Biophysical Journal Volume 69 August 1995 460–469

Effects of Chain Unsaturation on the Equation of State for Lipid Monolayers at the Air-Water Interface

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ABSTRACT An equation of state for lipid monolayers at the air-water interface is presented, which takes into account the effects of the conformation and the number and position of double bonds of the hydrocarbon chains. The total Hamiltonian of the monolayer is assumed to consist of three terms. Two of them are calculated exactly within the limitations of the formulation. These are the two-dimensional entropy of mixing of the lipid and water molecules at the surface and the conformational entropy of the chains using a model available from the literature. These two terms give rise to positive surface pressure. The third term, which includes all energies that are not amenable to calculation, was obtained as the difference between the sum of the two calculated terms and experimental data and is found to represent an approximately area-independent tension. The effects of chain unsaturation on the equation of state were modeled in the present theory in two ways; the chain bend caused by *cis* double bonds increases the minimal molecular area, and the double bond linkage on a chain decreases the degrees of freedom of the chain. Calculations revealed that the former is highly significant whereas the latter is negligible. The deduced equation of state reproduces experimental data with appropriate values for three parameters, which represent the collapse area, the overlap of adjacent chains, and the combined effects of the intra- and intermolecular interactions other than the surface mixing entropy and the chain conformational energy.

INTRODUCTION

Experimental measurement and theoretical or empirical prediction of the relationship between surface pressure and surface molecular area have been important aspects in the study of monolayers of amphiphilic substances. These have provided essential information about molecule structure and intra- and intermolecular interactions and have also contributed useful information on various functions of biomembranes. There is a considerable amount of experimental data for monolayers of various lipids in the literature, from which several empirical equations of state have been constructed (Gaines, 1966, 1978; MacDonald and Simon, 1987; Menger et al., 1988; Wolf and Brockman, 1988; Birdi, 1989; Smaby and Brockman, 1991). In general, equations of state in the literature are of an empirical or phenomenological form. Such equations of state were usually deduced either from a two-dimensional kinetic theory of gases approach or from a phenomenological treatment of a two-dimensional solution, both of which avoid a detailed model of molecular structure (Lucassen-Reynders, 1981). Their value in explaining the physical behavior of monolayers on the basis of the molecular structure and the intra- and intermolecular interactions is therefore limited. It is clear that the form of the equation of state for a monolayer should be completely determined by the properties of the monolayer, which should be dominated by the microscopic structure of the surfactant and solvent molecules and by the mode of the interactions among molecules.

Several equations of state have been deduced from analyses of surfactant configurational structure and surfactant-solvent interaction. For example, Cantor and Dill (1986) developed a statistical mechanical theory for the interactions among the surfactant molecules in monolayers at the oil-water interface and deduced a state equation applicable over a wide range of surface densities, temperatures, and chain lengths. It was assumed in their study that the interfacial free energy per molecule is the sum of four contributions, which are all molecule surface area dependent and have physical origins in the two-dimensional translational freedom of the molecules on the surface plane, the headgroup interactions, the hydrocarbon chain configurations, and the volume dependence of chain packing. Their approach predicted pressure-area isotherms and other thermodynamic properties of monolayers at the oil-water interface. Their treatment for the surfactant position partition did not explicitly consider the solvent molecules as a component, and the empirical formulation for the headgroup interactions was extrapolated from experimental data for monolayers in low density (surface area per molecule $a > 200$ Å^2) at the oil-water interface. Subsequently, a general statistical mechanical theory was developed to describe structural and thermodynamic properties of surfactant monolayers at the interface between water and a hydrophobic solvent (Cantor and McIlroy, 1989a,b). In this theory, the total energy was assumed to include three terms: one is due to the translational entropy of the ideal two-dimensional mixing of the

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Received for publication 14 June 1994 and in final form 9 May 1995.

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Abbreviations used: PE, phosphatidylethanolamine; PC, phosphatidylcholine; DLPE, dilaureoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PLPC, 1-palmitoyl-2-linoleoylphosphatidylcholine; PlnPC, 1-palmitoyl-2-α-linolenoylphosphatidylcholine; SOPC, 1-stearoyl-2-oleoylphosphocholine; SLPC, 1-stearoyl-2-linoleoylphosphocholine; SLnPC, 1-stearoyl-2-α-linolenoylphosphocholine.

0006-3495/95/08/460/10 $2.00

solvent with the chains and of chains with each other; the second is the chain conformation energy; and the third represents the interaction energies among chain, solvent, and water molecules. The treatment of Popielawski and Rice (1988), for water-surfactant mixing at the air-water interface, was applied to the two-dimensional mixing of the solvent and the surfactant molecules at the oil-water interface. The water molecule, however, was still not considered as an active element of the oil-water interface. The surfactant chains were characterized by the position and orientation of their surface area available for nearest neighbor contacts. The interaction energies among chain, solvent, and water were determined by two parameters, one related to the solvent-water interfacial tension and the other involving the difference between the chain-water and the solvent-water interfacial tensions. The pressure-area isotherms were predicted by their theory, which was implemented by using a cubic lattice approximation. This mean-field theory was then extended to examine monolayers of mixed chain length (Cantor, 1989). A rigorous theoretical approach to include intermolecular interactions for monolayers of low to intermediate surfactant densities is possible through the method of the virial expansion. The second virial coefficient and its temperature dependence could be predicted by a simple model in which the headgroup is considered to be a rigid dipole, which leads to strong lateral repulsion among PC molecules increasing with temperature and to much less repulsion and negligible temperature dependence for PE molecules (Dill and Stigter, 1988). The theory was generalized to treat the broader range of area densities of surfactants by including both the second and the third virial coefficients. In addition to the steric repulsion and the dipole interactions, hydrogen bonding between PE and hydrophobic interaction between PC headgroups in water were also introduced. The theory was consistent with the data for both the second and the third virial coefficients, demonstrating the applicability of the headgroup model to semiconcentrated monolayers (Mingins et al., 1992; Stigter et al., 1992). All of these studies were for monolayers at the oil-water interface, and the extent to which the surface pressure as a function of the chain length is affected by the oil-water interfacial interactions remains unknown. Effects of chain unsaturation were not included in these lattice-based theories.

Several workers developed statistical mechanical models to predict membrane properties from the lipid chain structure (Ben-Shaul and Gelbart, 1985, 1994; Fattal and Ben-Shaul, 1994; Nagel, 1973, 1975; Schindler 1979, 1980; Szleifer et al., 1988, 1990). For example, MacDonald and Simon (1987) proposed an equation of state for lipid monolayers at pressures >10 dyn/cm. They assumed that the thermodynamic behavior of a monolayer is governed only by the surface molecular area dependence of the entropy of the lipid tails. This elementary analysis was, however, approximate as it neglected hydration and all nonentropic sources of intra- and intermolecular interactions. Nevertheless, a relatively simple molecular theory can account quite well for many of the important conformational characteristics of lipid chains in monolayers (see, for example, Ben-Shaul, 1994; Ben-Shaul and Gelbart, 1994). In these chain packing and conformational statistics for monolayers and bilayers, however, the electrostatic interactions prevailing in the interfacial (aqueous headgroup) region were not dealt with in detail, particularly in comparison with the treatment for the hydrophobic chain region. In addition, the effects of double bonds were not included in any of these treatments. There has been no equation of state development in which the effects of both the headgroup structure and the chain unsaturation are included.

In their study of chain melting phase transitions, Jacob et al. (1975) and Berde et al. (1980) developed a statistical mechanical description for the partition function of aqueous dispersions of bilayers and monolayers, in which the effects of chain conformation are explicitly recognized and the consequences of the chain unsaturation could be assessed. The formulation of the chain conformation partition function is different for different chain structures. It is this variation in partition function formulation for various chain structures that allows us to analyze the effects of the number and the location of *cis* double bonds on the equation of state.

Here we present a state equation, formulated from a theoretical model for molecular structure and intra- and intermolecular interactions, of lipid monolayers in the liquid-expanded state at the air-water interface. Our goal is to understand the effects of the number and position of *cis* double bonds in the hydrocarbon chains on the equation of state. The total Hamiltonian of the monolayer is assumed to consist of three primary parts: the two-dimensional mixing entropy of water and lipid molecules on the monolayer surface, the chain conformational energy, and a residual part containing all other intra- and intermolecular interaction energies. The description of the energy due to the chain conformations given by Jacob et al. (1975) and Berde et al. (1980) is utilized to formulate the effects of the number and location of *cis* double bonds. Comparison with available experimental data of our computation for monolayers of various lipid molecules having different extents of unsaturation shows that the residual component of the total surface pressure is approximately area independent. The effects of *cis* double bonds on the monolayer appear in the present theory in two aspects; a bend due to a *cis* double bond increases the minimal molecular area, and the presence of a double bond in a chain decreases the degrees of freedom of the chain. Our computation shows that the former effect is very significant whereas the latter is negligible. The equation of state is well described with appropriate values for three parameters, which represent (i) the effect of the double bond bend on the minimal molecular area, (ii) the degree of overlap of the projected area increment due to a gauche bend, and (iii) the combined effect of all intra- and intermolecular interactions other than the surface mixing entropy and the chain conformational energy. The described equations of state are shown to reproduce the π-a isotherms of DLPE (Möhwald, 1990), DPPC

(Albrecht et al., 1978), POPC, PLPC, and PLnPC (Evans et al., 1987).

THEORETICAL ANALYSIS

Following the treatment by Jacobs et al. (1975) and Berde et al. (1980) of the thermotropic phase transition of phospholipid bilayer dispersions, we construct a statistical mechanical description of monolayers composed of PC or PE molecules at the air-water interface. Our goal is to reproduce the principal features of the π-a diagram and to elucidate the effects of chain unsaturation. The system considered has N lipid molecules, each of which occupies a surface area a and has two chains, either or both of which may be saturated, *trans*-unsaturated, or *cis*-unsaturated. The degrees of freedom of the molecules in the system are divided into two groups: those of the molecules as a whole, denoted by X_i ($i = 1, 2, 3, \ldots, N$) for the ith molecule, and those of the hydrocarbon chains, denoted by Y_j ($j = 1, 2, 3, \ldots, 2N$) for the jth hydrocarbon chain. We decompose the total Hamiltonian of the monolayer system into three parts: the two-dimensional entropy of mixing of the lipid molecules (chain conformations at the maximal density) with the water molecules at the interface, the conformational energy due to the changes in chain conformation away from the maximal density state, and a residual term that lumps together all other intra- and intermolecular interaction energies. The residual term in the total Hamiltonian includes the energy of interactions between surfactant molecules and between the surfactant and the water molecules, as well as energies due to headgroup configurational and molecular rotational entropies:

$$H(X^N, Y^{2N}) = Nh_m(a) + \sum_{j=1}^{2N} h_c(Y_j, a) + Nh_3(a)\,, \quad (1)$$

where h_m, h_c, and h_3 are the Hamiltonians due to the two-dimensional mixing entropy for a single lipid molecule, the conformational energy for a single chain, and the residual term of all other interaction energies for a single lipid molecule, respectively. We specifically excluded an energy term for van der Waals forces. This choice is based on our assumption that a monolayer expands by thinning, not as a consequence of molecular separation. The three-dimensional density is assumed to remain constant; the molecules are expected to maximize their contact with each other by increased average tilt and not separate except in the gaseous phase. Chain-chain interactions are reduced upon monolayer expansion by thinning, but because alkane-water adhesion is slightly stronger (~10%) than alkane-alkane cohesion, inclusion of van der Waals forces would, if anything, increase rather than reduce the calculated surface pressure. Furthermore, if the van der Waals forces do contribute a small component to the surface energy, this would be subsumed in the residual term. van der Waals forces do change through phase transitions, but the latter are not considered here.

Accordingly, the partition function $Q = Q(N, A, T)$ has the following form:

$$Q(N, A, T) = Q_m(N, A, T) \times [q_c(a, T)]^{2N} \times \exp[-Nh_3(a)/kT]\,, \quad (2)$$

where Q_m and q_c are the partition functions due to the two-dimensional mixing for the entire system and to the conformational energy for a single chain, respectively. $A = Na$ is the total surface area of the system and T is the absolute temperature. The Helmholtz free energy, $F = F(N, A, T)$, and the surface pressure, $\pi = \pi(N, A, T)$, are found from Eq. 2 as a sum of three terms corresponding to the three components of the total Hamiltonian, i.e.,

$$F(N, A, T) = -kT \ln Q = -kT \ln Q_m - 2NkT \times \ln q_c - Nh_3(a)\,, \quad (3)$$

$$\pi(N, A, T) = -\left(\frac{\partial F}{\partial A}\right)_{N,T} = \frac{kT}{Q_m}\frac{\partial Q_m}{\partial A} + \frac{2kT}{q_c}\frac{\partial q_c}{\partial a} + \frac{\partial h_3}{\partial a}\,. \quad (4)$$

Two-dimensional mixing entropy

The entropy due to the mixing of lipid and water molecules at the interface of area A can be found from the distribution function of the lipid molecules on the monolayer surface. Previous treatments of this problem either did not explicitly consider water molecules as a component of the surface phase or did not consider the effect of the size difference between the lipid and the water molecules (Cantor and Dill, 1986; Defay and Prigogine, 1966). The partition function for the two-dimensional mixing of lipid and water molecules, which includes the effects of mixing of both the lipid and the water molecules as well as the size difference between the lipid and the water molecules, can be formulated as follows. We suppose there are N lipid molecules in the monolayer system of a total surface area of A. The area per molecule is thus $a = A/N$. The molecular area at maximal density is denoted by a_m, so that when multiplied by the total molecule number N, $Na_m = A_m$, is the minimal surface area into which the N lipid molecules of the monolayer system can be compressed. a_m is also the molecular surface area for monolayers at collapse. The relative surface area change from the maximal density to the given molecular area a is

$$\alpha = \frac{a - a_m}{a_m}\,. \quad (5)$$

The surface area occupied by a single water molecule, a_w, is taken as $a_w = 9.65$ Å^2 (Fowkes, 1962). The total number of water molecules at the interface is thus $N_w = N(a - a_m)/a_w$. The problem is to find the distribution function for the mixture of N surfactant molecules and N_w water molecules on the interface of the surface area Na. Let us consider how one surfactant molecule and $(a - a_m)/a_w$ water molecules partition themselves on the surface area a. In one of the two perpendicular coordinate directions, there are $\sqrt{a/a_w}$ possible positions for water molecule, among which $\sqrt{a_m/a_w}$ positions are occupied by the lipid molecule. There are thus $(1 + \sqrt{a/a_w}) - \sqrt{a_m/a_w}$ positions available for the surfactant molecule. Accordingly, there are $[(1 + \sqrt{a/a_w}) - \sqrt{a_m/a_w}]^2$ positions available for this surfactant molecule in the area a. Then the positional distribution function for the monolayer system is

$$Q_m = \left[\left(1 + \sqrt{\frac{a}{a_w}}\right) - \sqrt{\frac{a_m}{a_w}}\right]^{2N}. \qquad (6)$$

The component of the surface pressure, nondimensionalized by the factor kT/a_m, which is due to the distribution of both the lipid and the water molecules on the surface, is given for all lipid molecule densities, i.e., for all values of α from 0 to ∞, as

$$\frac{\pi_m(\alpha)a_m}{kT} = 1\bigg/\left[\sqrt{1+\alpha}\left(\sqrt{1+\alpha} - 1 + \sqrt{\frac{a_w}{a_m}}\right)\right]. \qquad (7)$$

This pressure corresponds to a two-dimensional osmotic pressure; partitioning of water molecules into the monolayer represents an increase in entropy, hence the monolayer tends to expand (Davies and Rideal, 1980; Prigogine and Defay, 1962).

The minimal molecular surface area, a_m, is a parameter in Eqs. 6 and 7. We shall see below that the value of a_m can be found from experimental measurement and has a different value for monolayers of lipids with different headgroup size, chain number and length, and chain unsaturation. The mixing entropy in the present model is affected by the number and position of double bonds via the parameter a_m.

Fig. 1 plots the partial surface pressure due only to the mixing of the lipid and the water molecules at the interface versus the lipid molecular area for monolayers of various lipid molecules. The molecular areas at collapse are taken from experimental measurements. Curve 1 is for the DLPE monolayer at T = 44.2°C, for which $a_m = 46$ Å^2 (Möhwald, 1990). Curve 2 is for the DPPC monolayer at T = 42°C, for which $a_m = 52$ Å^2 (Albrecht et al., 1978). Curves 3, 4, and 5 are π_m-a plots for the monolayers of POPC, PLPC, and PLnPC lipid molecules, respectively, at T = 22°C for which $a_m = 56$, 60.8, and 70 Å^2 (Evans et al., 1987), respectively. From this figure, it is clear that the minimal molecular area, a_m, to which a lipid molecule can be compressed is an important factor in determining the magnitude of the partial surface pressure due to the two-dimensional mixing. Another factor is the relative size of lipid and water molecules; chain bends at double bonds increase the projected area of the lipid molecules on the interface and, therefore, increase this partial surface pressure.

It is interesting to note that, for monolayers at collapse ($\alpha \to 0$), the surface pressure component due to the two-dimensional mixture of the surfactant and the water molecules at the interface has a limiting value of $\pi_m(0) = kT/\sqrt{a_m \cdot a_w} \doteq 18.8$ (dyn/cm) at typical values of T = 27°C and $a_m = 50$Å^2. We will see that this pressure is of the same order of magnitude as that of the residual surface pressure, which includes all intra- and intermolecular interactions other than the interface mixing entropy and the chain conformational energy, and is found to be almost independent of the lipid molecular area for liquid-expanded monolayers. This means that, at the maximal density, the surface pressure component due to the two-dimensional mixing of the lipid and water molecules is almost balanced by the residual component due to all other intra- and inter molecular interactions. We anticipate, therefore, that the chain conformation partition will become the dominant factor in the thermodynamic behavior of monolayers at the vicinity of the maximal density.

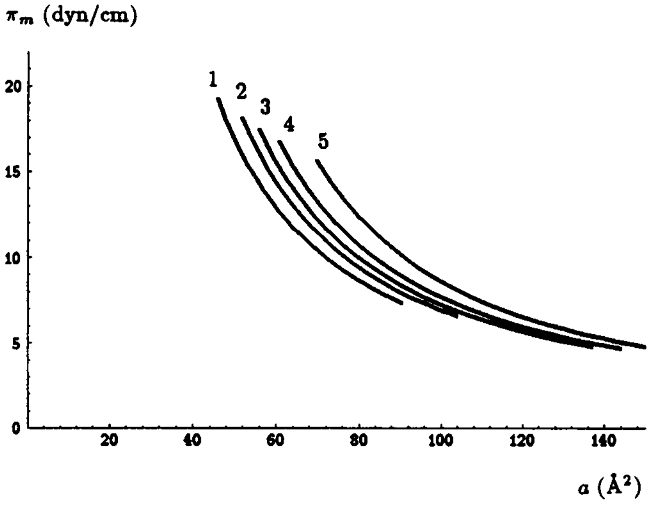

FIGURE 1 The partial surface pressure due to the two-dimensional entropy of mixing of lipid and water molecules at the interface plotted versus the lipid molecular area for monolayers of various lipid molecules with the molecular area at collapse taken from experimental measurement. *Curve 1*: DLPE ($c_{12:0}/c_{12:0}$ PE) at T = 44.2°C, $a_m = 46$ Å^2 (Möhwald, 1990). *Curve 2*: DPPC ($C_{16:0}/C_{16:0}$ PC) at T = 42°C, $a_m = 52$ Å^2 (Albrecht et al., 1978). *Curves 3, 4,* and *5*: POPC ($C_{16:0}/C_{18:1,n-9}$ PC), PLPC ($C_{16:0}/C_{18:2,n-6}$ PC), and PLnPC ($C_{16:0}/C_{18:3,n-3}$ PC) at T = 22°C, a_m = 56, 61.8, and 70 Å^2, respectively (Evans et al., 1987).

Chain conformational energy

Jacobs et al. (1975) and Berde et al. (1980) considered two contributions to the chain conformational energy of molecules with no double bonds. In addition to the expected steric energy of $\epsilon = 500$ cal/mol for each gauche bond, there is an energy due to the work of expansion of a given chain against the collective force of other chains during conformation changes that involve area changes. If it is assumed that the effective area increase for such a conformation change is $\Delta a_e^b(\alpha)$, then the corresponding Hamiltonian per chain is

$$h_c(\alpha) = \sum_{j=1}^{m} |\alpha_j| \epsilon + \pi(\alpha)\Delta a_e^b(\alpha), \qquad (8)$$

where $\pi(\alpha)$ is the total lateral surface pressure opposing the relative area increase α. The pressure-area relationship $\pi = \pi(\alpha)$ is our goal. For its computation, we initially approximate the lateral surface pressure as that due to the two-dimensional mixing entropy of the lipid and water molecules at the interface, $\pi_m(\alpha)$. Iteration is then used to find the chain conformational surface pressure to higher precision. In Eq. 8 the α_j ($j = 1, 2, \ldots, m$) specify the conformations of the single bonds, $\alpha_j = +1, -1, 0$ for gauche+, gauche−, and *trans* conformations, respectively, and $m = n - 2$ is the bond number for which bond rotation generates different conformations.

The effective area increase, Δa_c^b, differs from the projected area increase because of chain overlap. However, it can be assumed that there is a proportional relationship between these two area increments (Berdeetal., 1980). If γ^b is the proportionality factor,

$$\Delta a_c^b(\alpha) = \gamma^b \Delta a^b(\alpha), \quad (0 < \gamma^b < 1). \tag{9}$$

This permits the computation that, for a gauche bend at the *i*th bond on a chain, $\Delta a^b = (m - i + 1)wL \sin 60° = 0.14(m - i + 1)a_0$, where $w = 4.85$ Å is the diameter of the projection of an all-*trans* chain onto the plane of the surface (the diameter of the cross-sectional area of the chain), $L = 1.25$ Å is the length per methylene group along the chain axis, and $a_0 = 2 \times \pi w^2/4$ is twice the area per chain in the all-*trans* state. Therefore, the partition function for a single saturated chain attributable to conformations at the maximal density is

$$q_c = 1 + \sum_{i=1}^{m} \left[1 + 2\exp\left(-\frac{\epsilon}{kT}\right)\right]^{m-i}$$
$$\times 2\exp\left\{-\left[\frac{\epsilon}{kT} + \frac{\pi(\alpha)a_m}{kT}\left(\frac{a_0}{a_m}\right)\gamma^b \times 0.14(m - i + 1)\right]\right\}. \tag{10}$$

For chains with different degrees of unsaturation, the chain conformation partition function q_c should differ from Eq. 10 according to the number and position of *trans* or *cis* double bonds. It is this variation in the formulation of the chain conformational partition function that allows us to quantify the effects of number and location of double bonds in the molecule. For an unsaturated chain with a *cis* double bond at the *k*th bond position, only one conformation is allowed at this position and, at the adjacent $(k \pm 1)$th bond, only the states with torsional bond angles of 0° and ±60° are allowed. The energies associated with the conformations at these positions are determined by the torsional energy about a single bond and the steric interaction of pendant hydrogens at carbons $(k - 1)$ and $(k + 2)$. $\mu = 0$ kcal/mol, $\lambda = 0.8$ kcal/mol, and $\kappa = 1.6$ kcal/mol are used to denote the energies assigned to the pairs of bond angles at $k - 1$ and $k + 1$ of (±60°, ±60°); (0°, ±60°) or (±60°, 0°), and (0°, 0°), respectively (Flory, 1969). For example, for oleic acid (9-octadecenoic acid) the chain conformation partition function is

$$q_c = q_1 + q_2 + q_3, \tag{11}$$

where

$$q_1 = (e^{-\kappa/kT} + 4e^{-\mu/kT} + 4e^{-\lambda/kT})$$

$$q_2 = \sum_{i=1}^{7} 2q_1[1 + 2e^{-\epsilon/kT}]^{16-i-3}$$
$$\times 2\exp\left\{-\left[\frac{\epsilon}{kT} + \frac{\pi(\alpha)a_m}{kT}\left(\frac{a_0}{a_m}\right)\gamma^b \times 0.14(16 - i + 1)\right]\right\}$$

$$q_3 = \sum_{i=11}^{16} 2q_1[1 + 2e^{-\epsilon/kT}]^{16-i}$$
$$\times 2\exp\left\{-\left[\frac{\epsilon}{kT} + \frac{\pi(\alpha)a_m}{kT}\left(\frac{a_0}{a_m}\right)\gamma^b \times 0.14(16 - i + 1)\right]\right\}.$$

For linoleic acid (9,12-octadecenoic acid) the partition function is

$$q_c = q_1 + q_2 + q_3, \tag{12}$$

where

$$q_1 = (e^{-\kappa/kT} + 4e^{-\mu/kT} + 4e^{-\lambda/kT})^2$$

$$q_2 = \sum_{i=1}^{7} 2q_1[1 + 2e^{-\epsilon/kT}]^{16-i-6}$$
$$\times 2\exp\left\{-\left[\frac{\epsilon}{kT} + \frac{\pi(\alpha)a_m}{kT}\left(\frac{a_0}{a_m}\right)\gamma^b \times 0.14(16 - i + 1)\right]\right\}$$

$$q_3 = \sum_{i=14}^{16} 2q_1[1 + 2e^{-\epsilon/kT}]^{16-i}$$
$$\times 2\exp\left\{-\left[\frac{\epsilon}{kT} + \frac{\pi(\alpha)a_m}{kT}\left(\frac{a_0}{a_m}\right)\gamma^b \times 0.14(16 - i + 1)\right]\right\}.$$

And for linolenic acid (9,12,15-octadecenoic acid) the partition function is

$$q_c = q_1 + q_2 + q_3, \tag{13}$$

where

$$q_1 = (e^{-\kappa/kT} + 4e^{-\mu/kT} + 4e^{-\lambda/kT})^3$$

$$q_2 = \sum_{i=1}^{7} 2q_1[1 + 2e^{-\epsilon/kT}]^{16-i-9}$$
$$\times 2\exp\left\{-\left[\frac{\epsilon}{kT} + \frac{\pi(\alpha)a_m}{kT}\left(\frac{a_0}{a_m}\right)\gamma^b \times 0.14(16 - i + 1)\right]\right\}$$

$$q_3 = 0.$$

The rule in composing the conformational partition function for an unsaturated chain is (1) the power index in the expression for q_1 is the number of double bonds; (2) the sum for q_2 is taken from 1 to $k_1 - 2$, where k_1 is the carbon atom number immediately before the first double bond location and the power index in the expression for q_2 is $(m - i - 3) \times n_d$, where n_d is the number of double bonds in the chain; (3) the sum for q_3 is taken from $k_2 + 2$ to m, where k_2 is the carbon atom number immediately after the last double bond location.

Fig. 2 shows the theoretical curves for the partial surface pressure, π_c, produced from changes in the chain conformational energy versus the lipid molecular area for monolayers of five lipid molecules: DLPE at T = 44.2°, DPPC at T = 42°C, and POPC, PLPC, and PLnPC at T = 22°C. The values of the overlap coefficient γ^b, which is a parameter in the formulation of the chain partition function, are taken from Table 1, which are obtained by fitting the theory to the experimental π-a curves. From Fig. 2 we can see that a small number of factors determine the magnitude of this partial surface pressure. These are the chain number and the chain length, the bond energy, and the number and the position of the double bonds in a chain. The minimal molecular area a_m, however, is also an important factor, because work is needed to overcome the lateral surface pressure for chains to gain more available conformations and, as mentioned before, a_m is the key factor that determines the partial surface pressure due to the two-dimensional mixing. Also, compared with Fig. 1, this figure shows that the partial surface pressure due to the chain conformation energy is much larger than that due to the interfacial mixing entropy. This is especially true for monolayers near collapse. In contrast, for monolayers at low density, the chain conformation energy has little effect on the surface pressure, whereas the two-dimensional mixing entropy plays an important role in balancing the other intermolecular interactions.

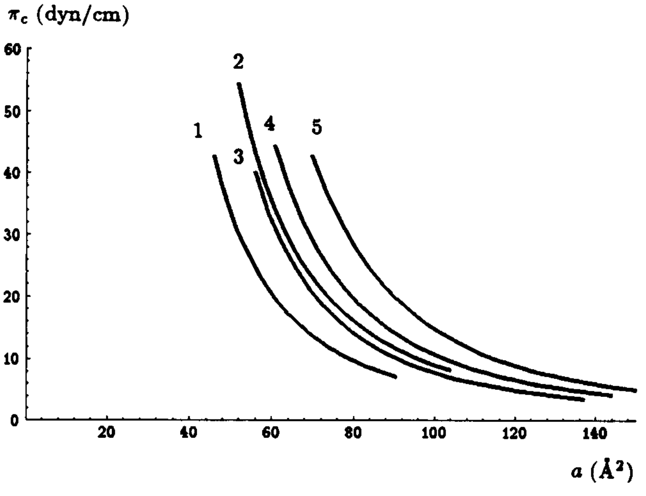

FIGURE 2 The partial surface pressure due to the chain conformational energy plotted versus the lipid molecular area for monolayers of various lipid molecules. The values of the overlap coefficient γ^b are taken from Table 1. From left to right: DLPE ($c_{12:0}/c_{12:0}$ PE) at T = 44.2°C (Möhwald, 1990); DPPC ($C_{16:0}/C_{16:0}$ PC) at T = 42°C (Albrecht et al., 1978); and POPC ($C_{16:0}/C_{18:1,n-9}$ PC), PLPC ($C_{16:0}/C_{18:2,n-6}$ PC), and PLnPC ($C_{16:0}/C_{18:3,n-3}$ PC) at T = 22°C (Evans et al., 1987).

TABLE 1 Values of the three parameters of the equation of state, γ^b, a_m/a_0, and π_3*

Lipid	γ^b (fitted)	a_m/a_0 Coolbear et al., 1984	a_m/a_0 Measured	π_3 (fitted, dyn/cm)
DLPE	0.685	1.0	1.0	−15.0
DPPC	0.685	1.15	1.13	−15.0
POPC	0.545	1.26	1.22	−6.0
PLPC	0.685	1.39	1.32	−9.0
PLnPC	0.800	1.45	1.52	−9.0

* As determined by fitting the theory to the experimental measurement for DLPE at T = 44.2°C (Möhwald, 1990), DPPC at T = 42°C (Albrecht et al., 1978), and POPC, PLPC, and PLnPC at T = 22°C (Evans et al., 1987). Also listed are the values of a_m/a_0 converted from those given by Coolbear et al. (1984).

The model of Berde et al. (1980) reduces the functional dependence of the partition function on the chain conformation (which is a function of coordinates) to a function related to the molecular surface area, a, by considering the mean effects of the chain conformations on the molecular energy. The advantage of such a treatment is that the effect of chain unsaturation is explicitly included in an analytical expression and can be separately formulated for chains with different unsaturation.

Although the theory of Berde et al. (1980) provides a convenient framework with which we can examine the double bond effects on the state equation, it is incomplete. It is clear that not all bond angles are possible under the constraint of a given molecular area. The contribution of the intramolecular energy for each gauche bond to the chain Hamiltonian should be a function of the molecular area but, in fact, is ignored in the calculation of the bond energy in Eqs. 10–13. Also, the effect of chain unsaturation in the bond energy calculation is not taken to be dependent on molecular area and is therefore underestimated in this theory. Finally, each possible chain conformation should have a different probability as a function of surface pressure. The larger the projected area of a given configuration, the less probable; moreover, its probability will diminish with increasing surface pressure. The volume of a monolayer in the liquid-expanded state can be considered constant, so that conformations of smaller projected area should be available at higher pressure. This effect is also omitted.

The residual part of the molecular interaction energy

The residual part in the total Hamiltonian is presumed here to include all other intra- and intermolecular interactions among lipid and water molecules besides those considered in the two sections above. Consequently, the corresponding residual part in the total surface pressure, π_3, is the difference between the measured surface pressure and the surface pressure due to the two-dimensional mixing and the chain conformational energy. It can be obtained by subtracting the theoretical computation of $\pi_m + \pi_c$ from the experimental measurement of the total surface pressure for a given lipid monolayer of specified chain length and chain unsaturation, π_e:

$$\pi_3(a) = \pi_e(a) - \{\pi_m(a) + \pi_c(a)\}. \quad (14)$$

Our computation revealed that this residual surface pressure is approximately independent of the molecular area, although its magnitude may depend on the headgroup size, chain length, and chain unsaturation. In principle, the dependence of π_3 on lipid structure can be experimentally investigated and formulated empirically. At present, the available experimental data for lipids with different chain lengths and unsaturation are insufficient to provide much insight into the mechanism of the intra- and intermolecular interactions. This confines us to specific quantitative formulations, and the value of the constant π_3 is thus available for individual cases only.

Once determined, this residual surface pressure π_3 is then put back into the theory. The equation of state for a monolayer of lipid molecules with a specified headgroup, chain lengths, and chain unsaturation is then formulated and can be used to reproduce an experimental measurement. For example, a constant residual component $\pi_3 = -15$ dyn/cm and $\gamma^b = 0.685$ with $a_m/a_0 = 1$ provides a good fit of the theoretical prediction of equation of state to the experimental π-a relationship for the DLPE monolayers at the air-water interface. Similarly, the values of the parameters γ^b, a_m/a_0 and π_3 in Table 1 generate equations of state in the form of Eq. 4, which correspond very well to the experimental data for monolayers of the DPPC, POPC, PLPC, and PLnPC, i.e., a range of double bonds from zero to three per molecule.

COMPUTATIONAL RESULTS

Determination of parameters

There are three parameters in the present description of an equation of state for lipid monolayers at the air-water interface, a_m/a_0, γ^b and π_3, which are to be determined by fitting the theory to the experimental π-a curve for lipid molecules with specific headgroups, chain lengths, and chain unsaturation. The physical significance of each of these three parameters is as follows. The first parameter a_m/a_0 is the ratio of the minimal molecular surface area to the cross-sectional area of the two chains of a single lipid molecule. It reflects the effects of the *cis* double bonds on the molecular surface area of the monolayer at its maximal density. For PE monolayers we assume $a_m/a_0 = 1$ as there is no inclination between the chain and the headgroup. For PC monolayers, however, the headgroup is large enough compared with the chain cross-sectional area to cause a chain tilt. It is suggested that there is a 60° inclination with respect to the monolayer plane for saturated PC molecules (Tardieu et al., 1973; Janiak et al., 1976). We therefore assumed that $a_m/a_0 = 1/\cos 60° = 1.1547$. The value of a_m for monolayers of lipids with unsaturated chains can be directly measured from the experimental π-a curve as the molecular area for the monolayer at collapse. The experimental values of a_m for DLPE, DPPC, POPC, PLPC, and PLnPC are thus measured to be 46, 52, 56, 60.8, and 70 Å^2 and, consequently, the corresponding values of a_m/a_0 are equal to 1.0, 1.13, 1.22, 1.32, and 1.52, respectively, as listed in the fourth column in Table 1. Also listed in the third column in Table 1 are the values of a_m/a_0 converted from experimental measurements given by Coolbear et al. (1983). The data in these two columns are in rather good agreement.

The second parameter, γ^b, is the ratio of the effective to the actual projected surface area increment due to a gauche bond bend. γ^b reflects the degree of overlap of the projected areas of adjacent chains, which is increased by gauche conformations. It is not clear what factors affect this overlap coefficient, but it is evident that chain length and the number and position of the double bonds are involved. The value of $\gamma^b = 0.685$ for a DLPE monolayer at the air-water interface is obtained by fitting the theory to the experimental measurement. It should represent the magnitude of γ^b values in general. For a DPPC monolayer at the air-water interface, the same values of $\gamma^b = 0.685$ and $\pi_3 = -15$ dyn/cm provide a good fit of the theory to the experimental measurement when the measured value of $a_m/a_0 = 1.13$ is used. The fitted values of γ^b of 0.545, 0.685, and 0.80 reflect the double bond effect on the degree of the projection area overlap (See Table 1).

The third parameter π_3 is the residual part in the total surface pressure, that component due to all intra- and intermolecular interactions besides the two-dimensional mixing entropy and the chain conformational energy. Our computations show that it can be treated as constant for liquid-expanded lipid monolayers; $\pi_3 = -15, -15, -6, -9$, and -9 dyn/cm, as listed in the fifth column in Table 1, are the fitted values for monolayers of DLPE, DPPC, POPC, PLPC, and PLnPC molecules, respectively, at the air-water interface, which reflect the double bond effects on the residual intra- and intermolecular interactions.

The first two rows of Table 1 list the fitted values of γ_b, a_m/a_0, and π_3 for monolayers of DLPE at T = 44.2°C (Möhwald, 1990), and DPPC at T = 42°C (Albrecht et al., 1978). The first two curves from the left in Fig. 3 show that the equation of state with these three fitted parameters reproduces the experimental π-a relationship.

Effects of chain unsaturation

To study double bond effects on the π-a relationship, we calculated the equations of state for monolayers of POPC, PLPC, and PLnPC at T = 22°C (Evans et al., 1987). The values used for a_m/a_0, γ^b and π_3 are listed in the third to fifth rows in Table 1.

The largest effect among those considered is that of double bonds on the collapse area, i.e., on the value of a_m or, equivalently, on the value of a_m/a_0. These values can be directly measured from the experimental π-a curves. From

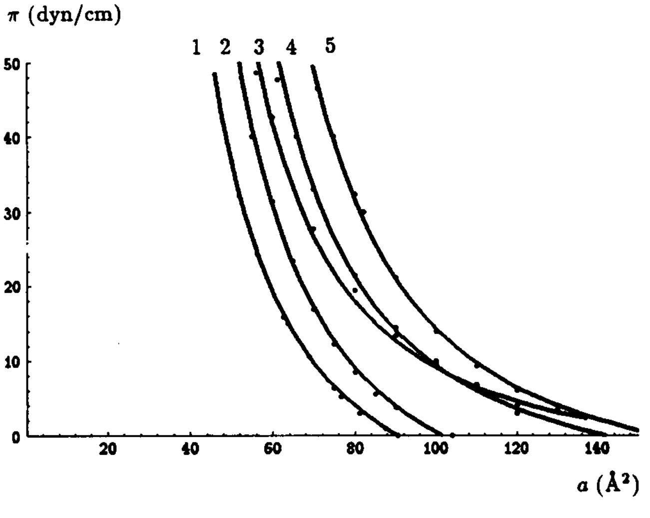

FIGURE 3 The experimental data of surface pressure versus surface area for monolayers of various lipid molecules is fitted by the theoretical prediction of the equation of state with the values of the parameters listed in Table 1. Symbols represent the experimental values and the five solid curves represent the corresponding theoretical predictions of the equation of state with the parameter values listed in Table 1. From left to right: DLPE ($c_{12:0}/c_{12:0}$ PE) at T = 44.2°C (Möhwald, 1990); DPPC ($C_{16:0}/C_{16:0}$ PC) at T = 42°C (Albrecht et al., 1978); and POPC ($C_{16:0}/C_{18:1,n-9}$ PC), PLPC ($C_{16:0}/C_{18:2,n-6}$ PC), and PLnPC ($C_{16:0}/C_{18:3,n-3}$ PC) at T = 22°C (Evans et al., 1987).

the data in the fourth column of Table 1 we can see that (1) the chain inclination of PC molecules causes a 13% increment in the minimal molecular area a_m, and (2) the first, second, and third double bond introduced at the 9th, 12th, and 15th atom position cause a 22, 32, and 52% increment, respectively, in the minimal molecular area a_m. The effect of increasing a_m is a shift in the π-a curve toward larger areas and is depicted in Fig. 1.

Coolbear et al. (1983) suggested that, related to $a_m/a_0 = 1$ for saturated PC monolayers, $a_m/a_0 = 1.31$, 1.55, and 1.64 for SOPC, SLPC, and SLnPC monolayers, respectively. These data can be converted to those listed in the third column of Table 1 (a_m/a_0), which are related to $a_m/a_0 = 1$ for saturated PE monolayers and adjusted for monolayer molecules each with two chains of different unsaturation. The data of Coolbear et al. (1983) are close to those we obtained from the experimental measurements of Möhwald (1990), Albrecht et al. (1978), and Evans et al. (1987).

Although a_m/a_0 is the parameter that is of prime importance in the effect of double bonds on the equation of state, calculation of the a_m/a_0 value from the structure of the lipid molecule and the number and the position of the double bonds for a specific unsaturated lipid molecule can be complicated. Berde et al. (1980) considered that, for monolayers of lipid molecules with unsaturated chains, the minimal molecular area is the cross-sectional area of the two chains of the molecule, a_0, plus a partial increment in the projection area caused by the chain bend due to the introduction of *cis* double bonds, $\gamma^c \Delta a^c$, i.e.,

$$a_m = a_0 + \gamma^c \times \Delta a^c , \qquad (15)$$

where Δa^c is the actual increment in the projection area of the chain caused by the *cis* double bonds and $0 < \gamma^c < 1$ is a coefficient. γ^c reflects the degree of overlap on adjacent molecules of the increased projected area of the *cis* double bond bend. This γ^c, therefore, is an alternative fitting parameter, equivalent to a_m/a_0. It is difficult, however, to calculate Δa^c of a double bond bend from the structure of a chain of given unsaturation. To compute a_m/a_0 from γ^c, more information is needed about the molecular structure of chain unsaturation. Hence, we prefer to use the parameter a_m/a_0 as obtained from experimental values. In this way we reduce the number of the fitting parameters from three (a_m/a_0, γ^b, and π_3) to two (γ^b and π_3) and, therefore, reduce the ambiguity of the problem.

The second influence of the chain unsaturation on the equation of state is the effect of the number and position of double bonds on the chain conformational energy. This effect actually comprises two different mechanisms that have opposite consequences for the π-a relationship. First, introduction of double bonds reduces the availability of chain configurations. This mechanism is addressed here in the formulation of the partition function for the unsaturated chains, which is given by Eqs. 11, 12, and 13 for POPC, PLPC, and PLnPC monolayers, respectively. We see from these equations that the introduction of each additional double bond in the chain causes the chain partition function to contain three terms fewer in the summation over the total number of chain conformations and, therefore, decreases the conformational energy of the chain. Second, introduction of double bonds increases the mechanical work needed to overcome the collective lateral forces for given changes in chain configuration. This mechanism is also included in Eqs. 11, 12, and 13, in which the surface pressure function $\pi(a)$ for monolayers with chain unsaturation has a larger value than that for saturated chains at the same value of a. The former effect is opposite to the latter and, as computations showed, is of a different order of magnitude. The decrement in the number of available chain configurations has a slight effect of decreasing the chain conformational energy. The increment in surface pressure due to chain unsaturation, however, has a significant effect of increasing the molecular area. This qualitative conclusion was also revealed in the computer simulations of the Langevin molecular dynamics of the lipid molecules with various degrees of unsaturation (Pearce and Harvey, 1993). There is also considerable experimental evidence in the literature that introduction of double bonds increases the surface area (Evans and Tinoco, 1978; Evans et al., 1987; Smaby and Brockman, 1991).

Another effect of chain unsaturation on the equation of state is on the residual intra- and intermolecular interactions. The different values of the $\pi_3 = -6.0$, -9.0, and -9.0 dyn/cm are obtained for monolayers of POPC, PLPC, and PLnPC molecules at the air-water interface by fitting the described equation of state to the corresponding experimental π-a curves measured by Evans et al. (1987). We do not currently have an adequate basis to formulate this difference in π_3 due to the different unsaturation of these three lipid molecules. It can be seen, however, that in comparison with $\pi_3 = -15.0$ dyn/cm for the saturated DLPE and DPPC, the introduction of the first *cis* double bond produces significant effects in the residual intra- and intermolecular interaction, whereas the introduction of the second and third *cis* double bond has little effect on the value of the constant residual pressure π_3. This is qualitatively in agreement with experiment.

It has been reported (van Deenen, 1965; Cornwell and Patil, 1977; Albrecht et al., 1978; Evans and Tinoco, 1978; Evans et al., 1987) that introduction of the first *cis* double bond at the middle of the chain produces a large effect on the π-a relationship, whereas introduction of the second double bond produces little effect and introduction of the third again produces another large effect. This has been hard to explain by any theory. Our computational model, however, provides a qualitative explanation. It may be that the introduction of the first *cis* double bond produces a large increment in the projection of the minimal molecular area by bending the chain at the middle and also results in a large decrement in the tension due to the residual intra- and intermolecular interactions, whereas the introduction of the second double bond brings little increment and the introduction of the third one again brings a second large increment in the projection area of the chain on the interface by further bending the chain at the 12th and 15th carbon atom position, respectively, and so on for the decrement in the residual tension. Although the appearance of the second and the third double bonds also further decreases the conformational energy due to the decrement in available chain configurations, this effect on the equation of state is minor.

DISCUSSION

The mechanism by which double bonds affect equations of state provides a basis for understanding the consequences chain unsaturation has for the mechanical and thermodynamic properties of biological membranes. The present approach decomposes the total partition function of a monolayer at the air-water interface into three parts: the two-dimensional mixing of water and lipid molecules at the interface, the chain conformational energy, and a residual part that includes all other intermolecular interactions. The three corresponding parts in the effects of double bonds can then be modeled. Double bond bends of the lipid chains increase the minimal compressible area occupied by lipid molecules at the interface and thus change the translational entropy of the water-lipid mixing. This causes an increase in the surface pressure and, therefore, an increase in the work needed to overcome the lateral pressure. Also, the double bond bend of the chains decreases the available chain conformations and the bond energy. This is, however, only a minor effect. Finally, double bonds change the residual term, i.e., change all other intermolecular interactions between chains, between water molecules, between chain and water molecules, etc. by a small amount. The degree of these three effects depends on the number and position of the double bonds in the chain. Although π-a curves have been measured for monolayers of various lipid molecules (Mingotaud et al., 1993), further elucidation of double bond effects requires additional experimental data, especially those data measured under the same experimental conditions for a series of different chain unsaturations. However, we can see from Fig. 3 that the predicted equations of state for the monolayers of lipid molecules that have one saturated chain and one unsaturated chain with one, two, and three double bonds at the 9th, 12th, and 15th carbon positions, respectively, reproduce the experimental data fairly well. These equations of state can be used to further study the thermodynamic behavior of monolayers and bilayers of these particular degrees of unsaturation, for example, to obtain quantitative descriptions for the free energy, the chemical potential, the surface activity of lipids, etc., of monolayers and bilayers as functions of the surface density of lipid molecules. It is essential, of course, that π-a curves to be analyzed theoretically are obtained under quasi-equilibrium conditions so that dynamic effects are absent. The present treatment can be extended to include the double bond effects for monolayers of charged surfactants with dipole interactions and for monolayers at the oil-water interface.

This work was supported by research grants R01-GM38244 and P01-HL45168 from the National Institutes of Health.

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