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Langmuir monolayers and Langmuir-Blodgett films of pH-sensitive lipid

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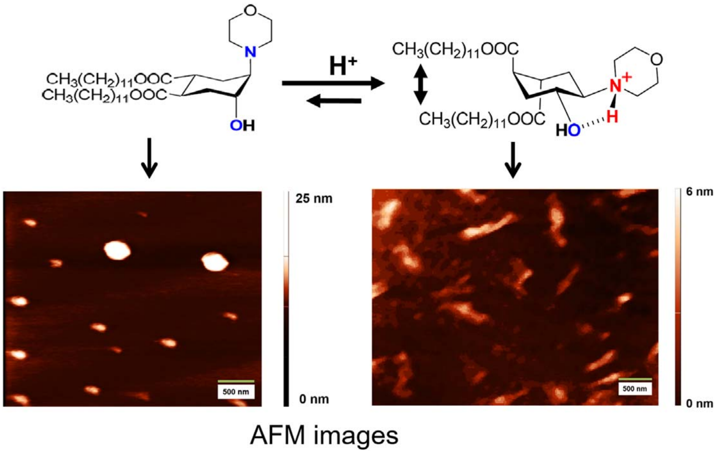
GRAPHICAL ABSTRACT



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ABSTRACT

Colloidal membranous vesicles based on the functionalized liposomes are promising for development of novel efficient systems of controlled and stimuli-triggered drug delivery. In particular, the specific metabolism of cancer cells results in significant acidification in tumors, and thus the corresponding pH value changes can be used as a stimulus for drug release specifically in the tumor areas. pH-sensitive liposomes can be formed via incorporation of special pH-sensitive amphiphilic compounds into the liposomal membrane. Molecules of such compounds undergo a conformational transition caused by certain changes in the pH value of aqueous environment and resulting in corresponding membrane perturbations with rapid leakage of drug content from those liposomes into the areas with corresponding acidity. In the present work the pH-induced changes of Langmuir monolayer compression isotherm and of the structure of corresponding Langmuir-Blodgett films of pH-sensitive synthetic lipid trans-4,5,didodecyloxycarbonyl-trans-2-morpholinocyclohexanol have been observed and studied. The substantial expansion of Langmuir monolayer of that lipid caused by change of the aqueous subphase pH value from 7 to 5 was observed. Corresponding monolayer Langmuir-Blodgett films were deposited on mica substrate surface and studied using AFM technique. The substantial differences in the structure

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of Langmuir-Blodgett films deposited at pH values 7 and 5 were found. The observed effects directly demonstrate the pH-induced structural transformations in organized condensed planar molecular systems formed by pH-sensitive lipid molecules which underlie the pH-sensitivity of corresponding liposomal bilayer membranes containing such lipids.

1. Introduction

Colloidal aqueous systems of various nature including liposomes, polymeric micelles, polymeric vesicles and capsules, dendrimers and composite particles are currently actively studied as potential means for capsulation and controlled drug delivery. [1–5]. Due to the biocompatibility and relative ease of preparation the colloidal bilayer membranous vesicles – liposomes are now actively used in bio-medical studies and pharmacological developments of advanced drug carriers [2,6]. The main unresolved challenging problem in that field now is creation of novel efficient stimuli-triggered drug delivery systems that can deliver the encapsulated substances on-demand in response to external physical and/or chemical stimuli. The external physical stimuli can include thermal, electromagnetic, light, magnetic, electric, ultrasound effects [7–10]. Chemical stimuli are currently used in pH-responsive, redox-responsive and enzyme-responsive drug delivery systems [7–10]. Different tissues and cellular compartments have different pH values in various physiological states what makes the pH value a suitable stimulus for controlled drug release in a number of drug therapy approaches. In particular, the specific metabolism of cancer cells (dominance of aerobic glycolysis) results in significant acidification in tumors, and thus the pH value changes can be used as a stimulus for drug release specifically in the tumor areas. To date a number of pH-sensitive compounds were used for development of pH-responsive colloidal drug carriers [11–13].

pH-sensitive liposomes can be formed via incorporation of special pH-sensitive amphiphilic compounds into the liposomal membrane. Molecules of such compounds can undergo a conformational transition caused by protonation under certain changes in the pH value of aqueous environment and resulting in corresponding membrane perturbations with rapid leakage of drug content from those liposomes into areas with corresponding acidity [14–16].

Because liposomes are colloid particles the direct studies of pH-induced structural transformations in situ in their bilayer membranous matrix containing pH-sensitive lipids are rather problematic. However, related effects in interfacial monolayer systems of the same molecular composition and organization can be studied readily using Langmuir monolayer technique and Langmuir-Blodgett (LB) films [17,18]. The structure and properties of compressed lipid Langmuir monolayer in its condensed state correspond most closely to the lipid matrix properties in bilayer membrane [19].

The aim of the present work was direct visualization and study of pH-induced changes of structure and properties of planar model systems – Langmuir monolayer and corresponding LB films formed by pH-sensitive synthetic lipid trans-4,5-di(dodecyloxycarbonyl)-trans-2-morpholinocyclohexanol (MOCH-flipid) previously used for preparation of pH-responsive liposomes [14–16]. That lipid molecule contains the trans-2-morpholinocyclohexanol conformational switch which enables a substantial conformational change in the molecule upon amine group protonation at pH values below 5.5 resulting in corresponding changes of the liposomal membrane structure and increase in permeability for a number of encapsulated compounds including anticancer drug methotrexate [15,16]. For the first time the Langmuir monolayer of that lipid was obtained and substantial expansion of the monolayer caused by the change of the aqueous subphase pH value from 7 to 5 was observed. The substantial differences in the structure of corresponding Langmuir-Blodgett films deposited at pH values 7 and 5 were found using atomic force microscopy (AFM) technique. The observed effects directly demonstrate the pH-induced structural transformations in organized condensed planar systems formed by pH-sensitive lipid molecules which underlie the pH-sensitivity of corresponding liposomal bilayer membranes containing such lipids.

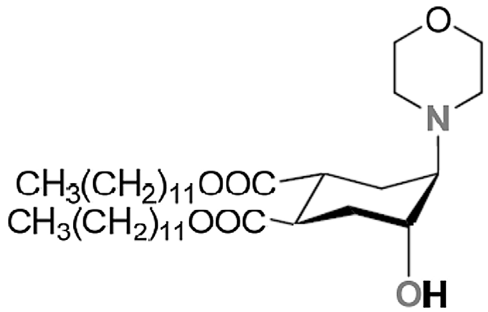

Fig. 1. The structural scheme of pH-sensitive lipid trans-4,5,didodecyloxycarbonyl-trans-2-morpholinocyclohexanol (MOCH-flipid).

2. Material and methods

Milli-Q integral water purification system for ultrapure water was used to produce water with an average resistivity of 18 MΩ-cm for all experiments. The pH-sensitive lipid MOCH-flipid was synthesized as described in Refs [14,15]. The structural scheme of the molecule of that lipid is shown on Fig. 1.

The formation of Langmuir monolayers of pH-sensitive MOCH-flipid and deposition of corresponding Langmuir–Blodgett films were carried out with the use of KSV-Nima LB Trough Medium KN 1003 device at ambient conditions (room temperature about 24 °C). Langmuir monolayer of MOCH-flipid was formed by the dropwise deposition of solution of that lipid in chloroform (concentration 5×10^{-4} M) onto the surface of aqueous subphase with definite pH value. To maintain the desired pH value the 1 mM buffer solution of TRIS (pH = 7) and 10^{-2} M acetate buffer solution (pH = 5) were used. After 10 min necessary for chlorophorm evaporation and uniform distribution of lipid molecules onto the aqueous subphase surface the compression isotherm (the dependence of surface pressure value on the monolayer area) was measured with the moving barrier speed 5 mm/min. LB film samples for AFM measurements were prepared by Langmuir monolayer transfer onto the freshly prepared mica substrate surface using conventional Langmuir-Blodgett vertical substrate lifting method with the substrate rate 5 mm/min. Because we were interested in the study of monolayer structure in the condensed state the deposition of LB films was carried out at surface pressure value 40 mN/m. The monolayer transfer ratio was very high (about 1) which is characteristic for deposition of amine-containing amphiphile LB films onto the mica substrates. Some details of our experiments with Langmuir monolayers and LB films can be also found in our previous related publications [20].

The structure of LB films deposited onto the atomically flat mica substrate surfaces was studied by the AFM technique using Solver P47 SPM MDT device (NT-MDT, Russian Federation) in tapping and contact AFM imaging modes. Images were measured in air at ambient temperature (24 °C) and were highly stable and reproducible. The tips were used with total curvature radius about 10 nm. The reproducible topographic images with more resolved structural details were obtained in the contact mode of operation.

3. Results and discussion

3.1. PH-induced changes in Langmuir monolayer of pH-sensitive lipid

We have found that MOCH-flipid forms stable Langmuir monolayer at the air/aqueous phase interface. Such amphiphilic compound previously was used as a pH-sensitive functional component of the liposomal membrane to obtain the dependence of permeability of such membranes on the pH value of the aqueous liposome suspension. Decreasing the pH value of the suspension below a certain value (pH = 5.5) caused a significant increase of liposomal membrane permeability to model compounds (fluorescent dye, 8-aminonaphthalene-1,3,6-trisulfonic acid and a quencher, *p*-xylene-bis-pyridinium bromide) and anionic anticancer drug methotrexate [15,16]. This effect was attributed to the conformation change of the lipid molecule at low pH values with spatial reorganization representing the substantial increase in separation of alkyl chains caused by the protonation of the nitrogen atom in the amino group and formation of intramolecular hydrogen bond [14,15], as shown schematically in Fig. 2.

We have studied pH-induced changes in the compression isotherm of Langmuir monolayer of MOCH-flipid. For this purpose, Langmuir monolayers of MOCH-flipid were formed on water subphases with pH values above and below the characteristic pH value corresponding to the pK of the amino group of the molecule MOCH-flipid (~ 5,5-6.0 [14]). It was found that the characteristic compression isotherm changes of MOCH-flipid Langmuir monolayer occurred at the aqueous phase pH values from 5 to 6.5 and the maximum difference between the monolayer compression isotherms was observed when monolayers were formed on subphases with pH values 5.0 and below, and 6.5 and above, correspondingly. The aqueous subphase in the experiments with different pH values was a TRIS buffer solution (pH = 6.0 or higher) and acetate buffer (pH = 5.5 or lower). In the special experiments it was found that the presence of the 10 mM buffer in an aqueous subphase did not cause noticeable changes in the compression isotherm of MOCH-flipid at corresponding pH. At pH values of the aqueous phase below 5.5 the amino groups of MOCH-flipid molecules in Langmuir monolayer become predominantly protonated with molecule conformation different from the “unprotonated” conformation at pH = 7.0 (as shown on Fig. 2) causing a corresponding effect of the “expansion” of the monolayer at lower pH values, as it is shown schematically on Fig. 3 and experimentally on Fig. 4.

Typical experimental compression isotherms of MOCH-flipid Langmuir monolayer measured at aqueous subphase pH values 5.0 and 7.0 are shown on Fig. 4. As it is seen from the compression isotherm on Fig. 4 at pH value 7 (unprotonated electroneutral monolayer) the monolayer area per molecule at the monolayer collapse (~55 $\acute{\text{Å}}^2$/molecule) corresponds to that value for conventional electroneutral zwitterionic lipids with two hydrocarbon chains [21].

It follows from Fig. 4 that the decrease in the aqueous phase pH value from pH = 7 to pH = 5 actually leads to a substantial “expansion” of the Langmuir monolayer of MOCH-flipid with corresponding shift of the compression isotherms to the higher values of monolayer area per molecule. Quantitatively, such pH-induced increase in the monolayer area per molecule in a close-packed Langmuir monolayer of MOCH-flipid in condensed liquid phase at the surface pressure values of about 30–40 mN/m is about 25%. It is known that the increase in surface charge and/or surface potential of Langmuir monolayer resulting in the increase of electrostatic surface energy can cause an increase of surface pressure value (decrease of surface tension) resulting in corresponding “expansion” of the monolayer. The increase in positive surface charge of MOCH-flipid Langmuir monolayer due to the protonation of its amino groups can also contribute to the observed effect of pH-induced monolayer expansion. For Langmuir monolayer of simple monoalkyl amine amphiphilic molecule stearylamine (octadecylamine) the monolayer area per molecule in the compressed monolayer state (at about 30 mN/m) is ~20 $\acute{\text{Å}}^2$/molecule in unprotonated state at pH = 10, and change of pH value of the aqueous subphase from pH = 10 to pH = 5 resulted in compressed octadecylamine monolayer expansion of about 10% caused by protonation of amino groups and monolayer surface charging [22].

Molecules of dialkyl monoamine pH-sensitive lipid MOCH-flipid have two hydrocarbon chains and occupy more than twice the area in a close-packed compressed Langmuir monolayer as compared with the monoalkyl molecules of octadecylamine. Thus, surface charge density of positively charged protonated amino groups in compressed close-packed Langmuir monolayer of MOCH-flipid can not exceed half the surface density of charged amino groups in compressed octadecylamine Langmuir monolayer and the effect of monolayer expansion due to the interfacial electrostatic interactions must be correspondingly less for pH-sensitive dialkyl lipid. Thus, the observed substantial pH-induced increase in the area of Langmuir monolayer of MOCH-flipid was due, apparently, mainly the effect of pH-induced conformational changes of such lipid molecules. This effect of pH-induced changes of thermodynamic parameter (surface pressure) of organized monomolecular system of pH-sensitive lipid molecules caused by a change in conformation of the molecules is observed first and is directly related to the pH-induced structural rearrangements in a liposome membrane containing such pH-sensitive molecules.

3.2. PH-induced changes in Langmuir-Blodgett film of pH-sensitive lipid

The data presented in Section 3.1. indicate to significant pH-induced changes of the conformational state of pH sensitive lipid molecules resulting in corresponding changes of the characteristics of their Langmuir monolayer as a whole. Therefore, one can expect a significant difference in the structure of the compressed Langmuir monolayers of pH-sensitive lipid and in corresponding LB films and bilayer membranes at appropriate different pH values of the aqueous medium. With the aim of direct visualization of such differences we have studied experimentally the pH-induced changes in the structure of monolayer LB films of MOCH-flipid molecules deposited at aqueous subphase pH values 7 and 5 on atomically flat solid mica substrates. Characteristic topographical AFM images of such monolayer LB films are presented on Figs. 5 and 6. Fig. 5 shows a topographic analysis of monolayer LB film of unprotonated MOCH-flipid deposited on the surface of mica substrate at aqueous subphase pH = 7.0 and the surface pressure value 40 mN/m.

Fig. 5 shows that a regular quasi-spherical and ellipsoidal structures with diameter of about 200–300 nm and height of 20–30 nm are present in the structure of such LB film. Such structures, apparently, are the result of 2D/3D transitions in a monomolecular film with collective molecular processes of formation of supramolecular structures depending on the nature of the amphiphile and the Langmuir monolayer state (compression degree). Thus, in particular, so-called surface micelles are known which can be formed under certain conditions in Langmuir monolayers of amphiphilic compounds with appropriate molecular structure [23,24]. The 2D/3D transition processes in monolayers can lead to the formation of non-planar structures and are directly related to the collapse processes in Langmuir monolayers [21].

Fig. 6 demonstrates characteristic height topographic image of monolayer LB film of protonated MOCH-flipid deposited on the surface of mica substrate at aqueous subphase pH = 5.0 and the surface pressure value 40 mN/m.

Fig. 7 shows the line profiles corresponding to the dotted lines **a** and **b** on topographic image on Fig. 6.

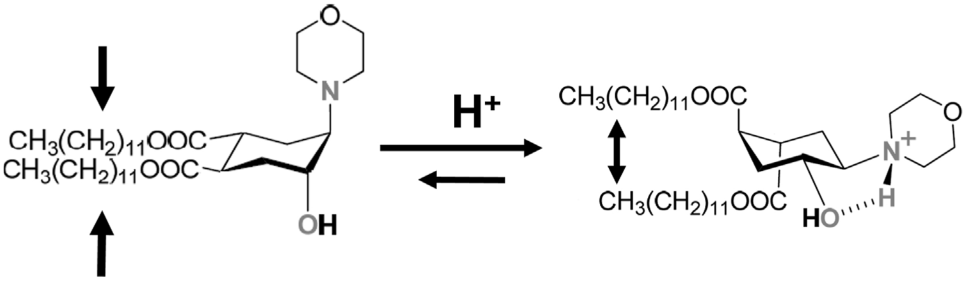

Fig. 2. Schematic representation of the pH-induced conformational transition in the molecule of MOCH-flipid caused by the protonation of the nitrogen atom in the amino group.

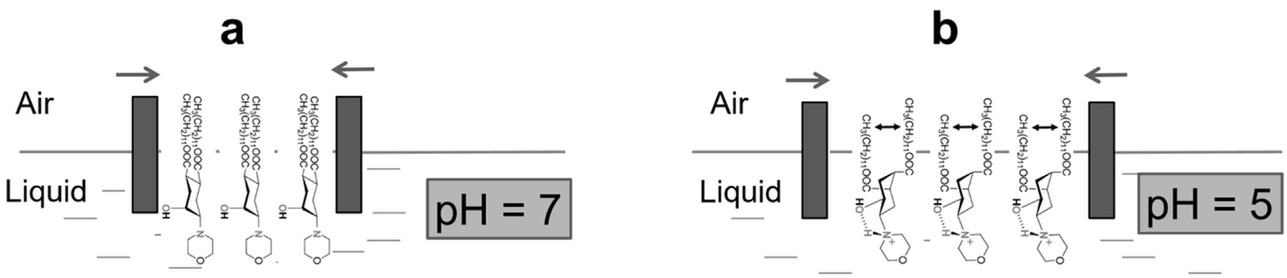

Fig. 3. Schematic representation of Langmuir monolayer of MOCH-flipid molecules with different pH-dependent conformations. a: Aqueous subphase pH = 7; b: Aqueous subphase pH = 5.

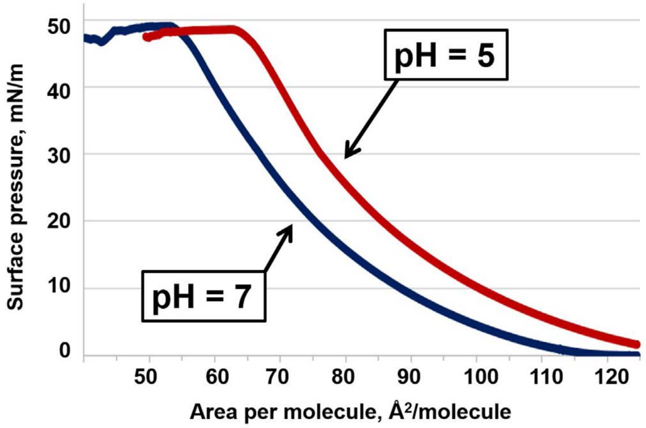

Fig. 4. Typical compression isotherms of MOCH-flipid Langmuir monolayer measured at aqueous subphase pH values 7.0 and 5.0. Aqueous subphase contained 1 mM buffer TRIS (pH = 7.0) and 10^{-2} M acetate buffer solution (pH = 5).

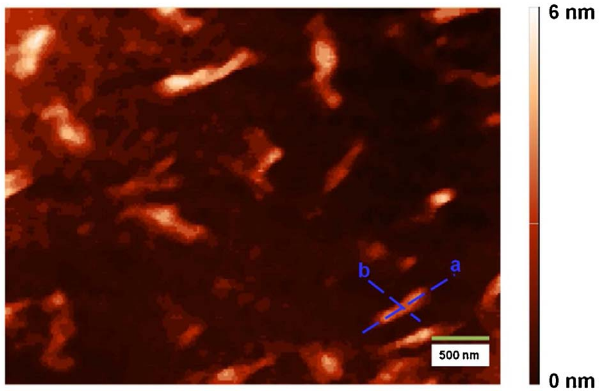

Fig. 6. Height topographic image of monolayer LB film of MOCH-flipid deposited on the surface of mica substrate at aqueous subphase pH = 5.0 and the surface pressure value 40 mN/m. Image was obtained by AFM technique in contact mode of operation at ambient conditions.

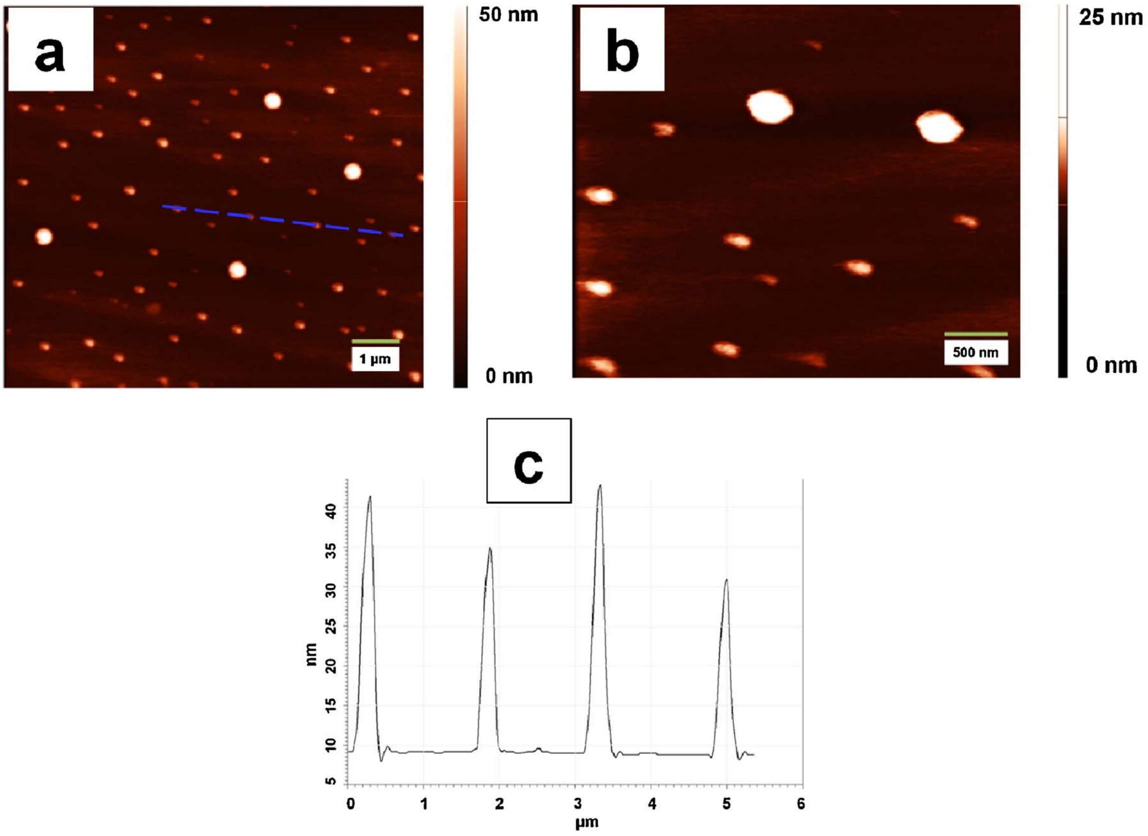

Fig. 5. Typical AFM topographic images of monolayer LB film of MOCH-flipid deposited on the surface of mica substrate at aqueous subphase pH = 7.0 and surface pressure 40 mN/m. The topographic images a and b are different in scale. c: line profile (dotted line in a). Images were obtained by AFM technique in a contact mode of operation at ambient conditions.

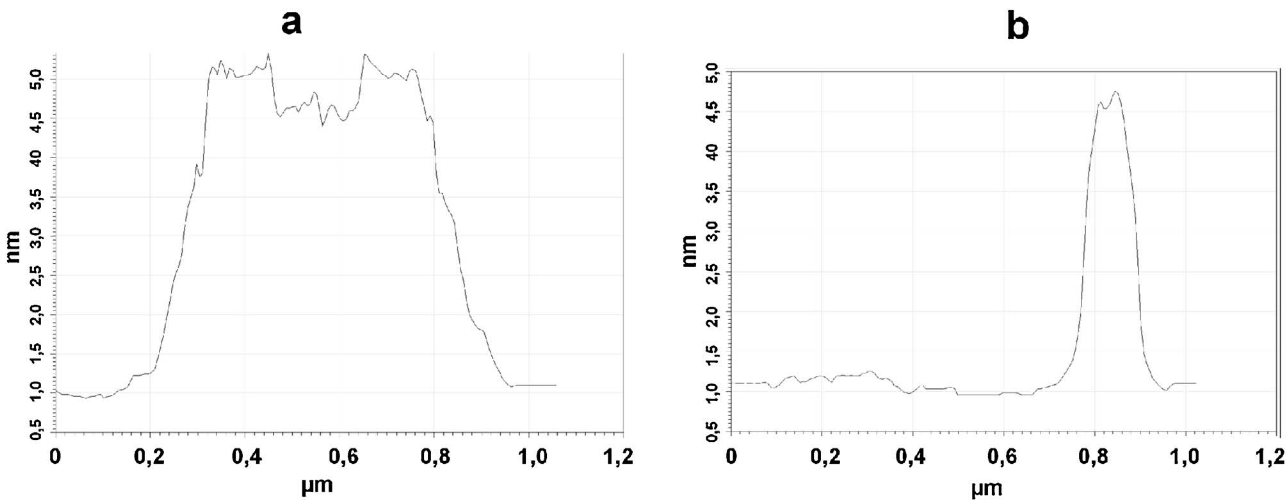

Fig. 7. Line profiles corresponding to dotted lines a (a) and b (b) on topographic image on Fig. 6.

It is seen from Figs. 6 and 7 that the structure of monolayer LB film of MOCH-flipid deposited on the mica surface at aqueous subphase pH = 5.0 is significantly different from the structure of the film obtained at pH = 7 and shown in Fig. 5. The character of structural organization of the protonated monolayer at pH = 5 changed significantly – the anisotropic elongated structures about 4 nm in height with 500–600 nm length and 150 nm width are present in the film. The obtained experimental data show that pH-induced protonation of the MOCH-flipid molecule and corresponding conformation transition, as well as electrostatic interactions of charged protonated molecules in the monolayer, change the character of their formation of 3D supramolecular structures resulting in significant changes in their close-packed planar ensembles – Langmuir monolayers and films. The same interactions apparently cause the pH-induced structural changes in the corresponding bilayer liposomal membranes resulting in pH-sensitivity of MOCH-flipid containing liposomes.

4. Conclusions

Langmuir monolayer of pH-sensitive synthetic lipid trans-4,5,didodecyloxycarbonyl- trans-2-morpholinocyclohexanol was obtained for the first time and the effect of pH-induced expansion of the monolayer was observed corresponding to increase about 25% in the monolayer area per molecule at the surface pressure values of ∼30–40 mN/m caused by the change of the aqueous subphase pH value from 7 to 5. The substantial differences in the structure of corresponding monomolecular Langmuir-Blodgett films deposited at pH values 7 and 5 were found using AFM technique. The observed effects directly demonstrate the pH-induced structural transformations in condensed planar close-packed systems formed by pH-sensitive lipid molecules which underlie the pH-sensitivity of corresponding liposomal membranes containing such lipids. Such molecules can be the basis for the creation of new prospective pH-sensitive smart materials and colloidal systems for stimuli-triggered drug delivery.

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