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Authors

Dr. A.G. Bykov

Affiliations
- St.Petersburg State University, 199034 St. Petersburg, Russia

Mr. E.A. Tsyganov

Affiliations
- St.Petersburg State University, 199034 St. Petersburg, Russia

Miss E.A. Levchuk

Affiliations
- St.Petersburg State University, 199034 St. Petersburg, Russia

Prof. B.A. Noskov
Corresponding Author
Submitting Author

ORCiD
https://orcid.org/0000-0001-8117-1490

Affiliations
- St.Petersburg State University, 199034 St. Petersburg, Russia

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Influence of a lipid monolayer on adsorption of oat globulin fibrils

A.G. Bykov, E.A. Tsyganov, E.A. Levchuk, B.A. Noskov

St.Petersburg State University, 199034 St. Petersburg, Russia

Abstract

The adsorption of a plant protein, oat globulin, and its fibrils at the liquid/air interface with and without a monolayer of an oppositely charged lipid was studied by the oscillating barrier method, ellipsometry and atomic force microscopy. Spreading of a monolayer of dipalmitoyl phosphatidyl glycerol (DPPG) on the liquid surface decreases the electrostatic adsorption barrier accelerating strongly the adsorption, and increases the surface concentration of fibrils. The resulting formation of a dense and thick layer of fibrils at the interface can be used for the effective stabilization of liquid dispersion systems.

Keywords: amyloid fibrils, oat globulin, lipid monolayer, multilayer formation, dilational surface rheology

Introduction

Amphiphilic macromolecules, in particular, the most of proteins, can stabilize foams and emulsions more efficiently than the surfactants of low molecular weight, because the macroomolecules can form a thicker shell around droplets or bubbles preventing coagulation due to the formation of a steric barrier. As a result the proteins, mainly animal proteins, are widely used in industry to stabilize liquid disperse systems. Therefore, an important problem arises of the substitution of the animal proteins by cheaper and more ecologically friendly proteins of plant origin. It is not a simple problem because the functional properties of plant proteins are usually worse, for example, their surface activity is lower, and their solubility in water is very low in the concentration range close to the isoelectric point. Meantime, the problem can be solved, at least partially, if protein nano- and microaggregates, mainly amyloid fibrils, are used instead of native proteins. This is one of the reasons why special attention has been paid recently to the investigation of the properties of plant protein fibrils and their aqueous dispersions (1-9) (Afkhami et al., 2024; Amagliani and Schmitt, 2017; Han et al., 2023; Herneke et al., 2021; Li et al., 2023; Liu et al., 2024; Peydayesh et al., 2023; Sagis and Yang, 2022; Xu et al., 2024). In the case of fibril dispersions the dissolution problem disappears to a significant extene and the fibrils can form larger and stronger shells around emulsion droplets even larger than the globules of native proteins. Moreover, the surface tension of fibril dispersions can be lower than that of native protein solutions and their dynamic surface elasticity can be higher leading to the higher stability of the emulsions and foams containing fibrils. At the same time, there are some difficulties of the use of fibrils of both plant and animal proteins as stabilizers of disperse systems. The preparation of fibrils is frequently connected with the formation of peptides of low molecular weight and high surface activity influencing strongly the surface properties of fibril dispersions. Although it is almost impossible to get rid entirely of these impurities, their concentration can be significantly reduced by dialysis or the dispersion centrifugation (10-12) (Gowda et al., 2021; Noskov et al., 2023, 2022). Another difficulty can be connected with a strong electrostatic adsorption barrier for charged fibrils slowing down their adsorption (13, 17)(Noskov and Mikhailovskaya, 2013; Wierenga et al., 2005). The interest in the surface properties of protein fibrils has appeared only recently and the corresponding information is quite scarce. First of all this relates to the surface rheological properties, which determine the stability of liquid disperse systems (1, 21-25) (Amagliani and

Schmitt, 2017; Chutinara et al., 2024; Grasberger et al., 2024; Kontogiorgos and Prakash, 2023; Langevin, 2023; Mileti et al., 2022). This study is devoted to the dynamic surface properties of the solutions of oat globulin (OG) and aqueous dispersions of its fibrils, which were purified from the most of peptide impurities by centrifugation. To reduce the adsorption barrier in the course of adsorption of protein globules or fibrils, a small amount of a lipid of an opposite charge was spread on the surface. As far as we know, this is the first study of the properties of a mixed layer of fibrils of a plant protein and a lipid.

Materials

The oat groat was grounded and defatted before the extraction of OG according to the procedure described by Zhou et al. (14) (Zhou et al., 2022). Protein fibrils were prepared from purified by dialysis and freeze-dried OG dissolved in triple distilled water. To this aim pH of OG aqueous solution was adjusted to 2 by the addition of hydrochloric acid and the solution was heated at 90 °C with stirring for 18 hours. The dispersion of prepared mature OG fibrils was centrifuged (12000 g) after that for 4,5 hours to obtain to obtain purified fibrils (pFOG). The investigated solutions of native OG and fibril dispersions were prepared by dilution of a stock solution or dispersion. The final fibril concentration was determined by the gravimetric method. Fibril dispersions were stored in a refrigerator for no longer than 1 month. The temperature during all the measurements was set to 25 °C.

DPPG with purity of 99% (Sigma-Aldrich Germany) was used as received. Chloroform and methanol (Sigma-Aldrich Germany) were purified by distillation before use. DPPG solution (1 mg/ml) in a methanol/chloroform mixture (1/3 v/v) was spread onto an aqueous surface. After that OG solution or pFOG dispersion were injected into the subphase using two syringes to obtain the desired concentration.

Methods

Surface Tension and Compression Isotherms

The surface tension was measured by the Wilhelmy plate method using a piece of filter paper as a plate. The accuracy of the measurements was approximately ±0.2 mN/m. The surface pressure was recorded in the course of the spread layer compression in a Langmuir trough using an ISR instrument KSV NIMA (Finland). The surface area between two barriers in the trough was adjusted by the synchronized movement of the barriers. The compression isotherms were recorded at a constant compression rate of 100 mm/min. The Langmuir trough was placed inside a plexiglass cabinet to maintain high humidity.

Dynamic Surface Elasticity

The dynamic dilatational surface elasticity was determined using the oscillating barrier method using the ISR instrument KSV NIMA (Finland), as previously described (11, 12) (Noskov et al., 2023, 2022). Symmetrical oscillations of the two barriers in the Langmuir trough resulted in the oscillations of the surface area, leading to the corresponding variations of the surface tension. The frequency and amplitude of the surface area oscillations were set 0.03 Hz and 4%, respectively. Only the real part of the complex surface elasticity is discussed below because the imaginary component is significantly smaller. The real part of the surface elasticity was determined with an accuracy of approximately ±5%.

Ellipsometry

To estimate the changes in surface concentration during adsorption, a null ellipsometer NTEGRA Prima instrument (Optrel-GBR, Berlin, Germany) with a laser wavelength of 623.8 nm was utilized. The measurements were taken at an angle close to the Brewster angle. For a single solute,

the ellipsometric angle Δ is expected to be proportional to the surface concentration (26) (Motschmann and Teppner, 2001).

Atomic force microscopy (AFM)

To estimate the micromorphology of the adsorption layer it was transferred from the liquid surface onto a freshly cleaved mica plate using the Langmuir–Schaeffer method, and the plate with the layer was dried in a desiccator. After that the layer morphology was studied by the atomic force microscope (NTEGRA Prima instrument, NT-MDT, Moscow, Russia) in a semicontact mode with a cantilever having an approximate curvature radius of 10 nm.

Results and discussions

Native OG

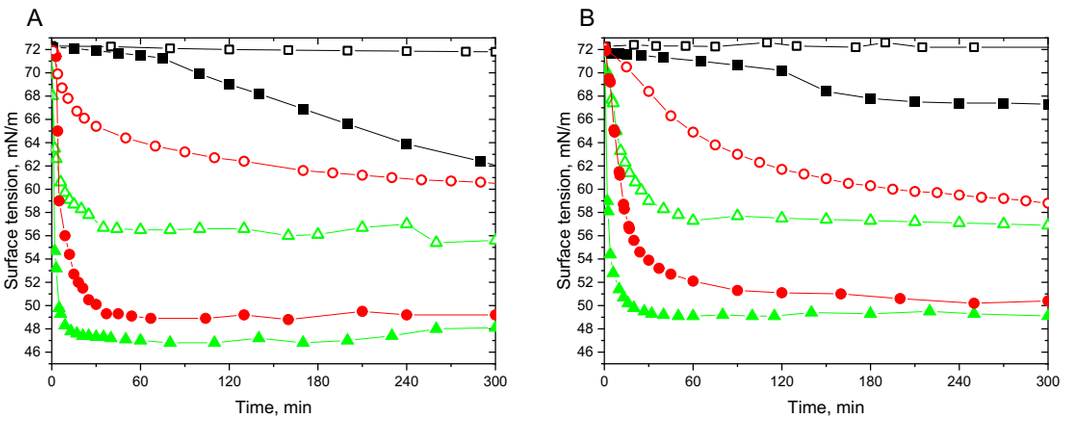

Figure 1. Kinetic dependencies of the dynamic surface tension of native OG solutions (A) and pFOG dispersions (B) at pH 2 (open symbols) and with DPPG monolayer at the surface (closed symbols) at protein concentrations 0.00004 mass % (black squares), 0.0005 mass % (red circles) and 0.0017 mass % (green triangles).

Figure 1A shows the kinetic dependences of the surface tension of native oat globulin solutions with and without a monolayer of DPPG on its surface. In both cases the increase of the protein concentration leads to an increase the adsorption rate. If there is no lipid monolayer on the surface, the surface tension at the lowest investigated OG concentration does not change for more than five hours after the surface formation. The surface tension decreases much faster in the system with a monolayer on the surface than without it. Although, the used DPPG surface concentration is small and not sufficient for noticeable changes of the surface tension of pure subphase, the spreading of DPPG monolayer accelerates significantly the adsorption rate of native OG. Note that at pH 2 OG and DPPG molecules are oppositely charged. It has been shown previously that the protein adsorption accelerates significantly if an oppositely charged lipid is spread onto the solution surface (15, 16) (Junghans et al., 2010; Zhang et al., 2003). The electrostatic adsorption barrier leads to slow changes of the surface properties with the surface age (13, 17) (Noskov and Mikhailovskaya, 2013; Wierenga et al., 2005), but even small additions of an oppositely charged lipid decreases the barrier and accelerates the adsorption. Besides, the steady-state surface tension for the system with a lipid monolayer is lower than for the solution without it indicating the influence even of small amounts of the lipid in the surface layer on the measured surface pressure (Figure 1A).

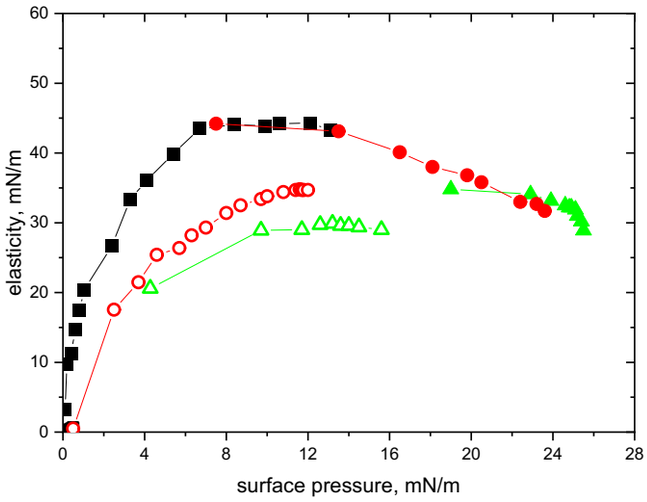

Figure 2. Dependencies of dynamic surface elasticity on surface pressure for the native OG solutions at pH 2 (open symbols) and with DPPG monolayer at the surface (closed symbols) at protein concentrations 0.00004 mass % (black squares), 0.0005 mass % (red circles) and 0.0017 mass % (green triangles).

Although the dynamic surface elasticity of native protein solutions increases monotonically with the surface pressure, the dynamic surface elasticity as a function of the surface pressure goes through a local maximum in the systems with a DPPG monolayer on the solution surface (Figure 2). In previous studies, the maximum of the dependences of the dynamic surface elasticity of protein solutions on the surface pressure was associated with the unfolding of protein globules in the surface layer (18, 19) (Mikhailovskaya et al., 2011; Noskov, 2014). It is possible to assume that the interactions between the protein globules and lipid molecules lead to the unfolding of OG molecules in the surface layer.

Fibrils of oat globulin

It has been shown recently that oat fibrils can reduce the surface tension to 57-60 mN/m, lower than the values for the native protein solutions of the same concentrations (20) (Khrebina et al., 2025). The purification of fibrils by centrifugation led to a decrease in the adsorption rate as a result of a decrease in the concentration of peptide impurities but not to a noticeable increase of the surface tension. After the purification the rate of adsorption of pFOG was close to that of native OG (20) (Khrebina et al., 2025). The obtained results show that the rate of pFOG adsorption is also close to the adsorption rate of native OG molecules and increases with the increase of pFOG concentrations (Figure 1B). The spreading of a lipid monolayer accelerates the adsorption of pFOG. As a result, the surface tension for dispersions of pFOG with the DPPG monolayer reaches steady – state values in a shorter time, than for solutions without the monolayer. Since the fibrils have the same charge as native OG molecules, it is possible to assume that the acceleration of fibril adsorption is also caused by a decrease of the electrostatic adsorption barrier in presence of an oppositely charged lipid monolayer. Moreover, as in the case of native OG solutions the steady-state values of the surface tension of fibril dispersions in presence of the lipid monolayer are lower than the values for the dispersions without the monolayer.

While the dynamic surface elasticity was below 30 mN/m for the mixed layer of the native protein with lipid in the region of high surface pressures, the higher values of the surface elasticity were observed for the fibril dispersions at similar conditions (Figures 2 and 3). Therefore, the elasticity

of the adsorption layer of fibrils turns out to be greater than that for the native protein layer at similar weight concentrations of the native protein and fibrils, (Figure 4). Moreover, an increase of the surface elasticity at high surface pressures (more than 20 mN/m) for the mixed adsorption layer of fibrils and lipids agrees with the corresponding increase in the ellipsometric delta angle (Figure 3). In the absence of the lipid monolayer, the adsorption of fibrils leads to the increase of surface pressure to 18 mN/m and the ellipsometric angle Δ remains lower than 2.3. Since the angle Δ can change proportionally to the surface concentration, it is possible to conclude that the spreading of a DPPG monolayer not only increases the fibril adsorption rate but also increases the surface concentration of fibrils.

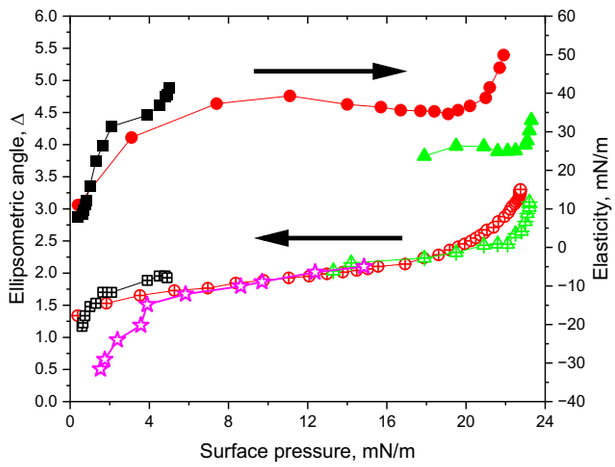

Figure 3. Dependencies of ellipsometric angle delta (open crossed symbols) and dynamic surface elasticity (closed symbols) on surface pressure for spread DPPG monolayers on the surface of pFOG dispersions at pH 2 and protein concentrations 0.00004 mass % (black squares), 0.0005 mass % (red circles) and 0.0017 mass % (green triangles) and at pH 3 with protein concentration of 0.0005 mass % (open magenta asterisks).

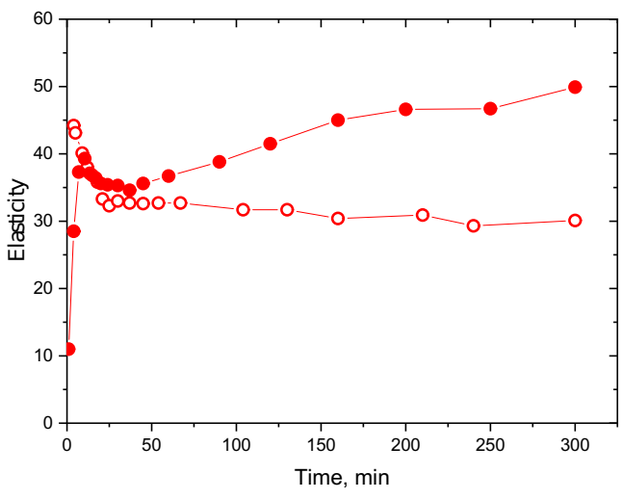

Figure 4. Kinetic dependencies of the dynamic surface elasticity for spread DPPG monolayers on the surface of native OG (open red circles) and pFOG (closed red circles) dispersions at pH 2 and protein concentrations of 0.0005 mass %.

An increase in the surface concentration of fibrils, if an oppositely charged lipid is spread on the surface, is also corroborated by AFM images of the adsorbed layer transferred onto a mica surface by the Langmuir-Schaeffer method (Figure 5). In the case of an adsorption pFOG layer on the surface without a monolayer only a few separate fibrils are visible, which can presumably interact only weakly. This means that even after the purification of fibrils, their surface concentration remains low. If the lipid layer is spread on the surface, the surface concentration of fibrils increases significantly, and they become intertwined forming a net. The addition of an oppositely charged lipid leads to a significant increase in the surface concentration of fibrils.

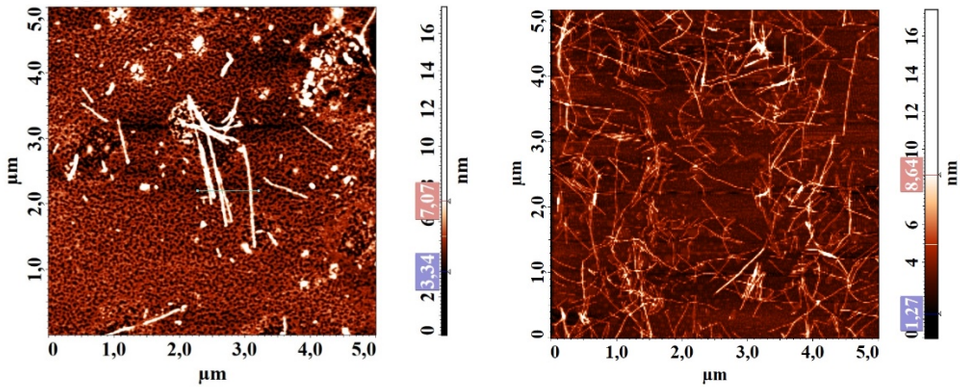

Figure 5. AFM images for adsorbed pFOG layers at the surface of pFOG dispersion without (left) and with a monolayer of DPPG (right).

The surface layer was compressed after the adsorption of fibrils and the equilibration. The compression isotherm of a mixed layer of pFOG and DPPG can be divided into two parts (Figure 6). At the beginning of compression, the surface pressure slightly increases as in the case of the adsorption layer of pure fibrils. At the end of compression the isotherm almost coincides with the results for a pure lipid monolayer. It looks like that only the lipid molecules remain in the surface layer as the surface area decreases, and the fibrils are displaced from it. At the same time, the AFM results show that the fibrils remain close to the surface after compression (Figure 7). The surface concentration of fibrils increases significantly during the surface compression leading presumably to a fibril network below the lipid monolayer. Such a structure can be formed, for example, on the surface of droplets or bubbles in emulsions and foams preventing their destruction.

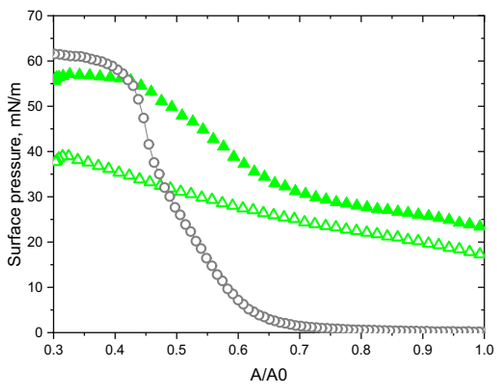

Figure 6. Compression isotherms of native OG adsorption layer at pH 2 without (open green triangles) and with DPPG monolayer at the surface (closed green triangles) at protein concentration of 0.0017 mass %, and of a spread DPPG monolayer on water at pH 2 (open grey circles). The initial area is 93 Å per molecule.

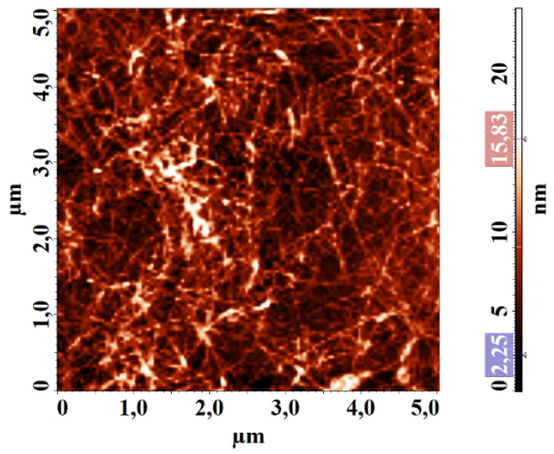

Figure 7. AFM image for adsorbed pFOG layers at the surface of pFOG dispersion with a DPPG monolayer at pH 2 and protein concentration of 0.0017 mass % after compression.

Conclusions

The mixed layers of fibrils of a plant protein and an oppositely charged lipid at the liquid/air interface have been studied for the first time according to our knowledge. A spread lipid monolayer at the surface of aqueous fibril dispersions leads to a decrease of the adsorption barrier and accelerates significantly the adsorption of fibrils and native protein molecules. Moreover, the lipid monolayer leads to a significant increase of the surface concentration of fibrils forming an elastic network in the surface layer. It is possible to assume that the mixed fibril/lipid layer can provide a strong steric barrier for the coalescence of bubbles and drops in liquid dispersion systems.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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