SPECIAL TOPIC — Physical research in liquid crystal

Analysis of the induction of the myelin basic protein binding to the plasma membrane phospholipid monolayer*

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(Received 11 May 2016; revised manuscript received 29 June 2016; published online 20 July 2016)

Myelin basic protein (MBP) is an essential structure involved in the generation of central nervous system (CNS) myelin. Myelin shape has been described as liquid crystal structure of biological membrane. The interactions of MBP with monolayers of different lipid compositions are responsible for the multi-lamellar structure and stability of myelin. In this paper, we have designed MBP-incorporated model lipid monolayers and studied the phase behavior of MBP adsorbed on the plasma membrane at the air/water interface by thermodynamic method and atomic force microscopy (AFM). By analyzing the pressure–area (π–A) and pressure–time (π–T) isotherms, univariate linear regression equation was obtained. In addition, the elastic modulus, surface pressure increase, maximal insertion pressure, and synergy factor of monolayers were detected. These parameters can be used to modulate the monolayers binding of protein, and the results show that MBP has the strongest affinity for 1,2-dipalmitoyl-sn-glycero-3- phosphoserine (DPPS) monolayer, followed by DPPC/DPPS mixed and 1,2-dipalmitoyl-sn-glycero-3-phospho-choline (DPPC) monolayers via electrostatic and hydrophobic interactions. AFM images of DPPS and DPPC/DPPS mixed monolayers in the presence of MBP (5 nM) show a phase separation texture at the surface pressure of 20 mN/m and the incorporation of MBP put into the DPPC monolayers has exerted a significant effect on the domain structure. MBP is not an integral membrane protein but, due to its positive charge, interacts with the lipid head groups and stabilizes the membranes. The interaction between MBP and phospholipid membrane to determine the nervous system of the disease has a good biophysical significance and medical value.

Keywords: myelin basic protein, liquid crystal monolayers, synergy factor, surface morphology

PACS: 05.70.–a **DOI:** 10.1088/1674-1056/25/9/090507

1. Introduction

Biological membrane is a supramolecular structure constituted by phospholipids and proteins, which are essential components for cellular activities.[1–3] Biological membranes have molecule alignment similar to crystal structure, and have the fluidity of liquid.[4–7] Therefore, the bio-membrane is a typical structure of the liquid crystalline state. Biological membrane of the lipid content and water system that lyotropic liquid crystal containing two components.[8,9] Here, we mainly focus on the lyotropic liquid crystal, which is regarded as the simple model of biological membrane: the system of an amphiphilic molecule and water. On the one hand, the monolayer of an amphiphilic may be considered as half of a membrane and is the simplest model system that can be discussed.[10,11] On the other hand, the monolayer of a liquid crystal on air–water interface can reveal the richness of phases and an amazing diversity of structures, which plays an important role in living cells.[12] Various techniques of membrane mimicking have been developed. Among these the measurement of pressure–area curves of a Langmuir monolayer is most often used.[13,14] The relationship between bio-membrane with liquid crystal has been noticed.[9] When people study the structure of living cells, myelin shape has been described as liquid crystal structure.

Myelin is the insulating, multi-lamellar membrane discontinuously wrapped around the nerve axon. Myelin integrity is the foundation of the fast salutatory conduction of the signal along the axon in the central nervous system (CNS). One of the major CNS compact myelin proteins is MBP, which accounts for about 30%.[15–17] The main physiological role of MBP is to maintain the structural integrity of the myelin sheath via adhesion to cytoplasmic leaflets of the oligodendrocyte membrane.[18] MBP has vital significance in many neurological diseases,[19–21] such as multiple sclerosis (MS) and experimental cerebral spinal cord inflammation (EAE). A great deal of research[22,23] has demonstrated that the major isoform of MBP in mature myelin has a molecular weight of 18.5 kDa and a net positive charge of 19. MBP has a high net positive charge which is likely to be crucial to its interaction with the phospholipids in model membranes. Numerous studies have now shown that MBP causes adhesion of the cytosolic surfaces and maintain the structural integrity of myelin by binding to

*Project supported by the National Natural Science Foundation of China (Grant Nos. 21402114 and 11544009), the Natural Science Basic Research Plan in Shaanxi Province of China (Grant No. 2016JM2010), the Fundamental Research Funds for the Central Universities of China (Grant No. GK201604004), and the National University Science and Technology Innovation Project of China (Grant Nos. 201610718014 and cx16018).

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phospholipids contain electrostatic and hydrophobic.[24] That is, minor changes in lipid composition in myelin membrane will alter myelin membrane domain structure, size, and the adsorption characteristics of the intermembrane. Therefore, the study of the interaction mechanism of MBP with different lipid compositions is important in understanding the myelination process.

To better study the detailed interaction mechanism, we use a monolayer of myelin lipids at the air–water interface to mimic the actual myelin membrane. Recently, we characterized the adsorption of MBP into model lipid monolayers, composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-dipalmitoyl-sn-glycero-3-phosphoserine (DPPS), as representative of neutral and charged lipids. The structures are shown in Fig. 1.

Adsorption of MBP onto phospholipid monolayers at the air–subphase interface has been extensively studied in the past by Langmuir–Blodgett (LB) techniques, atomic force microscopy (AFM), scanning electron microscope (SEM), surface forces apparatus (SFA), immune-electron microscopy, and x-ray diffraction.[15,25–28] Some studies[29] demonstrated the importance of hydrophobic interaction between MBP and lipids. Other studies[30,31] have shown a temporal evolution of the structure of subphase penetrating the lipid phase together with the protein. In these studies, the possible origin and regulation of the domain segregation induced by MBP was previously observed in monolayers. In particular, the interface parameters (adsorption time, surface pressure, and type of lipid) behavior of MBP with lipids by LB techniques would be studied. The method enables us to visually provide information to better understand the interfacial behavior of MBP with lipids.

In this paper, we have concentrated on the change of the lateral organization induced by the MBP and lipids (DPPC, DPPC/DPPS, DPPS) when spread as a monolayer at the air–subphase interface. Here, we have used Langmuir–Blodgett (LB) technology and atomic force microscopy (AFM)[32] to investigate the membrane interaction of MBP and lipids. The lipid monolayers were formed in a Langmuir trough at various initial surface pressures (π_{initial}). Although there have been many reports on the interaction of MBP and lipids, as far as we are aware, this is the first study on the interaction of MBP and DPPC/DPPS (the volume ratio of 7:3) at different initial surface pressures. The measurement of the maximum insertion pressure (MIP) can provide additional useful information for evaluating the binding parameters of MBP such as the synergy. In this article, the procedure to determine the $\Delta\pi$, MIP, and synergy will be described and the experimental values of $\Delta\pi$, MIP, and synergy obtained with MBP will be presented and discussed.

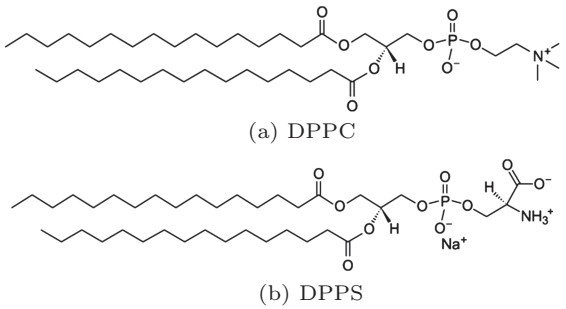


Fig. 1. Chemical structure of DPPC (a) and DPPS (b).

2. Experiment

2.1. Materials

MBP was extracted from bovine brain and purchased from Merck (Darmstadt, Germany). MBP has been diluted against pure water while at the same time used as the working solution in the concentration of 5×10^{-9} M. 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoserinesodium salt (DPPS) from bovine heart was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA) and used without further purification. Spreading solutions of DPPC and DPPS were prepared in chloroform/methanol 3:1 (v/v) mixture at a concentration of 0.1 mg/mL. For the mixed lipid solution, DPPC and DPPS were used in a volume ratio of 7:3, which is the ratio of these lipids typically found in the plasma membrane. The ultrapure water used as subphase was distilled and purified with a Millipore purification system (electrical resistivity ≥ 18.2 MΩ·cm).

2.2. Surface pressure–area measurements

Surface pressure–area (π–A) isotherms were measured on a KSV Minitrough system (Helsinki, Finland). It was operated on a Wilhelmy platinum plate with a dynamic surface pressure range of 0–150 mN/m and a resolution of 0.01 mN/m.

Monolayers have been formed by spreading an aliquot of lipid solution on 10 mM Tris (hydroxyethyl) amino-methane titrated to pH 7.2 with HCl. Certain volumes of the lipid solutions were spread on the subphase containing the moderate MBP using a Hamilton micro syringe. 15 minutes were allowed for evaporation of the chloroform to occur. The isotherm for DPPC/DPPS was then recorded from online measurements and plotted by compressing the barriers at a forward rate of 10 mm/min. The temperature of the subphase was maintained within $20 \pm 1^{\circ}$ with the aid of a water circulator bath. The trough and barriers were thoroughly cleaned with absolute ethyl alcohol and ultrapure water between each separate isotherm.

Compression of the DPPC monolayer occurred until the target surface pressure of 10 mN/m was reached. Monolayers have been transferred to the surface of mica substrate (transfer ratio is 1) with a method of vertical pulling transferring. In order to keep the area of the monolayers, pressure which changes

with time has been recorded so as to obtain π–T isotherms when the lipid monolayer is compressed to a surface pressure. The whole experiment was repeated three times.

2.3. Atomic force microscopy observation

AFM images were acquired in air at room temperature using a SPM-9500-J3 AFM (Shimadzu Corporation, Japan), which provided both a topographical image and a phase contrast one. The sample imaging has been carried out in the contact mode through a Micro-V-shaped Cantilever probe (Olympus Corporation, Japan) with cantilever spring constant about 0.06 N/m. A 55 μm×55 μm tube-type piezoelectric scanner and a Si_3N_4 probe were used and images in height mode were collected simultaneously with 512×512 points with scan rate of 1.0 Hz per line.

3. Results and discussion

3.1. Surface pressure isotherm (π–A)

The LB techniques become perceived as desirable model systems for investigating the important role of the interaction between lipids and proteins in the structure and function of biological cell membrane. The typical pressure–area isotherm can be written as a two-dimensional van der Waals equation of state analogous to the three-dimensional real gas[33,34]

$$(\pi + a/A_0^2)(A_0 - b) = k_B T, \tag{1}$$

where π stands for the applied surface pressure, A_0 is the average area occupied per amphiphilic molecule, T and k_B represent the absolute temperature and Boltzmann constant, respectively, a and b are the constant attraction between the molecules (van der Waals force) and the finite size of amphiphilic molecules, respectively. Properties of the liquid crystal state in an amphiphilic molecular monolayer related to its enthalpy and geometry (A_0). If the amphiphilic molecule monolayer was seen as a lyotropic liquid crystalline phase, then the orientation orders to thermotropic liquid crystal were replaced.[35] This is the unique feature of amphiphilic molecules and water system.

To get insight into the physical state of the investigated monolayer, we first discussed the π–A isotherms. It is possible to determine the dilational elasticity, C_s^{-1} under quasi-static compression of surface area. The values of the foregoing parameters were calculated from the isotherm data points, at a given monolayer composition[36]

$$C_s^{-1} = -A(\mathrm{d}\pi/\mathrm{d}A), \tag{2}$$

where A is the experimentally evaluated area per molecule at a given value of π. The higher the values of this parameter, the more ordered the monolayer is.[36,37]

Figures 2(a), 2(b), and 2(c) show that the interactions of MBP with different lipid monolayers (DPPC, DPPC/DPPS, and DPPS) were characterized by measuring the π–A curves before and after the protein adsorption at the air–subphase interface under physiological conditions (Tris-HCl buffer at pH 7.2). Figure 2(a) reports the π–A isotherms obtained for DPPC monolayer spread on buffer without or with MBP (concentration: 5 nM). The monolayer of DPPC shows the presence of liquid expanded–liquid condensed (LE–LC) phase transition plateau at about 3–6 mN/m, and collapses at pressure higher than 65 mN/m, which is similar to previous results obtained by others.[38,39] For mixed DPPC-MBP monolayers, the curve was shifted to a larger area per molecule when the concentration of the MBP is 5 nM. Compared with the phase transition of pure DPPC monolayer, an increase of mixed MBP/DPPC monolayer phase transition occurred for surface pressures higher than 3 mN/m. MBP has a high positive charge, making the π increase at the LC–LE phase coexistence for DPPC (see insert of Fig. 2(a)). This means that the MBP molecules penetrate the monolayer and interact with the phospholipid at about 10 mN/m.

The isotherms of DPPS monolayer measured in the presence of MBP are shown in Fig. 2(b), where the curve is mainly shifted toward higher molecular area than the isotherm of pure DPPS. At lift-off, the curve with the MBP mixtures shows higher mean molecular area (MMA) of 149.5 ±1.0 Å^2, followed by the pure DPPS at 61.5±1.5 Å^2. The monolayer of DPPS shows the presence of liquid expanded–liquid condensed (LE–LC) phase transition plateau at about 10–20 mN/m. It can be seen in the inset of Fig. 2(b) that when the surface pressure is lower than 38 mN/m, the compression modulus of the mixed film is slightly lower than pure DPPS monolayer. In contrast, when the surface pressure is greater than 38 mN/m and less than 70 mN/m, DPPS pure compressive modulus is slightly higher in the mixed monolayer. This means pure DPPS cannot easily be compressed when the surface pressure is lower than 38 mN/m. When the surface pressure is greater than 38 mN/m, the protein is absorbed onto the membrane lipid monolayer, the monolayer is so difficult to compress. This implies that MBP will remain combined with the DPPS monolayer at high surface pressure.

A qualitatively similar behavior was observed with the mixed monolayer of DPPC/DPPS (7:3, w/w) containing MBP. DPPC/DPPS mixed membrane has not undergone liquid-expanded to liquid-condensed transition in the plateau-like region between 60 and 38 Å^2. This result is consistent with the insert of Fig. 2(c). The area increase is larger in the presence of MBP than for DPPC/DPPS, and the isotherm of the mixed monolayer is parallel to that of DPPC/DPPS mixed.

From experimental results shown in Figs. 2(a), 2(b), and 2(c) we find that the isotherms shift to a larger area, due to

the interaction between MBP and lipids. From C_s^{-1} versus π plot of the DPPC and DPPC/DPPS monolayer containing the moderate MBP, we observe that the mixed monolayer membrane elastic modulus is higher than pure lipid compression modulus. The higher compression modulus makes it more difficult for the monolayer to be compressed. This shows that the pure lipids membrane has good liquidity, i.e., easily compressed, whereas the monolayer cannot easily be compressed when the protein is absorbed onto the lipid monolayer. This behavior indicates that the MBP molecules have been bound to the lipids.

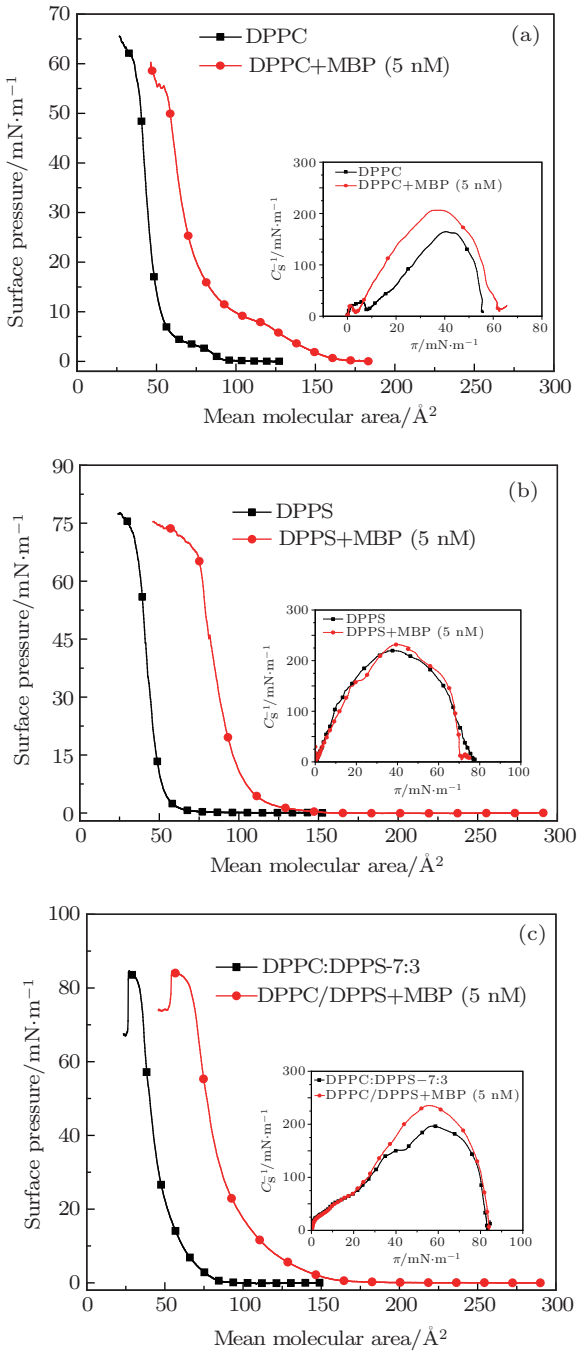


Fig. 2. (color online) π–A isotherms of lipid monolayers in the absence and in the presence of MBP in the subphase (5 nM): (a) DPPC, (b) DPPS, and (c) DPPC/DPPS mixed monolayers. Inset: compressibility modulus (C_s^{-1}) as a function of area per molecule for the same compositions.

3.2. Penetration kinetics

In order to study the relation of MBP molecules with DPPC, DPPC/DPPS, and DPPS monolayers spreading over the air–water interface, we measured the surface pressure-time (π–T) isotherms and determined the insertion ability of MBP into lipid monolayer in the presence of lipids (DPPC, DPPC/DPPS, DPPS) at three initial surface pressures (π_{initial}) equal to 10, 20, and 30 mN/m. Insertion can be measured by the increase in surface pressure ($\Delta\pi = \pi_{\text{final}} - \pi_{\text{initial}}$). Previous research[40] has shown that the monolayer is not affected by surface pressure without protein insertion into the lipid monolayer. Figure 3 shows an increase of surface pressure, as the DPPC, DPPC/DPPS, and DPPS are spread over the interface with an initial surface pressure of 20 mN/m. We find the absorbed behaviors on the three types of lipid monolayers in the order of DPPC>DPPC/DPPS>DPPS. This implies that the strength of protein–lipid interactions is not the only determinant of the lipid head groups, and the value of the surface pressure can also be used to analyze the extent of protein/lipid interactions.[41] Table 1 shows that the larger an initial surface pressure is, the smaller the increase in surface pressure is, which means that MBP was mainly in the LE phase embedded in the lipid monolayers. For the lipid molecules arranged more loosely, with lower surface pressure, the MBP is easily inserted into monolayers.

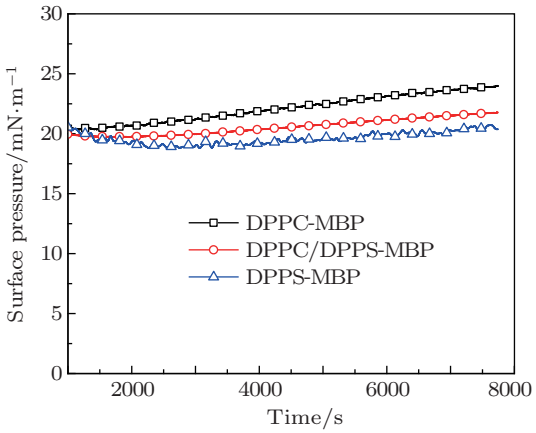


Fig. 3. (color online) Insertion of the MBP into lipid monolayer π–T isotherms. Variation of surface pressure with time after spread DPPC, DPPC/DPPS, and DPPS over the same subphase concentration, which was spread at the initial surface pressure of 20 mN/m.

Table 1. The increases in surface pressure ($\Delta\pi$) of the DPPC, DPPC/DPPS, and DPPS monolayers at different initial surface pressures (π_{initial}) of the monolayers at the same subphase concentration of 5 nM.

π_i/mN·m^{-1}	DPPC ($\Delta\pi$)	DPPC/DPPS = 7/3 ($\Delta\pi$)	DPPS ($\Delta\pi$)
10.00	3.84	1.30	0.30
20.00	1.99	0.90	0.18
30.00	1.03	0.52	0.10

The lipid monolayer is an interesting and useful model membrane system for investigating the three parameters ($\Delta\pi$,

synergy, and maximum insertion pressure (MIP)) responsible for a particular protein with different lipid monolayers.[42,43] To characterize MBP adsorption and lipid specificity without the need of radio labels or other tags, figure 4 shows the plot of $\Delta\pi$ as a function of π_{initial}. The $\Delta\pi$ and MIP can be determined by extrapolating the regression of the plot to the y axis and x axis, respectively. The term "synergy" has been calculated because the slope of the linear regression+1 corresponds to the synergy of the monolayer at which the interaction of MBP with the lipids is energetically favorable. In addition, the synergy factor provides additional information on the insert of protein for the lipid monolayers. The value of negative synergy suggests that the protein disfavors combination to the lipid monolayers. In contrast, the positive synergy means the strong interaction between protein and lipids. On the basis of the relationship between $\Delta\pi$ and π_{initial}, the linear regression for MBP in the presence of DPPC, DPPC/DPPS (7:3, v/v), and DPPS monolayers, results in

$$y = -0.01x + 0.40, \tag{3}$$

$$y = -0.04x + 1.68, \tag{4}$$

$$y = -0.14x + 5.10. \tag{5}$$

The largest values of MIP 40.00±1.80 mN/m have been obtained for the MBP combined to the DPPS monolayer which also suggested the greatest ability to remove the DPPS from the cell membrane, followed by DPPC/DPPS and DPPC monolayers. Values of synergy factor of 0.99±0.01, 0.94±0.02, and 0.86±0.01 have been respectively obtained for DPPS, DPPC/DPPS, and DPPS monolayer which correlate with a favorable binding of MBP to these phospholipid monolayers. Therefore, the values of $\Delta\pi$, synergy, and MIP depend on the lipid head groups and initial surface pressures of phospholipid monolayers. The fact that the highly positively charged MBP could aggregate is adsorbed onto the net negatively charged DPPS and the zwitterionic phospholipids via electrostatic and hydrophobic interactions.

This partly has demonstrated that the MBP penetrates very slightly into DPPC monolayer. (i) When the MBP concentration in the subphase is 5 nM, the average molecular area increase of the DPPC monolayer reaches ~ 50 Å^2, followed by DPPC/DPPS (~ 70 Å^2) mixed and DPPS (~ 80 Å^2) monolayers. (ii) MBP insert, into DPPS and DPPC/DPPS monolayers suggesting that the presence of MBP induces phase separation and perturbation of lipid configuration. Instead, MBP had no significant effect on the surface morphology of DPPC monolayer. (iii) From three parameters of linear equation we can see that MBP interacts preferentially with the net negatively charged DPPS followed by DPPC/DPPS, and finally DPPC monolayers.

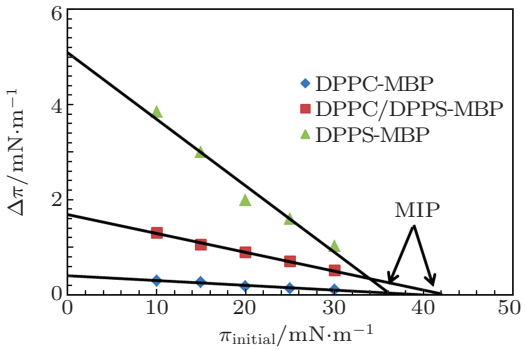


Fig. 4. (color online) The binding parameters of MBP onto the DPPC, DPPC/DPPS, and DPPS monolayers determined by plot of $\Delta\pi$ as a function of π_{initial}. The subphase concentration of 5 nM, pH = 7.2.

3.3. AFM observation of different lipid monolayers in the presence of MBP

To further characterize the effect of the mixture of MBP on the DPPS, DPPC/DPPS (7:3, V/V), and DPPC monolayers, AFM was employed to view selected surface morphologies of the mixture of the different lipid monolayers and MBP deposited on mica substrates at low (10 mN/m), intermediate (20 mN/m), and high (30 mN/m) surface pressure. Figure 5 shows images of the effect produced by the MBP at various initial surface pressure. Therefore, there are two aspects to understanding the morphologies observed. Firstly, how the molecules orient themselves at the air–subphase interface when the monolayers are deposited at various surface pressures. In the presence of MBP, with the increase of surface pressure, the mixture structure of monolayers yielded a densely packed structure and holes are clearly visible, regardless of the lipid conformational change (Figs. 5(c), 5(f), and 5(i)). It implied MBP is squeezed out of the monolayer at high surface but remains associated to the interface, which is consistent with the π–T isotherm shown in Fig. 3. Furthermore, the incorporation of MBP into the lipid monolayers had exerted a significant effect on the domain structure (Figs. 5(b), 5(e), and 5(h)) at a given surface pressure (e.g., 20 mN/m in this case). We found that MBP induces the phase segregation, the mixture of DPPS/MBP forms small watercress sheet domains and the MBP embedded in it, with the height of around 12 nm. The mixture of DPPC/DPPS monolayers in the presence of MBP forms two-phase morphology, one is homogeneous sheet structure from DPPC/MBP monolayer, another is watercress linear region from DPPS/MBP. This behavior is consistent with previous studies on the π–A curves of the interaction between DPPS, DPPC/DPPS, and MBP at 10–20 mN/m. For DPPC/MBP monolayer the formation of relatively loose micro petals is indicated, along with the existence of granular-like domains (Fig. 5(g)). The attraction and binding of MBP to monolayers of different lipid compositions is modulated by hydrophobic interactions and electrostatic interactions with the amphiphilic and acidic lipid headgroups.

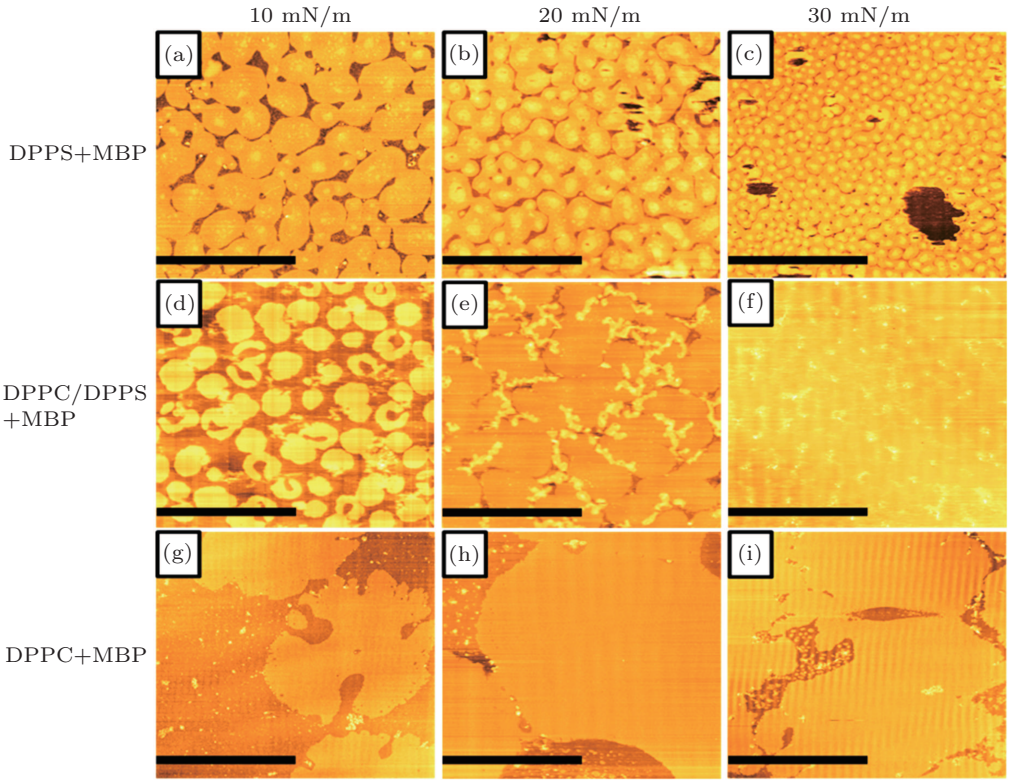


Fig. 5. (color online) AFM image (10×10) of DPPS (a)–(c), DPPC/DPPS (7:3, V/V) (d)–(f), and DPPC (g)–(i) in the presence of MBP (5 nM) at low, medium, and high surface pressure: 10 mN/m ((a), (d), (g)), 20 mN/m ((b), (e), (h)), 30 mN/m ((c), (f), (i)). Scale bar: 5 μm.

4. Conclusions

The combination of surface pressure–area isotherm and atomic force microscopy allows us to study MBP-incorporated DPPC, DPPS, and DPPC/DPPS monolayers. We can draw these conclusions. (i) The phase behavior of DPPC, DPPS, and DPPC/DPPS in the presence of MBP was significantly different from that of the pure lipid. In addition, the higher C_s^{-1} values proves that the presence of the positively charged MBP makes the DPPC, DPPS, and DPPC/DPPS monolayers more incompressible, and reduces the membrane fluidity. (ii) Then, the kinetics of binding of MBP onto lipid monolayers has been measured by three parameters of linear regression (MIP, $\Delta\pi$, and synergy). The magnitude of the maximum surface pressure, surface pressure change, and the synergy can thus be concluded that MBP has a high affinity for the DPPC, DPPS, and DPPC/DPPS monolayers. Among them, the strongest absorption of MBP on the DPPS monolayer has been obtained by the synergy factor, followed by DPPC/DPPS and DPPC monolayers. (iii) AFM image studied in monolayers of DPPS and DPPC/DPPS containing MBP showed that MBP induces the segregation of two-phase in LE–LC. Monolayers became increasingly close with the increase of surface pressure, and MBP was squeezed out of the monolayer at high surface pressure. These may be significant from the viewpoint of the biological function of MBP in the native membrane.

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