Surface dipole moments of lipids at the argon-water interface

Similarities among glycerol-ester-based lipids

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ABSTRACT Surface potential-surface pressure-area isotherms at the argon-buffer interface have been determined for 38 lipid species comprising 19 chemical classes. These lipids all exhibited a finite range of liquid-expanded surface pressure-area behavior. For most species, the linearity of surface potential with reciprocal area was excellent, but nonzero intercepts were obtained. This suggests a lipid-induced reorganization of interfacial water molecules which is area independent. The linearity of the data permits calculation of the surface dipole moment, $\mu_\perp$, for each lipid. The values of $\mu_\perp$ for a series of oleoyl-containing acylglycerols, dioleoyl phosphatidylcholine, and dioleoyl phosphatidylethanolamine exhibit acylglycerol ester group $\mu_\perp$'s which are generally consistent with known conformational properties of such lipids. The values are 132 mD for the perpendicular oleoyl glycerol-ester group and 252 mD for that in the kinked-chain conformation. Comparison of $\mu_\perp$'s calculated using these values with homologues confirms the approximate independence of $\mu_\perp$ from aliphatic chain length and permits identification of exceptions with possible conformational or orientational differences. Notably, diphytanoyl phosphatidylcholine shows a 45% larger $\mu_\perp$ than predicted. Differences in $\mu_\perp$ among lipid classes allow estimation of the electrical consequences of lipid metabolism and exchange. Calculations show that reactions such as the generation of 1,2-diacylglycerol from diacyl glycerophosphocholine or diacyl glycerophosphoinositol should produce surface potential changes of -127 and $+42$ mV, respectively. Thus, the two phospholipids are not simply alternative sources of diacylglycerol with respect to processes dependent on surface potential.

INTRODUCTION

An ubiquitous property of natural and model lipid membranes in aqueous solutions is the existence of an electrical potential profile across the interface between the bulk aqueous phase and the hydrocarbon region (for reviews see 1 and 2). This potential profile arises in part from uncompensated head group charges and dipoles, but a major portion is attributable to dipoles in the glycerol-ester region of the membrane lipids (1, 3–6). The lipid dependence of surface potential has biological consequences. In particular, any metabolic event which alters lipid composition on one side of a membrane, such as lipid hydrolysis, can regulate lipid conformation (5), gated ion transport (2), phospholipase activity (7), membrane adhesion (8) or repulsion (9), protein conformation (2), protein adsorption (10), and anesthesia (11). Although these potential-dependent processes are generally considered on a global basis, it should be recognized that local reactions on a membrane face can generate transient or metastable concentrations of particular species and, hence, potential changes at a specific site or region. For example, the hydrolyses of phosphatidylinositol (12) and phosphatidylcholine (13) by phospholipase C generates diacylglycerol which may be further metabolized. Global, steady-state concentrations of diacylglycerol up to 11% have been measured in stimulated *ras*-transformed fibroblasts (14) and in model bilayers metastable concentrations of 60–80 mol% have been observed under mild conditions (15).

Such compositional changes can be modeled using lipid monolayers at the air-water interface. This system has the advantage that lipid composition and packing density can be varied not only over a wide range but also independently of each other. Moreover, potential changes across the gas-liquid interface due to the presence of lipids can be measured readily (16). Hence, monolayer measurements can be used to predict the size and direction of potential changes which will occur as a consequence of lipid metabolism or changes in packing density. In this report we examine the surface potential (ΔV)-surface pressure (π)-molecular area (A) behavior of a number of lipids exhibiting liquid-expanded (17) π-A behavior. This monolayer state is analogous to the liquid-crystalline state of bilayer membranes (e.g., 18, 19). The results show that in the liquid-expanded state lipids exhibit regularities of behavior which allow the prediction of changes in ΔV

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which accompany lipid metabolism. The data also support the notion of a common molecular conformation and interfacial orientation of the glycerol-ester region of glycerol-based lipids at lipid-water interfaces.

MATERIALS AND METHODS

Materials

Monooleoyl-, dioleoyl-, 1,3-dicaproyl-, and triacylglycerols, oleic acid, 13,16-*cis*-*cis*-docosadienoic acid, oleyl alcohol, oleyl methanol, and oleyl nitrile were purchased from NuChek Prep, Inc. (Elysian, MN).Dilauroylglycerols were purchased from Sedary Research Laboratories, Ind. (London, Ontario, Canada). The 1,2-dicaproylglycerol was prepared by partial digestion of tricaproylglycerol with pancreatic triglyceride lipase-colipase. The triglyceride (1 g) was sonicated in 50 ml of 1 mM Tris, pH 7.5, containing 0.1 M NaCl, 1 mM $CaCl_2$, and 5 mM sodium taurocholate. Porcine pancreatic lipase (50 μg) and colipase (220 μg) were added and pH was maintained titrimetrically. After 9 min, the reaction was stopped by addition of HCl to give a pH of 3.0 and the lipids were extracted by the method of Bligh and Dyer (20). Diacylglycerols were isolated by column chromatography using Unisil and the isomers separated by preparative thin-layer chromatography using boric acid-impregnated silica gel H (21). As judged by analytical thin-layer chromatography, the final product was >99% dicaproylglycerol of which >97% was the 1,2-isomer. *N*-oleyl ethanolamine was a generous gift from H.H.O. Schmid of the Hormel Institute. Most phospholipids were purchased from Avanti Biochemical (Birmingham, AL). The synthesis and purification by thin-layer chromatography of 1-2-diacyl, 1-alkyl-2-acyl and 1-alkenyl-2-acyl glycerophosphocholines, and glycerophosphoethanolamines has been previously described (22). The same purification procedure was used to repurify diphytanoyl phosphatidylcholine. The purity of each lipid was checked by thin-layer chromatography and from measured detection limits were shown to be >99%.

Petroleum ether was purified as previously described (23), ethanol was distilled from KOH and zinc, and hexane, nonspectro grade was from American Burdick and Jackson (Muskegon, MI). Water was purified by reverse osmosis, mixed bed deionization, adsorption on activated charcoal and filtration through a 0.22-μm Durapore membrane (Millipore Corporation, Bedford, MA). Buffer was degassed and filtered through 0.4- and 0.2-μm polycarbonate filters (Nucleopore, Pleasanton, CA) or a Diaflo hollow fiber with a molecular weight cutoff of 10,000 (Amicon Corporation, Danvers, MA). After filtration, the buffer was maintained in an argon atmosphere. Phospholipids were dissolved in purified petroleum ether or hexane containing 5 vol% ethanol and other lipids were dissolved in petroleum ether or hexane alone. Phospholipid concentration was determined by assaying aliquots for phosphorus (24). Other lipid concentrations were determined by weighing the lipids before solvent addition. All other chemicals were reagent grade and used without further purification.

Surface pressure was calibrated by reference to equilibrium spreading pressures of lipids as described (25). Surface pressure and potential were measured as a function of area using a fully automated Langmuir film balance system with a ^{210}Po ionizing electrode (26, 27). Unless otherwise indicated, films were spread from 50 or 51.67 μl of solvent onto an aqueous subphase. After standing at a large molecular area for 4 min, the films were compressed, generally at $\leq$5 Å^2/min/molecule. Phase transitions were identified using second and third derivatives as described previously (26).

RESULTS

As a part of an ongoing effort to understand the behavior of lipids in surface phases, π-A-ΔV isotherms have been obtained for monolayers of many biologically relevant species under varying experimental conditions (Table 1). All isotherms considered here show liquid-expanded π-A behavior over a finite range of A. Limited analysis of some of the π-A isotherms has appeared in an earlier publication (28) but only the qualitative ΔV-A behavior of a few of the lipids has been reported (22).

Ideally, π-A-ΔV data should be amenable to analysis over the entirety of the liquid-expanded state, i.e., the end of the gaseous to liquid-expanded phase transition (typically π < 1.0 mN/m) to π_t, the surface pressure of the transition of the film to a more condensed monolayer or nonmonolayer state. Values of π_t for the lipids are given in Table 1. In practice, however, data obtained at the limits of a phase region may be particularly influenced by the dynamic nature of the experiment and trace impurities (e.g., 29). We have noted that experimental π-A isotherms generally exhibit positive values of $d^2\pi/dA^2$ through most of the liquid-expanded state but this parameter typically becomes negative at a π value 10–20% below π_t (26). The π value at which $d^2\pi/dA^2$ goes from positive to negative, designated π_d in Table 1, was used to set an upper limit for obtaining the most reliable π, A and ΔV data for subsequent analysis. The lower limit for data analysis was selected as the values of ΔV and A at π = 1.0 mN/m (28). This π is sufficiently high to eliminate any possible contribution of the gaseous state but low enough to insure a range of data which encompasses most of the liquid-expanded state.

The classical, quantitative model for the analysis of ΔV-A data has been the treatment of the surface as a parallel plate capacitor. This yields the familiar Helmholz equation,

$$\Delta V = 37.70\,\mu_\perp/A, \quad (1)$$

where ΔV is the measured change in surface potential in millivolts relative to the absence of lipid, A is the total surface area divided by the number of lipid molecules present, and $\mu_\perp$ in millidebye units is the component of the molecular dipole moment perpendicular to the lipid-water interface (16). Examples of ΔV vs. $1/A$ plots over the range $1.0 \leq \pi \leq \pi_d$ are shown in Fig. 1. Although they are reasonably linear (Table 1), they do not show the proportionality predicted by Eq. 1, i.e., they intercept the ordinate at nonzero potentials, ΔV_0. Thus, the data better obey a relationship of the form,

$$\Delta V = \Delta V_0 + 37.70\,\mu_\perp/A, \quad (2)$$

TABLE 1 Properties of lipids exhibiting liquid-expanded surface pressure-area behavior

Lipid	Subphase*, Temp °C	$\mu_\perp$	ΔV_o	Correlation coefficient	π_d	π_t	A_t	ω_o	f_1	q
		mD	*mV*		*mN/m*	*mN/m*	$Å^2$	$Å^2$		
Trioleoylglycerol	A, 24	701	113	0.9997	10.6	11.9	101.3	68.2	1.16	2.56
Tricaproylglycerol	E, 24	661	122	0.9996	14.2	16.4	78.2	53.4	1.16	2.10
1,2-diphytanoyl GPC‡	F, 24	702	49	0.9999	36.8	45.8	73.2	58.8	1.16	2.94
1,2-diphytanoyl GPC	A, 24	653	59	0.9998	39.6	46.4	70.9	56.1	1.16	3.08
1,2-diphytanoyl GPC (repurified)	A, 24	649	64	0.9998	39.9	46.5	71.8	55.9	1.15	3.30
1,2-dioleoyl GPC	F, 24	486	97	0.9997	38.4	44.9	59.0	45.0	1.16	2.84
1,2-dioleoyl GPC	A, 24	463	122	0.9999	37.2	45.3	57.9	42.7	1.15	3.04
1-oleoyl-2-stearoyl GPC	A, 24	473	126	0.9994	40.8	46.9	54.9	40.2	1.15	2.99
1-stearoyl-2-oleoyl GPC	A, 15	475	116	0.9998	42.3	49.5	52.6	39.4	1.16	2.97
1-stearoyl-2-oleoyl GPC	A, 24	489	127	0.9998	41.2	47.6	56.7	43.0	1.15	2.84
1-palmitoyl-2-oleoyl GPC	A, 15	444	127	0.9997	40.5	48.8	52.3	39.8	1.17	2.81
1-palmitoyl-2-oleoyl GPC	F, 24	485	94	0.9998	38.6	46.3	54.7	42.8	1.17	2.50
1-palmitoyl-2-oleoyl GPC	A, 24	468	126	0.9998	40.0	47.6	55.1	42.3	1.16	2.72
1-palmitoyl-2-oleoyl GPC	B, 24	505	121	0.9997	42.7	51.1	55.2	41.5	1.14	2.99
1-palmitoyl-2-oleoyl GPC	A, 30	465	118	0.9997	40.0	46.2	55.9	41.4	1.16	2.93
1-palmitoyl-2-oleoyl GPC	A, 37	468	112	0.9995	39.9	46.4	56.4	40.7	1.16	3.13
1,2 diacyl GPC	A, 24	490	118	0.9996	39.4	47.6	57.6	42.6	1.15	3.14
1,2-dipalmitoyl GPC	A, 24	469	119	0.9997	8.6	9.7	69.3	32.5	1.16	2.62
1,2-dimyristoyl GPC	F, 24	501	93	0.9999	36.5	47.8	51.2	40.0	1.17	2.42
1,2-dimyristoyl GPC	A, 24	471	118	0.9996	34.2	47.0	51.6	40.1	1.16	2.42
1,2-dilauroyl GPC	A, 24	409	111	0.9990	39.6	47.9	44.4	36.3	1.19	1.83
1,2-dicaproyl GPC	A, 24	505	94	0.9975	35.8	45.7	53.3	45.5	1.14	1.61
rac-1-0-alkyl-2-acyl GPC	A, 24	294	143	0.9994	37.5	47.5	51.8	39.3	1.17	2.67
1-0-alkenyl-2-acyl GPC	A, 24	234	124	0.9982	33.2	47.1	52.1	42.1	1.20	2.24
1,2-dioleoyl GPE	A, 24	484	95	0.9998	39.2	46.3	55.1	43.0	1.18	2.59
1-palmitoyl-2-oleoyl GPE	A, 24	467	93	0.9999	35.4	40.5	50.2	40.7	1.21	1.87
1,2-diacyl GPE	A, 24	464	89	0.9999	28.6	33.8	51.0	39.8	1.21	1.85
1-0-alkyl-2-acyl GPE	A, 24	342	112	0.9996	27.8	33.4	51.5	40.3	1.21	1.80
1-0-alkenyl-2-acyl GPE	A, 24	172	118	0.9970	37.3	45.8	48.6	42.2	1.26	1.58
1,2-dioleoyl GPS	C, 24	440	6	0.9999	39.8	45.6	57.4	42.5	1.15	2.98
1-palmitoyl-2-oleoyl GPS	C, 24	459	2	0.9999	38.7	47.4	53.7	41.5	1.15	2.52
Bovine liver diacyl PI	C, 24	312	27	0.9999	38.0	46.0	51.8	36.9	1.14	2.95
1,2-dioleoyl PG	D, 24	324	56	0.9999	41.7	45.8	54.7	38.2	1.13	3.18
1-palmitoyl-2-oleoyl PG	C, 24	335	44	0.9995	40.1	46.3	52.7	37.3	1.13	3.01
1-palmitoyl-2-oleoyl PA	A, 24	476	−9	0.9999	31.5	35.3	54.4	43.3	1.18	1.80
1,2-dioleoylglycerol	A, 24	439	98	0.9999	27.3	30.6	58.4	44.7	1.20	2.07
1,2-dilauroylglycerol	E, 24	413	122	0.9997	20.0	24.1	51.4	36.8	1.21	1.80
1,2-dicaproylglycerol	E, 24	401	112	0.9987	33.2	38.5	44.4	36.0	1.22	1.60
1,3-dioleoylglycerol	A, 15	311	113	0.9994	26.3	29.4	60.0	41.8	1.18	2.74
1,3-dioleoylglycerol	A, 24	319	116	0.9997	24.9	27.9	63.6	44.4	1.18	2.68
1,3-dioleoylglycerol (neat)	A, 24	305	119	0.9997	24.4	27.6	63.1	43.8	1.18	2.68
1,3-dioleoylglycerol	A, 30	326	102	0.9997	24.1	26.4	65.0	44.8	1.17	2.67
1,3-dioleoylglycerol	A, 37	342	99	0.9997	22.0	25.6	67.6	45.6	1.17	2.76
1,3-dilauroylglycerol	E, 24	286	129	0.9988	22.8	26.8	52.6	35.7	1.19	2.25
1,3-dicaproylglycerol	E, 24	307	130	0.9939	29.2	32.9	53.6	39.4	1.17	2.16
1(3)-monooleoylglycerol	A, 24	165	103	0.9977	37.8	44.4	33.8	25.0	1.20	1.87
13,16-docosadienoic acid	A, 24	94	−49	0.9793	32.4	38.3	28.2	26.2	1.34	0.61
13,16-docosadienoic acid	B, 24	53	−2	0.8770	37.8	46.7	27.9	26.7	1.26	0.56
Oleic acid	A, 24	136	−75	0.9986	34.1	40.4	27.2	21.9	1.30	1.23
Oleic acid	B, 24	112	−36	0.9872	40.9	48.3	27.3	23.6	1.27	1.08
Oleyl alcohol	A, 24	144	35	0.9992	28.5	32.1	28.5	24.3	1.38	0.87
Oleyl methanol	B, 24	192	144	0.9972	14.0	15.8	34.9	25.1	1.24	0.79
Oleyl nitrile	A, 24	145	115	0.9994	13.6	15.8	28.1	20.8	1.41	0.74
N-oleylethanolamine	A, 24	194	43	0.9992	37.6	44.2	31.0	22.4	1.23	1.90

*Subphases used are: *A*, 0.01 M phosphate, 0.1 M NaCl, pH 6.6; *B*, 0.01 M phosphate, 2 M NaCl, pH 6.6; *C*, 0.01 M phosphate, 0.1 M NaCl, 0.1 mM EGTA, pH 6.6; *D*, 0.15 mM KCl, 0.1 mM EGTA, pH 7.0; *E*, 1 mM phosphate, 0.1 M NaCl, pH 7.5; *F*, H_2O.

‡Abbreviations: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; GPS, *sn*-glycero-3-phosphoserine; GPI, *sn*-glycero-3-phosphoinositol; PG, *sn*-3-phosphoglycerol; PA, *sn*-glycero-3-phosphate; $\mu_\perp$, surface dipole moment (Eq. 2); ΔV_o, area-independent surface potential (Eq. 2); π_d, surface pressure at which $d\pi/dA$ becomes negative; π_t, surface pressure at transition from the liquid-expanded film to a more condensed or collapsed state; A_t, lipid molecular area at π_t; ω_o, lipid molecular area at $\pi = \infty$ (Eq. 3); f_1, rationalized activity coefficient for interfacial water (Eq. 3); q, interaction parameter (Eq. 3).

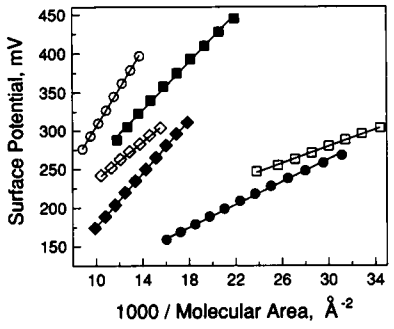

FIGURE 1 Area dependence of surface potential for representative lipids in the liquid-expanded state. Films of (○) 1,2-diphytanoyl GPC, (■) 1,2-dilauroyl GPC, (◇) 1,3-dioleoylglycerol, (◆) 1-palmitoyl-2-oleoyl GPS, (□) oleyl nitrile, and (●) *N*-oleylethanolamine were spread and compressed on a subphase of 0.01 M phosphate, 0.1 M NaCl, pH 6.6, at 24°C. For 1-palmitoyl-2-oleoyl GPS, 0.1 mM EGTA was also present. The straight lines were fitted using 400–500 data points although only selected points are shown for identification. Abbreviations are as in Table 1.

which was used to obtain the values of ΔV_0 and $\mu_\perp$ given in Table 1.

Because of the relatively small range of areas which constitute the liquid-expanded state and the extrapolation to $1/A = 0$, the non-zero intercepts could reflect calibration or other experimental errors. Accordingly, a number of control experiments were performed. Obvious possibilities were nonlinearity in ΔV and A calibration. The trough of the film balance is rectangular but the lipid-containing surface has an irregular shape around the π-measuring barrier which separates it from the reference surface. To correct for this irregularity, an empirical calibration method can be used. Using different numbers of molecules, π-A isotherms for oleoyl alcohol were obtained. For each, the relative position of the moving barrier was determined at three selected π values and plotted vs. the quantity of lipid spread. As shown in Fig. 2,

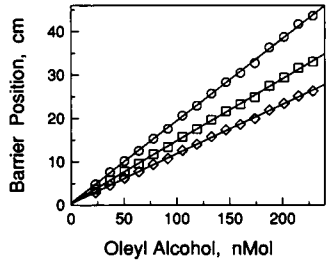

FIGURE 2 Calibration of monolayer trough area at three surface pressures. (○) $\pi = 1$, (□) $\pi = 10$, (◇) $\pi = 30$ mN/m. Data are from surface pressure-area isotherms obtained from films of oleyl alcohol spread and compressed on 0.01 M phosphate, 0.1 M NaCl, pH 6.6. Lines were drawn using a least squares fit.

the data obtained at each π are linear and have a common intercept on the ordinate. Using this relative barrier position as $A = 0$, together with the known width of the trough, insured that A calibration was proportional over the entire working range of the instrument. We also demonstrated that our digital volt meter response was proportional to input voltage and that ΔV readings were independent of electrode height above the aqueous subphase. Hence, calibration errors cannot explain the non-zero values of ΔV_0.

Another likely source of error is the presence of surface active impurities in solvents, subphases, or argon. As noted by Gershfeld (30), no monolayer experiment is devoid of such contamination. To determine if the level in our studies was sufficient to produce potential changes of the order of ΔV_0, a sham isotherm was obtained by placing only solvent in the sample vial. The subphase was 0.01 M potassium phosphate buffer, pH 6.6, 0.1 M NaCl at 24°C. The maximum usable surface area available for lipid in the apparatus was employed to accentuate the presence of contaminants. In addition, a teflon insert of 39.12 cm^2 was placed in the trough to obtain a large relative area change upon surface compression. This allowed a 38.5-fold area change without interference with ΔV measurment, yet the maximum change in ΔV was 19 mV and π was 0.08 mN/m. The elapsed time from spreading of solvent until these values were reached was 36 min. If Eq. 1 were obeyed or even approximated, these small changes could not reasonably explain the large ΔV_0 values shown in Table 1.

To determine if ΔV_0 could arise from contaminants in the lipids themselves, a sample of diphytanoyl phosphatidylcholine was repurified and its surface properties compared with those of the starting material. As shown in Table 1, the values of ΔV_0 and $\mu_\perp$ obtained using Eq. 2 are, within error, identical. Thus, if present, impurities in the lipid copurify with it. Additionally, if such impurities were present, they would be expected to vary from lot to lot, or if introduced by handling, from solution to solution. Table 2 summarizes the π-A-ΔV properties of lipids which were obtained with multiple lots and/or solutions used over a period of years. The number of lots used, solutions made, and isotherms obtained are indicated in brackets after the compound name. Reasonably, the percent standard deviations of ΔV_0, which correspond to absolute standard deviations of 2–14 mV, suggest that random contamination is not responsible for large, non-zero values of ΔV_0. Also, Tables 1 and 2 show class species specific values of ΔV_0 as well as $\mu_\perp$ which would not be expected if contamination were random.

Although prior studies have shown that solvent effectively evaporates from monolayers in a short time (31), solvent residues might in some way determine ΔV_0. To test this possibility, we obtained values of ΔV_0 and $\mu_\perp$

TABLE 2 Reproducibility of parameters describing liquid-expanded monolayer behavior

Lipid	$\mu_\perp$	ΔV_0	π_t	A_t	ω_o	f_1	q
	mD	*mV*	*mN/m*	$Å^2$	$Å^2$		
Triacylglycerol [2, 2, 12]	671 (6.5)	116 (11.7)	11.9 (0.8)	101.0 (0.7)	68.5 (1.7)	1.16 (0.4)	2.51 (5.4)
1,2-diphytanoyl GPC* [2, 3, 12]	657 (2.7)	62 (13.4)	46.1 (0.9)	71.9 (1.0)	57.0 (2.0)	1.16 (0.7)	3.07 (5.9)
1,2-dioleoyl GPC [1, 2, 6]	461 (2.9)	121 (3.9)	44.9 (0.6)	58.1 (1.6)	42.2 (4.7)	1.15 (0.5)	3.16 (7.0)
1-palmitoyl-2-oleoyl GPC [3, 5, 14]	471 (2.7)	122 (3.1)	47.0 (0.9)	55.0 (0.8)	41.9 (1.2)	1.16 (0.2)	2.75 (2.6)
1,2-dimyristoyl GPC [2, 2, 11]	476 (3.1)	115 (2.6)	47.3 (1.3)	50.8 (2.4)	39.4 (2.5)	1.17 (0.4)	2.41 (2.9)
1,2-dioleoyl GPC [1, 1, 4]	498 (1.9)	91 (3.4)	46.1 (0.7)	55.4 (0.7)	43.2 (0.4)	1.18 (0.3)	2.60 (2.5)
1,2-dioleoylglycerol [1, 1, 4]	442 (0.5)	95 (2.3)	30.5 (0.5)	58.0 (0.8)	44.5 (0.9)	1.21 (0.3)	2.05 (0.8)
1,3-dioleoylglycerol [3, 6, 12]	328 (3.8)	111 (4.5)	27.6 (0.5)	63.6 (0.8)	44.5 (1.1)	1.17 (0.2)	2.62 (1.9)
1(3)-monooleoylglycerol [1, 2, 6]	164 (4.1)	105 (4.4)	44.2 (0.5)	33.3 (3.7)	24.4 (4.7)	1.20 (0.7)	1.89 (2.5)
Oleic acid [2, 4, 10]	136 (5.2)	−78 (4.6)	40.8 (0.7)	26.8 (2.3)	21.9 (3.1)	1.31 (0.5)	1.18 (3.7)
Oleyl alcohol [2, 3, 10]	149 (5.2)	30 (22.6)	32.1 (1.8)	28.8 (1.4)	24.4 (0.8)	1.37 (0.8)	0.89 (2.8)

Values in brackets indicate number of lots, solutions and isotherms used to obtain averages shown. Values in parentheses () are percent standard deviations. Subphase for all experiments 0.01 M phospate, 0.1 M NaCl, pH 6.6, temperature 24°C.

*Abbreviations: GPC, *sn*-glycero-3-phosphocholine; GPE, *sn*-glycero-3-phosphoethanolamine; $\mu_\perp$, surface dipole moment (Eq. 2); ΔV_o, area-independent surface potential (Eq. 2); π_t, surface pressure at transition from the liquid-expanded film to a more condensed or collapsed state; A_t, lipid molecular area at π_t; ω_o, lipid molecular area at $\pi = \infty$ (Eq. 3); f_1, rationalized activity coefficient for interfacial water (Eq. 3); q, interaction parameter (Eq. 3).

using delays of 60–1920 s between spreading of 1,3-dioleoylglycerol solution and the onset of film compression. No significant differences were observed (data not shown). Next, we compared an isotherm obtained by spreading neat 1,3-dioleoylglycerol to one obtained by normal, automated solution spreading. Areas were matched at π_t to determine the number of molecules spread without solvent. Comparison of the potential parameters for the isotherms (Table 1) shows that unusual solvent effects are not responsible for the large ΔV_0 values. We also replaced the gas phase of argon with nitrogen for a phospholipid monolayer but observed no change in ΔV_0 or $\mu_\perp$ values.

Having reasonably eliminated material origins of ΔV_0, it was of interest to determine if the lipid films were, possibly, metastable. As shown in Fig. 3, a compression-expansion cycle of a typical lipid with $\pi < \pi_t$ shows only a small hysteresis in π-A behavior. We also compared the values of π_t (Table 1) for representative lipids with their equilibrium spreading pressures measured by the Wilhelmy technique. As shown in the inset to Fig. 3, there is a good correspondence. For the experiment shown in Fig. 3, ΔV behavior in the liquid-expanded state ($1{,}000/A > 10$) was also reasonably reversible (Fig. 4). However, at larger areas ($1{,}000/A < 10$), differences were quite large. Furthermore, we observed that during compression, for experiments analogous to that shown in Figs. 3 and 4, the region of rapid ΔV increase at $A > 100$ $Å^2$/molecule was very dependent on the initial value of A chosen. However, this may be due in part to differences in compression rate per molecule necessitated by instrumental limitations. During expansion, ΔV was always higher (e.g., Fig. 4) at any $A > 100$ $Å^2$/molecule than for the preceding compression and was approximately constant at 290–300 mV from 100–200 $Å^2$/molecule. In all experiments, however, ΔV values in the liquid-expanded state were essentially the same as shown previously in Fig. 4. Lastly, we spread a film at an initial $A = 90$ $Å^2$, i.e., in the liquid-expanded state itself, and obtained $\Delta V_0 = 138$ mV and $\mu_\perp = 452$ mD. These values are comparable to the

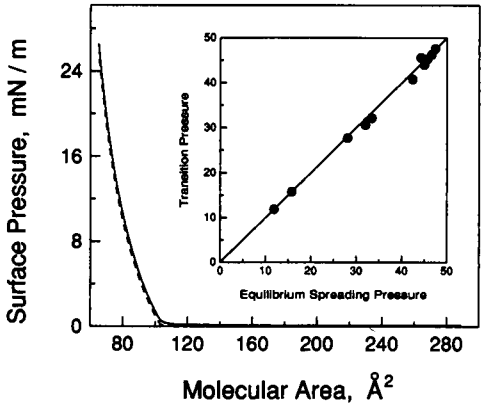

FIGURE 3 Surface pressure-area isotherms of 1-palmitoyl-2-oleoyl GPC. The film was compressed (—) from an area of 290–65 $Å^2$/molecule at a rate of 6 $Å^2$/min/molecule, and then expanded (----) over the same range. The subphase was 0.01 M phosphate, 0.1 M NaCl, pH 6.6, 24°C. The insert shows equilibrium spreading pressures obtained for representative lipids measured by the Wilhelmy technique vs. transition pressures, π_t, from Table 1. Lipids from left to right are trioleoylglycerol, oleyl methanol, 1,3-dioleoylglycerol, 1,2-dioleoylglycerol, oleyl alcohol, oleic acid, 1(3)-monooleoylglycerol, 1,2-dioleoyl GPS, 1,2-dioleoyl GPC, 1,2-dioleoyl GPE, and 1-palmitoyl-2-oleoyl GPC. Subphase as above except for the GPS isotherm which also contained 0.1 mM EGTA.

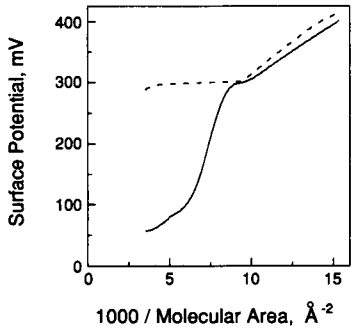

FIGURE 4 Surface potential-area behavior of 1-palmitoyl-2-oleoyl GPC. Conditions were the same as shown in Fig. 3. Compression (—); expansion (----).

values of 126 mV and 468 mD (Table 1) obtained with a typical initial A of 120 Å^2/molecule (Table 1) and with the values of 127 mV and 465 mD obtained from the compression data in Fig. 4. Overall, this set of experiments suggests that under our typical experimental conditions (data in Tables 1 and 2), ΔV-A behavior in the liquid-expanded state is reasonably independent of the previous history of the film, i.e., the system is at or near equilibrium. This is definitely not true at larger areas where, presumably, liquid expanded and gaseous phases coexist. This suggests that ΔV_0 arises as a consequence of film rearrangement occurring concomitant with the gaseous to liquid-expanded phase transition.

In the liquid-expanded state, the data are well described by Eq. 2, with which they may be reduced to two parameters. Thus, if A is known, ΔV can be calculated. If the value of ΔV at a particular π is desired, A must be determined from the π-A isotherm. It has been shown recently that for $\pi \geq 1$ mN/m up to near π_t that π-A isotherms for liquid-expanded films are well described by

$$A = \omega_0 + \omega_1/(f_1 e^{\pi\omega_1/qkt} - 1), \quad (3)$$

where ω_0 is the totally dehydrated area of the lipid, ω_1 is the cross-sectional area of water, f_1 is the rationalized activity coefficient of interfacial water, and q is a cumulative interaction parameter (28). Application of this model to π-A data in the range $1.0 \leq \pi \leq \pi_d$ yields the values of ω_0, f_1, and q given in Tables 1 and 2. Using these, A, and hence ΔV, can be calculated at any π. In addition, the value of A at π_t, i.e., A_t, was calculated (Tables 1 and 2). Averages of the π-A fitting parameters (Table 2) show excellent reproducibility. Like the reproducibility of the $\mu_\perp$ and ΔV_0 parameters, the lack of variation of π-A parameters between lots and solutions suggests minimal effects of contaminants.

DISCUSSION

Our results show clearly that many lipids of biological interest exhibit linearity of ΔV with respect to their surface concentration in the liquid-expanded state. A total of 54 isotherms were obtained with 38 ionic, nonionic, and zwitterionic species which represent 19 chemical classes (Table 1). This linearity, but not proportionality, indicates the appropriateness of Eq. 2 to summarize ΔV-A behavior, rather than Eq. 1 or more complex, nonlinear forms (e.g., 32). It should be pointed out that both Eqs. 1 and 2 must be regarded as approximate in that they reflect the aggregate behavior of water and multipolar molecules in an anisotropic medium (33). To a good approximation, however, lipids and their associated water in the liquid-expanded state behave as if they are point dipoles which do not reorient with changing concentration. In addition, in the liquid-expanded state there exists a concentration-independent potential which can be >100 mV.

The origins of constant potential term, ΔV_0, are not specifically indicated by the experimental data. Its values are too large, reproducible, and species specific to be explained by random contamination or systematic calibration errors. Moreover, the contribution of ΔV_0 to ΔV arises nonlinearly in the gaseous to liquid-expanded transition region during film compression and persists upon expansion of the film (Fig. 4). Thus, ΔV_0 seems to occur as a consequence of the organization of the molecules into the liquid-expanded state. Because it occurs with water alone as a subphase and with nonionic lipids, it is not simply attributable to subphase counterions. As noted above, the linearity of ΔV with $1/A$ suggests that the orientation of the dipoles which determine $\mu_\perp$ is unaltered over the ~ twofold change in areas encompassing the liquid-expanded state. Because ΔV_0 is species dependent, but independent of lipid concentration, it may reflect an organization of interfacial water by these lipid dipoles. Specifically, for any given area of the surface phase some of the lipid-organized water molecules reside between the lipid head groups and some between the head group region and bulk water. The former will be displaced in a stepwise fashion with changing lipid concentration and, hence, will contribute to $\mu_\perp$. The existence of the latter water region has been established by the direct measurement of repulsive hydration forces between lipid layers (e.g., 34). Because the partial specific volume of interfacial water is not significantly different from bulk water (35), changes in lipid concentration should not alter the two-dimensional concentration of this water layer. Its depth, i.e., the effective depth to which water is organized by the lipid dipoles, could change with lipid concentration. However, a recent study demonstrating an A-

independent decay length for the hydration force (9) suggests that the effective depth is constant. It is this structured water in the region between the lipid head groups and bulk water which could give rise to ΔV_0.

The partitioning of interfacial water into two pools is consistent with the approach of Simon and McIntosh to the relation of ΔV to the hydration repulsion (36). They define zero distance between bilayers on a line which separates head groups and some water molecules from the remainder. For bilayers, the overall thickness of this organized water layer is not known with certainty because it is induced by an exponentially decaying force. However, it is reasonably 8–13 Å (37), or several water molecule diameters. Simon and McIntosh also suggest that their use of monolayer-derived ΔV values to predict bilayer properties could be skewed by the existence of an A-independent potential term (36). They further note that dipole potential measurements for bilayers are 100–150 mV lower than for monolayers. For a typical phosphatidylcholine, the size and effect of ΔV_0 on ΔV (Table 1) is consistent with this monolayer-bilayer discrepancy if ΔV_0 is a unique property of monolayers. Alternatively, it could simply reflect the location of the probes used to measure the dipole potential in bilayers.

The A-dependent contribution to ΔV reflects the orientations and strengths of lipid dipoles, charges, and some interfacial water molecules. As noted, linearity of ΔV vs. $1/A$ implies that the conformation and orientation of all contributing dipoles remain unchanged throughout the linear range (38), i.e., only their concentration changes, and permits calculation of $\mu_\perp$ using Eq. 2 (Tables 1 and 2). Traditionally, $\mu_\perp$ has been subdivided into the contributions of lipid-reoriented water molecules (μ_1), lipid polar head groups (μ_2), and the chain terminal dipole (μ_3) in the form, $\mu_\perp = \mu_1 + \mu_2 + \mu_3$ (39). In light of the discussion above, μ_1 would represent the contribution of those water molecules between the lipids which are displaced by changing lipid concentration. In more condensed films, the value of μ_3 is substantial, having been estimated at −300 mD (40) or, recently, at +350 mD (41) per chain-terminal CH_3. If μ_3 were ±300-900 mD/molecule for lipids having 1–3 aliphatic chains, $\mu_\perp$ would be expected to vary greatly with molecular area due to changes in methyl group mobility and orientation. That it does not suggests that in the liquid-expanded monolayer state either $\mu_3 \ll \mu_1 + \mu_2$ or that CH_3 group reorientation does not change significantly with A.

The components which make up $\mu_\perp$ are not normally separable (41, 42) unless admittedly questionable assumptions are employed (43, 44). However, attempts have been made to separate $\mu_\perp$ into partial molecular or bond components based on lipid structure (45–48). Generally, this work preceded recognition that glycerol-based lipids tend to have similar conformations at bilayer interfaces and in crystals (49, 50), that phospholipid head groups and charges may contribute little to $\mu_\perp$ in monolayers and bilayers (4, 6, 42), and that condensed monolayers of phospholipids and 1,2-diacylglycerols exhibit similar $\mu_\perp$'s (51).

The data in Table 2 allow the partial dipole concept to be extended in a different form than in earlier studies. Average values of $\mu_\perp$ are available for six lipids containing only oleoylglycerol groups (Table 2). It can be assumed, as done previously (52), that the 1,2-dioleoylglycerol and 1,2-dioleoyl phospholipids share a common conformation with the same nonequivalent chain arrangement as in bilayers and crystals (49, 50). In that conformation, the *sn*-1-acyl moiety is essentially linear, whereas the *sn*-2-acyl moiety is kinked 90° between carbons 2 and 3. If the head group and associated water contribution (μ_h) is constant, then

$$\mu_\perp = \mu_h + n_a(\mu_a) + n_b(\mu_b), \qquad (4)$$

where μ_a and μ_b refer to the contributions of oleoylglycerol groups, their associated water, and chain terminal dipoles for chains having the linear and kinked conformations, and n is the number of chains in a molecule having each conformation. Based on previous arguments (52), 1,3-dioleoylglycerol is assumed to have type a ester conformation in both the 1 and 3 positions. 1(3)-Monooleoylglycerol is assumed to have type a also. Comparison of ^{13}C-NMR studies (53) of trioleoylglycerol in liquid-crystalline bilayers and monolayers (54, 55) and the construction of molecular models suggests that trioleoylglycerol has type b esters at the 1 and 3 positions and type a at carbon 2. With these assignments and the values of $\mu_\perp$, a set of linear equations of the form of Eq. 4 can be written. Solving these by stepwise linear regression using commercial software (Plotit, Scientific Programming Enterprises, Haslett, MI) gives values of 132 ± 50 mD for μ_a, 252 ± 27 mD for μ_b, and 65 mD for μ_h. The average absolute residual was 38 mD. A plot of $\mu_\perp$ values for the six oleoylglycerol esters (Table 2) vs. theoretical $\mu_\perp$ values calculated using the structural assumptions noted and the fitted values of μ_a, μ_b, and μ_h in Eq. 4 are shown in Fig. 5. Considering the simplifying assumptions of conformation and orientation used, the agreement with the solid line having a slope of 1.00 is quite reasonable.

It is stated that the contribution of internal CH_2 groups to $\mu_\perp$ is negligible (41), although this may not be true in states condensed more than the liquid expanded (56). If for lipid films the effective chain terminal dipole is small, the values of μ_a and μ_b represent the contributions of the glycerol-ester groups and their associated water. These values of μ_a and μ_b could, in theory, be used to calculate bond orientations or, using those from the literature (49, 50), to calculate effective dielectric constants for the

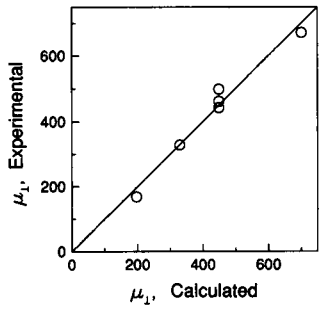

FIGURE 5 Comparison of experimental and calculated surface dipole moments for glycerol-ester lipids containing oleoyl groups. Experimental values are from Table 2 and calculated values were determined using estimated group dipole moments, μ_a, μ_b, and μ_h, as described in the text.

environment of each group (e.g., 44). However, given the approximate nature of the μ_a and μ_b values and no knowledge of the contribution of water dipoles to μ_a and μ_b (39), such calculations would be speculative at present. If the chain terminal dipole contribution is significant, then its orientation and value would also have to be considered, increasing the complexity. In either case, we note that $\mu_a < \mu_b$ which is consistent with the carbonyl group being more parallel to the interfacial phase in the a or extended conformation compared with the b or kinked form (49, 50).

The values of μ_a and μ_b, though approximate, are useful to identify glycerol-ester lipids which might have a different orientation or conformation at the interface from their homologues. Because the length of the aliphatic chain contributes little to $\mu_\perp$, its value for all diacyl phosphatidylethanolamines and phosphatidylcholines and 1,2-diacylglycerols at neutral pH and 0.1 M NaCl should be 449 mD. For most of such lipids listed in Table 1 having long acyl moieties, this is approximately correct. Note also, the similarity of these with $\mu_\perp$ values for the phosphatidic acid and phosphatidylserines, suggesting little effect of the head group charge. A conspicuous exception is diphytanoyl phosphatidylcholine with a $\mu_\perp$ of 653 mD, 45% greater than predicted. It also exhibits a ΔV_o approximately half that of other phosphatidylcholines. These values suggest that the presence of methyl branches, particularly at the α-position on each fatty acyl moiety may perturb glycerol-ester conformation or orientation, and hence, ΔV. This is of interest because of several phosphatidylcholines tested, the diphytanoyl species alone inhibited the activity of lecithin/cholesterol acyltransferase (57). The more soluble, medium chain 1,2-diacylglycerols and phospholipids have lower $\mu_\perp$'s than their long-chain counterparts, suggesting more flexible or altered conformations.

The values of $\mu_\perp$ and ΔV_o in Table 1 allow ΔV at any A to be predicted using Eq. 2 or at any π using Eqs. 2 and 3. These ΔV values can be used to estimate how metabolic events might alter the surface potential profile on one face of a lipid bilayer. Although such potential changes have been previously considered on a global basis (1), the action of an enzyme can produce transient, local differences. One relevant example is the generation of 1,2-diacylglycerols from different phospholipids. Using values of A_t (Table 1) for A in Eq. 2, the widely studied cleavage of phosphatidylinositol by phospholipase C to yield 1,2-diacylglycerol should increase ΔV from 254–381 mV at 24°C, a change of 127 mV more negative relative to the membrane center. In contrast, metabolic conversion of 1,2-dioleoyl phosphatidylcholine to 1,2-dioleoylglycerol (13) should change ΔV from 423–381 mV, a change of 42 mV more positive. Thus, with respect to local surface potential, phosphatidylinositol, and phosphatidylcholine are not simply alternative sources of 1,2-diacylglycerol. Such differences may be important in the recruitment and binding of ions like Ca^{2+} and of proteins like protein kinase C. It is also of interest to note the markedly different effects of 1,3- and 1,2-diacylglycerols on vesicle fusion (58). This could be related to their surface potentials which at π_t and A_t differ by 74 mV. Another process which can significantly alter surface potential, as noted previously (51), is lipid exchange. Replacing the 1,2-diacyl-*sn*-3-glycerophosphocholine by 1-0-alkenyl-2-acyl-*sn*-3-glycerophosphocholine should change the local surface potential from 439–293 mV, a change of 146 mV more positive at the lipid-water interface. Such changes may have marked effects on the activities of enzymes like phospholipase A_2 (7), hydration repulsion (36), and other processes noted in the Introduction.

Whereas the electrical consequences of metabolic and transport processes described above are only estimations, they should be indicative of the values and directions of surface potential changes which can occur in vivo. As shown in this study, monolayers of biologically relevant lipids can be readily analyzed to provide values of $\mu_\perp$, ΔV_o, and other parameters which allow predictions of surface potentials of bilayers and identification of structurally perturbed subspecies.

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