Ding S.-T. A novel chicken model of fatty liver disease induced by high cholesterol and low choline diets. Poultry Science 2020; In Press. https://doi.org/10.1016/j.psj.2020.11.046

[36] Bourlieu C, Paboeuf G, Chever S., Pezennec S, Cavalier JF, Adsorption of gastric lipase onto multicomponent model lipid monolayers with phase separation. Colloids and Surfaces B: Biointerfaces, Elsevier, 2016, 143, pp.97-106. https://doi.org/10.1016/j.colsurfb.2016.03.032

[37] Bénarouche A, Sams L, Bourlieu C, Vié V, Point V, Cavalier JF, Carrière F. Studying Gastric Lipase Adsorption Onto Phospholipid Monolayers by Surface Tensiometry, Ellipsometry, and Atomic Force Microscopy. Methods Enzymol. 2017; 583: 255-278. doi: 10.1016/bs.mie.2016.09.039. Epub 2016 Nov 14.PMID: 28063494

[38] Bourlieu C, Mahdoueni W, Paboeuf G, Gicquel E, Ménard O, Pezennec S, Bouhallab S, Deglaire A, Dupont D, Carrière F, Vié V. Physico-chemical behaviors of human and bovine milk membrane extracts and their influence on gastric lipase adsorption. Biochimie. 2020 Feb;169:95-105. doi: 10.1016/j.biochi.2019.12.003. Epub 2019 Dec 20.PMID: 31866313

[39] Zaitsev SYu. Dynamic surface tension measurements as general approach to the analysis of animal blood plasma and serum. Advances in Colloid and Interface Science 2016; (235): 201–213. https://doi.org/10.1016/j.cis.2016.06.007

[40] Zaitsev SYu, Savina AA, Garnashevich LS, Tsarkova MS, Zaitsev IS. Effect of Some Charged Polymers on the Activity of Pancreatic Porcine Lipase. BioNanoScience 2019; 9 (4): 773-777. https://doi.org/10.1007/s12668-019-00677-1

[41] Savina AA., Garnashevich LS. Zaitsev IS, Tsarkova MS, Zaitsev SYu. Changes in lipase activity in the presence of synthetic and natural polymers. Moscow University Chemistry Bulletin. Series 2: Chemistry 2019; 74(6): 306–309. https://doi.org/10.3103/S0027131419060130

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