How Membrane Chain-Melting Phase-Transition Temperature Is Affected by the Lipid Chain Asymmetry and Degree of Unsaturation: An Effective Chain-Length Model†

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ABSTRACT: Hydrocarbon effects on the lipid chain-melting phase-transition temperature are analyzed. The membrane fluidization temperature is shown to increase with the effective chain length, which is proportional to the thickness of the well-packed hydrocarbon region. The latter, as a rule, increases with the length of the longest ordered and aligned segment on each chain. This conclusion is independent of the cause for the reduced chain packing in membrane interior: chain unsaturation (which effectively decouples the two hydrocarbon segments disjoined by a double bond) or chain asymmetry (which causes the terminal hydrocarbon segments to lose close contact) both affect the bilayer chain-melting phase-transition temperature comparably on the effective chain-length scale. Thermodynamic consequences of the trans unsaturation are approximately 50% smaller than the effects of the double bonds in the cis conformation, owing to the smaller membrane perturbation by the former double bonds. A simple quantitative model is introduced for the analysis of the phospholipid chain-melting phase behavior. This new model permits quantitative predictions of the chain-melting transition temperature solely on the basis of the known lipid chemical composition. It also explains lipid sensitivity to the hydrocarbon type and attachment. The model agreement with the experimental data is usually better than to within 99% and thus comparable to experimental scatter, even when only a few or no adjustable parameters are used. The membrane fluidization temperature is calculated for a number of potentially interesting, also as yet unexplored, phospholipids, and the biological significance of the effective chain-length concept is discussed.

Lipid bilayers are the backbone of all biological membranes. Lipids act as a "solvent" for the integral membrane proteins; lipids, such as phosphatidylinositol, can play a role as second messengers; lipids serve as anchors for many receptors and act as catalytic factors and as an energy storage system, etc. Lipids, moreover, are progressively gaining importance as the basic component of innovative pharmacological and biotechnological applications. It is, therefore, important to understand how the physicochemical properties of the lipid bilayers, such as the phase state, are controlled by the structural variations at the molecular level.

The effect of the hydrocarbon chain length on the lipid phase behavior is well established and understood. It has been hardly attempted to date, however, to calculate the gel-to-fluid phase-transition temperature, even of most common phospholipids, as a function of the chain unsaturation or asymmetry. One of the few successful attempts was the approach of Berde et al. (1980), who have theoretically reproduced the experimental data of Barton and Gunstone (1975) with a somewhat better than 7% accuracy.

In previous contributions to this and other journals, I have discussed the effects of the polar lipid segments on the bilayer phase behavior influenced by the polar part of the membrane (Cevc, 1987, 1988). In this work I wish to present a method for the calculation of the lipid chain-melting phase-transition temperature as a function of the lipid chain length, position of attachment, or degree of unsaturation. For the sake of brevity, most of the following discussion will be limited to phosphatidylcholines. But this is solely a matter of convenience and all specific statements, with only minor modifications, also hold for any other lipid class with related structure.

Membrane Fluidity

The most important interactions between the hydrocarbon chains in a lipid bilayer are the dispersion (van der Waals) attraction and the excluded volume repulsion [see, for example, Cevc and Marsh (1987) and references therein]. Such interchain forces, which depend on the chain length and, very strongly, on the relative chain order, are both short ranged and highly anisotropic. This causes the lipid hydrocarbons, in many cases, to be in an ordered state.

At some lipid-dependent characteristic temperature, the thermal excitations overcome this hydrocarbon ordering tendency so that long-range orientational order within lipid bilayers is lost. The resulting molten fluid state is typically found in all biological membranes (Overath et al., 1975).

Membrane fluidity and dynamics thus may exert an effect on and depend upon the phase condition of the constituent lipids. On the one hand, lipid chain length, class, and degree of unsaturation all play an important role; on the other hand, the head-group type, hydration, and degree of (de)protonation (Cevc & Marsh, 1985) or ionization (Cevc, 1987, 1988, 1989) are quite influential. Short-chain lipids are very strongly sensitive to the head-group effects, but this influence as well as chain-length effects both level off with increasing number of the carbon atoms in each chain (see further discussion).

Chain Melting

Thermodynamic consequences of the variation of the hydrocarbon linkage with the glycerol lipid backbone can be operationally ascribed to the interfacial region. Consequently, they are not the topics of this report. Also the effects of the chain attachment also relatively small, at least when only fluid

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lamellar phases are concerned. The difference between the chain-melting phase-transition temperature of the diester, diacylphosphatidylcholines and of the corresponding dialkyl, diether lipids is about 50% of the shift caused by the chain prolongation by a single CH_2 unit. For example, for dioctadecylphosphatidylcholine, an ether (dialkyl) phospholipid with 18 carbons per chain, the chain-melting temperature is approximately 3.5 °C higher than that of the corresponding diacylphospholipid, distearoylphosphatidylcholine (Cevc, 1989), being 59 and 55.5 °C, respectively.[1]

The role of the chain asymmetry is greater and more complex. Reducing the number of the carbon atoms on either of the two saturated hydrocarbons causes the chain-melting transition temperature to decrease (for experimental data see, e.g., the insert to Figure 2). For C_{18} phosphatidylcholines, the resulting shift of the transition temperature is up to 42 °C (Mattai et al., 1987).

Even more dramatic are the consequences of the chain unsaturation. A single double bond in a cis configuration in each hydrocarbon chain may evoke, for example, a downward shift of the chain-melting phase-transition temperature of up to 63 °C, in the case of the dioctadecenoylphosphatidylcholines. This effect is maximal if the double bonds are approximately in the middle of the chain for the cis as well as trans double bonds, but the shift magnitude for the latter is smaller by nearly a factor or two (Silvius et al., 1979).

In the following, I will show how the measured chain dependence of the hydrocarbon fluidization temperature can be understood and reproduced accurately by estimating and considering the effective chain length of the lipids. I will discuss how such an approach can be used to predict the thermodynamic properties of other, as yet unexplored, lipids and will explain how the tilt of the ordered lipid hydrocarbons can be gauged from the known effective chain-length data.

The prerequisite for all this is to find a suitable means for the evaluation of the order–disorder bilayer phase-transition temperature as a function of the hydrocarbon chain length.

Hydrocarbon Effects

This goal can be achieved in several ways. Most sophisticated approaches make use of the exact statistical mechanical chain models in combination with the extensive numerical calculations and some input knowledge about the nature and characteristics of the investigated lipids (Marčelja, 1974; Meraldi & Schliter, 1981). Simpler statistical mechanical bilayer theories rely on the use of some special lattice models (Caille et al., 1980; Nagle, 1980; Pink, 1982); still others represent the hydrocarbon chains as hard cylinders (Jacobs et al., 1975) or elastic rods (Jähnig, 1979), for example.

In this work, I deliberately apply the most unpretentious approach. To calculate the lipid chain-melting phase-transition temperature, simple polynomial expressions, suitable for daily use, are used. Such heuristic usage of the model equations should not mislead the reader, however, to the false conclusion that in this work a simple polynomial regression analysis is being performed. The polynomial in eq 1, which is the basic working expression of the present model, can be traced back to the detailed descriptions of interlipid interactions at the molecular level (Ishinabe, 1980). Its specific form can also be justified by using certain thermodynamic considerations (Seddon et al., 1983) or phenomenological arguments (Broadhurst, 1962). At the present stage, I prefer to leave

[1] A significant proportion of this difference stems from the disparities in molecular hydration and is not a direct effect of the modified chain–chain interactions.

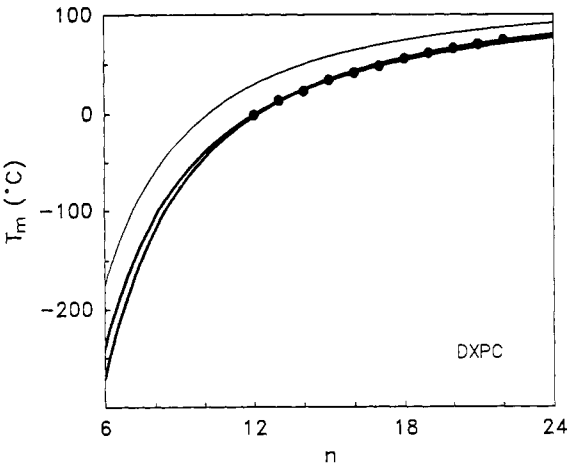

FIGURE 1: Lipid chain-melting phase-transition temperature T_m as a function of the chain length n for bilayers with tilted chains (thick curve) and for the membranes with nontilted hydrocarbons (thin curve) in the gel phase. Symbols denote the corresponding measured chain-melting transition temperatures [from Lewis et al. (1987) and Cevc (1990)] of fully saturated symmetric diacylphosphatidylcholines. Appropriate model calculations (thick curves) are based on eq 2. The results are obtained from alternative equations $T_m(n) = 414(1 - 2.81/n - 15.89/n^2) - 273$ and $T_m(n) = 414(1 - 3.20/n - 10.64/n^2) - 273$, which have been optimized for both experimental data sets, respectively. They deviate from each other in the short chain length region, indicative of the extrapolation problems. The thin curve is described by $T_m(n) = 414(1 - 2.29/n - 11.03/n^2) - 273$ and fits nearly perfectly the chain-melting phase transition ($L_\beta \rightarrow L_\alpha$) of phospholipids with nontilted chains such as phosphatidylethanolamines.

the model at this level. It would be rather straightforward, while also being lengthy and tedious, to implement the concepts advocated in this work into a detailed and fanciful theoretical model. But such theoretical excercise would add little to the basic scientific message of this work.

Chain Length. The simplest form of the phenomenological polynomial expression that describes adequately the chain-melting phase transition temperature of an arbitrary phospholipid as a function of the number of carbon atoms per chain, n, reads

$$T_m(n) = T_m(\infty)(1 + n_m/n + n_h/n^2 + ...) \quad (1)$$

The term n_m in the above expression corresponds approximately to the length of the shortest segment for which a first-order chain-melting phase transition is possible (Nagle & Wilkinson, 1982); the n_h term allows phenomenologically for the head-group and other end effects (Cevc & Marsh, 1987). The parameter T_m (∞) refers to the chain-melting transition temperature of a hypothetical lipid with infinitely long chains. In principle, its value should coincide with the chain-melting transition temperature of infinitely long simple hydrocarbons; in this work, it is thus assigned a value of 414 K (Broadhurst, 1962).

Application of eq 1 to the chain-melting phase-transition data (Lewis et al., 1987; Cevc, 1990) of fully saturated diacylphosphatidylcholines suggests

$$T_m(n) = 414(1 - 3.20/n - 10.64/n^2)\ \text{K} \quad (2)$$

This reproduces the available experimental data for phosphatidylcholines to within 2 °C (0.5%) (Figure 1 and Table I).

Specific values of the model parameters n_m and n_h suitable for the description of the chain-melting phase transitions of other common diacylphospholipids are given in Table II.

Chain Unsaturation. Chain unsaturation depresses the orientational chain order in the hydrophobic membrane interior, as concluded from deuterium nuclear magnetic reso-

Table I: Measured and Calculated Chain-Melting Transition Temperatures of Fully Saturated Symmetric and Asymmetric Chain Phosphatidylcholines as a Function of the Chain Length and Composition[a]

chain length	expt		eq 2	
12	−1	(−2.1)[b]	0.1	(−1.7)[b]
13	13.5	(13.7)	13.1	(12.5)
14	23	(23.9)	24.0	(24.3)
15	34	(34.7)	33.2	(34.2)
16	41.5	(41.4)	41.1	(42.7)
17	48	(49.8)	47.9	(49.8)
18	55.5	(55.3)	53.9	(56.0)
19	61	(61.8)	59.1	(61.5)
20	65.5	(66.4)	63.8	(66.4)
22	74.5	(74.8)	75.1	(74.5)

chain comp	expt	eq 6	chain comp	exp	eq 6
17:17	49.0	48.3	11:17	12	7.6
16:18	48.8(51)[c]	48.3	12:18	21(23.5)	18.7
15:19	44.8	44	13:19	30	28.3
18:16	44.4(44)	42.4	14:20	38	36.5
14:20	40.0	41.4	15:21	44	43.7
19:15	39.0	38.9	16:22	50.5	50
13:21	34.1	40.1	17:23	56	55.6
21:13	(34.1)	36.1	18:24	61	60.5
20:14	33.2	36.9	19:25		65
			20:26	68.5	69

[a] Note that the model uncertainty is always comparable to and not greater than the experimental scatter. [b] Values in brackets pertain to the data by Lewis et al. (1987) and to the results of appropriate model calculations, respectively. For the corresponding model parameters, see footnote a to Table II. [c] Values in brackets give the data by Mattai et al. (1987).

Table II: Effective Model Parameter Values for the Evaluation of the Chain-Melting Transition Temperatures of Various Phospholipids at pH 7 by Means of eq 1

lipid[a]	diacyl		dialkyl	
	n_m	n_h	n_m	n_h
phosphatidylethanolamine (dry)	−1.72	−3.58		
phosphatidylethanolamine + $2H_2O$	−2.42	0.14		
excess water or buffer with phosphatidylethanolamine	−2.29	−11.03	−2.11	−11.31
phosphatidylserine	−2.30	−17.12	−2.52	−12.50
phosphatidic acid	−2.25	−10.92	(−0.98)	(−27.05)
phosphatidylglycerol	−3.03	−13.07	−2.80	−14.09
cardiolipin	−2.93	−5.54		
phosphatidylcholine[b]	−3.20	−10.64	−3.20	−9.12

[a] Parameter variability is significant only if the root of the sum of the differences $(\delta n_m + \delta n_h)^{0.5}$ differs from the individual difference δn_m by more than a factor of 2, approximately. If so, smaller absolute values of the model parameter suggest a smaller chain tilt and/or lower degree of the head-group hydration (and/or ionization). [b] Determined from the data given in Cevc (1990). If other sets of data are used, slightly deviant optimal model parameter values are obtained; for example, for the results by Lewis et al. (1987) $n_m = -2.81$ and $n_h = -15.89$, the chain-length-dependent correction term factor for eq 6 being 0.209.

nance data by other authors (Seelig & Seelig, 1977). Concomitantly, it also lowers the chain-fluidization temperature. Double-bond position is extremely important for the magnitude of this effect; the measured differences between the gel-to-fluid transition temperatures of various dialkenoylphosphatidylcholines may be anywhere between 10 and nearly 80 °C, for example.

Figure 2, and especially its insert, provide evidence for this. The predominance of double bonds near the chain middle, $n_{cis} \sim n/2$, is obvious. The approximate symmetry of the T_m vs n curve around the resulting minimum is also well documented (see also Figure 3 for comparison). Last but not least, the transition temperature for the phosphatidylcholines with a

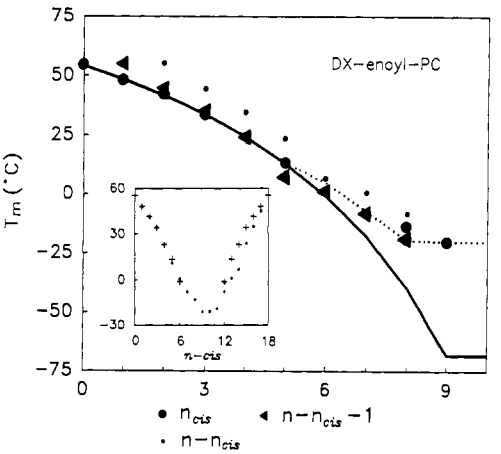

FIGURE 2: Effect of the position of cis-double bonds, n_{cis}, on the chain-melting phase-transition temperature of symmetric monounsaturated dioctadecenoylphosphatidylcholines. The filled circles show the transition-temperatures values reported by Barton and Gunstone (1975) for $n_{cis} < 0.5n$; original data (bullets), supplemented with the corresponding $T_m(n_{ef})$ data of the saturated phosphatidylcholines (crosses) are shown in the insert to the main figure body. Dots in the main figure pertain to the corresponding $n_{cis} > 0.5n$ data mirrored around the $n = 10$ point. The triangles directed toward the left were obtained by shifting the points toward the left by one, in order to conform with the rule $n_{ef} = n - n_{cis} - 1$. The thick curve, calculated by means of eq 2, and the data measured for diacylphospholipids of variable chain length agree well except when the effective chain length is comparable to half of the nominal hydrocarbon length and the two segments differ in length by less than 3–4 methylene groups. Equation 6 can be used to find the correct transition temperature values in the latter case (see dotted line and Figure 3).

cis-unsaturated double bond at the second carbon atom in an 18-carbon chain coincides with the transition temperature of the corresponding fully saturated lipid with 16 carbons per chain. In fact, the chain-melting temperature of most phosphatidylcholines with one double bound per chain is strikingly similar to the corresponding transition temperature values of the saturated phosphatidylcholines that fulfill the condition $n_{saturated} = n_{ef} \equiv n_{unsaturated} - n_{cis}$, when $n_{ef} > n_{unsaturated}/2$ (cf. insert to Figure 2). This means that that as far as their chain-melting characteristics are concerned all monounsaturated phospholipids behave as if they had saturated chains with an effective chain length identical with the length of the longer of the two chain segments that are separated by a double bond. [The latter is given by the difference $n_{unsaturated} - n_{cis}$, when the value of this expression exceeds half of the nominal chain length (cf eq. 6)]. I hypothesize that the shorter of the two segments is normally fluid at all accessible temperatures. Consequently, it should not exhibit an observable chain-melting phase transition. Its effect on the remainder of the chain is minimal owing to the size disproportions of the long- and short-chain parts.

To conform with this rule, the transition-temperature data (crosses) for various diacylphosphatidylcholines in the insert to Figure 2 are placed side by side with the original data for dioctadecenoylphosphatidylcholines at $n_{saturated} = n_{ef}$ (bullets). It will have been seen that the harmony between the two sets of results is nearly perfect for the positive values of the effective chain length. But for the negative values the agreement is worse. Both sets of data can be brought in agreement, however, by a reduction of the equivalent chain length in the latter case by one ($n_{ef} \equiv n - n_{cis} - 1$) (see the main figure body).

This suggests that the chain-melting polymorphism of phospholipids with a single cis double bond per chain is governed by the longer of the two segments that are disjoined by the double bond. This explains a posteriori why the effective

chain length must be reduced by one whenever the upper segment is the longer of the two chain segments: since the upper part of each chains is "unfree" at both ends, it must have a slightly different value of the minimal critical chain length n_m (cf. eq 1). In other words, the upper segment lacks one methylene group capable of undergoing orientational isomerization; correspondingly, it should melt at a relatively low temperature.[2]

Predictions based on such considerations are in good agreement with the exerimental data as long as the extension of the shorter segment is only a fraction of the long-segment length. Figure 2 corroborates this. Model accuracy is less satisfactory, however, if the double bonds are relatively close to the chain middle. Model extrapolations in such a situation are too low, by approximately 47 °C in the case of dioctadecenylphosphatidylcholines.

One of the reasons for this is the uncertainty of the extrapolation in the short-chain regions (see left part of the thick curves in Figure 1).[3] But even greater error arises from the neglect of the contributions from the shorter chain segment. For the chains that are nearly halved by double bond(s), the latter is quite significant.

In order to account for this model imperfection, and to improve the accuracy of eq 2, one can introduce a correction term; this must conpensate for the effects of the shorter of the two hydrocarbon segments. To retain model consistency, but without an obvious molecular rationale, another equation of the type of eq 1 can be used for this purpose.[4] Optimization of such an equation on the basis of the data shown in Figure 2 yields

$$\Delta' T_{m,kor}(18,n_{cis}) = 281\left(1 - \frac{0.176}{|10 - n_{cis}|}\right) \text{K} \qquad (3)$$

This equation, obtained by the adjustment of theoretical and experimental data in combination with eq 2, brings the calculated transition temperatures of phosphatidylcholines in a better than 0.5% (2 °C) agreement with the experimental data for dioctadecenoylphosphatidylcholines (compare full symbols and dotted curve in Figure 2).

In order to become applicable to other lipids with different chain lengths, eq 3 should allow for the hydrocarbon length. The desired scaling, which considers the fact that the significance of the short-segment contribution is relatively more important for the lipids with short hydrocarbons, can be achieved in many ways. Perhaps the most obvious one is to normalize the magnitude of the correction term with regard to the chain-length variability of the chain-melting transition temperature at a given value of n.

Let me exemplify this for dipalmitoleoylphosphatidylcholine ($n = 16, n_{9cis}$) and dioleoylphosphatidylcholines ($n = 18, n_{9cis}$). Incremental variation of the corresponding chain-melting phase-transition temperature of diacylphosphatidylcholines with 16 and 18 carbons per chain has relative magnitudes of $T_m(n) - T_m(n-1)/T_m(n) = 0.027$ and 0.019, for $n = 16$ and 18, respectively; the appropriate scaling factor is thus

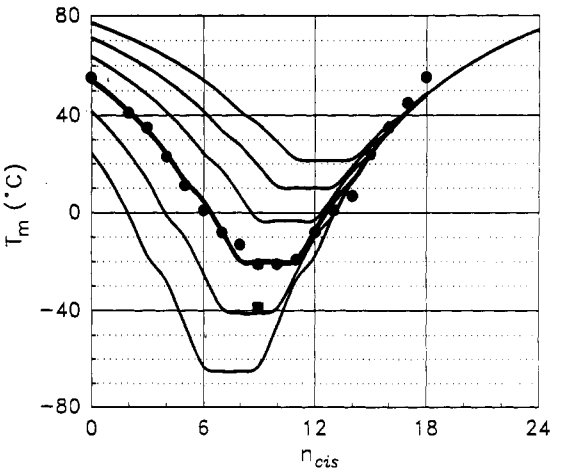

FIGURE 3: Chain-melting phase-transition temperature of symmetric unsaturated dialkenoylphosphatidylcholines as a function of the hydrocarbon length and position of the individual double bonds. The full curves correspond to phosphatidylcholines with 14, 16, 18, 20, 22, and 24 carbon atoms per chain, from the bottom to the top, respectively. They were calculated by means of eq 6 with minor corrections in the central region. The thick line pertains to dioctadecenoylphosphatidylcholines ($n = 18$) and agrees well with the corresponding experimental data (filled circles). The experimental result for $n = 16$ (square) is in nearly perfect accord with the model extrapolation.

0.027/0.019 = 1.42. This suggests that the correction terms, given by an adjusted eq 3, should have a magnitude of 47 and 33.3 °C, respectively. In the context of our model, this implies the corresponding transition temperatures to be −34 and −20 °C. Appropriate experimental values for the chain-melting phase-transition temperature are −36 and −21 °C (Faucon et al., 1976).

The generalized form of the phenomenological correction term thus can be written in the following apparently lengthy but extremely simple form:

$$\Delta T_{m,kor}(n,n_{cis}) = 281\left(1 - \frac{0.176}{|10 - n_{cis}|}\frac{T_m(n) - T_m(n-1)}{T_m(18) - T_m(17)}\frac{T_m(18)}{T_m(n)}\right) \text{K} \qquad (4)$$

with numerical factors stemming from eq 3; the transition temperatures are given by eq 3.

The gel-to-fluid phase-transition temperature of symmetric-chain phosphatidylcholines as a function of the chain length and degree of unsaturation can thus be calculated as

$$T_m(n,n_{cis}) = T_m(n_{ef}) + \Delta T_{m,kor}(n,n_{cis}) \qquad (5)$$
$$\simeq T_m(n_{ef}) \qquad n_{ef} \geq 0.5n + 4$$

where the effective chain-length is defined as

$$n_{ef} = \begin{cases} n - n_{cis}, & \text{terminal segment longer} \\ n - n_{cis} - 1, & \text{upper segment longer} \end{cases}$$

A correction term needs to be considered only when the two chain segments disjoined by a double bond are less than four methylene groups different.

Within the framework of present model, one can explain or foresee chain-melting phase-transition temperatures of arbitrary polyunsaturated phosphatidylcholines and, by analogy, of all other phospholipids. For example; the experimental chain-melting phase-transition temperatures of phosphatidylcholines with 18 carbon atoms per chain and one, two, or three double bonds at positions 9, 12, and 15 are −21, −53, and −63 °C, respectively. From eq 6, one gets −21, −58, and −67 °C. The measured T_m values for $n = 22$, 24, and 26

[2] Obviously, by simply reversing the argument, one could declare the effective chain length initially to be given by the expression $n_{ef} = n - n_{cis} - 1$ and argue that for the lower part this value should be increased by one owing to the free end of the hydrocarbon chain.

[3] Short-chain phosphatidylcholines form metastable gel phases; it will, consequently, probably never be possible to measure the required values directly.

[4] This is the only ad hoc assumption of the present model. Fortunately, it must be used only when the length difference between the longer and the shorter chain segments is less than 3–4.

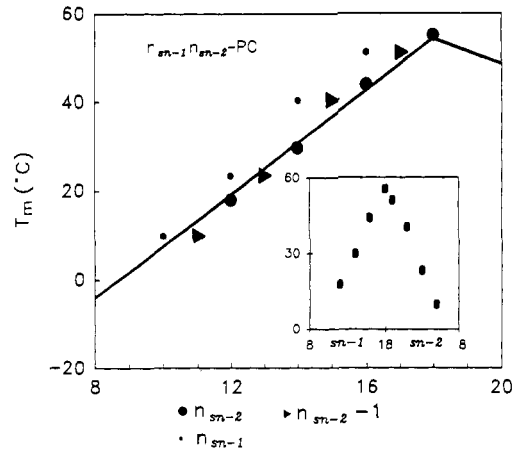

FIGURE 4: Effect of the chain asymmetry on the fluidization temperature of phospholipid bilayers. The filled circles give the transition temperatures published by Mattai et al. (1975) for $10 < n_{sn-1} \leq 18$; dots give the corresponding n_{sn-2} data mirrored around the $n = 18$ point. To obtain the positions of triangles, the latter data set was shifted toward the right by one, as required by the rule discussed in the text: $n_{sn-2,ef} = n_{sn-1} + 1$. Corresponding model predictions, obtained from eq 6, are shown as a straight full line. Nonmirrored data are shown in the insert.

in a system with two cis double bonds at positions 5 and 9 are 7 ± 3, 29 ± 2, and 42 °C, respectively (Ayanoglu et al., 1990); the calculated chain-melting transition temperatures are 1, 26, and 44 °C, respectively.

Equation 6 thus offers a simple, easy to use, and quite reliable tool for predicting the chain-melting transition temperatures of common phospholipids. Its implications for different phosphatidylcholines as a function of the hydrocarbon length and double-bond position are given in Figure 3. The thick curve pertains to the dioctadecenoylphosphatidylcholines. The thiner curves correspond to other phosphatidylcholines with one unsaturated bond per chain and biologically reasonable chain lengths. Double bonds in a cis configuration in all cases are assumed to be at identical positions in both chains.

The thermodynamic consequences of the double bonds in trans configurations are smaller than the effects of cis double bonds. On the basis of published data by other authors (Silvius et al., 1979), I conclude that a double bond of the trans type on each hydrocarbon chain affects the chain-melting phase-transition temperature approximately half as strongly as a single double bond in the cis configuration at the corresponding position on the *sn*-1 chain (not shown). The effects of trans unsaturation, therefore, will not be discussed separately; appropriate model equations for treating the lipids with trans unsaturation are similar to those given in the next section for the modeling of polymorphism of lipids with two unlike chains.

For lipid mixtures with identical head groups and diverse hydrocarbon chains, the temperature of the gel-to-fluid phase transition, in the simplest approximation, is given by an arithmetic mean of the individual transition temperatures, except when the chain lengths differ by more than 3–4 methylene groups.

Chain Asymmetry. A complete set of the transition temperature data for phosphatidylcholines with two different chains and between 10 and 18 carbon atoms per hydrocarbon has been published by Mattai et al. (1987); it is also reproduced in the insert to Figure 4. Less extensive and complete, but confirmatory, results have been reported by other authors (Keough & Davis, 1979; Chen & Sturtevant, 1981; Mason et al., 1981; Stümpel et al., 1981, 1983; Coolbear et al., 1983; Eriksson et al., 1985; Fragata et al, 1985).

All of these thermodynamic data convey, despite their apparent complexity, quite straightforward features. With a growing discrepancy between the lengths of the chains in the *sn*-1 and *sn*-2 positions, the total interchain attraction diminishes; consequently, the chain packing density in the intercalated terminal hydrocarbon regions decreases. Ultimately, this lowers the stability of the ordered bilayer phase and shifts the chain-melting transition temperature downward.

Within the framework of the new effective chain-length concept introduced in this work, it is quite easy to paraphrase such conclusions mathematically. It suffices to postulate that the terminal segment of the longer chain, a priori, is (partly) fluid. This suggests that the ordering contribution from the terminal segment will be negligible, its contribution to the chain-melting transition temperature thus being nonsignificant.

Effects of the chain attachment, however, normally play a marked role. To make allowance for the difference between the *sn*-1 and *sn*-2 chains, one can take the equivalent length of the *sn*-2 chain to be nominally shorter by one CH_2 group, for example. This compensates for the reduced ability of the uppermost hydrocarbon segment (Hauser et al., 1978), which is in the bent region, to participate in the chain fluidization.

Subsequently, simple linear extrapolation and eq 2 can be used. The temperature of the chain fluidization for the phospholipids with asymmetric chains is then identified with the chain-melting temperature of the corresponding lipids with two identical chains reduced by the shift that originates from the nonoverlapping of the terminal intercalated segments; the effective hydrocarbon length hereby corresponds to the length of the longer of the two chains.

The magnitude of the correction shift may be evaluated in a perturbative manner in order to avoid the usage of adjustable parameters. For example, it may taken to be proportional to the difference $\Delta n = n_{sn-1} - n_{sn-2}$ and to the slope of the corresponding transition temperature variation at the given chain length

$$T_{m,as}(n,\Delta n) = T_m(n) - \Delta n[T_m(n) - T_m(n-1)] \quad (6)$$

where $T_m(n)$ is again given by eq 2 and the length of the "noninteracting" segment is

$$\Delta n = \begin{cases} n_{sn-1} - n_{sn-2}, & \text{sn–1 chain longer} \\ n_{sn-2} - n_{sn-1} - 1, & \text{sn–2 chain longer} \end{cases}$$

The straight line in Figure 4 illustrates graphically how well eq 6 works for the desired purpose. Similar data pertaining to phosphatidylcholines with 18 carbons on each of the longer chains are also shown in Figure 5 in an alternative representation together with the corresponding model calculations (thick curves). The thin lines in the same plot give the chain-melting temperature of the asymmetric phosphatidylcholines with chain lengths ranging between 14 and 24.

Further supporting evidence comes from two recent publications (Lin et al., 1990; Wang et al., 1990) that present data for the order–disorder phase-transition temperature of synthetic phosphatidylcholines with unequal acyl chains. These published results are in nearly perfect agreement with the results of this paper. The corresponding values are given in Table I.[5]

In light of the fact that hydrocarbons of asymmetric lipids in the gel state tend to interdigitate when one chain is approximately half the length of the other (Shah et al., 1990), it remains to be shown by some direct experimental method,

[5] Also, the set of subtransition temperature data contained in the latter article can be analyzed by using the ideas of this work.

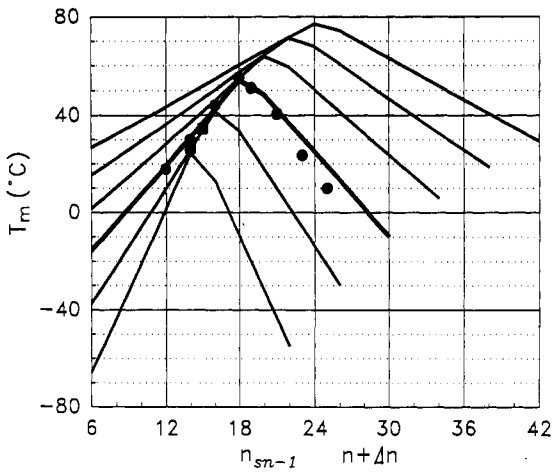

FIGURE 5: Effect of the hydrocarbon length and chain asymmetry on the chain-melting phase-transition temperature of phosphatidylcholine membranes in water calculated by means of eq 6. The thick curve represents in an exemplary fashion the results for phosphatidylcholines with 18 carbon atoms in the longer chain, the corresponding experimental transition-temperature values being shown as filled circles. Results pertaining to the *sn*-2 chain are mirrored around the $n = 18$ position, this is, they are located at $n_{ef} = n + \Delta n$. The chain-fluidization temperature is predicted to decrease with the increasing disparity of the *sn*-1 and *sn*-2 chain lengths; the shorter are the hydrocarbons the stronger is this effect. Because of the specific choice of data presentation, this gives rise to bell-shaped curves with different widths. The transition temperatures of asymmetric phosphatidylcholines with 16 carbons per chain are shown as squares and lie nearly precisely on the calculated curve. Despite the fact that eq 6 contains no adjustable parameters, the agreement between theory and experiment is nearly quantitative.

such as deuterium nuclear magnetic resonance, how the "fluidity profiles" throughout the bilayer look out in such a phase. Perhaps the deviation for the extremely asymmetric phosphatidylcholines of the chain-melting transition temperature data from the straight-line dependence (not shown) is also indicative of this effect.

Asymmetric Unsaturated Chains. Most lipids from the biological sources have asymmetric as well as unsaturated chains. To estimate the chain-melting phase-transition temperatures of such phospholipids, one can combine the results of the last two sections.

Head-Group and Solvent Effects

Hitherto, I have only discussed the polymorphism of the fully hydrated phosphatidylcholines in water. But the question also arises as to whether or not the effective chain length model can be used to analyze and predict the chain-melting properties of other phospholipids or even of the incompletely hydrated systems.

It can. To evaluate the temperature at which an order–disorder phase transition of a phospholipid other than phosphatidylcholine will take place one can, on the one hand, start with eq 6. The known published head-group-dependent phase-transition shifts (Cevc, 1987) can then be subtracted from or added to the phosphatidylcholine transition temperatures to get the desired transition temperature values.

Alternatively, a more pragmatic approach can be taken. Equation 2 can be reoptimized so as to become directly applicable to the lipid class under study. Results of several such optimizations are summarized in Table II.

Both methods work comparably well and guarantee better than 97.5% accuracy in every case. In practical terms, this means than one can theoretically guage the chain-melting phase-transition temperature of any phospholipid quite reliably, solely on the basis of its chemical composition.

With biological lipid extracts the situation is less easy. Such lipids are frequently quite complex, and the site of attachment of individual chains is often ambiguous. The best that one can do for such systems is to rely on the arithmetic means of corresponding transition temperatures of the "virtual" subcomponents. An illustrative example is given in the Appendix.

Transition-temperature data for the lipid anhydrides are scarce and relatively inaccurate. From the chain-length dependence of the chain-melting temperature of dry phosphatidylethanolamines (Cevc, 1988), which I believe to be representative for other phospholipids as well, one gets

$$T_{m,anh}(n) = 414(1 - 1.72/n - 3.58/n^2)\ \text{K} \qquad (7)$$

This result can be used to estimate the transition temperature of the incompletely hydrated phospholipids in combination with a suitable model of membrane hydration. Nonlocal electrostatic approximation is suitable for this (Cevc, 1985, 1989). For completely dry phosphatidylcholines with one, two, or three double bonds at the positions 9, 12, and 15, eq 7 in combination with eq 6 suggests the chain-melting temperature values to be 62.5, 48, and 44, respectively; for the corresponding dihydrates, one gets from the same expression 44, 24, and 20 °C. This is not too far from the experimental values of 48, 27 and 17 °C (Lynch & Steponkus, 1989).

It may appear surprising that solely by considering the hydrocarbon mean interaction length quite reliable estimations of the lipid chain-melting transition temperatures are obtained. The reason for this becomes clear, however, if one remembers that the chain-packing density in all gel-phase bilayers is approximately the same, owing to the strength and the orientational anisotropy of van der Waals interchain attraction. Interchain separation does not vary appreciably from lipid to lipid and even hydrated lipids in a crystalline phase retain very much the same *average* chain–chain distance perpendicular to the long-chain axis. The head-group size, hydration, ionization, and other end effects, consequently, are prone to induce chain tilt or interdigitation and to modify the transverse chain packing without a major variability of the hydrocarbon lateral packing density. This permits evaluation of the hydrocarbon chain tilts on the basis of appropriate thermodynamic data.

Chain Tilt

Chain tilt is often claimed to be essential for the differences between various lipid properties. In fact, such a tilt, by and large, is a consequence rather than the source of the lipid polymorphism variability. In anhydrous lipid bilayers, it is caused by the packing constraints in the polar molecular region; in hydrated lipid membranes it is additionally, and often dominantly (Seddon et al., 1987; Cevc & Seddon, 1987), affected by water binding to the lipid head groups.

In spite of this, useful predictions can be made, starting with only a few simple theoretical postulates, if only the hydrocarbon tilt angle ϕ is known. Take, for example, that the effective chain length for a given tilted-chain lipid is defined as $n_{ef} = n \cos \phi$. If the value of the chain-melting transition temperature of such lipid in an untilted lamellar bilayer is known, the transition temperature for the corresponding tilted-chain membrane can be evaluated from eq 2 and vice versa. Alternatively, in the absence of more firm structural informations, the tilt value can *approximated* from the known transition-temperature data by comparing the nominal and the effective chain lengths of a (hypothetical) bilayer with nontilted chains. For example, the chain-melting phase-transition temperatures of dry phosphatidylethanolamines with tilted chains are 42 and 54.5 °C for $n = 12$ and 14, respectively. From eq 7, one deduces that dry lipids with untilted

chains would have to have substantially shorter chains in order to melt at these temperatures. From a comparison of the nominal and effective chain lengths, one gets $\phi = 40°$ and 45°; X-ray diffractometry implies a value of approximately 40°. By comparing the gel-phase transition-temperature data of phosphatidylcholines ($L_{\beta'}$) and phosphatidylethanolamines (L_{β}), one, furthermore, can conclude that the chain tilt of the former lipid should be between 33° and 35° in the gel phase; experimental values for this lipid are 28–36°. For dry phosphatidylserine in excess solution of lithium chloride, one calculates 42° and measures 40° (Cevc et al., 1985). More examples could be given. They all support the notion that the effective chain length, indeed, is important for the structure of lipid membranes.

Biological Implications

For optimal performance, biological membranes are in the fluid state. But why do living organisms not achieve this by synthesizing short-chain lipids? And why are double bonds of common unsaturated hydrocarbons concentrated in the central region?

Incorporation of short hydrocarbons into lipid bilayers would bring about at least three handicaps. Fluid membranes with short-chain hydrocarbons, to begin with, would be relatively thin; optimal insertion and regulation of the integral membrane proteins or other molecules with an extended hydrophobic region in such membranes would be difficult. Secondly, the water solubility of short hydrocarbons is quite high; the on–off rates for the lipids with such hydrocarbons, consequently, would also be appreciable. And, last but not least, bilayers with predominantly short-chain components are thermodynamically unstable in the fluid phase (Lewis et al., 1987; Finegold & Singer, 1987). Dilauroylphospholipids, for example, revert spontaneously from the fluid bilayer phase into lipid crystals; such spontaneous change, no matter how seldom, obviously could not be tolerated well by living organisms. (In this connection it is worth remembering that lauric acid derivatives, even dilauroylphosphatidylcholine, are toxic for cells.)

Concentration of the double bonds in the middle of a chain, conversely, brings the advantage of maximizing the fluidization effect while minimizing the danger of chain oxidation; several double bonds in other positions would be required to guarantee similar fluidity; this would be detrimental for the chemical membrane stability. Moreover, the chains with double bonds half way down along the chain are symmetrical and have equally long chain segments separated by a double bond. This ensures a relatively homogeneous fluidity profile throughout the membrane. The idea of a membrane interior with three subregions[6] does not seem to make much sense for a system that has to behave as one functional entity.

Practical Applications

Lipid polymorphism and bilayer fluidity are important issues for most practical applications. It is therefore appropriate to first screen the suitability of a given lipid for certain application on paper prior to actually performing experimental tests. Results presented in this work provide a good starting point for such rational membrane design, as far as the chain effects are concerned (see Figures 3 and 5).

For example, in order to construct relatively impermeable liposomes with maximal encapsulation of the amphiphilic drugs, which preferentially partition in the interfacial region, I suggest using lipids with double bonds in the upper part of the hydrocarbon chain; these are expected to form membranes with relatively fluid interfacial segments (to act as a "drug solvent") embracing a well-packed rigid membrane core.

On the contrary, to form bilayers suitable for the encapsulation of strongly hydrophobic substances, it is more appropriate to use lipids that are unsaturated in the central or terminal hydrocarbon regions.

To get very flexible highly permeable lipid membranes, highly asymmetric (phospho?)lipids might prove useful; these are likely to give rise to thin interdigitated bilayers. It is possible to extend these examples to other and more specific applications.

Conclusions

In summary, I have shown that the biologically and structurally dominant part of the lipid chain consists of long nonintercalated hydrocarbon segments. Chain unsaturation efficiently decouples both hydrocarbon parts disjoined by (a) double bond(s). Effects of the chain unsaturation are the greatest when the resulting chain segments are comparably long. Double bonds in a cis configuration are approximately twice as efficient in promoting chain fluidization as the corresponding double bonds in a trans configuration; *sn*-1 chains are more influential in this respect than the hydrocarbons attached at *sn*-2 positions. The main reason for the dependence of the lipid chain-melting transition temperature on the lipid chain asymmetry is the loss of coupling between the terminal interdigitated hydrocarbon parts. These termini are likely to be (partly) fluid already below the overall chain-melting transition temperature.

An effective chain length can be used to explain and quantify the effects of chain unsaturation as well as the consequences of chain asymmetry on the bilayer-melting phase transition. If the length of the first hydrocarbon segments exceeds the extension of the second part of the chain by more than 3–4 carbon atoms, the lipid chain-melting polymorphism is governed by the length of the former; the chain-melting phase-transition temperature in such a situation is determined chiefly by the longer hydrocarbon part. From the thermodynamic point of view, the effective chain length of a lipid with asymmetric chains is always approximately equal to the length of the shorter chain, plus one.

A extremely simple but quite potent phenomenological model, which incorporates this concept, has been shown to predict the lipid chain-melting transition temperatures of arbitrary phospholipids with better than 3% accuracy, in the worst case. Normally, model accuracy is better than to within 0.75% (2 °C), however. By a similar rationale, the lipid chain tilt in the gel phase can be estimated on the basis of known transition-temperature data.

Equations presented in this work are, in a way, trivial; they are important mainly for the practical applications. But the concept that they incorporate is new, excitingly simple, and has far reaching truths to convey.

Appendix

Estimation of the Chain-Melting Temperature for Complex and Biological Lipids. To illustrate how the present model works for the biological lipids in this appendix the chain-melting phase-transition temperature of egg-yolk phosphatidylcholine is calculated. The hydrocarbon composition of this lipid is typically 34% n = 16:0, 13% n = 18:0, 35% n = 18:1c9, and approximately 17% n = 18:cc9,11 (Hawthorne & Ansell, 1982). To determine the appropriate transition-temperature

[6] A bilayer consisting of phospholipids with (a) double bond(s) close to one end would tend to form fluid-gel-fluid or gel-fluid-gel sandwiches, depending on whether the terminal or the upper part of the chain would be the shorter one.

value, one can start with the results for phosphatidylcholines with homologous symmetric chains. Calculation of the corresponding transition temperature by means of eq 6 is then followed by the appropriate contributions, weighing factors being identical with the relative chain concentrations. This yields $T_m(n_{ef}) = 0.34T_m(n = 16{:}0) + 0.13T_m(n = 18{:}0) + 0.35T_m(n = 18{:}1c9) + 0.17T_m(n = 18{:}cc9,11) = 2$ °C. But half of all chains reside in the *sn*-1 positions on the glycerol backbone. All such chains, consequently, stabilize the ordered phase less strongly than anticipated. To correct for this, a calculation similar to the one just made is repeated for the phosphatidylcholines with a nominal chain length shorter by one, $n - 1$, as discussed previously. This yields an estimated transition temperature of −8 °C. To obtain the chain-melting temperature of the original lipid, an arithmetic mean of both particular values is taken, $T_m = [T_m(n_{ef}) + T_m(n_{ef} - 1)]/2 = -6$ °C. This is somewhat higher than the experimental value of −15 °C but still accurate to within 3.5%, on the absolute temperature scale.

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