CHAIN ARRANGEMENTS IN THE GEL STATE AND THE TRANSITION TEMPERATURES OF PHOSPHATIDYLCHOLINES

P. J. DAVIS AND K. M. W. KEOUGH
Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1B 3X9

ABSTRACT Measures of chain length, inequivalence of chain length, and chain position have been incorporated into a parameter, D, which we call the "relative chain inequivalence." D has been calculated for a number of saturated phosphatidylcholines (PC) containing one type of chain (homoacid PC), saturated PC containing two different acyl chains (heteroacid PC), and heteroacid PC containing one saturated and one unsaturated chain. The gel to liquid-crystalline transition temperatures and D are related in a regular pattern, which suggests similarity of chain packing in the gel. This pattern may have useful predictive value.

INTRODUCTION

Phospholipids in hydrated bilayers can undergo a transformation from a state of high order and low inter- and intramolecular mobility (gel state) to a state of lower order with high inter- and intramolecular motion (liquid crystalline state). The gel to liquid-crystalline transition temperatures (T_c) of individual molecular species of phosphatidylcholines (PC) are dependent upon chain position, chain length, differences in chain length, and number and position of double bonds.

For a given head group class of lipid, the total van der Waals interactions between chains are major determinants of T_c. In calculating D, the measure of these interactions has been taken as the depth in the bilayer of the lesser-penetrating chain in the all-*trans* conformation (1, 2), taking into account the fact that the chains of the *sn*-1 and *sn*-2 positions are out of register (3) (Fig. 1). When the chains of a lipid penetrate the bilayer to different depths, packing irregularities in the center of the bilayer may be expected to influence T_c. The difference in the penetration or "chain inequivalence," was taken as a measure of this effect. The greater the chain inequivalence is as a proportion of the bilayer thickness, the more it may influence T_c (4, 5); therefore the inequivalence was expressed as a proportion of the depth of the shorter chain. Long range interactions between the acyl chains and the head group might play a role in determining T_c. In the gel, alignment of glycerol backbones might occur so that intermolecular chain contacts may be nonrandom. Therefore chain position was considered as potentially influencing T_c, and accounted for by taking the chain inequivalence as the algebraic sum with its sign in the numerator of the equation for D. These measures were combined in one parameter

$$D = \frac{(\text{depth of } sn\text{-1 chain}) - (\text{depth of } sn\text{-2 chain})}{\text{depth of lesser-penetrating chain}}.$$

This parameter is similar to those used by others (4, 5) in analyses of entropies of transition, but differs in the use of the depth of the lesser-penetrating chain instead of the longer chain in the denominator and in that the influence of chain position was accommodated by taking the algebraic value of the numerator, not its absolute value.

EXPERIMENTAL

Depths were measured using CPK models as shown in Fig. 1. When unsaturated chains were measured, three conformations were considered. In one (the "one-rotamer" conformation) a rotamer was introduced at the bond either $n \pm 1$ from the double bond at position n, and a 30° twist was introduced in the adjacent bond at either n ± 2 (2, 6). In the second (the "two-rotamer" conformation) gauche rotamers were introduced at both bonds $n \pm 1$ to the double bond and 30° twists were introduced at both adjacent bonds at $n \pm 2$. Both these conformations have unsaturated chains with the major portions of their lengths parallel to the bilayer normal. In the third ("bent") conformation, both chains were assumed to be bent at the double bond like the conformation shown in Fig. 1 *D* of reference 7. For the "bent" conformation, measurements of chain depths were made both along the chains and parallel to the bilayer normal. Only measurements of the first type gave values of D and T_c that fit the pattern in Fig. 2 *a*.

RESULTS AND DISCUSSION

Fig. 2 *a* shows the values of T_c plotted as a function of D. A regular pattern of two families of straight lines was observed. All three conformations of saturated-unsaturated PC gave straight lines, but the "bent" conformation

Dr. Davis's present address is Department of Physiology, University of Alberta, Edmonton, Alberta Canada T6G 2H7
Requests for reprints should be addressed to Dr. Keough.

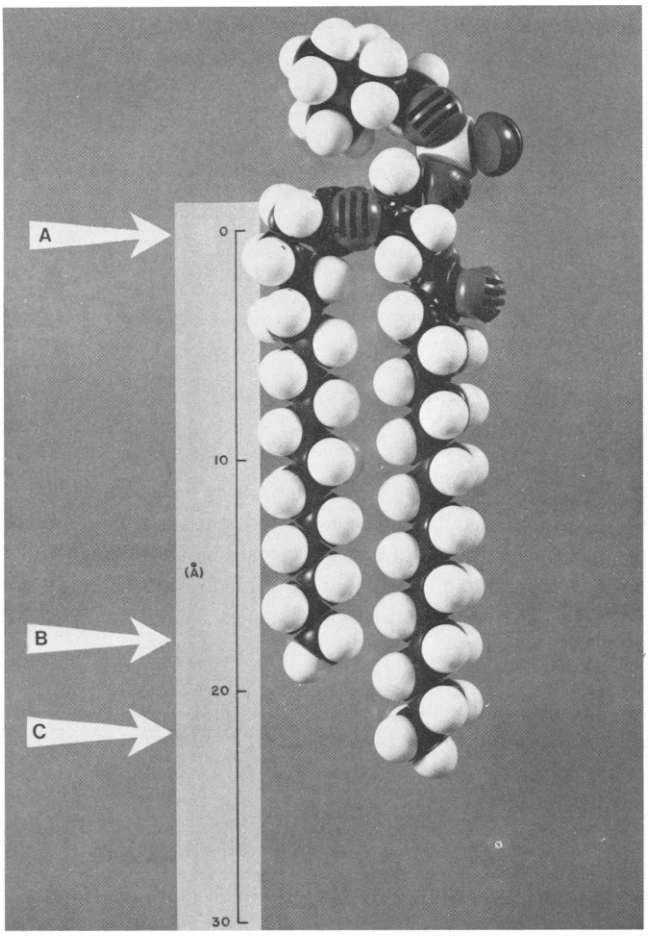

FIGURE 1 CPK model showing how the measurements of chain penetration were made. Models were arranged with chains as shown and depths were measured with the centre of the 1'-carbon of the *sn*-2 chain as the zero point. In this model of 16:0–16:0 PC the depth of the *sn*-1 chain is given by the distance AC and the depth of the *sn*-2 chain is given by the length AB. In this case the lesser-penetrating chain is the *sn*-2 chain, but in some lipids it is the *sn*-1 chain.

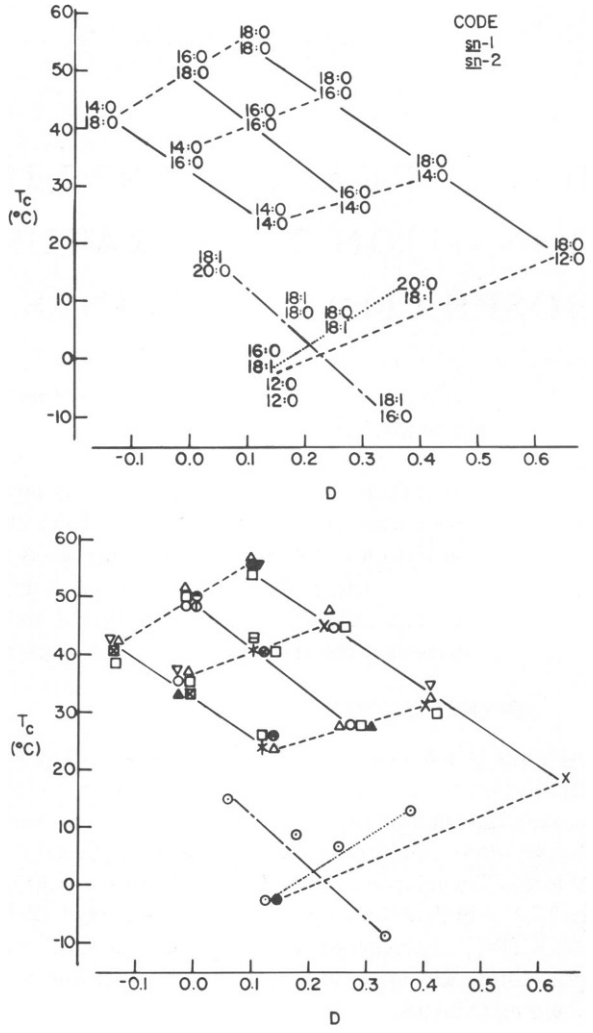

FIGURE 2 Relationship between transition temperature (T_c) and the relative chain inequivalence parameter, *D*. Fig. 2 *a* shows actual data points from the literature. Fig. 2*b* shows specific PC at the appropriate points on the lines. Lines are linear regressions whose parameters are given in Table I. The chain in the *sn*-1 position is designated first, that in the *sn*-2 position second. Chains are indicated by (number of carbons: number of double bonds). All 18:1 chains were oleate, i.e., the double bonds were at position 9 or *n*-9.

The following data were used to draw Fig. 2 *a* and to compute regression lines in Table I. The list is given in the order—PC: *D*; T_c (reference); T_c (reference); etc.

18:0–12:0 PC: 0.65; 18.5 (5).
18:0–14:0 PC: 0.41; 29.9 (5); 34 (11); 29.6 (12); 33 (13).
18:0–16:0 PC: 23; 44.0 (1); 44.1 (5); 43.9 (12); 48 (13).
18:0–18:0 PC: 0.09; 54.2 (12); 56 (13); 54.9 (14); 54.4 (15).
16:0–14:0 PC: 0.27; 27.2 (1); 27.5 (12); 27 (13); 27 (16).
16:0–16:0 PC: 0.11; 41.1 (12); 41 (17); 42 (18), 41.5 (19).
16:0–18:0 PC: −0.02; 47.4 (1); 49.0 (12); 52 (13); 48 (20); 48.4 (K. Keough, and N. Kariel, unpublished).
14:0–14:0 PC: 0.12; 23.6 (12); 23 (13); 23 (17); 24 (19).
14:0–16:0 PC: −0.02; 35.3 (1); 37 (11); 35.1 (12); 37 (13); 34 (16); 34 (21).
14:0–18:0 PC: −0.15; 42 (11); 38.6 (12); 42 (13); 41 (21).
12:0–12:0 PC: 0.14; −1.8 (14).
16:0–18:1 PC "bent": 0.13; −2.6 (2); "1-rotamer": 0.005; −2.6 (2); "2-rotamer": 0.02; −2.6 (2).
18:0–18:1 PC "bent": 0.25; 6.3(2); "1–rotamer": 0.12; 6.3 (2); "2–rotamer": 0.14; 6.3 (2).

gave slopes closest to those for the lines for saturated PC, and data from this conformation was used for statistical comparisons.

One family had negative slopes (e.g., 18:0–18:0 PC ↔ 18:0–12:0 PC and related lines). Each line in this family describes the T_c of a group of PC where the *sn*-1 chain was constant and the *sn*-2 chain was varied. Analysis of covariance (8) indicated that the slopes were not significantly different from one another. Moving downward to the right of these lines corresponds to decreasing carbon number, decreasing length of *sn*-2 chain and increasing *D*, all of which combine to lower T_c.

The second family of lines, those with positive slopes, corresponds to series of PC with constant *sn*-2 chains and varying *sn*-1 chains. Analysis of covariance indicated not all the slopes were statistically equivalent, but subsequent Newman-Keuls testing was ambiguous and specific significant differences were not identified (8). Since T_c might also depend on differences in chain conformational states

TABLE I
PARAMETER OF THE LINES IN FIGURE 2.

Line	Regression	Subgroup
	Family with *sn*-2 chains fixed, *sn*-1 chains variable	
14:0–18:0 PC↔18:0–18:0 PC	$T_c = 49.84 + 58.47D$	18:0 in *sn*-2 position
14:0–16:0 PC↔18:0–16:0 PC	$T_c = 36.48 + 38.76D$	16:0 in *sn*-2 position
14:0–14:0 PC↔18:0–14:0 PC	$T_c = 19.42 + 28.32D$	14:0 in *sn*-2 position
12:0–12:0 PC↔18:0–12:0 PC	$T_c = -7.37 + 39.79D$	12.0 in *sn*-2 position
16:0–18:1 PC↔20:0–18:1 PC	$T_c = -9.90 + 60.42D$	18:1 in *sn*-2 position; "bent" chain conformation
16:0–18:1 PC↔20:0–18:1 PC	$T_c = -2.44 + 64.59D$	18:1 in *sn*-2 position; "one-rotamer" conformation
16:0–18:1 PC↔20:0–18:1 PC	$T_c = -3.43 + 63.13D$	18:1 in *sn*-2 position; "two-rotamer" conformation
	Family with *sn*-1 chains fixed, *sn*-2 chains variable	
18:0–18:0 PC↔18:0–12:0 PC	$T_c = 60.64 - 68.32D$	18:0 in *sn*-1 position
16:0–18:0 PC↔16:0–14:0 PC	$T_c = 48.12 - 75.04D$	16:0 in *sn*-1 position
14:0–18:0 PC↔14:0–14:0 PC	$T_c = 32.43 - 65.15D$	14:0 in *sn*-1 position
18:1–20:0 PC↔18:1–16:0 PC	$T_c = 20.88 - 87.84D$	18:1 in *sn*-1 position; "bent" conformation
18:1–20:0 PC↔18:1–16:0 PC	$T_c = 7.89 - 105.86D$	18:1 in *sn*-1 position; "one-rotamer" conformation
18:1–20:0 PC↔18:1–16:0 PC	$T_c = 5.62 - 109.77D$	18:1 in *sn*-1 position; "two-rotamer" conformation

FIGURE 2 (continued)
20:0–18:1 PC "bent": 0.37, 11.9 (22); "1-rotamer": 0.25; 11.9 (22); "2-rotamer": 0.23; 11.9 (22).
18:1–16:0 PC "bent": 0.33; −9.3 (2); "1-rotamer": 0.15; −9.3 (2); "2-rotamer": 0.12; −9.3 (2)
18:1–18:0 PC "bent": 0.17; 8.6 (2); "1-rotamer"; 0.02; 8.6 (2); "2-rotamer": 0.005; 8.6 (2).
18:1–20:0 PC "bent": 0.04; 15.9 (22); "1-rotamer": −0.09; 15.9 (22); "2-rotamer": −0.11; 15.9 (22).

between the gel and liquid crystal, and since these will be a function of chain length and double bond number and position, it is perhaps not surprising that all the slopes were not identical. Moving downward to the left along a line in this family corresponds to decreasing carbon number and shortening the *sn*-2 chain, both of which would reduce T_c. In this case, however, the value of D decreases, and that would not be expected to lower T_c. The countervailing effects of decreasing chain length and decreasing D are likely the reason why the absolute values of the slopes in this family are generally lower than those in the family of lines with negative slopes.

The difference in the magnitudes of the slopes for the two families emphasizes the need for caution when interpreting changes in acyl chain composition of lipids in terms of potential physical changes in membranes.

The regularity of the pattern in Fig. 2 gives us some comfort in the thought that the assumptions in the calculation of D are reasonable. Other formulations (e.g., using absolute values, or using the longer chain in the denominator) did not produce a regular pattern encompassing as many PC as did the formulation used.

The pattern in Fig. 2 supports the idea that there is similarity in the packing of the various types of PC in the gel unless chain length differences are great. The saturated-unsaturated PC yield straight lines that also fit the pattern. While the double bond causes some disruptions in packing, it would appear that a substantial number of the interactions in the gel state of unsaturated heteroacid PC are nearly equivalent to those in the saturated PC.

Two heteroacid PC, 18:0–10:0 PC [$D = 0.98$; $T_{c\ (predicted)}^* = -6.3°C$; $T_{c\ (observed)}^* = 19.7°C$ (5)] and 10:0–18:0 PC [$D = -0.55$; $T_{c\ (predicted)}^* = 17.7°C$; $T_{c\ (observed)}^* = 10.0°$ (5)] do not fit the pattern, suggesting that these lipids, especially 18:0–10:0 PC, might pack differently from other PC in the gel state. This prediction was borne out recently by the x-ray studies of PC (9, 10) that indicated that in the gel state 18:0–10:0 PC has a fundamentally different packing arrangement from that of other saturated PC. The models for the packing of 18:0–10:0 PC in the gel indicate extensive chain interdigitation so that each polar head group is associated with three acyl chains, its own two plus the methyl end of the longer chain of the molecule on the opposite side of the bilayer. Both papers (9, 10) presented models for the packing of 18:0–14:0 PC that were more "conventional" in that they had two chains per head group, although the models differed somewhat in their picturing of packing in the bilayer interior. It was suggested (10) that 18:0–12:0 PC might be packed like 18:0–10:0 PC in the gel except that there may be links near the end of the *sn*-2 chains at the center of the bilayer.

We hope the pattern will lead to further investigation,

especially of heteroacid PC, to elucidate the exact nature of packing in these lipids. Since D is easily calculated the regularity of the pattern may prove useful for predicting T_c of PC, and aid in the interpretation of data on fatty acid and molecular species compositions in biological membranes.

We thank the Medical Research Council of Canada for financial support, and Drs. D. A. Pink, R. L. Biltonen, and G. G. Shipley for their valuable suggestions about the manuscript. This pattern was presented at the 16th FEBS (Federation of European Biochemical Societies) Meeting, June 1984 (Abstract XV-157).

REFERENCES

1. Keough, K. M. W., and P. J. Davis. 1979. Gel to liquid-crystalline phase transitions in water dispersions of saturated mixed-acid phosphatidylcholines. *Biochemistry.* 18:1453–1459.
2. Davis, P. J., B. D. Fleming, K. P. Coolbear, and K. M. W. Keough. 1981. Gel to liquid-crystalline transition temperatures of water dispersions of two pairs of positional isomers of unsaturated mixed-acid phosphatidylcholines. *Biochemistry.* 20:3633–3636.
3. Seelig, J., and A. Seelig, 1980. Lipid conformation in model membranes and biological membranes. *Q. Rev. Biophys.* 13:19–61.
4. Mason, J. T., and C. -h. Huang. 1981. Chain length dependent thermodynamics of saturated symmetric-chain phosphatidylcholine bilayers. *Lipids.* 16:604–608.
5. Mason, J. T., C. -h. Huang, and R. L. Biltonen. 1981. Calorimetric investigations of saturated mixed-chain phosphatidylcholine bilayer dispersions. *Biochemistry.* 20:6086–6092.
6. Huang, C. -h. 1977. A structural model for the cholesterol-phosphatidylcholine complexes in bilayer membranes. *Lipids.* 12:348–356.
7. Berde, C. B., H. C. Andersen, and B. S. Hudson. 1980. A theory of the effects of head-group structure and chain unsaturation on the chain melting transition of phospholipid dispersions. *Biochemistry.* 19:4279–4293.
8. Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall Inc., Englewood Cliffs, N.J. 228–235.
9. McIntosh, T. J., S. A. Simon, J. C. Ellington, Jr., and N. A. Porter. 1984. New structural model for mixed-chain phosphatidylcholine bilayers. *Biochemistry.* 23:4038–4044.
10. Hui, S. W., J. T. Mason, and C. -h. Huang. 1984. Acyl chain interdigitation in saturated mixed-chain phosphatidylcholine bilayer dispersions. *Biochemistry.* 23:5570–5577.
11. Stümpel, J., A. Nicksch, and H. Eibl. 1981. Calorimetric studies on saturated mixed-chain lecithin-water systems. Nonequivalence of acyl chains in the thermotropic phase transition. *Biochemistry.* 20:662–665.
12. Chen, S. C., and J. M. Sturtevant. 1981. Thermotropic behavior of bilayers formed from mixed-chain phosphatidylcholines. *Biochemistry.* 20:713–718.
13. Stümpel, J., H. Eibl, and A. Nicksch. 1983. X-ray analysis and calorimetry of phosphatidylcholine model membranes. The influence of length and position of acyl chains upon structure and phase behavior. *Biochim. Biophys. Acta.* 727:246–254.
14. Mabrey, S., and J. M. Sturtevant. 1976. Investigation of phase transitions of lipids and lipid mixtures by high sensitivity differential scanning calorimetry. *Proc. Natl. Acad. Sci. USA.* 73:3862–3866.
15. Lentz, B. R., E. Freire, and R. L. Biltonen. 1978. Fluorescence and calorimetric studies of phase transitions in phosphatidylcholine multilayers: kinetics of the pretransition. *Biochemistry.* 17:4475–4480.
16. Serrallach, E. N., G. H. de Haas, and G. G. Shipley. 1984. Structure and thermotropic properties of mixed-chain phosphatidylcholine bilayer membranes. *Biochemistry.* 23:713–720.
17. Ladbrooke, B. D., and D. Chapman. 1969. Thermal analysis of lipids, proteins and biological membranes. A review and summary of some recent studies. *Chem. Phys. Lipids.* 3:304–367.
18. Janiak, M. J., D. M. Small, and G. G. Shipley. 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyllecithin. *Biochemistry.* 15:4574–4580.
19. Silvius, J. R., B. D. Read, and R. N. McElhaney. 1979. Thermotropic phase transitions of phosphatidylcholines with odd-numbered *n*-acyl chains. *Biochim. Biophys. Acta.* 555:175–178.
20. Davis, P. J., and K. M. W. Keough. 1984. Phase diagrams of bilayers of dimytistoyl lecithin plus heteroacid lecithins. *Chem. Phys. Lipids.* 35:299–308.
21. Lewis, B. A., S. K. Das Gupta, and R. G. Griffin. 1984. Solid-state NMR studies of the molecular dynamics and phase behavior of mixed-acid phosphatidylcholines. *Biochemistry.* 23:1988–1993.
22. Davis, P. J., and K. M. W. Keough. 1984. Scanning calorimetric studies of aqueous dispersions of bilayers made with cholesterol and a pair of positional isomers of 3-*sn*-phosphatidylcholine. *Biochim. Biophys. Acta.* 778:305–310.