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Membrane effects of lysozyme amyloid fibrils

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ABSTRACT

The influence of mature lysozyme fibrils on the structural and physical properties of model membranes composed of phosphatidylcholine (PC) and its mixtures with cardiolipin (CL) (10 mol%) and cholesterol (Chol) (30 mol%) was studied using fluorescent probes DPH, pyrene, Laurdan and MBA. Analysis of pyrene fluorescence spectra along with the measurements of DPH fluorescence anisotropy revealed that the structure of hydrocarbon chains region of lipid bilayer is not affected by the fibrillar aggregates of lysozyme. In contrast, probing the membrane effects by Laurdan and MBA showed the rise of both the generalized polarization of Laurdan and the MBA fluorescence anisotropy, suggesting that amyloid protein induces reduction of bilayer hydration and increase of lipid packing in the interfacial region of model membranes.

1. Introduction

A vast number of severe disorders including Parkinson's, Alzheimer's and Huntington's diseases, type II diabetes, systemic amyloidosis, etc. are currently associated with the deposits of insoluble protein aggregates (amyloid fibrils) in various human organs and tissues (Glabe, 2006; Stefani, 2004). The discovery of this correlation has led to drastic upsurge of interest in protein folding and aggregation. Amyloid fibrils were found to share common structural features, regardless of amino acid sequence (Tycko, 2004; Kayed et al., 2003). Typically, amyloid fibrils are straight, 6–12 nm in width and about 1 μm in length. They are formed by a variable number of elementary filaments (1.5–2.0 nm in diameter) twisted around each other (Stefani, 2004). A defining characteristic of amyloid fibrils is the presence of cross-β structure as revealed by X-ray diffraction, circular dichroism, solid-state NMR and EPR measurements (Tycko, 2004; Kayed et al., 2003; Chiti et al., 1999; Naito and Kawamura, 2007). The dyes, such as Congo red and Thioflavin T (ThT), are traditionally used as optical markers for amyloid fibrils.

At present more than 30 proteins are reported to form toxic aggregates, each of which is associated with a particular disease. Increasing evidence supports the idea that amyloid formation is a generic property of polypeptide chain, determined by the nature of peptide backbone, independently of the specific amino acid sequence (Chiti et al., 1999; Stefani, 2008). It was suggested that protein folding and aggregation are competing pathways (Stefani, 2008). The factors, such as acidic pH, high temperature, lack of ligands or moderate concentrations of salts, reduce conformational stability of a protein molecule and may cause aggregation. Numerous studies indicate that surfaces, in particular, cell membranes, can promote protein misfolding and fibril formation (Stefani, 2008; Gorbenko and Kinnunen, 2006; Relini et al., 2009). Furthermore, it is becoming increasingly recognized that cell membrane is a primary target for amyloid proteins (Butterfield and Lashuel, 2010; Wang et al., 2008; Friedman et al., 2009). The exact molecular mechanisms of membrane damage by toxic protein aggregates have not been ascertained yet, but several hypotheses based on the experimental data have been proposed. There are some disagreements as to three aspects of amyloid toxicity, viz.: (i) whether mature amyloid fibrils or their precursors (oligomeric prefibrillar aggregates) are the most cytotoxic species; (ii) whether amyloids increase lipid bilayer conductance or form discrete pores and channels; (iii) what kind of cell death, apoptosis or necrosis is induced by the protein aggregates.

Kayed et al. (2004) and Sokolov et al. (2006) have reported reduction of the bilayer dielectric barrier under the influence of oligomeric amyloid β-peptide without any evidence of the single channel formation. The observations, such as the lack of selectivity, increase of the induced bilayer conductance with solution conductivity for a variety of ions, and the absence of current jumps typical for single channels, have been interpreted in terms of Aβ-induced increase of the area per lipid molecule, followed by the bilayer thinning and lowering the dielectric barrier (Sokolov et al., 2006). The validity of this viewpoint is confirmed also by atomic force

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microscopy images (Green et al., 2004). In contrast to the above idea, the 'channel hypothesis' is strengthened by the observation of stepwise ion flux across planar lipid bilayers and cell membranes induced by oligomeric Aβ-peptide. Capone and coworkers have attributed the observed gradual thinning of membranes with concomitant growth of transmembrane current to the residues of the solvent hexafluoroisopropanol, which was used for the preparation of amyloid samples (Capone et al., 2009). The hypothesis of discrete pore formation as a key permeabilization process is proposed in the reviews by Stefani (2004), Butterfield and Lashuel (2010). Membrane permeabilization induced by amyloid oligomers can initiate increased production of reactive oxygen species (ROS) (Cecchi et al., 2005).

As regards to the membrane damage induced by mature amyloid fibrils, there is some experimental evidence demonstrating the toxicity of fibrillar aggregates (Gharibyan et al., 2007; Novitskaya et al., 2006). Novitskaya and coauthors showed that both the β-oligomers and amyloid fibrils of recombinant mammalian prion protein are highly toxic to cultured cells, hippocampal and cerebellar neurons. The percent of cell death in cultured cells treated with β-oligomers was lower than that in the cells treated with fibrils (Novitskaya et al., 2006). In this study amyloid fibrils and the β-oligomers of prion protein were found to induce apoptosis. However, Gharibyan and coworkers reported that lysozyme fibrils produce necrosis-like cell death, while the cells treated with protein oligomers undergo the apoptotic changes (Gharibyan et al., 2007). Another pathway for the cytotoxicity of amyloid fibrils was proposed by Huang and coworkers, who observed hemolysis and aggregation of erythrocytes accompanied by disulfide cross-linking under the influence of lysozyme protofibrils and fibrils (Huang et al., 2009). Recent studies revealed that fibrillar lysozyme induced liposome leakage resembling the effect of carbon nanotube conjugates with adsorbed lysozyme (Hirano et al., 2010). Along with direct membrane-disrupting action of mature fibrils, their cytotoxicity has been proposed to be associated with fibril growth at lipid matrix (Engel et al., 2008). Another possibility involves fibril fragmentation leading to production of shorter fibrillar aggregates, which possess a higher cytotoxic activity comparing to their longer counterparts (Xue et al., 2009). Nevertheless, in spite of the rapidly growing amount of experimental data on the role of aggregated proteins in the amyloid related diseases, the nature of the toxic species still remains obscure.

In the present study we focused our efforts on the investigation of the influence of fibrillar aggregates of lysozyme, a protein possessing bactericidal and immunomodulatory activities, on the structural and physical properties of the model membranes composed of phosphatidylcholine (PC) and its mixtures with cardiolipin (CL) (10 mol%) and cholesterol (Chol) (30 mol%). Cardiolipin, an important component of bacterial and mitochondrial membranes, was used to create model systems with negative charge. Examining the effects of amyloid fibrils on CL-containing membranes seems to be of importance in the context of recent reports indicating that mitochondrial membrane perturbation leading to enzyme release is one of the possible mechanisms of amyloid toxicity (Maratan et al., 2011). The use of Chol-containing membranes was performed in view of increased interest in specific influence of cholesterol, which has an ability to modify lipid bilayer parameters (such as thickness, molecular packing, conformational freedom of acyl chains, headgroup hydration, membrane excitability in neurons and membrane fluidity), on amyloid–lipid interactions.

To evaluate the extent of lipid bilayer modification, we used fluorescence spectroscopy, one of the most accurate and powerful tools for monitoring the changes in membrane structure. Our goal was to ascertain what kind of structural alterations fibrillar protein species can produce in the lipid bilayer. To achieve this purpose, four fluorescent probes with different spectral properties and bilayer location have been employed, viz. pyrene and 1,6-diphenyl-1,3,5-hexatriene (DPH), distributing in the acyl chain region, as well as Laurdan and 3-methoxybenzantrone (MBA) residing at the lipid–water interface.

2. Materials and methods

2.1. Materials

Egg yolk phosphatidylcholine (PC), cardiolipin (CL) and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL). Chicken egg white lysozyme and pyrene were obtained from Sigma (St. Louis, MO, USA). Laurdan (6-Lauroyl-2-dimethylaminonaphthalene) was from Invitrogen Molecular Probes (Eugene, OR, USA). MBA was from Zonde (Latvia), DPH was from EGA Chemie (Steinheim, Germany).

2.2. Preparation of lysozyme fibrils

The reaction of lysozyme fibrillization was initiated using the approach developed by Holley et al. (2008). Protein solutions (3 mg/ml) were prepared by dissolving lysozyme in deionized water with subsequent slow addition of ethanol to a final concentration 80%. Next, the samples were subjected to constant agitation at ambient temperature. This resulted in the formation of lysozyme fibrils over a time course of about 30 days. The amyloid nature of fibrillar aggregates was confirmed in ThT and electron microscopy assays.

2.3. Preparation of lipid vesicles

Large unilamellar lipid vesicles were prepared by the extrusion technique from PC and its mixtures with CL (10 mol%) and Chol (30 mol%). The thin lipid film was obtained by evaporation of lipids' ethanol solutions and then hydrated with 1.2 ml of 5 mM Na-phosphate buffer (pH 7.4) to yield final lipid concentration 2 mM. Lipid suspension was extruded through a 100 nm pore size polycarbonate filter.

2.4. Fluorescence measurements

Steady-state fluorescence spectra were recorded with LS-55 spectrofluorimeter (Perkin Elmer, Great Britain) equipped with magnetically stirred, thermostated cuvette holder. Fluorescence measurements were performed at 20 °C using 10 mm path-length quartz cuvettes. Emission spectra were recorded with excitation wavelengths of 340 nm (pyrene), 364 nm (Laurdan). Anisotropy measurements were performed with excitation wavelengths of 420 nm (MBA) and 350 nm (DPH).

The ratio of vibronic bands in the pyrene fluorescence spectra (I_I/I_{III}) was calculated from the intensities at 371 nm (I_I) and 382 nm (I_{III}). The excimer-to-monomer fluorescence intensity ratio (E/M) was determined by measuring fluorescence intensity at the monomer (391 nm) and excimer (466 nm) peaks.

The generalized polarization (GP) of Laurdan fluorescence was determined as (Parasassi et al., 1998)

$$GP = \frac{I_B - I_R}{I_B + I_R} \quad (1)$$

where I_B and I_R are the maximum fluorescence intensities of the blue (440 nm) and red (490 nm) spectral components, respectively.

Deconvolution of MBA fluorescence spectra was performed with Origin 7.0 (OriginLab Corporation, Northampton, USA), with

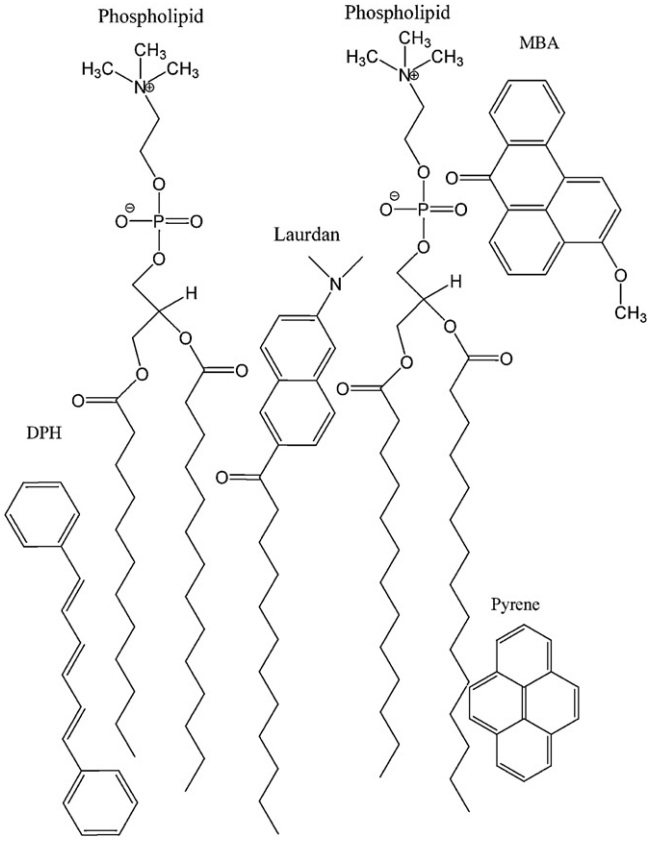

Fig. 1. Schematic representation of the location of the employed fluorescent probes in a lipid bilayer.

individual spectral components being approximated by a log-normal function:

$$I(\lambda) = \frac{I_1}{\sqrt{2\pi w_1 \lambda}} \exp\left\{-\frac{(\ln\ (\lambda/\lambda_1^{max}))^2}{2w_1^2}\right\} + \frac{I_2}{\sqrt{2\pi w_2 \lambda}} \exp\left\{-\frac{(\ln\ (\lambda/\lambda_2^{max}))^2}{2w_2^2}\right\} \quad (2)$$

where λ_1^{max} = 520 nm and λ_2^{max} = 560 nm are maxima of the first and the second spectral bands, respectively; w_1 and w_2 are parameters characterizing the width of the spectrum of the first and the second components; I_1 and I_2 are fluorescence intensities at λ_1^{max} and λ_2^{max}, respectively.

3. Results and discussion

3.1. Effect of the lysozyme fibrils on the hydrocarbon core of lipid bilayer

To monitor the membrane modifications occurring at the level of acyl chains we used fluorescent probes pyrene and DPH, localized in the hydrophobic bilayer region (Fig. 1). Pyrene is a classical nonpolar fluorescent probe whose spectra have characteristic vibronic structure in the wavelength region 370–400 nm. A distinctive feature of pyrene is an ability of excited probe molecule to form a complex with unexcited one. Such a complex is called an excimer and is recognized by the appearance of a new fluorescent band at longer wavelengths compared to the monomer fluorescence (Lakowicz, 2006; Novikov et al., 2000). Excimer-to-monomer intensity ratio (E/M) reflects the extent of pyrene excimerization, determined by the frequency of collisions between pyrene monomers in the lipid bilayer, which, in turn, is a function of molecular packing density (Samoilenko et al., 1999; L'heureux and Fragata, 1989). As seen in Table 1, excimer-to-monomer intensity ratio in all types of investigated lipid vesicles was not markedly influenced by the fibrillar aggregates of lysozyme, suggesting that membrane fluidity remained practically unchanged.

In addition to E/M value, analysis of pyrene fluorescence spectrum yields another significant parameter, the intensity ratio of the first (I_I) to third peaks (I_{III}). It was proved experimentally that I_I/I_{III} quantity characterizes the polarity of the probe microenvironment (Kalyanasundaram and Thomas, 1977; Karpovich and Blanchard, 1995). For example, I_I/I_{III} in hydrocarbon solvents is about 0.6, in ethanol ~1.1, and in water ~1.96 (Karpovich and Blanchard, 1995). Similarly to E/M, the magnitude of I_I/I_{III} ratio was not affected by the protein (Table 1), indicating that lysozyme fibrillar aggregates have no effect on the hydration level in the acyl group membrane region.

Thus, using fluorescent probe pyrene, we did not observe any influence of lysozyme mature fibrils on the structural state of nonpolar region of lipid bilayer, composed of PC and its mixtures with CL (10 mol%) or Chol (mol%). Notably, similar results were obtained in the study of fibrillar lysozyme effects on the parameters of pyrene fluorescence in liposomes containing phosphatidylglycerol or phosphatidylserine instead of CL (data not shown).

To confirm or disprove this fact, another environmentally sensitive probe DPH has been employed. This probe is widely used to gain insights into molecular organization and dynamics of the membrane hydrocarbon core. The DPH fluorescence anisotropy characterizes the rate of the probe rotational diffusion and is correlated with the membrane microviscosity (Lakowicz, 2006). DPH anisotropy measurements, performed in a number of studies into cytotoxic effects of amyloid proteins, yielded controversial results (Muller et al., 1998; Kremer et al., 2000, 2001; Muller et al., 2001; Ma et al., 2002). In the works of Kremer et al., aggregated β-amyloid peptide was reported to reduce the fluidity of unilamellar liposomes composed of anionic, cationic, and zwitterionic phospholipids as judged from the increased DPH fluorescence anisotropy. This finding suggests that Aβ aggregates interact with the hydrophobic core of lipid bilayer (Kremer et al., 2000, 2001). Muller and coauthors found similar effects of Aβ peptides on human cortex membranes. The influence of Aβ peptide on membrane fluidity at the lipid–water interface was quantitatively less than at the level of acyl chains (Muller et al., 1998, 2001). However, according to the data obtained by Xiaocui Ma, aggregated Aβ exerts influence mainly on the bilayer surface and has little effect on the hydrophobic membrane region (Ma et al., 2002). The disagreements between the above data may arise from amyloid heterogeneity and polymorphism. A number of recent studies suggest that

Table 1
Effect of lysozyme fibrils on vibronic structure of pyrene fluorescence spectra and excimer-to-monomer fluorescence intensity ratio.

Liposome composition	Without protein		In the presence of fibrillar lysozyme (0.96 μM)	
	I_I/I_{III}	E/M	I_I/I_{III}	E/M
PC	0.95 ± 0.05	0.39 ± 0.02	0.94 ± 0.05	0.37 ± 0.02
PC:CL (10%)	0.96 ± 0.05	0.52 ± 0.03	0.96 ± 0.05	0.50 ± 0.03
PC:Chol (30%)	0.94 ± 0.05	0.34 ± 0.02	0.93 ± 0.05	0.32 ± 0.02

different destabilizing conditions may alter the folding (or misfolding) of the same protein in different ways, resulting in production of oligomers with different conformational features, which, in turn, grow into polymorphic mature fibrils. The presence of structural polymorphism in amyloid assemblies was found to modulate their interactions with plasma membranes and the following membrane permeabilization (Stefani, 2010).

In our study DPH fluorescence anisotropy remained practically changeless with the growth of amyloid lysozyme concentration (Fig. 2). These results indicate that fibrillar protein does not interact with the hydrocarbon core of the lipid bilayer and has no effect on the membrane microviscosity in the nonpolar region.

3.2. Influence of the fibrillar lysozyme on the interfacial membrane layer

To monitor the membrane perturbations at lipid–water interface, two other fluorescent probes, MBA and Laurdan have been employed. The fluorescent probe MBA has a number of features, which make it a suitable reporter molecule in examining the membrane properties. MBA is poorly soluble in water and easily binds to the membranes in aqueous suspensions, with concomitant significant enhancement of its fluorescence intensity. Since MBA fluorescence is strongly quenched by water molecules, it displays high sensitivity to the degree of membrane hydration. In highly anisotropic systems, such as lipid bilayer, the population of the probe molecules is not homogeneous (Dobretsov et al., 1978). MBA, similar to other probes, was found to distribute between two membrane sites, located presumably at the level of phosphate groups and glycerol backbone. Accordingly, MBA fluorescence spectra in liposomes can be represented as a sum of two separate bands with the maxima around 520 and 560 nm. The shape and maximum of the overall MBA spectrum are determined by the relative contributions of these components. Shown in Fig. 3 are MBA fluorescence

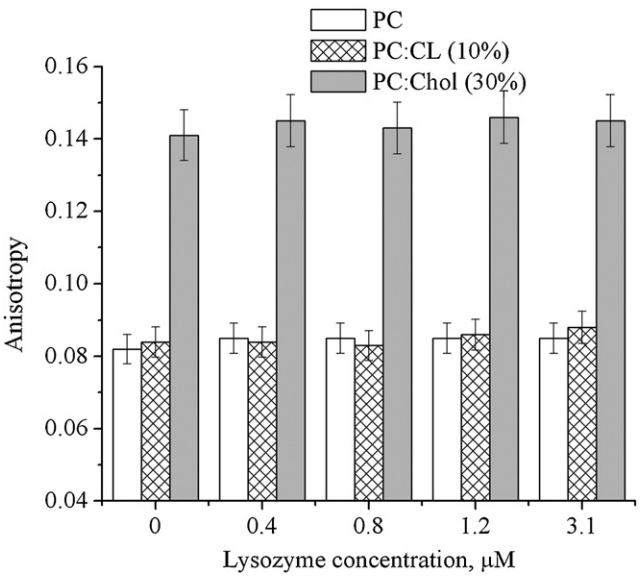

Fig. 2. Anisotropy of DPH fluorescence in lipid vesicles.

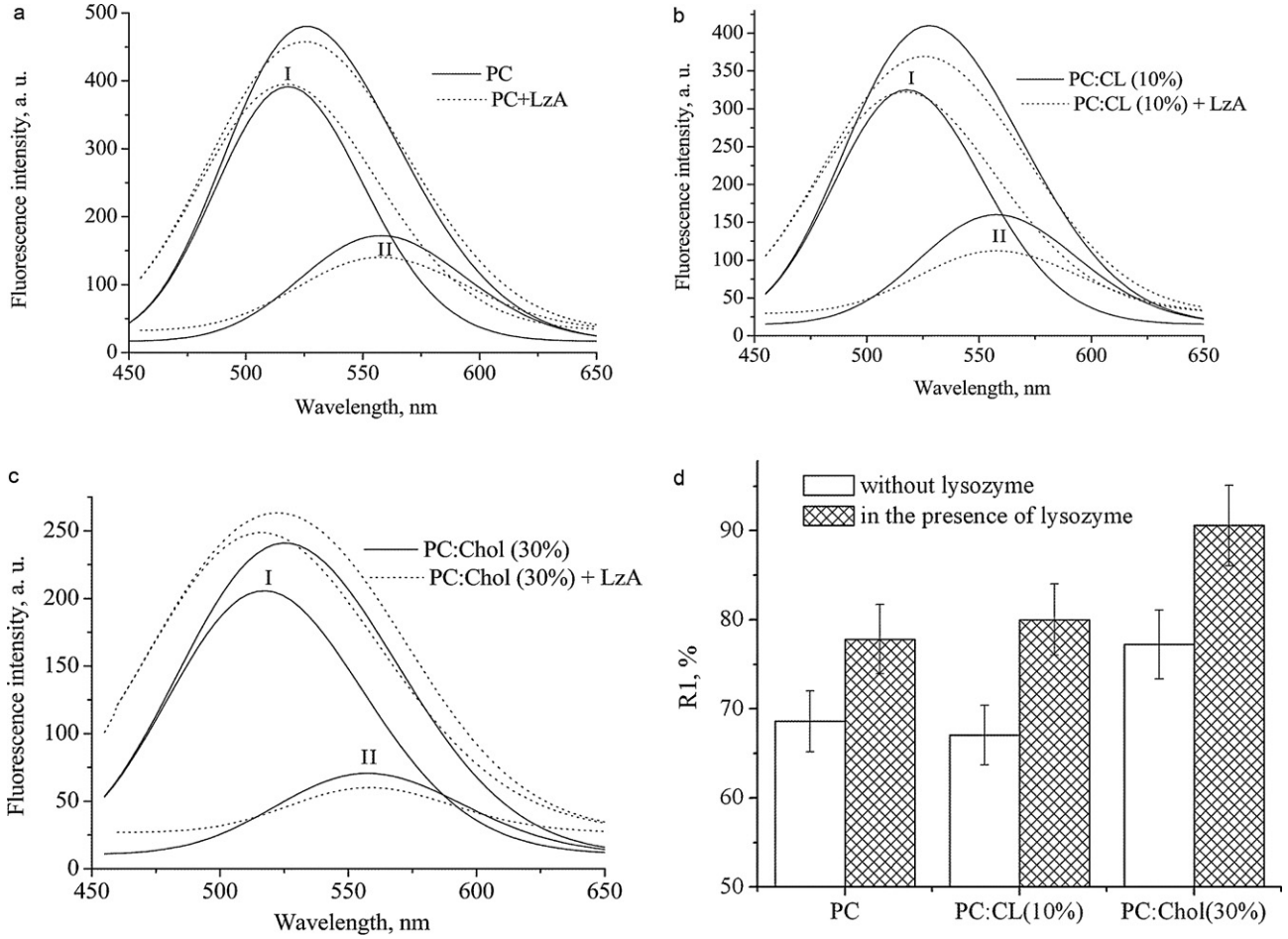

Fig. 3. Deconvolution of MBA emission spectra in lipid and lipid-lysozyme systems. A – PC, B – PC:CL (10%), C – PC:Chol (30%), D – relative contributions of the short-wavelength spectral component into the total MBA spectra. LzA – amyloid lysozyme. Protein concentration was 3.1 μM.

Table 2
The changes in anisotropy of MBA fluorescence under the influence of amyloid lysozyme.

Concentration of fibrillar lysozyme (μM)	PC	PC:CL (10%)	PC:Chol (30%)
0	0.035 ± 0.001	0,038 ± 0.002	0.052 ± 0.002
0.4	0.044 ± 0.001	0.050 ± 0.002	0.065 ± 0.001
0.8	0.053 ± 0.001	0.064 ± 0.001	0.080 ± 0.001
1.2	0.062 ± 0.001	0.077 ± 0.001	0.092 ± 0.001
3.1	0.156 ± 0.001	0.122 ± 0.001	0.162 ± 0.001

spectra deconvoluted into two bands and relative contribution of the short-wavelength spectral component (R1) in different lipid vesicles. Spectra deconvolution revealed small but statistically significant increase of R1 value in all types of the model membranes under the influence of fibrillar lysozyme. The rise of the short-wavelength spectral component of MBA can be caused by the decreased concentration of water molecules in the interfacial layer, leading to the increase of quantum yield of this component, and/or relocation of the probe molecules from the phosphate groups to glycerol backbone region. Since we found comparable effects in PC, PC:CL (10 mol%) and PC:Chol (30 mol%) liposomes, the most probable explanation for the increased R1 value involves bilayer dehydration under the influence of lysozyme fibrils.

At the next step of the study, the anisotropy measurements of MBA fluorescence were carried out. Fluorescence parameters, such as anisotropy and polarization, provide information about the relative angular displacement of the fluorophore between absorption and emission. As mentioned above, they depend on the rate of rotational diffusion during fluorescence lifetime. Diffusion movements, in turn, depend on the solvent viscosity. The changes in anisotropy of MBA fluorescence under the influence of amyloid lysozyme are shown in Table 2 and Fig. 4.

As seen from the presented data, fibrillar lysozyme brought about significant increase of MBA fluorescence anisotropy even at the lowest protein concentration (0.4 μM) – about 26% in PC liposomes, 32% in PC:CL (10 mol%) and 25% in PC:Chol lipid vesicles. With the growth of lysozyme concentration up to 3.1 μM anisotropy value was found to increase by a factor of ~4 in PC liposomes and ~3 in the other types of model membranes. The rise of anisotropy reflects the decrease of the probe mobility in the presence of amyloid fibrils and, consequently, increase of the membrane microviscosity under the influence of lysozyme fibrils.

For deeper understanding of the bilayer-modifying propensities of lysozyme fibrils, fluorescent probe MBA was complemented by the probe Laurdan, sensitive to a variety of membrane processes. Laurdan is an amphiphilic fluorescence probe, synthesized by Gregorio Weber to study the dipolar relaxation processes. A remarkable feature of this probe lies its ability to respond to the environmental changes by the shift of emission maximum (Bagatolli et al., 1998; Mukherjee and Chattopadhyay, 2003). The red spectral shift of Laurdan in solvents of high polarity is induced by partial consumption of the probe excited state energy on reorientation of the surrounding dipoles. This reorientation occurs due to increase of the dipole moment of Laurdan naphthalene moiety upon excitation. The sensitivity of this probe to solvent polarity can be illustrated by the fact that emission maximum of Laurdan in dodecane is near 380 nm, in dimethylsulfoxide ~460 nm, and in methanol ~490 nm (Parasassi et al., 1998).

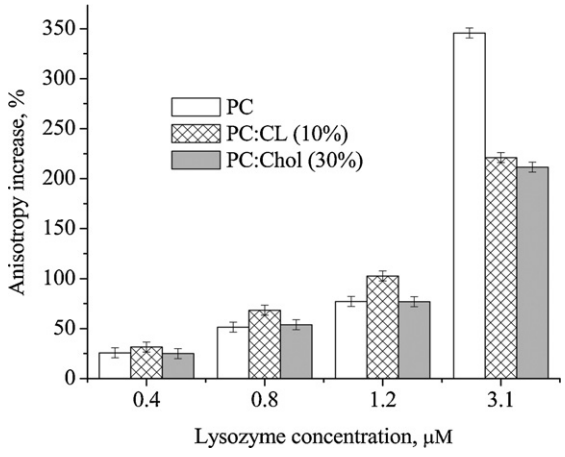

Fig. 4. Relative increase of MBA fluorescence anisotropy under the influence of amyloid lysozyme.

In a lipid bilayer this probe is localized at the glycerol backbone level with the lauric acid tail anchored in the acyl chain region (Fig. 1) (Volinsky et al., 2006). The emission maximum of Laurdan was found to depend strongly on the packing of lipid chains. In the gel state emission maximum is near 440 nm and in the liquid crystalline state it is near 490 nm. At the temperatures above the phase transition the foregoing long-wavelength shift of the Laurdan emission maximum is observed with increasing temperature. This red shift originates from the increased concentration and mobility of water molecules in the lipid bilayer at the level of glycerol backbone. At the temperatures below the phase transition (gel phase) lipid chains are tightly packed and red shift of the emission is not observed.

The spectral changes of Laurdan fluorescence can be quantitatively characterized by the steady-state fluorescence parameter known as the generalized polarization (GP) (Parasassi et al., 1991). This parameter is determined by the dipolar relaxation processes, occurring while Laurdan is in an excited state. To evaluate the extent of alterations in bilayer hydration and lipid packing density upon addition of lysozyme fibrils, we traced the Laurdan GP changes. To calculate the value of this parameter, fluorescence intensities at 440 (I_B) and 490 (I_R) nm were used (Eq. (1)). The GP value of Laurdan in three types of lipid vesicles as a function of amyloid protein concentration is shown in Fig. 5.

As evident from the presented data, in pure PC liposomes and vesicles containing CL, the GP value was negative (about −0.10 and −0.08, respectively), indicating the predominance of the Laurdan molecules located in a more polar environment, while it turned out to attain positive values in the Chol-containing vesicles (about 0.16), reflecting an opposite distribution of the probe species, which tend to occupy mainly less polar bilayer region in this type of liposomes. Addition of fibrillar lysozyme resulted in the increase of the GP value in all types of the model membranes. The rise of GP value could be interpreted in terms of bilayer dehydration and/or increase of the lipid packing density. Remarkably, in PC:Chol (30 mol%) vesicles the effect of amyloid protein was much less pronounced (about 18%) compared to PC liposomes (about 37%). This finding indicates that ability of fibrillar aggregates to alter physical properties of the interfacial membrane region is hampered by cholesterol. The preventing role of cholesterol in the membrane disordering effects induced by oligomeric amyloid proteins is reported in a number of studies (Cecchi et al., 2005; Muller et al., 2001; Sponne et al., 2004). Particularly, it was found that increase of cholesterol content inhibited aggregation and fusion of liposomes

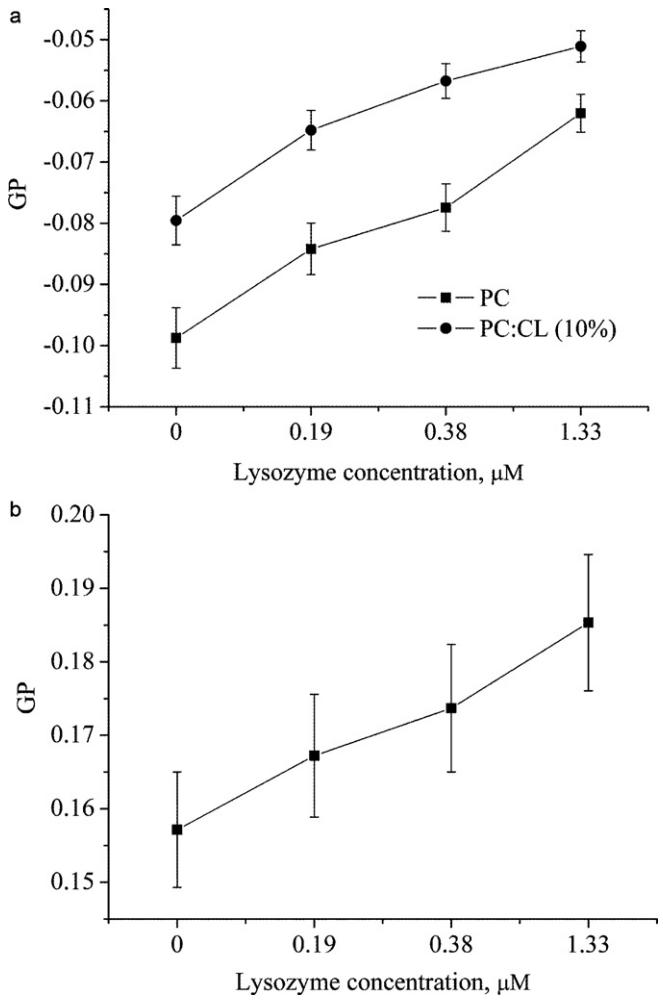

Fig. 5. Generalized fluorescence polarization (GP) of Laurdan emission in the lipid vesicles of various compositions as a function of amyloid lyzozyme concentration. A – PC, PC:CL (10%), B – PC:Chol (30%).

induced by Aβ(1–40) peptide (Sponne et al., 2004). The cell membranes rich in cholesterol were found to possess a strong resistance to prefibrillar aggregates (Cecchi et al., 2005). In our work this ability of cholesterol was observed for mature amyloid fibrils.

Thus, using fluorescent probes MBA and Laurdan we demonstrated that lysozyme fibrils cause structural alterations in the interfacial membrane region. The insertion of the fibrillar aggregates into the membrane hydrophobic region is impeded. This fact can be explained by the structural properties of mature lysozyme fibrils. To penetrate into hydrocarbon core of the lipid bilayer protein aggregates should have exposed hydrophobic surfaces. It is noteworthy in this regard that protofibrils have been proposed to possess amphiphilic properties: the hydrophobic fibril surface associates efficiently with the membrane hydrocarbon core and the hydrophilic surface is responsible for structural, membrane permeabilizing defects (Kinnunen, 2009). A strong correlation among conformational, structural (exposure of hydrophobic patches and flexibility) and stability features of amyloid assemblies and their ability to permeabilize the cell membrane has been recently reported. Highly flexible, less compact HypF-N oligomers with extended exposed hydrophobic surface area were found to be substantially more toxic (Campioni et al., 2010). Furthermore, it has been recently shown that structural and dynamic properties and, in particular, the presence of substantial non-core regions in lysozyme fibrils determinate their cytotoxicity. More specifically, lysozyme fibrils formed at physiological pH were less ordered in the cross-β structure and generated a substantial cytotoxic effect while the highly organized fibrils, which were formed at acidic pH, had a negligible effect on cell viability (Mossuto et al., 2010). Likewise, a significance of the surface hydrophobicity of protein aggregates in amyloid-induced decrease of membrane fluidity was demonstrated for aggregated Aβ peptide (Kremer et al., 2000). In view of these findings, it can be proposed that high ordering and stability as well as minor quantity of the exposed hydrophobic patches and surface charge make it difficult for fibrillar lysozyme to insert into the membrane hydrocarbon core. The data presented here indicate that mature amyloid fibrils interact mainly with the polar membrane region, inducing structural changes at the level of phospholipids headgroups and initial acyl chain carbons.

The results presented here are consistent with recently reported inability of Aβ fibrils to penetrate into lipid monolayer of different composition (Zhang et al., 2012). Besides, it was previously demonstrated that fibrillar IAPP is unable to insert into DOPC/DOPS (7:3) membrane. In contrast to fibrillar species, monomeric IAPP was found to display strong penetration, leading to the fibril formation at the membrane (Engel et al., 2006).

4. Conclusions

To summarize, the present study provides evidence for modifying effect of fibrillar lysozyme on the physical properties of model membranes composed of PC and its mixtures with CL and Chol. The changes in viscosity of lipid bilayers of various composition under the influence of amyloid lysozyme fibrils were characterized by measuring the DPH fluorescence anisotropy and the extent of pyrene excimerization. Both these fluorescence parameters were not markedly affected by fibrillar protein in all types of the model membranes. The effect of amyloid fibrils on the bilayer properties at lipid–water interface was analyzed by the generalized polarization value of Laurdan fluorescence (GP) and by the anisotropy of MBA fluorescence. It was found that addition of amyloid lysozyme resulted in the increase of both Laurdan GP value and MBA anisotropy. The data presented here suggest that mature lysozyme fibrils may cause bilayer dehydration and/or increase of the lipid packing density. Cholesterol was found to reduce membrane-modifying propensity of lysozyme fibrils. Our results add fuel to the notion that not only prefibrillar oligomeric species, but also mature fibrils may display membranotropic activity, giving rise to the structural changes of lipid bilayer.

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