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Structural models and surface equation of state for pulmonary surfactant monolayers

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Abstract

A simple surface equation of state is proposed to describe π-A isotherms of pulmonary surfactant monolayers. The monolayer is considered as undergoing three characteristic states during the compression: the disordered liquid-expanded (LE) state, the ordered liquid-condensed (LC) state and the collapse state. Structural models of pure protein (SP-B and SP-C) monolayer are proposed to interpret the behavior characteristics of monolayer in the states. The area, A_{LC}, is defined as an instantaneous LC-state area when the monolayer is under the complete LC state. The area, A_t, is defined as a transition area from the ordered LC state to the collapse state. And the collapse pressure, π_{max}, is defined as the maximum surface pressure that the monolayer can bear before collapse. The ideal equation of state is revised by A_{LC}, A_t and π_{max}, and a new equation of state is obtained, which is applicable for pure components of pulmonary surfactant. The theoretical π-A isotherms described by the equation of state are compared with the experimental ones for SP-B, SP-C, DPPC and DPPG, and good agreements are obtained. The equation of state is generalized to protein–lipid binary mixtures by introducing mixing rules. The predicted π-A isotherms agree with the experimental ones for various pulmonary surfactant components and the average deviation is about 9.2%.

Keywords: Surface equation of state; Pulmonary surfactant; Surfactant protein; Structural model

1. Introduction

Pulmonary surfactant (PS) is a complex mixture of approximately 90% lipids and 10% proteins present at air–liquid interface of lungs. The main function of PS is to reduce the surface tension at the alveolar air–liquid interface in order to avoid alveolar collapse at the end of expiration and to facilitate the work of breathing [1]. The deficiency or inactivation of PS in premature infants is responsible for respiratory distress syndrome (RDS), which is a major cause of neonatal morbidity and mortality [2]. Therefore, the studies on the action mechanism and molecular biology of PS components have clinical importance.

The surfactant lipids mainly include saturated dipalmitoylphosphatidylcholine (DPPC) and phosphatidylglycerol (PG), where DPPC is the most abundant (40 wt.% of PS) and the most surface-active component [3]. DPPC is an amphiphilic molecule that can generate ordered films and pack tightly to reduce the surface tension to less than 1 mN/m [4]. The very low surface tension value enables the alveolar space to contract during expiration without collapse. Besides, other phospholipids, such as dipalmitoylphosphatidylglycerol (DPPG), can help DPPC facilitate the re-spreading of monolayer [5].

There are four specific surfactant proteins: hydrophilic SP-A and SP-D, hydrophobic SP-B and SP-C. SP-B and SP-C are two small proteins synthesized by the alveolar type II epithelial cells, accounting for approximately 1% to 2% (wt) of total PS [6]. SP-B is a disulphide-linked homodimer composed of two 79-residue polypeptide chains. As a member of the saposin-like family, SP-B is the only protein which is a hydrophobic covalent dimer [7,8]. SP-C is a 35-residue lipopeptide expressed only in lung tissue and is one of the most hydrophobic polypeptides so far known [8]. SP-B and SP-C have been implicated as important contributors to the surface activity of PS. SP-B can enhance the surface tension-reducing properties of PS films, the rate of adsorption and surface spreading of phospholipids [3]. Similarly, SP-C can interact with

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phospholipids and promote the phospholipids to be absorbed to the monolayer surface. And SP-C is more effective in promoting the reinsertion of lipids squeezed out of the surface monolayer during the compression [4]. In order to understand the breathing process and function of PS in a deeper way, it is necessary to learn more details about structural properties of PS monolayers, as well as the effect of SP-B/SP-C.

Another important point to understand the breathing process is the determination of surface tension of the alveolar surface in vivo, because surface tension or surface pressure is one of the most important characteristics for the surface behavior of PS. Most studies on surface pressure (π) ~ area (A) isotherms for insoluble surfactant monolayers were focused on the phase transition from a fluid phase of low density (liquid-expanded or LE phase) to a condensed phase (liquid-condensed or LC phase) [9]. Some surface equations of state for describing π-A isotherms have been proposed by Israelachvili [10], Fainerman [11], Ruckenstein [9,12], Zeng et al. [13]. For example, Fainerman et al. assumed that the monolayer was in the formation of two-dimensional aggregates and an equation of state was theoretically derived to describe the main phase transition between the gaslike and the condensed phases, however, the equation is complex in form and limited in the practical use. The surface equation of state proposed by Ruckenstein et al. could be used to interpret the LE–LC phase transition. They treated the monolayer as a two-dimensional mixture consisting of LC domains, disordered molecules in the LE state, and free sites, however, only pure phospholipid monolayers were studied. All of these equations [9–13] only had good agreements in pure monolayers, and the mixtures were not taken into account, especially the protein–lipid mixtures. Furthermore, they didn't consider about the relations between structural properties of PS components and surface features of surfactant monolayer. In this paper, structural models of pure protein monolayer are suggested, and a new simple surface equation of state is derived based on the structural models. The surface equation of state is applicable for both pure components and binary mixtures of PS.

2. The structural models of pure protein monolayer

2.1. The structural model of pure SP-C monolayer

The three-dimensional structure of SP-C molecule in chloroform/methanol solutions has been determined by NMR spectroscopy [14]. SP-C is composed of a short palmitoylated N-terminal region and a valyl-rich α-helical transmembrane domain. The N-terminal eight residues are conformationally disordered and haven't determined yet. The C-terminal α-helix encompassing residues 9–34 is nearly ideal helix geometry with a length of 3.7 nm and it is very rigid and hydrophobic [8]. It was showed that SP-C α-helix situated in a DPPC monolayer made a 20° tilt angle to the interface [15]. Based on the SP-C structure above, a structural model of pure SP-C monolayer is proposed as follows:

(1) At low surface pressure, the monolayer is in a disordered LE state with large free space among SP-C molecules (Fig. 1 (a)-I).
(2) In the compressing process, the monolayer turns to an ordered LC state, where SP-C molecules are in good order and close together. The N-terminal residues are compressed into the underside of the surrounding C-terminal of α-helix (Fig. 1(a)-II). There exists LE–LC transitional state in the compression, and a LE–LC plateau is showed in the sketch of π-A isotherm (Fig. 1(c)). For pure protein monolayer, it is difficult to get the LE-LC plateau from the experimental π-A isotherms (see Fig. 4), while it is easy for pure lipid monolayer (see Fig. 3). When the monolayer is under the complete LC state, an area, A_{LC}, is defined as an instantaneous LC-state area per "residue" (where "residue" denotes an amino acid residue of protein or a phospholipid

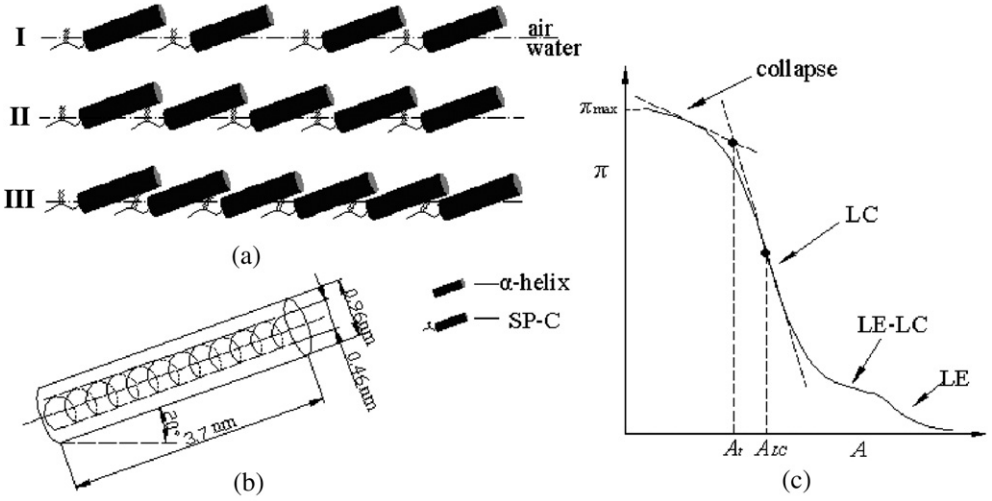

Fig. 1. (a) The structural model of pure SP-C monolayer in the compression. (I) The disordered LE state, (II) The ordered LC state, (III) The collapse state. (b) The structural analysis of α-helix in SP-C molecule. (c) Sketch of π-A isotherm of protein or lipid monolayer. A_{LC} is the instantaneous LC state area. A_t is the transition area. π_{max} is the maximum surface pressure.

molecule). A_{LC} is obtained by the corresponding area of a "pseudo inflection point" from the linear LC-state part of π-A isotherm (Fig. 1(c)).

(3) When the monolayer is further compressed, the surface pressure increases sharply and then steadily until a "pseudo plateau" appears. C. S. Song et al. showed that it was a transition from monolayer to multilayer on acidic subphase and a molecular area, A_t, was defined as a transition area [16]. In this paper, a similar definition is made that A_t is a transition area per "residue" from the ordered LC state to the collapse state. When the compression continues in the collapse state, the space among SP-C molecules reduces rapidly (Fig. 1(a)-III). Many investigations suggested that the SP-C molecules with other PS components were selectively squeezed out at high surface pressure [17–20], and these squeezed-out structures were called "surface-associated surfactant reservoir". Amrein et al. considered that the reservoir was organized in the formed of stacks of bilayers [17]. Dan Li et al. suggested that the squeezed-out matters formed lipid–protein aggregations in the subphase [20]. For pure SP-C monolayer, at high surface pressure, the structure of SP-C molecules in the film may change greatly during the compression until the film collapses. A collapse pressure, π_{max}, is defined as the maximum surface pressure that the monolayer can bear before collapse (Fig. 1(c)).

According to the geometrical characteristics of regular α-helical structure and the molecular size of one SP-C molecule, an estimated theoretical value of A_{LC} is obtained (Fig. 1(b)). Regular α-helical radius is about 0.23 nm [21], so the main diameter of α-helix column is 0.46 nm. Suppose the side chain length (mainly isopropyl in Valine) is about 0.25 nm (because the bond length and angle of carbon–carbon bonds is 0.154 nm and 109°, respectively [22], $0.154 \times \mathrm{Sin}\ (109° \div 2) \times 2 = 0.25$ nm), so the whole diameter is 0.96 nm. Based on the helical length (3.7 nm) and tilt angle (20°), the area of α-helix structure in the monolayer is 3.34 nm^2, so the area per amino acid residue in the α-helical region is 0.124 nm^2 (suppose there are 27 amino acid residues in α-helix structure of SP-C [14]). It has been calculated that the area per amino acid residue in a film of α-helices was 0.128 nm^2 [23], which is very close to 0.124 nm^2 (see Table 2).

In this paper, we assume that there is partially overlapping phenomenon among SP-C molecules and N-terminal residues are compressed into the underside of the surrounding C-terminal α-helix in the complete LC state (Fig. 1(a)-II). So for the whole SP-C molecule, the available area in the monolayer is given by α-helix structure and the mean area per residue, A_{LC}, is 0.0954 nm^2.

2.2. The structural models of pure SP-B monolayer

SP-B is a hydrophobic homodimer by joining two SP-B monomers via the interchain Cys48–Cys48′ disulphide [24]. Each monomer contains three intrachain disulfides and associates with phospholipid bilayers by three or four amphipathic helices [25]. Several experimental studies showed that SP-B interacted preferentially with superficial parts of lipid membranes and lacked transmembrane parts [26–28]. Based on the structure of SP-B above, a structural model of pure SP-B monolayer is proposed.

In the whole compressing process, the action mechanism of pure SP-B monolayer is similar to SP-C's (Fig. 2(a)). There is also an instantaneous LC-state area, A_{LC}, in the complete LC state (Fig. 2(a)-II), which is calculated as follows: for one SP-B monomer with 79 amino acid residues, its two-dimensional geometrical structure is supposed to be a rectangle (Fig. 2(b)). The diameter of α-helix is 0.96 nm, so the width of rectangle is about 1.92 nm. SP-B exhibits an overall content of 27–45% α-helical structure [8], in this paper, we choose 36% as the mean value and suppose the retained secondary structure is β-sheet, accounting for 64%. The rising length for each residue in α-helix and β-sheet structure is about 0.15 nm and 0.31 nm, respectively [21]. Considering that β-sheet chain is not straight, and suppose it bend like a semicircle, so a bending coefficient of β-sheet chain is defined in this paper as $\pi/2$ (about 0.637). So the rectangle length is about 6.17 nm $(79 \times 36\% \times 0.15 + 79 \times 64\% \times 0.31 \times 0.637 - 1.92) \div 2 = 6.17$ nm). Therefore, for the mean area per residue of SP-B, A_{LC} is 0.150 nm^2. According to Malcolm's study [23], the area per amino acid residue was 0.128 nm^2 for α-helices and

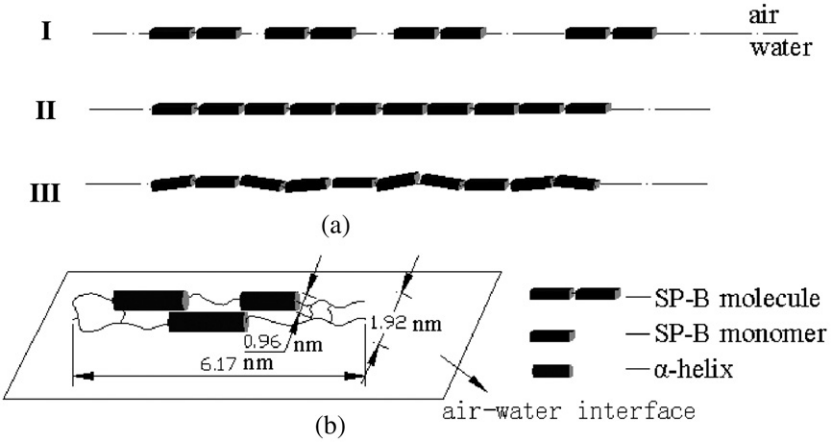

Fig. 2. (a) The structural model of pure SP-B monolayer in the compression. (I) The disordered LE state, (II) The ordered LC state, (III) The collapse state. (b) The structural analysis of SP-B monomer.

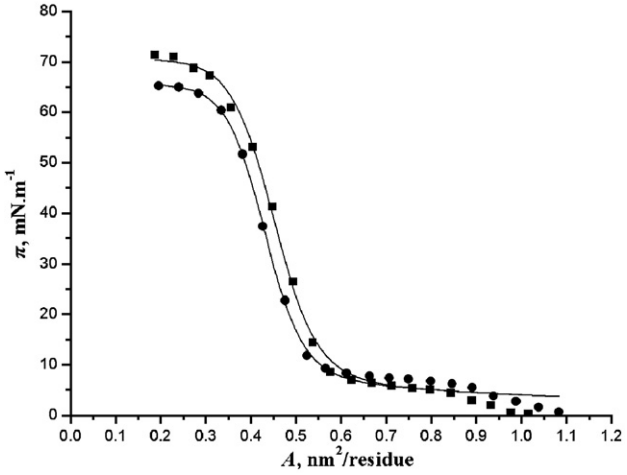

Fig. 3. π-A isotherms of pure DPPC (■) and DPPG (●) monolayers. The points represent the experimental data obtained from references [29,30], and the solid lines represent the regression results of Eq. (13) by the method of least minimum square.

0.168 nm^2 for β-conformations, so A_{LC} can be calculated as 0.128×36%+0.168×64%=0.154 nm^2, which is very close to the theoretical value of A_{LC} from the structural model (see Table 2).

3. The surface equation of state for pulmonary surfactant monolayers

At a very low surface pressure, surfactant molecules (except for macromolecules, such as protein molecules) in the monolayer behave like ideal gas, which obey the ideal equation of state:

$$\pi A = kT \tag{1}$$

where π is surface pressure, A is the area of each residue in the monolayer, k is the Boltzmann constant, and T is the Kelvin temperature.

For protein molecules, we treat them as aggregates consisting of amino acid residues according to Taneva's study [29,30]. However, the protein residues can not behave as independent molecules even at very low surface pressure. So a correction parameter Z is introduced into the ideal equation of state:

$$\pi A = ZkT \tag{2}$$

Eq. (2) could describe the behavior of surfactant molecules (protein and phospholipid) at low surface pressure. For phospholipid molecules, the value of Z is 1, while for protein molecules, it is given by the following formula:

$$Z = \frac{1}{Nr} \tag{3}$$

where Nr is the number of residues for each protein molecule. So Z is 0.029 for SP-C molecule and 0.0063 for SP-B molecule.

The surface behavior of surfactant molecules (protein and phospholipid) deviates from Eq. (2) when surface pressure of the monolayer increases. So a correction function $F(A,T)$ is introduced into Eq. (2), and a modified equation of state is obtained, as follows:

$$\pi A = ZkT + F(A,T) \tag{4}$$

According to the structural models above, corrections from the collapse-state and LC-state are taken into account. $F(A,T)$ is composed of two parts: correction function of collapse-state: $F_{CO}(A,T)$, and correction function of LC-state: $F_{LC}(A,T)$.

$F_{CO}(A,T)$: When the monolayer is under the collapse state, the film surface pressure is π_{max}, and Eq. (2) is not applicable. Take π_{max} into Eq. (2), an unbalance between the two sides of equation is obtained. $F_{CO}(A,T)$ is defined as the difference between two sides:

$$F_{CO}(A,T) = \pi_{max}A - ZkT \tag{5}$$

$F_{LC}(A,T)$: When the monolayer is under the LC-state, the film surface pressure changes rapidly under the compression. An exponential function is introduced as follows:

$$F_{LC}(A,T) = 1 + \exp\left[\frac{A - A_{LC}}{(\Delta A_{LC}/4)}\right] \tag{6}$$

where ΔA_{LC} is a change in area from the beginning of LC-state to its end in the compression, A_{LC} has been defined in Section 2.1. It is found that ΔA_{LC} has a physical meaning about the surface compressibility of monolayer. According to Eq. (7) [31], the isothermal compressibility of monolayer can be calculated from π-A isotherms:

$$Cs = -\frac{1}{A}\frac{d\pi}{dA} \tag{7}$$

where Cs is the film surface compressibility. As for the whole LC-state isotherm here (see Fig. 1(c)), the LC-state isotherm

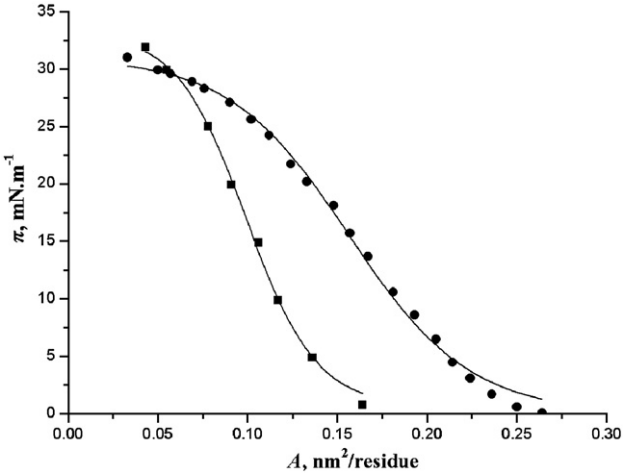

Fig. 4. π-A isotherms of pure SP-C (■) and SP-B (●) monolayers. The points represent the experimental data obtained from references [29,30], and the solid lines represent the regression results of Eq. (13) by the method of least minimum square.

Table 1
The regression values of pure component parameters in Eq. (13)

Component	π_{max} (mN m^{-1})	A_{LC} (nm^2/res)	A_t (nm^2/res)
DPPC	70.5	0.447	0.353
DPPG	65.5	0.427	0.343
SP-C	33.1	0.0987	0.0600
SP-B	31.0	0.156	0.0893

is almost straight, so the surface compressibility is defined as:

$$Cs_{LC} = -\frac{1}{A_{LC}}\frac{\Delta\pi_{LC}}{\Delta A_{LC}} \tag{8}$$

where $\Delta\pi_{LC}$ is a change in surface pressure corresponding to ΔA_{LC}. From Eq. (8), ΔA_{LC} is related to surface compressibility of the LC-state film, while $\Delta\pi_{LC}$ and A_{LC} also make contributions.

Furthermore, from Fig. 1(c), we find that the difference between A_{LC} and A_t is almost equal to the half value of $\Delta\pi_{LC}$, so there is:

$$\Delta A_{LC} = 2 \times (A_{LC} - A_t) \tag{9}$$

Substituting Eq. (9) into Eq. (6),

$$F_{LC}(A,T) = 1 + \exp\left[2 \times \left(\frac{A - A_{LC}}{A_{LC} - A_t}\right)\right] \tag{10}$$

F(A,T): $F(A,T)$ should tend to be zero when the film area goes to infinity, so $F(A,T)$ is established on the basis of function $F_{CO}(A,T)$ and function $F_{LC}(A,T)$ as follows:

$$F(A,T) = \frac{F_{CO}(A,T)}{F_{LC}(A,T)} = \frac{\pi_{max}A - ZkT}{1 + \exp\left[2 \times \left(\frac{A-A_{LC}}{A_{LC}-A_t}\right)\right]} \tag{11}$$

Substituting Eq. (11) into Eq. (4), a modified equation of state is obtained,

$$\pi A = ZkT + \frac{\pi_{max}A - ZkT}{1 + \exp\left[2 \times \left(\frac{A-A_{LC}}{A_{LC}-A_t}\right)\right]} \tag{12}$$

As for Eq. (12), if A goes to infinity, function $F(A,T)$ will be zero, Eq. (12) will change into Eq. (2). And for phospholipid monolayer, Eq. (12) would change into the ideal equation of state. The theoretical analysis results agree with the practical phenomenon. If the film area is infinite, the distance among molecules is so far that molecules in the monolayer would behave like ideal gas and obey the ideal equation of state.

Eq. (12) can be transformed to Eq. (13):

$$\pi = \frac{ZkT}{A} + \frac{\pi_{max} - ZkT/A}{1 + \exp\left[2 \times \left(\frac{A-A_{LC}}{A_{LC}-A_t}\right)\right]} \tag{13}$$

At a constant temperature, Eq. (13) can be used to describe π-A isotherms of various pure PS components. In this paper, the experimental π-A isotherms are obtained from references [29,30], and the experimental temperature is 22±1 °C. On the basis of the mixing rules, as well as pure component parameters (Z, π_{max}, A_{LC}, A_t) gotten from the simulation results of pure component's experimental data, the binary mixture parameters are obtained. So Eq. (13) is generalized to binary mixtures (see below).

Table 2
Comparison of A_{LC} from different methods

Component	The theoretical values from structural models A_{LC} (nm^2/res)	The regression values from equation of state A_{LC} (nm^2/res)	The reference values from Malcolm's study [23] A_{LC} (nm^2/res)
SP-B	0.150	0.156	0.154
SP-C	0.0954	0.0987	–
α-helix of SP-C	0.124	–	0.128

Table 3
The predicted values of binary mixture parameters according to mixing rules

Component	"Residual" fraction of protein ($x_{protein}$)	Parameters					AAD %
		π_{max} (mN/m)	Z	A_{LC} (nm^2/res)	A_t (nm^2/res)	λ	
SP-B/	0.28	70.5	0.72	0.350	0.235	0.9	9.2
DPPC	0.45	70.5	0.55	0.298	0.191		4.2
SP-C/	0.22	70.5	0.79	0.349	0.187	0.7	8.2
DPPC	0.48	70.5	0.53	0.248	0.127		15.6
SP-B/	0.61	65.5	0.40	0.246	0.101	0.6	9.0
DPPG	0.74	65.5	0.26	0.214	0.0837		13.0
SP-C/	0.39	65.5	0.62	0.272	0.103	0.5	7.9
DPPG	0.56	65.5	0.46	0.215	0.0780		6.5

4. Results and discussions

4.1. The regression results of pure component monolayers

Eq. (13) is used to correlate the experimental data of pure components (DPPC, DPPG, SP-B, SP-C, the points in Figs. 3 and 4) [29,30] with the method of least minimum square. The regression values of related parameters in Eq. (13) are showed in Table 1, and the theoretical regression isotherms (the solid lines) are showed in Figs. 3 and 4. The correlation coefficients (R^2) of the model for the four groups of experimental data all exceed 0.99.

From Figs. 3 and 4, it is clear that the regression π-A isotherms fit the experimental data of DPPC, DPPG, SP-C, SP-B well. In Table 1, the maximum surface pressure π_{max} in pure protein monolayer is much lower than that in pure phospholipid monolayer. Because compared with phospholipid molecules, protein molecules are much larger and have more complicated structures that can not pack sufficiently tightly under compression. Therefore, pure protein monolayer is more prone to collapse at lower surface pressure. As mentioned above, pure DPPC film can reduce surface tension to less than 1 mN/m [4], so the maximum surface pressure that the film can bear is about 71 mN/m. The regression value of π_{max} for DPPC in Table 1 is 70.5 mN/m, which is almost equal to the actual value.

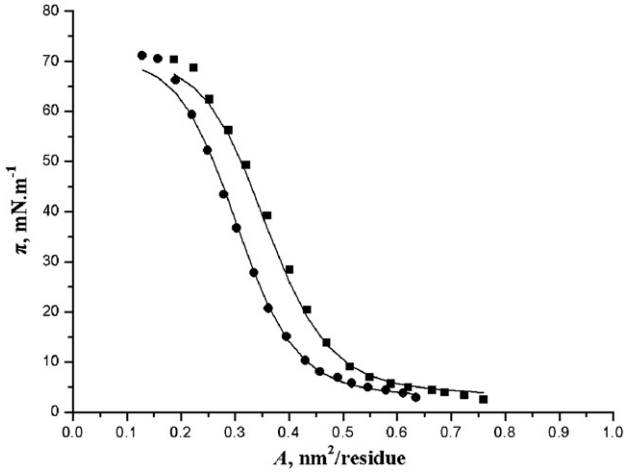

Fig. 5. π-A isotherms for SP-B/DPPC mixtures of two different protein residual fraction $x_{protein}$: 0.28 (■) and 0.45 (●). The points represent the experimental data obtained from references [29,30], and the solid lines represent the predicted theoretical π-A isotherms in this paper.

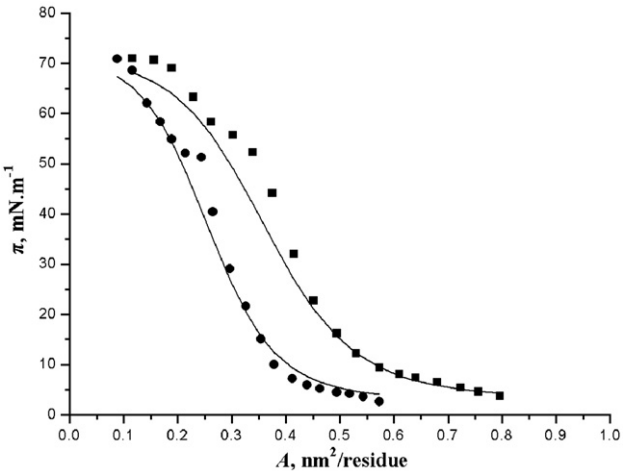

Fig. 6. π-A isotherms for SP-C/DPPC mixtures of two different protein residual fraction $x_{protein}$: 0.22 (■) and 0.48 (●). The points represent the experimental data obtained from references [29,30], and the solid lines represent the predicted theoretical π-A isotherms in this paper.

In Section 2, the theoretical values of A_{LC} estimated from structural models have been obtained, 0.150 nm^2/res for SP-B, 0.0954 nm^2/res for SP-C, which are close to the regression values (see Table 2).

4.2. Comparison with experiments for mixture monolayers

PS is the mixture of proteins and lipids, so studies on the surface equation of state for mixtures are more practical. The extension from pure components to mixtures is commonly achieved by introducing mixing rules for the parameters in Eq. (13).

$$Z_M = \sum_{i=1}^{N} x_i Z_i \tag{14}$$

$$A_{LC\_M} = \sum_{i=1}^{N}\sum_{j=1}^{N} x_i x_j A_{LC\_ij} \tag{15}$$

$$A_{LC\_ij} = \sqrt{A_{LC\_ii} \times A_{LC\_jj}} \tag{16}$$

$$A_{t\_M} = \lambda \times \sum_{i=1}^{N}\sum_{j=1}^{N} x_i x_j A_{t\_ij} \tag{17}$$

$$A_{t\_ij} = \sqrt{A_{t\_ii} \times A_{t\_jj}} \tag{18}$$

where Z_M, A_{LC_M}, A_{t_M} represent the corresponding parameters of mixtures; x_i, x_j are "residual" fraction of protein or lipid molecules in the protein–lipid monolayer [29]; A_{LC_ij}, A_{t_ij} are the corresponding binary interaction parameters for component i and j; A_{LC_ii}, A_{LC_jj}, A_{t_ii}, A_{t_jj}, Z_i are the corresponding parameters of pure component i or j. Besides, an experimental correction coefficient, λ, is introduced into the mixing rule of A_{t_M}. As we known, the surface properties of the monolayer is not only influenced by the film structure, but also by the experimental factors. λ is used to describe the effect of the experimental factors, and its value is obtained by regression analysis of the experimental data.For binary mixtures, Eqs. (14)–(18) can be transformed to Eqs. (19)–(21):

$$Z_M = x_1 Z_1 + x_2 Z_2 \tag{19}$$

$$A_{t\_M} = \lambda \times \left(x_1^2 A_{t\_11} + 2x_1 x_2 A_{t\_12} + x_2^2 A_{t\_22}\right) \tag{20}$$

$$A_{LC\_M} = x_1^2 A_{LC\_11} + 2x_1 x_2 A_{LC\_12} + x_2^2 A_{LC\_22} \tag{21}$$

On the basis of Eqs. (19)–(21) and the data of pure components in Table 1, parameters Z, A_t, A_{LC}, for mixtures with various "residual" fraction of protein are obtained (Table 3). Besides, many investigations suggested that SP-B and SP-C were selectively squeezed out at high pressure and could reinsert into PS monolayers during the expansion [4,17,18,29,30]. So in this paper, we assume the maximum surface pressure of mixture monolayer can reach to the pressure of pure phospholipid

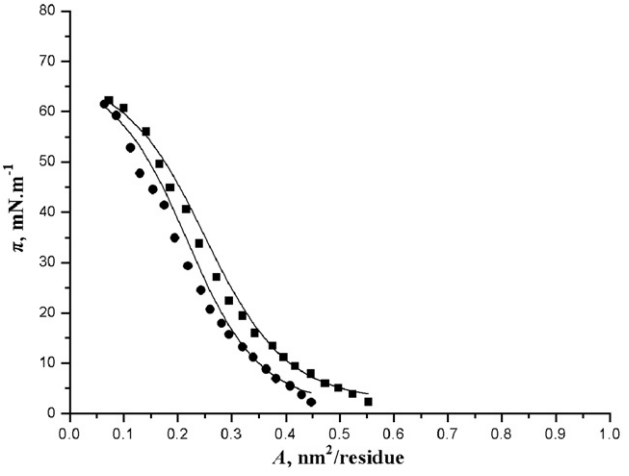

Fig. 7. π-A isotherms for SP-B/DPPG mixtures of two different protein residual fraction $x_{protein}$: 0.61 (■) and 0.74 (●). The points represent the experimental data obtained from references [29,30], and the solid lines represent the predicted theoretical π-A isotherms in this paper.

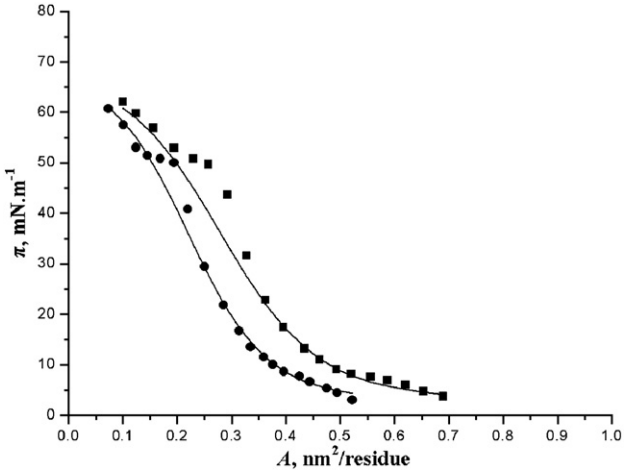

Fig. 8. π-A isotherms for SP-C/DPPG mixtures of two different protein residual fraction $x_{protein}$: 0.39 (■) and 0.56 (●). The points represent the experimental data obtained from references [29,30], and the solid lines represent the predicted theoretical π-A isotherms in this paper.

monolayer. In other words, π_{max} is 70.5 mN/m for monolayer including DPPC, and 65.6 mN/m for monolayer including DPPG. λ is a characteristic parameter related to the experimental factors, so it is determined by experimental data with a scale of 0.5 to 1.0.

Comparisons between the theoretical and experimental π-A isotherms for binary mixture monolayers (SP-B/DPPC, SP-C/DPPC, SP-B/DPPG, SP-C/DPPG) are presented in Figs. 5–8. From these figures, the theoretical π-A isotherms agree with the experimental ones for these four kinds of binary mixtures with different residual fraction of protein. The absolute average deviations (AAD%) are shown in Table 3. AAD% is defined as follows:

$$\mathrm{AAD\%}(\pi) = \frac{1}{N} \times \left(\sum_{i=1}^{N} \frac{|\pi_{i,\mathrm{exp}} - \pi_{i,\mathrm{calc}}|}{\pi_{i,\mathrm{exp}}} \right) \times 100 \qquad (22)$$

where N is the number of data points in each π-A isotherm, $\pi_{i,\mathrm{exp}}$ is the experimental value of π from data point i, $\pi_{i,\mathrm{calc}}$ is the calculated value from Eq. (13) with the same value of A as $\pi_{i,\mathrm{exp}}$.

The average AAD% between the experimental data and theoretical data in Figs. 5–8 are 9.2%, so the prediction of π-A isotherms from pure components to mixtures is acceptable.

In addition, a kink is present in all experimental isotherms in Figs. 6 and 8 for SP-C/lipids mixtures. It is because of the existence of "squeeze-out" phenomenon. According to Taneva's study [29,30], one SP-C molecule, associated with 8–10 phospholipid molecules, was squeezed out from the monolayer under the compression, while for SP-B molecule, it was 2–3 phospholipid molecules. So SP-C showed a higher efficiency for removing phospholipids from the protein–lipid monolayer than did SP-B. Therefore, a clear kink is given in π-A isotherms when SP-C exists. Because of "squeeze-out" phenomenon, the protein and phospholipid fractions in the monolayer change. However, we choose a constant "residual" fraction ($x_{protein}$) in our model. So a better fit between experimental and theoretical values is obtained for SP-B/lipids mixture monolayers than SP-C/lipids mixture monolayers.

5. Conclusions

Structural models of pure protein monolayer in the compression are proposed on the basis of molecular structures of SP-B and SP-C. And the structural models interpret three characteristic states of the monolayer: the disordered liquid-expanded (LE) state, the ordered liquid-condensed (LC) state and the collapse state. Besides, the simple surface equation of state is derived to describe the π-A isotherms of PS monolayers in the compressing process including these three states. The surface equation of state in this paper takes into account the relations between structural properties of PS components and surface features of surfactant monolayer, so it is more practical. The equation is applicable not only for pure component monolayers, but also for binary mixture monolayers. The results predicted by the theory are in good agreement with the experimental monolayer isotherms. However, our work is not enough to understand the whole lung surfactant π-A behavior, further work needs to be done and a more generalized surface equation of state may be obtained in future that can be applicable for multi-component monolayers or natural lung surfactant films.

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