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Accelerated Publications

How Membrane Chain Melting Properties Are Regulated by the Polar Surface of the Lipid Bilayer†

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ABSTRACT: The principle of regulation of various membrane properties by the hydrocarbon membrane interior is now well understood. The mechanism by which the interfacial membrane region including aqueous solution affects the state of the lipid bilayer matrix, however, is as yet unclear, despite its great biological and physiological significance. Data and analysis presented in this paper show that apart from the lipid chain type, length, and degree of unsaturation the main factors determining the characteristics of lipid membranes are surface polarity and interfacial hydration. These incorporate the effects of head group dipole and multipole moments as well as the head group ability for hydrogen bonding and can account for most of the changes in the physicochemical membrane state caused by the lipid head group structure, bulk pH value, salt content, solute adsorption, etc. The effects of membrane potential are much less, only 10–30% of the former. Variations in hydration thus not only govern the short- and medium-range intermolecular and intermembrane interactions but also provide a fast and energetically inexpensive regulatory mechanism for lipid membranes to adapt their characteristics, at least locally or transiently, to new requirements.

Phospholipids are one of the major constituents of biological membranes. They assemble into bilayers, which provide cells with permeability barriers and with matrices for the insertion of the nonlipid membrane components. The biochemical and biological functions of all membrane components, such as proteins, are therefore influenced by the local properties, or accumulation of lipids near such components [cf. Jost and Griffith (1982)], and varying the type or the physicochemical state of these lipids can thus contribute to controlling the state and functions of a given membrane region. The role of the apolar bilayer interior in this has long been recognized (Marčelja, 1973; Pink, 1982), but not even an elementary understanding of the principles existed to date that would allow an analysis of the effect of the polar membrane region, including the ionic solution, on the membrane characteristics.

Attempts have been made to explain the effect of lipid ionization on the bilayer chain melting phase transition temperature by using classical electrostatic double layer theory [cf. Träuble et al. (1976)]; however, it is now rather certain that only a fraction of the ionization-induced chain melting phase transition shift is indeed of simple electrostatic origin (Cevc et al., 1986; Cevc & Marsh, 1987; also further discussion). To interpret the effects of chemical head group modification, e.g., the consequences of methylation, on the membrane phase behavior, the differences in electrostatic moments and/or bulkiness of the lipid head groups have been

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Table I: Effect of Lipid Chain Length, Head Group Protonation, or Methylation on the Chain Melting Phase Transition Temperature (°C) of Lipid Bilayers or Its Shifts (in Italics)

	pH						pH				
lipid[a]	0		8		13	lipid[a]	0		8		13
DMPE[b]	54	*4.5*	49.5	*25.5*	24	DTPC	59	*4*	55	*26*	29
	6		*7.5*		*-1*		*6*		*8*		*-1*
$DMPE(CH_3)$	48	*5.5*	42.5	*17.5*	25	$DTPE(CH_3)$	53	*6*	47	*17*	30
	6		*11.5*		*0*		*6*		*11*		*0*
$DMPE(CH_3)_2$	42	*11*	31	*6*	25	$DTPE(CH_3)_2$	46	*10*	36	*6*	30
	6		*8*		*2*		*7*		*7.5*		*1.5*
DMPC	36	*13*	23	*0*	23	DTPC	39	*10.5*	28.5	*0*	28.5
DPPE	67	*3.5*	63.5	*21.5*	42	DHPE	71.5	*3*	68.5	*24.5*	44
	5.5		*5.5*		*-1*		*5.5*		*7*		*-1*
$DPPE(CH_3)$	61.5	*3.5*	58	*15*	43	$DHPE(CH_3)$	66	*4.5*	61.5	*16.5*	45
	5.5		*10*		*0*		*5.5*		*11*		*0*
$DPPE(CH_3)_2$	56	*8*	48	*5*	43	$DHPE(CH_3)_2$	60.5	*10*	50.5	*5.5*	45
	6		*6*		*1*		*6.5*		*7*		*1.5*
DPPC	50	*8*	42	*0*	42	DHPC	54	*10.5*	43.5	*0*	43.5

[a] DM = 1,2-dimyristoyl-*sn*-glycero; DT = 1,2-ditetradecyl-*rac*-glycero; DP = 1,2-dipalmitoyl-*sn*-glycero; DH = 1,2-dihexadecyl-*rac*-glycero; PE ≡ $P(CH_2)_2NH_3$ ≡ phosphatidylethanolamine; $PE(CH_3)$ ≡ phosphoryl-*N*-methylethanolamine; $PE(CH_3)_2$ ≡ phosphoryl-*N*,*N*-dimethylethanolamine; $PE(CH_3)_3$ ≡ $P(CH_2)_2N(CH_3)_3$ ■ PC ≡ phosphatidylcholine. [b] Some related data concerning methylated PE are also found in works by Mulukutla and Shipley (1984), Seddon et al. (1983a), and Vaughan and Keough (1974).

invoked. The former cannot be particularly relevant, as can be seen from the fact that various phospholipids with quite different transition temperatures in aqueous suspensions have similar head group conformations and consequently comparable effective dipole moments. The concept of the head group bulkiness is so trivial that it is essentially useless: it is a mere verbal description, neither capable of providing an insight into the molecular mechanism of the surface-induced chain melting phase transition shifts nor capable of permitting a quantitative analysis of the experimental data.

Here I present the first self-consistent, and yet simple, explanation of the mechanism underlying the control of membrane structural and phase behavior by the lipid head groups and the ionic solution, which provides means for quantitatively analyzing and predicting the corresponding experimental data. I demonstrate that particularly the variation of the surface polarity and interfacial hydration—as can arise, for example, from changing the lipid head group structure, bulk pH value, salt content, or solute adsorption—can dramatically alter the thermodynamic and thus the functional properties of lipid bilayer membranes. Changes in hydration thus not only govern the short- and medium-range intermolecular and intermembrane interactions (Rand, 1981; Parsegian & Rau, 1984), as has been discovered in the last few years, but also provide a fast and energetically inexpensive regulatory mechanism for lipid membranes to adapt their characteristics, at least locally and transiently, to new requirements.

Experimental Procedures

Lipids were (1) made by enzymatic transphosphatidylation (Comfurius & Zwaal, 1977) from the corresponding phosphatidylcholines or phosphatidylethanolamines [for $PE(CH_3)$[1] and for some PG or PS], (2) made by de novo synthesis (Eibl, 1980) [for certain $PE(CH_3)_2$], or (3) obtained from commercial sources [Fluka (Neu-Ulm, West Germany), Sigma (Munich, West Germany), and Novachem (Lüfelfingen, Switzerland), (PC and PE)]. Lipid purity was confirmed by thin-layer chromatography in at least two solvent systems just before and occasionally after the experiments.

Phase transition temperatures were determined calorimetrically with a Perkin-Elmer DSC 2B differential scanning calorimeter equipped with an Intracooler; alternatively, the chain melting was monitored with a Beckmann 3400 UV–vis spectrophotometer to measure the optical density at 400 nm of lipid solutions (pH 8 ≥12) or films deposited between silica or glass plates (pH 8, ≤1), with an accuracy of ±0.2 K and a respective reproducibility of ±1 or ±2 K. Temperature gradients in the latter case were shown to be ≤2 deg. The ionic strength of the solution was kept constant, $J = 0.1$, except at pH ≤1, where it was $0.1 \leq J \leq 1$.

Nonbilayer phase formation was detected by fluorescence methods or by optical microscopy.

Results and Discussion

On the absolute temperature scale the phase behavior of lipid bilayers is determined mainly by the hydrocarbon chains (Marčelja, 1973; Pink, 1982). On the physiological temperature scale, however, small thermodynamic differences, which arise from different chemical structures and from varying the physicochemical state of the lipid head groups or their bathing medium, are clearly equally important. I have therefore investigated systematically the effect of the polar membrane region including the aqueous subphase on the lipid chain melting phase transition temperature. I have determined the latter as a function of chemical structure and degree of head group methylation or protonation for a series of glycerophospholipids that are identical with those encountered in biological membranes except that their symmetric hydrocarbon chains had a known length, degree of unsaturation, and type of attachment.

Data obtained with the most ubiquitous noncharged lipids phosphatidylethanolamine and phosphatidylcholine and with some derivatives of the former are given in Table I; those pertaining to lipids that are charged under normal conditions are summarized in Table II. These data all indicate, first, that acidification of the solution, which leads to at least partial phosphate group protonation, causes the temperature at which

[1] Abbreviations: PA, phosphatidic acid; $PA(CH_3)$, phosphatidic acid methyl ester; PC, phosphatidylcholine; PE, phosphatidylethanolamine; $PE(CH_3)$, phosphoryl-*N*-methylethanolamine; $PE(CH_3)_2$, phosphoryl-*N*,*N*-dimethylethanolamine; PG, phosphatidylglycerol; PS, phosphatidylserine; J, ionic strength; T_m, chain melting phase transition; $\Delta T_{m,i}$ (i = el, h, bond, vdW), various contributions to the shift of lipid chain melting phase temperature; G_i, contributions to the free energy of the polar membrane region; $\Delta G_{i,m}$, shifts of the former associated with chain melting; $\Delta S_{ref,m}$, the change in entropy at the chain melting phase transition.

Table II: Effect of Lipid Ionization[a] and Chain Length on the Bilayer Chain Melting Phase Transition Temperature (°C) and Its Shifts (in Italics)

	pH							pH						
lipid[b]	0[d]		~3		8		13	lipid[b]	0[d]		~3		8		13
DMPG	42		*18*		24	*0*	24	DTPG	46		*18*		28	*0*	28
DMPS	52	*8*	44	*8*	36	*21*	(15)	DTPS	56	*8*	48	*7*	41	*21.5*	19.5
DMPA[c]	45	*-10*	55	*5*	50	*22*	28	DTPA	48	*-11*	59	*4*	55	*21*	34
	-3		*8*		*18*		*-4*		-4		8		18		-3
DMPA(CH_3)	48	*1*	47	*15*	32	*0*	32	DTPA(CH_3)	52	*1*	51	*14*	37	*0*	37
DPPG	58		*16*		42	*0*	42	DHPG	62		*16*		46	*0*	46
DPPS	68.5	*7*	61.5	*7.5*	54	*22*	32	DHPS	72	*8*	64	*8*	56	*21*	34
DPPA[c]	62	*-12*	74	*6*	68	*23*	45	DHPA	62	*-14*	76	*5*	71	*21*	50
	1		*12*		*20*		*-3*		*-4*		*11*		*19*		*-2*
DPPA(CH_3)	63	*1*	62	*16*	48	*0*	48	DHPA(CH_3)	66	*1*	65	*13*	52	*0*	52

[a] Ionization states are at pH 0 for PG, PA(CH_3), PA, and PS^+, at pH 3–4 for PA(CH_3), $PA^{0.5-}$, and PS^-, at pH 8 for PG^-, PS^-, PA(CH_3)$^-$, and PA^-, and at pH 13 for PG^-, PS^{2-}, PA(CH_3)$^-$, and PA^{2-}. [b] DM = 1,2-dimyristoyl-*sn*-glycero; DT = 1,2-ditetradecyl-*rac*-glycero; DP = 1,2-dipalmitoyl-*rac*-glycero; DH = 1,2-dihexadecyl-*rac*-glycero; PG = phosphatidylglycerol; PA = phosphatidic acid; PA(CH_3) = phosphatidic acid methyl ester; PS = phosphatidylserine. [c] For the data concerning PA, see also papers by Eibl and Blume (1979) and Blume and Eibl (1979). [d] Occasionally the transition temperature of the samples that have been stored for longer periods of time or prepared in highly concentrated salt solutions is 3–5 K higher. This indicates the possibility that lipid under such conditions has reverted into another state, which may imply that values given here are characteristic of the bilayers with tilted chains [cf. Cevc et al. (1985, 1986)].

the hydrocarbon membrane core begins to fluidize, T_m, to increase. Alkalization of the aqueous subphase or chemical lipid deprotonation, such as is caused, for example, by the head group methylation, has the reverse effect. Changes of pH can thus lead to an isothermal chain melting or freezing within a fraction of a second (data not shown).

Such behavior can be rationalized quantitatively in terms of the various contributions, $\Delta T_{m,i}$, from the polar membrane region to the corresponding total shift of the transition temperature, $\Delta T_{m,p}$. From perturbation theory (Träuble et al., 1976) it can be shown (Cevc & Marsh, 1985) that the latter has its origin in the overall change of the free energy of the polar membrane region at the phase transition, $G_{p,m}$, and is given by

$$\Delta T_{m,p} = \Delta G_{p,m}/\Delta S_{ref,m} \quad (1)$$

where $\Delta S_{ref,m}$ is the change in entropy at the phase transition of the lipid in the reference state; a reasonable choice of such state is that of the anhydrous lipid (Cevc & Marsh, 1985).

To a good approximation the free energy of the polar membrane part, G_p, is a sum of the energetic contributions from the electrostatic ion–lipid and certain ion–ion interactions, G_{el}, from the lipid (head group) hydration, G_h, from the interlipid bonds, G_{bond}, from the van der Waals forces, $G_{m,vdW}$, etc.: $G_p = \Sigma_i G_{p,i}$, with i = el, h, bond, vdW, ..., because various interactions between the lipid head groups and the solution are nearly independent. Correspondingly, the total shift of the lipid chain melting phase transition temperature $\Delta T_{m,p}$ can be represented as a sum of the partial contributions

$$\Delta T_{m,p} = \sum_i \Delta T_{m,i} =$$
$$\Delta T_{m,el} + (\Delta T^{PO_4}_{m,h} + \Delta T^{H}_{m,h}) + \Delta T_{m,bond} + \Delta T_{m,vdW} + \dots \quad (2)$$

the hydration shift being here subdivided into two parts (in parentheses) for the sake of convenience and clarity. The first of the latter two shifts, $\Delta T^{PO_4}_{m,h}$, is a result of the phosphate (de)protonation. The second, $\Delta T^{H}_{m,h} \sim 0.5\Delta T^{PO_4}_{m,h}$, represents the shift associated with creation of such new binding sites for water on a polar residue, e.g., on a carboxyl or ammonium group, that involves *strong* H^+ binding to some—most frequently charged—head group proton donor or acceptor.

By means of eq 2 the phase behavior of various lipids in excess solution can now be described parametrically, provided that the partial shifts, $\Delta T_{m,i}$, with i = el, h, bond, etc., are known.

The magnitudes of some of these partial phase transition shifts can be deduced directly from the experimental data. The electrostatic shift $\Delta T_{m,el}$, for example, is found by comparing the transition temperatures of dimethylated phosphatidylethanolamine at neutral and high pH (Table I) or, alternatively, by studying the variation of the T_m values for charged lipids with increasing salt concentration (Cevc, unpublished results; Cevc et al., 1980, 1981, 1986). In both instances a value of $\Delta T_{m,el} \sim 5$–6 K is found; around 50% of this is probably not a direct consequence of the surface electrostatics, however, but rather a manifestation of the "electrostatic hydration" (Cevc, 1985; Cevc & Marsh, 1987).

Similarly, the shift of the bilayer chain melting phase transition temperature caused by a change in the number of proton donors (or acceptors) on the head group by one, $\Delta T^{H}_{m,h}$, can be taken to be essentially identical with the difference between the chain melting transition temperatures of sequentially methylated phosphatidylethanolamines at low pH. This is because all such lipids have a similar net positive charge and possess little or no capacity for mutual hydrogen bonding under such conditions. The estimate for $\Delta T^{H}_{m,h}$ based on such an assumption is approximately 7 K (see also further discussion).

The remaining two parameters of eq 2, $\Delta T_{m,bond}$ and $\Delta T^{PO_4}_{m,h}$, must be established by a more laborious procedure. Concluding from the chemical lipid structure at pH 0, all of the titratable groups of the common glycerophospholipids are protonated, or nearly so. At pH 8 only the trimethylammonium and the amino groups and at pH ≥12 solely the trimethylammonium groups still carry a nitrogen-associated net charge. If now only the lipids that bear anionic oxygen(s) and possess at least one donatable proton on a cationic group, e.g., having two protons on a charged ammonium group, are assumed to be involved in the direct, mutual H bonds, this implies that none of the phosphatidylethanolamine derivatives in very acidic or very alkaline suspensions is likely to form such H bonds. By similar logic, phosphatidylcholines or dimethylated phosphatidylethanolamines can be argued not to be connected by H bonds at neutral pH, whereas phosphatidylethanolamines and its monomethylated derivatives can then be thought to be hydrogen bonded. The fact that hydrogen bonds exist between the ammonium and phosphate groups in anhydrous dilauroylglycerophosphodimethylethanolamine single cyrstals (Pascher & Sundell, 1986) is not in conflict with this assumption because in the absence of water the H bonds

Scheme I

	pH ≤1		pH ~8		pH ≥12	
head group		← protonation		deprotonation →		no. of H
PE		$-\Delta T_{bond} - \Delta T_{el} + \Delta T_h^{PO_4} + 0.5\Delta T_h^H$		$-\Delta T_{bond} - \Delta T_{el} - 0.5\Delta T_h^{PO_4} - 2\Delta T_h^H$		2
$PE(CH_3)$	$-\Delta T_h^H$	$-\Delta T_{bond} - \Delta T_{el} + \Delta T_h^{PO_4} + 0.5\Delta T_h^H$	$-\Delta T_h^H$	$-\Delta T_{bond} - \Delta T_{el} - 0.5\Delta T_h^{PO_4} - \Delta T_h^H$	$(-0.5\Delta T_h^H)$	1
$PE(CH_3)_2$	$-\Delta T_h^H$	$-\Delta T_{el} + \Delta T_h^{PO_4}$	$-\Delta T_{bond} - 0.5\Delta T_h^{PO_4} - \Delta T_h^H$	$-\Delta T_{el}$	nil	0
PC	$-\Delta T_h^H$	$-\Delta T_{el} + \Delta T_h^{PO_4}$	$-\Delta T_h^H$	nil	$-\Delta T_{el} + \Delta T_h^H$	0
	cationic		zwitterionic		(anionic)	

connect *interdigitated head groups of opposing bilayers* with an orientation perpendicular to the interfacial plane. For fully hydrated lipids, which are being dealt with here, the head group orientation is believed, in contrast to this, to be generally directed nearly parallel to the bilayer surface; in this latter case, circumstantial X-ray diffraction data and molecular models indicate that direct interlipid hydrogen bonds between the phosphate and dimethylated ammonium groups are quite unlikely.

From all this, a scheme emerges for the evaluation of the magnitude of the total chain melting phase transition shift $\Delta T_{m,p}$ (see Scheme I), for example, in the case of phosphatidylethanolamine derivatives either (1) as a function of the degree of head group methylation at a constant pH value (vertically) or (2) as a function of the degree of head group protonation at a constant number of methylene groups per ammonium (horizontally), the subscript "m" and the left-hand sides of the equations being omitted for brevity and the signs being defined relative to the neutral pH region. In these results it is explicitly considered (1) that the elimination of direct interlipid bonds makes those polar residues available for water binding that otherwise participate in mutual interlipid O–H–O networks (Cevc, 1985), (2) that such a liberation of a water binding group induces half of the shift that would arise from the direct (de)protonation of the same group, and (3) that the shift from the van der Waals interactions is negligibly small, $\Delta T_{m,vdW} \to 0$.

Scheme I and measured shifts of the bilayer chain melting phase transition temperature (Table I, italic values), for example, of dimyristoylphosphatidylethanolamine derivatives as a function of the lipid head group protonation and methylation, yield the following estimates: $\Delta T_{m,bond} \sim 1.5 \pm 1\ K \leq \Delta T_{m,el} \sim 5.5 \pm 0.5\ K \leq \Delta T_{m,h}^H \sim 7 \pm 1\ K \ll \Delta T_{m,h}^{PO_4} \sim 13 \pm 3\ K$. However, these values may vary with the detailed lipid type and state (see further discussion). The electrostatic shift arising from the second charge on a lipid molecule is relatively small: $\Delta T_{m,el}(++, --) - \Delta T_{m,el}(+, -) \leq 3\ K$.

Noteworthy is the fact that the chain melting phase transition temperature of dialkylglycerophospholipids is always slightly higher than that of the corresponding diacyl lipids but the T_m shifts in both cases are nearly the same (Tables I and II).

The difference between the transition temperatures of ester and ether lipids with 16 or 14 carbons per chain amounts to 3–5 K. Supported by indirect experimental evidence by Fringeli and Günthard (1976) and model calculations, I surmise that this is predominantly due to the increase in hydration of the carbonyl group of the ester bond upon chain melting and suggest that this effect can be allowed for, within the framework of the present model, by introducing an additional hydration term, $\Delta T_{m,h}^{C=O} \sim 4$ K, into eq 2 and Scheme I. The relatively small value of this parameter and the constancy of the head group dependent shifts $\Delta T_{m,i}$ imply, however, that the local excess charge and the dipole moment of the carbonyl group are of secondary importance for the overall lipid chain melting phase behavior; consequently, the term $\Delta T_{m,h}^{C=O}$ will not be discussed further.

The values given in the previous paragraph reproduce the experimental data of Table I with better than 85% accuracy. Moreover, they also provide a basis for analogously analyzing and describing the shifts of the chain melting transition temperature of other charged phospholipids upon pH titration or alkylation (Table II). However, the agreement between theory and experiment in this latter case is somewhat less satisfactory, albeit in the worst case still within 20%, owing to the lack of further parameter optimization.

Full titration of the chain melting phase transition of phosphatidylserine, for example, can be explained by realizing that this lipid possesses two acid-ionizable groups (Cevc et al., 1981) so that upon lowering the pH value from neutral first an upward shift of $(\Delta T_{m,el} + \Delta T_{m,h}^H) = 12.5 \pm 1.5$ K and subsequently an increase in the transition temperature by $(-\Delta T_{m,el} + \Delta T_{m,h}^{PO_4} - \Delta T_{m,bond} - 0.5\Delta T_{m,h}^H) = 5.5 \pm 2.5$ K occur, the experimental values being 12 and 4 K, respectively (Table II). The measured shift of −21 K upon deprotonation of the ammonium group also agrees quite well with the theoretical expectation, $(-\Delta T_{m,el} - 0.5\Delta T_{m,h}^{PO_4} - 2\Delta T_{m,h}^H - \Delta T_{m,bond}) = -23 \pm 2.5$ K, if one remembers that the second charge on a lipid head group has approximately half of the electrostatic effect of the first one.

The data of Table II suggest that bilayers of phosphatidic acid or its methyl ester are characterized by a comparable chain melting phase transition temperature in their states of maximal protonation or deprotonation but differ thermodynamically in the neutral pH region, where their ionization state is similar but the protonation state and the H-bonding capacity are dissimilar, methylated phosphatidic acid forming no strong mutual hydrogen bonds. The difference between the chain melting phase transition temperatures of both lipids then suggests that under such conditions $(1.5\Delta T_{m,h} + \Delta T_{m,bond}) = 19 \pm 1$ K so that with $\Delta T_{m,bond} = 1.5$ K one gets $\Delta T_{m,h} = 12 \pm 0.5$ K. [The mean value of the shift associated with the phosphate group deprotonation is thus $\Delta T_{m,h}^{PO_4} = 12.5\ (1 \pm 0.2)$ K, which gives an impression of the parameter spread with changing lipid type.]

The value of $\Delta T_{m,h}^{PO_4} \sim 12$ K can quite accurately reproduce the shifts observed for other charged lipids in their different states too. The change in the chain melting phase transition temperature upon protonation of the phosphatidic acid methyl ester, for example, 15 K, compares reasonably well with the theoretical estimate, $(\Delta T_{m,el} + \Delta T_{m,h}^{PO_4}) = 17.5 \pm 1.5$ K. Better agreement is probably hampered by the possibility that phosphate protonation might cause a structural change in the hydrocarbon region. An indication of this is the protonation-induced lowering of the transition temperature of the molecules in the anhydrous reference state by 30 K for this

and some other lipids,[2] indicative of chain tilt independent of hydration. The reason whey the expected large shift of the transition temperature of phosphatidic acid upon complete protonation, $(\Delta T_{m,el} + \Delta T_{m,h}^{PO_4} - \Delta T_{m,bond}) = 16 \pm 1.5$ K, is never observed in integro is probably the same. Conversely, the shift induced by the complete deprotonation of this latter lipid, −22 K, conforms with the expectation $(-\Delta T_{m,el} - 1.5\Delta T_{m,h}^{PO_4} - \Delta T_{m,bond}) = -22.5 \pm 2.5$ K if the value $\Delta T_{m,el} = 3$ K is used, owing to the double ionization. Similarly, the shift of the chain melting phase transition caused by the protonation of phosphatidylglycerol, 18–19 K, also can be reproduced by the present model quite accurately: $(\Delta T_{m,el} + \Delta T_{m,h}^{PO_4}) = 17.5 \pm 1.5$ K.

It would be an oversimplification to claim that the bilayer chain melting phase behavior is only a function of the lipid chain type and head group hydration, but it is important to realize (1) that the modulation of lipid hydration is the prevalent means of regulation of the membrane properties by the bilayer–solution interface and (2) that the thermodynamic and structural consequences of changing the ionization state, the chain tilt, or the "bulkiness" of lipid head groups may in part reflect the changes in hydration too.

All ions, not only protons, affect the membrane properties, but their results depend strongly on the ion charge density and lipid affinity for binding. Small (e.g., Li^+) (Cevc et al., 1985; Hauser & Shipley, 1981), divalent (e.g., Ca^{2+}) (Hauser & Shipley, 1983), or polyvalent ions [e.g., polyamines, polylysine (data not shown)] are therefore particularly efficient in this respect. It should be noted, however, that noncharged solutes can strongly modify the bilayer phase behavior too if they can interfere with the membrane–solution interface. Thermodynamic measurements with the biologically active disaccharide trehalose (Crowe et al., 1986), other carbohydrates, or the immunoactive carbohydrate derivative muramyl dipeptide illustrate this. Even millimolar concentrations of the chemical precursor of the latter, muramic acid, for example, can cause the chain melting phase transition of dimyristoylphosphatidylcholine to shift upward by more than 5 K (data not shown).

Two conclusions emerge from this. First, the interfacial hydration provides, apart from the lipid chains, the most important source of the regulation of the lipid and membrane phase behavior. Second, on the basis of the values for free energy changes associated with the measured chain melting phase transition shifts, $\Delta G_{bond,m} \sim 0.2$ kJ/mol $\leq \Delta G_{el,m} \sim 0.75$ kJ/mol $\leq \Delta G_{h,m} \sim 1.8$ kJ/mol (the total hydration free energy change being $\Delta G_{h,m} \geq 5 - 10$ kJ/mol), one can see that the regulation of the membrane properties by means of changing the properties of the lipid polar head groups and the ionic solution is energetically inexpensive. Molecular rationale for this (Cevc & Marsh, 1985) together with a detailed evaluation of the transitional free energy changes and the phases shifts from a molecular force theory is given elsewhere (Cevc, 1985; Cevc & Marsh, 1987; Cevc et al., 1986; Träuble et al., 1976).

The procedure used here for analyzing and explaining the chain melting properties of plain disaturated phospholipids also highlights the phase behavior of other, unsaturated, or les common lipids, provided that allowance is made for the variations in the relevant thermodynamic quantities, such as the entropy of chain melting. [More thorough discussion of this aspect can be found in previous works (Cevc & Marsh, 1985; Cevc et al., 1986).]

For example, increasing the chain length causes the phase transition shifts typically to become smaller (cf. eq 2 and Table I and II), owing to the proportionality between the chain melting entropy and the number of hydrocarbons per chain (Marčelja, 1973). Introduction of double bonds into the chain region, however, can more than compensate for this because it typically decreases such entropy change. The difference between the transition temperatures of phosphatidylcholine and phosphatidylethanolamine with two elaidic chains (C18:9t), 26.5 K (Gagne et al., 1985), is much larger than that of the corresponding fully saturated distearoylphospholipids (C18:0), 17.5 K (Seddon et al., 1983a). Likewise, the shifts caused by the protonation of the phosphate group or deprotonation of the ammonium group of phosphatidylethanolamines are greater for the unsaturated lipids, being 3 and 1.5 K or approximately 26 and 19 K for the C18:9t and C18:0 chains, respectively. If this chain length dependence of the transition entropy is allowed for by normalizing the measured shift values with respect to the measured $\Delta S_{m,ref}$ data by means of eq 2, the resulting renormalized magnitudes of $\Delta T_{m,i}$ become nearly independent of the chain length or type.

Chain unsaturation thus renders membranes more sensitive to the modulation of their properties by the lipid head group and ionic solution effects. This may be biologically relevant and part of the reason why living organisms in the long run maintain their membrane fluid by using unsaturated rather than short chain lipids.

Hitherto I have discussed solely the lipid chain melting phase transition, but a similar approach can also be used to explain the effects of the polar membrane region on other phase transitions such as the bilayer pretransition or the conversion from a lamellar to nonlamellar lipid state. Moreover, the somewhat smaller changes in entropy associated with the latter transitions make the effects even greater. Typically, the probability that a nonlamellar phase will emerge at somewhat elevated temperature increases with the stability of the ordered bilayer phase. For example, for the maximally protonated state of phosphatidylethanolamines and for the monomethylated derivatives of this lipid with two alkyl or alkenoyl chains of more than 16 carbons/chain, the chain melting is close to or identical with the critical temperature for the formation of nonlamellar lipid phase(s). Conversely, at pH 7 the chain melting transition temperatures and the temperature of transition into a nonbilayer phase differ substantially, by 27, 25, and 16 K for the distearoyl-, dielaidoyl- and dioleoylphosphatidylethanolamines. Also, alkylated derivatives of the former two lipids then no longer form nonlamellar phases in excess buffer under 100 °C.

In summary, lipid membranes and their bathing solutions have been shown here to represent one thermodynamic entity. The membrane properties are therefore strongly sensitive to the properties and characteristics of the bilayer–solution interface. This provides a regulatory mechanism for controlling the membrane structure and phase behavior, which is much faster and energetically less expensive than that based on the adaptation of the lipid chain composition but has been neglected to date. This lipid-dependent membrane control mechanism should be particularly relevant in cases in which

[2] Other charged lipid molecules, e.g., phosphatidylserine, can exhibit similar behavior (Cevc et al., 1985, and unpublished data), and comparable observations have been reported for uncharged lipids (Seddon et al., 1983b, and unpublished data). Perturbation theory that relies exclusively on the explanation of the lipid membrane phase behavior in terms of the fixed transition temperature shifts permits no allowance for this unless variable reference states are used, which is tantamount to the introduction of new, undesirable adjustable parameters. A simple perturbation approach, consequently, is inappropriate in cases where the structures of lipid anhydrides differ, which indicates that the procedure introduced in this work should be used with prudence.

only short-term, transient modification of the bilayer properties must be achieved or in such biological situations in which only parts of the membrane surface, e.g., the contact points, membrane complexes, or lipid–protein aggregates, are involved.

Some observed differences in the thermodynamic behavior of lipid and biological membranes might therefore reflect effects within the polar rather than in the apolar bilayer regions. This has been demonstrated here at least for the lipid chain melting and argued for the bilayer-to-nonbilayer phase transitions, stressing that the bilayer surface hydration effects in both cases are more important than surface electrostatics. Major modifications in the bilayer state can thus easily be achieved by modulating the effective interfacial polarity via the protonation or chemical modification (e.g., alkylation) of the surface polar residues or else can be a result of the adsorption to or desorption from the bilayer surface of various charged or noncharged solutes. It can even be envisaged that conformational changes involving nonlipid membrane components may induce similar effects. The latter two mechanisms of controlling the membrane properties are reported and explained here for the first time and provide further evidence for the paramount role of hydration phenomena at biological interfaces.

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