Thermotropic phase behavior of phosphatidylcholines with ω-tertiary-butyl fatty acyl chains

Ruthven N. A. H. Lewis,* Henry H. Mantsch,‡ and Ronald N. McElhaney*
*Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7; and ‡Division of Chemistry, National Research Council of Canada, Ottawa, Ontario, Canada K1A OR6

ABSTRACT The thermotropic phase behavior of a homologous series of phosphatidylcholines containing acyl chains with ω-tertiary butyl groups was studied by differential scanning calorimetry, Fourier transform infrared spectroscopy, and ^{31}P-nuclear magnetic resonance spectroscopy (^{31}P-NMR). Upon heating, aqueous dispersions of these lipids exhibit single transitions which have been identified as direct conversions from L_c-like gel phases to the liquid-crystalline state by both infrared and ^{31}P-NMR spectroscopy. The calorimetric data indicate that the thermodynamic properties of the observed transition are strongly dependent upon whether the acyl chains contain an odd- or an even-number of carbon atoms. This property is manifest by a pronounced odd/even alternation in the transition temperatures and transition enthalpies of this homologous series of lipids, attributable to the fact that the odd-numbered compounds form gel phases that are more stable than those of their even-numbered counterparts. The spectroscopic data also suggest that unlike other lipids which exhibit the so-called odd/even effect, major odd/even discontinuities in the packing of the polymethylene chains are probably not the dominant factors responsible for the odd/even discontinuities exhibited by these lipids, because only subtle differences in the appropriate spectroscopic parameters were detected. Instead, the odd/even alternation in the physical properties of these lipids may be attributable to significant differences in the organization of the carbonyl ester interfacial regions of the lipid bilayer and to differences in the intermolecular interactions between the terminal *t*-butyl groups of the odd- and even-numbered homologues. Our results also suggest that the presence of the bulky *t*-butyl groups in the center of the lipid bilayer reduces the conformational disorder of the liquid-crystalline polymethylene chains, and promotes the formation of L_c-like gel phases. However, these L_c-like gel phases are considerably less ordered than those formed by saturated, straight-chain lipids.

INTRODUCTION

The properties of lipid bilayers that contain hydrocarbon chains with bulky substituents at or near to their hydrophobic termini are of considerable interest from both the physical and biological perspectives. Naturally occurring examples of these molecules include the methyl iso- and anteiso-branched fatty acids, which are abundant and widespread constituents of the membrane lipids of many genera of bacteria (1), the ω-cyclohexyl fatty acids, which are major constituents of the membrane lipids of the thermoacidophilic microorganism *Bacillus acidocaldarius* (see reference 2 and references cited therein) as well as a mesophylic bacterium *Curtobacterium pusillum* (3), and the ω-cycloheptyl fatty acids, which have recently been discovered as major constituents of the membrane lipids of a moderately thermoacidophilic bacterium (4). Evidently this group of fatty acids possesses all of the physical properties needed to support the normal function of a bacterial membrane growing over a wide range of temperatures.

In recent years both model and natural membranes composed of lipids containing these classes of acyl chains have been studied by a variety of physical techniques (3, 5, 6–24, 8, 9, 10–24). Those studies have shown that the presence of substituents at or near to the hydrophobic ends of the acyl chains perturbs acyl chain packing in the gel state but, in the liquid-crystalline state, may actually reduce the degree of conformational disorder in the acyl chains. At this stage the extent to which these altered physical properties are useful to the organisms in which such fatty acids naturally occur is not entirely clear. However, in the case of the thermoacidophilic *B. acidocaldarius,* the reduced conformational disorder of the liquid-crystalline acyl chains confers a greater stability to thermal stress (25) and an increased resistance to permeation by small molecules (5–7), properties which may be key to its survival at high temperatures.

All of the information currently available on the effects of bulky ω-substituents on the physical properties of lipid bilayers has been obtained from studies on bilayers containing either methyl iso- and anteiso-branched fatty acyl chains or ω-cyclohexyl fatty acyl groups. Although these classes of fatty acids have different chemical structures, they can adopt all-*trans* conformations in which the substituent groups are all coplanar with the mean line

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TABLE 1 Precursors used in the synthesis of the ω-tertiary butyl fatty acids

Fatty acid*	Carboxyl fragment	Alkyl terminal fragment
11,11-*dm*-12	Monomethylsuberate	3,3-dimethyl-1-bromo butane
12,12-*dm*-13	Monomethylazelaeate	3,3-dimethyl-1-bromo butane
13,13-*dm*-14	1,10 Decanedioic acid	3,3-dimethyl-1-bromo butane
14,14-*dm*-15	Monomethylazelaeate	5,5-dimethyl-1-bromo pentane
15,15-*dm*-16	1,12 Dodecanedioic acid	3,3-dimethyl 1-bromo butane
16,16-*dm*-17	1,13 Tridecanedioic acid	3,3-dimethyl-1-bromo butane
17,17-*dm*-18	1,14 Tetradecanedioic acid	3,3-dimethyl-1-bromo butane
18,18-*dm*-19	1,13 Tridecanedioic acid	5,5-dimethyl-1-bromo-pentane
19,19-*dm*-20	1,16 Hexadecanedioic acid	3,3-dimethyl-1-bromo butane

*The fatty acids are described by the short hand notation *M,M-dm-N*. *N* denotes the length of the hydrocarbon chain, and *dm* denotes that there are two methyl groups attached to carbon atom *M*.

defined by the zig-zag of the polymethylene chain. This common structural feature could explain the common infrared spectroscopic properties exhibited by some phosphatidylcholines (PCs) containing these acyl chains (see reference 19). To investigate this further, we have synthesized a homologous series of ω-tertiary butyl (*t*-butyl) fatty acids and their accompanying PCs, and we report here the initial differential scanning calorimetric (DSC), Nuclear Magnetic Resonance (^{31}P-NMR) and infrared spectroscopic characterization of the thermotropic phase properties of the bilayers that they form. The availability of this new class of lipids permits additional studies of the effects of large bulky ω-end groups on the physical properties of bilayers and also provides the first opportunity to study a lipid bilayer containing branched hydrocarbon chains in which at least one of the branches cannot align with the polymethylene zig-zag. These *t*-butyl fatty acids can be considered as derivatives of the methyl isobranched fatty acids in which an additional methyl branch is attached to the same carbon atom containing the methyl iso-branch, and to our knowledge, they are not naturally occurring. However, these fatty acids can support the normal growth and function of the mycoplasma *Acholeplasma laidlawii B* when it is made fatty acid auxotrophic (unpublished experiments from this laboratory). Also, when fed appropriate metabolic precursors, the mesophylic bacterium *Bacillus subtulis* can synthesize and incorporate these fatty acids into their membrane and is apparently capable of normal growth under such conditions (26).

MATERIALS AND METHODS

The sources of commercially available chemicals, solvents, and chromatographic adsorbents and their pretreatment and/or purification were the same as previously used in this laboratory (8). 5,5-Dimethyl-1-bromohexane was synthesized from 3,3-Dimethyl-1-bromobutane (Aldrich Chemical Co., Inc., Milwaukee, WI) by the ethylene oxide chain-elongation procedure previously used in this laboratory (8). The ω-tertiary butyl fatty acids were synthesized from the precursors listed in Table 1 by the same chemical procedures used by Lewis and McElhaney (8), and were purified by silicic acid chromatography and crystallization from 70% ethanol. The procedures used for the synthesis and purification of the PCs used here were the same as those previously used in this laboratory to prepare highly purified samples of 1,2-diisoacyl PCs (8).

The thermotropic phase behavior of fully hydrated samples of the phospholipids was studied using MC-1 and MC-2 high sensitivity differential scanning calorimeters (Microcal, Inc., Amhearst, MA) and with a DSC-2C differential scanning calorimeter (Perkin-Elmer Corp. Instrument Div., Norwalk CT) equipped with a thermal analysis data station. The samples for DSC were prepared and quantified by the same methods previously used in this laboratory (8). The ^{31}P-NMR spectra were recorded on a NT-300WB spectrometer (Nicolet Scientific Corp., Northvale, NJ) (121.47 MHz for ^{31}P) using the data acquisition and data processing procedures used by Lewis et al., 1988 (29). The infrared spectra were recorded on a Digilab FTS-60 Fourier transform infrared spectrometer (Biorad, Digilab Division, Cambridge, MA) using data acquisition and data processing procedures that have been detailed elsewhere (9).

RESULTS

Differential scanning calorimetry

DSC heating and cooling thermograms of aqueous dispersions of the 9 ω-tertiary butyl PCs of acyl chain lengths ranging from 12 to 20 carbon atoms (14–22 total carbon atoms) are shown in Figs. 1 and 2. The lipids of acyl chain lengths shorter than 15 carbon atoms exhibit phase transitions at temperatures below 0°C and are not amenable to high sensitivity DSC measurements with our instruments. For the longer chain compounds ($N \geq 15$), the high sensitivity DSC measurements all show that with the possible exception of 19,19-*dm*-20 PC,[1] these compounds all exhibit single heating endothermic transitions that are fairly broad and somewhat skewed towards the low-

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[1] The fatty acyl chains on the lipids used here are described by the short hand notation *M, M-dm-N*. *N* denotes the length of the hydrocarbon chain, and *dm* (for dimethyl) denotes that there are two methyl groups attached to carbon atom *M*.

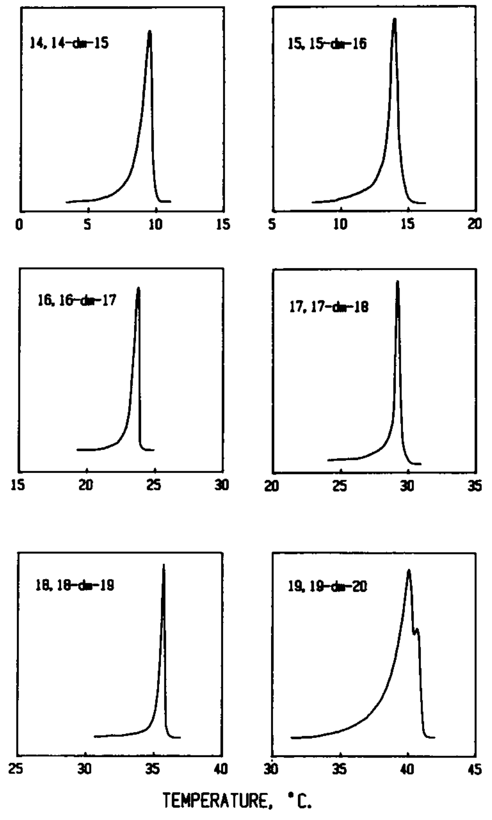

FIGURE 1 High-sensitivity DSC heating endotherms of the ω-tertiary butyl phosphatidylcholines in aqueous dispersion. The endotherms shown were all acquired at heating rates of 10–12°C per hour.

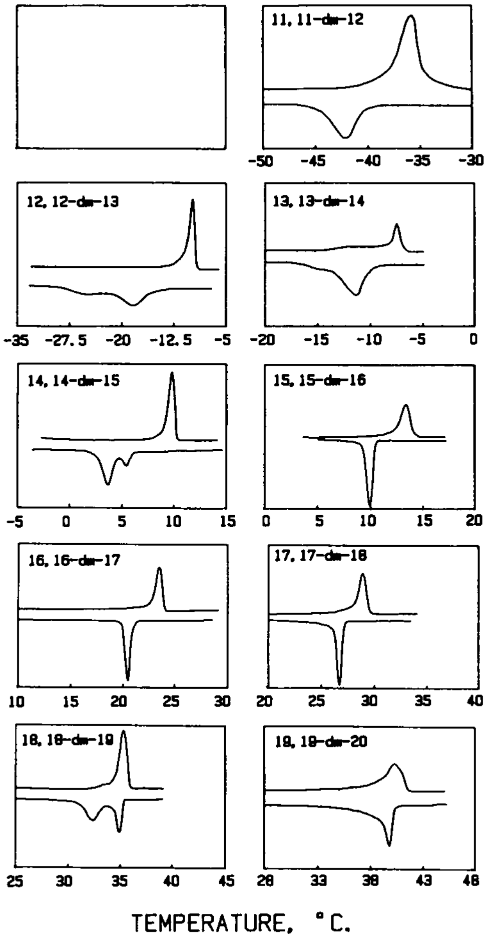

FIGURE 2 Low-sensitivity heating and cooling thermograms of the ω-tertiary butyl phosphatidylcholines in aqueous dispersion. The thermograms shown were all acquired at scan rates of 18.75°C per hour.

temperature end. These transitions have been shown by infrared and ^{31}P-NMR spectroscopy to be transitions from L_c-like gel phases (subgel-like phases) to the liquid-crystalline state. In the case of 19,19-*dm*-20 PC, DSC detects two partially resolved peaks that we have tentatively assigned to gel/gel (low temperature event) and gel/liquid-crystalline (high temperature event) phase transitions.

The complexity of the thermotropic phase behavior of these lipids becomes apparent when their cooling behavior is examined. As shown in Fig. 2, they all exhibit cooling exotherms that are observed at temperatures lower than those of the corresponding heating endotherms. For all the compounds except the two longer chain homologoes (N = 19, 20), this undercooling effect is neither the result of instrumental-lag time artifacts nor classical cooling hysteresis, since the observed behavior is dependent only on the history of the samples and is essentially unchanged by prolonged incubation at temperatures between those of the heating and cooling peaks. This behavior becomes less pronounced as the length of the acyl chain increases and is essentially absent with the two longer-chain homologues. Such behavior has also been observed with some homologues of other PC model membrane systems (2, 8, 27) in which the observed heating transitions are direct conversions from L_c-like gel phases to the liquid-crystalline state (i.e., L_c/L_α transitions). Fig. 2 also shows that there are some differences between the odd- and even-numbered homologues of this series of lipids. All of the odd-numbered homologues exhibit single cooling exotherms, whereas, with the exception of 16,16-dm-17 PC, the even-numbered homologues exhibit two exothermic transitions on cooling. Our experiments indicate that there is a significant kinetic component to these differences and that with some of the even-numbered compounds, two cooling exotherms are sometimes observed when DSC measurements are made at very fast cooling rates (≥10°C/min). Perhaps the differences in the observed behavior of the odd- and even-numbered homologues are a result of the different rates at which they form their respective subgel-like phases.

The transition temperatures and the enthalpy changes

TABLE 2 Thermodynamic characterization of the phase transitions exhibited by the ω-tertiary butyl phosphatidylcholines*

PC	Heating endotherms		Cooling endotherms	
	T_m (°)	ΔH_c	T_m (°)	ΔH_c
11,11-*dm*-12	−35.9	3.3	−42.3	1.6
12,12-*dm*-13	−9.7	7.0	−25.9	3.8
			−21.3	3.4
13,13-*dm*-14	−7.5	5.3	−11.2	5.3
14,14-*dm*-15	9.9	9.6	3.7	7.8
			5.7	1.8
15,15-*dm*-16	13.4	8.3	9.2	8.3
16,16-*dm*-17	23.6	14.0	20.5	12.0
17,17-*dm*-18	28.9	10.6	26.7	10.6
18,18-*dm*-19	35.6	13.8	32.7	8.0
			35.2	5.8
19,19-*dm*-20	40.2	11.6	38.7	11.6

*Transition temperatures (T_m) are in °C and transition enthalpies (ΔH_c) are in kcal/mol.

associated with the thermotropic phase transitions exhibited by these lipids are listed in Table 2. The expected trend towards increases in the transition temperatures and associated enthalpy changes with increases in chain length is evident with these lipids, but as shown in Fig. 3, the transition temperatures and transition enthalpies are not described by a smooth function of acyl chain length. The odd-numbered homologues exhibit higher transition temperatures and transition enthalpies than do neighboring even-numbered homologues, suggesting that the odd-numbered homologues form more thermodynamically stable gel-state assemblies. In addition, it is also apparent that the transition enthalpies of the two longer-chain compounds studied ($N = 19, 20$) are lower than what would be expected by extrapolation of the trends set by the shorter chain even- and odd-numbered homologues. This suggests that there may be some differences in the organization of the gel states formed by these longer-chain homologues. Moreover, it is also clear that the transition temperatures of these PCs are lower than are those of saturated *n*-acyl, isoacyl, and ω-cyclohexyl PCs of comparable acyl chain length, but are higher than those of the comparable anteiso-acyl PCs. Thus it would appear that the ω-tertiary butyl group is more disordering of the gel state packing of the acyl chains than are the ω-cyclohexyl ring or the methyl-iso branch but a lot less disordering than a methyl-anteiso branch.

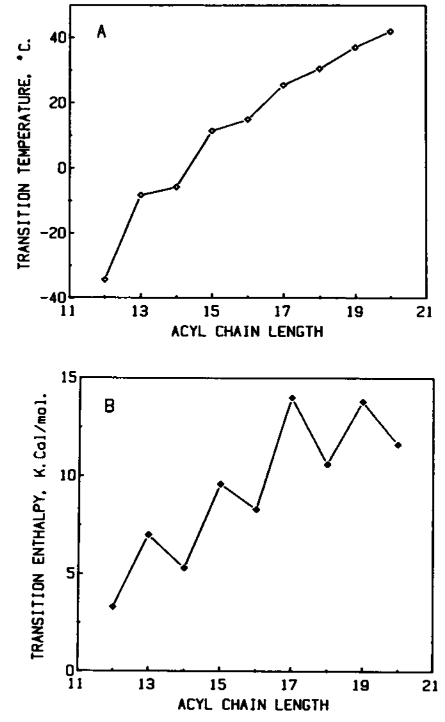

FIGURE 3 Effect of acyl chain length on the transition temperatures (*A*) and the transition enthalpies (*B*) of aqueous dispersions of the ω-tertiary butyl PCs.

^{31}P NMR spectroscopy

Illustrated in Fig. 4 are the ^{31}P-NMR spectra of 16,16-dimethyl 17:0 PC and 17,17-dimethyl 18:0 PC, representative members of the odd- and even-numbered homologues, respectively, of this series of lipids. The spectra shown were acquired at temperatures that bracket the single heating endothermic phase transition resolved by DSC. At temperatures above those of their respective

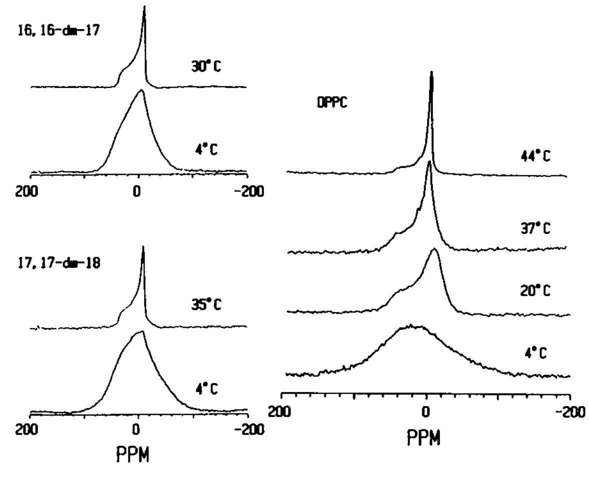

FIGURE 4 Proton-decoupled ^{31}P-NMR spectra of the gel and liquid-crystalline phases of 16,16-dm-17 PC and 17,17-dm-18 PC. The spectra were acquired at the temperatures indicated. To facilitate comparison of these spectra with those of straight-chain lipids, spectra of the various phases formed by DPPC are also shown.

phase transitions, these lipids all exhibit ^{31}P-NMR powder patterns that are typical of fast, axially symmetric motion of the phosphate head groups of phospholipid bilayers (see reference 28). Furthermore, the width (basal linewidth ≈ 60 ppm) and general contours of the powder patterns approach those of the limiting spectral line shape expected of fast, axially symmetric motion in the liquid-crystalline state of phospholipid bilayers (29). However, in the gel state, both compounds exhibit ^{31}P-NMR powder patterns with broad featureless contours (basal linewidth ≈ 120 ppm), that show none of the features of the axially symmetric motion which typifies the L_α and L_β phases of phospholipid bilayers (28). The ^{31}P-NMR spectroscopic properties exhibited by the gel states of these lipids are indicative of slow, axially asymmetric motion of the phosphate head groups on the ^{31}P-NMR time scale (28), and are typical of the subgel-like (L_c phases) formed by many PCs (3, 9, 14, 27, 29–31). The ^{31}P-NMR spectroscopic data thus suggest that the single heating endothermic transitions of these lipids are the net result of transitions from subgel-like phases to the liquid-crystalline state. However, it is also clear that the broad gel-phase ^{31}P-NMR powder patterns of these lipids are considerably narrower than those exhibited by the subgel phase of dipalmitoylphosphatidylcholine (DPPC). Thus, in the subgel or subgel-like phases formed by these ω-tertiary butyl PCs, the phosphate head groups must be subject to fewer motional restrictions than is the case with DPPC. This suggests that the subgel-like phases formed by the ω-tertiary butyl PCs are probably significantly less ordered than those formed by the saturated straight-chain PCs.

Infrared spectroscopy

In these studies infrared spectra of 15,-15-dimethyl 16:0 PC, 16,16-dimethyl 17:0 PC, 17,17-dimethyl 18:0 PC, and 18,18-dimethyl 19:0 PC were acquired at temperatures that bracket the calorimetrically observed phase transitions. The single thermotropic phase transition observed on heating these lipids is accompanied by diagnostic changes in the parts of the infrared spectrum attributable to the methylene (CH_2) stretching, CH_2 scissoring and carbonyl (CO) stretching vibrational modes. These infrared spectral parameters are sensitive to changes in the conformation of the acyl chains, changes in the interactions between the acyl chains, and changes in the structure of the polar/apolar interface of the lipid bilayer, respectively. With all of these lipids the observed transition coincides with an increase in the frequency of the CH_2 symmetric stretching mode from 2,848 to 2,851 cm^{-1}. Because CH_2 stretching frequencies above 2,850 cm^{-1} are typical of conformationally disordered, melted polmethylene chains (32), the observed increase in the CH_2 stretching frequency clearly identifies the respective thermotropic transitions as chain-melting events. However, with these lipids, the frequency of the CH_2 symmetric stretching band is in the low end of the range of frequencies expected of melted polymethylene chains and is certainly lower than that observed for saturated, straight-chain PCs (32). This suggests that despite being melted, the conformational disorder of the liquid crystalline hydrocarbon chains must be less than that of comparable straight-chain PCs. It is also apparent that the increase in frequency observed upon melting of the acyl chains (3.3 cm^{-1}) is considerably greater than that which accompanies the gel/liquid-crystalline phase transition of saturated, straight chain lipids (1.5 – 2 cm^{-1}, see reference 32). Thus the structural transformations occurring at the single thermotropic transition observed calorimetrically must be more complex phenomena. This conclusion is consistent with the ^{31}P-NMR data, which suggest that the transitions observed are L_c/L_α type transitions and not the L_β/L_α type transitions normally exhibited by most saturated, straight-chain PCs.

These lipids also exhibit a sharp decrease in the frequency of the CH_2 scissoring vibrational mode at their respective phase transitions. The thermotropic phase transition observed upon heating the even-numbered homologues coincides with a decrease in frequency from 1,470.5 to 1,468 cm^{-1}, whereas with the odd-numbered homologues a larger decrease in frequency is observed (1,471.5 to 1,468 cm^{-1}). Methylene scissoring frequencies near 1,468 cm^{-1} are typical of melted or hexagonally packed polymethylene chains (33). Thus the observed decrease in frequency to 1,468 cm^{-1} is consistent with the data obtained from the CH_2 stretching vibrational mode, which clearly showed that the lipids are liquid-crystalline at the higher temperatures. With the odd-numbered compounds, the gel state CH_2 scissoring frequency (1,471.5 cm^{-1}) is close to that expected of ordered, triclinically packed, all-*trans* polymethylene chains (34). Similar CH_2 scissoring bands have been observed in the subgel-like (L_c) phases formed by many phospholipid dispersions (9, 15, 19, 35), indicating the L_c-like character of the gel phases formed by these odd-numbered ω-tertiary butyl lipids. In the case of the even-numbered homologues, the gel state CH_2 scissoring frequency (1,470.5 cm^{-1}) is also consistent with the presence of ordered all-*trans* polymethylene chains, a result again indicating the L_c-like character of the gel phases that they form. However, the properties exhibited by this scissoring band are somewhat unusual. The frequency of this band is lower than that which typifies triclinically packed all-*trans* polymethylene chains and the observed value is in the range observed for the high-frequency component of the CH_2 scissoring frequency of orthorhombically packed hydrocarbon chains. However, in the usual cases where there is orthorhombic packing of polymethylene chains, factor group splitting of the CH_2 scissoring mode gives rise to two bands at frequencies near 1,470 and 1,466 cm^{-1}, respectively (see reference 36). With these even-numbered ω-tertiary butyl lipids there is only one band at 1,470.5 cm^{-1} and we found no evidence for a low-frequency component to the CH_2 scissoring vibration. As a result, it seems unlikely that the hydrocarbon chains of these even-numbered lipids are orthorhombically packed. We suspect that the acyl chains of these even-numbered lipids could also be triclinic-like and that the difference between them and their odd-numbered homologues could be the result of a distorted chain-packing lattice, presumably as a result of unfavorable alignments of their terminal *t*-butyl groups. However, despite this unusual property of the even-numbered homologues, it is clear that like their odd-numbered counterparts, these lipids also form L_c-like gel phases and that the data are consistent with the identification of the observed calorimetrically observed heating endothermic event as a L_c/L_α transition.

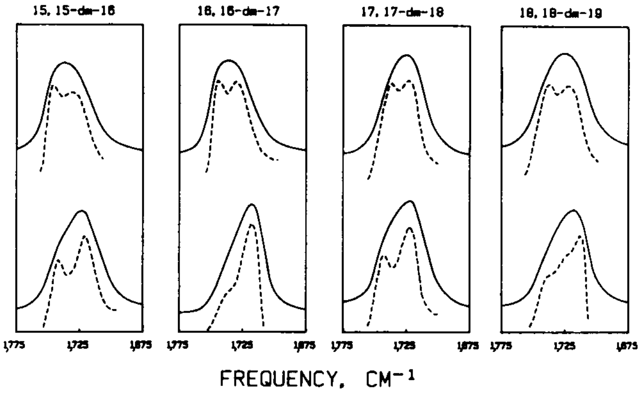

FIGURE 5 Characteristic infrared absorption band contours in the region of the CO stretching modes of the ω-tertiary butyl PCs. The spectra were acquired at the temperatures indicated. In each panel the solid line represents the observed band contour while the dashed line represents the same spectra after Fourier self deconvolution leading to a reduction of band width by a factor of 2.5.

The changes in the frequencies of the CO stretching vibrational modes at the phase transition temperatures of all of these lipids are generally similar. However, this observation can be misleading, because the broad CO stretching band contour exhibited by diacyl glycerolipids is, in fact, a composite of at least two bands, as is readily demonstrated by resolution-enhancement techniques (see reference 37, a review). After Fourier self-deconvolution, these bands usually have maxima near 1,743 and 1,728 cm^{-1} and have been assigned to the stretching vibrations of the sn-1 and sn-2 ester carbonyl groups, respectively (38, 39). The fine structure of the CO stretching band contours of these lipids revealed after the deconvolution (see Fig. 5) indicates that there are some unusual properties exhibited by this homologous series of lipids. In the liquid-crystalline state, the broad CO band contour of all of the compounds studied resolves into two bands at 1,743 and 1,728 cm^{-1}. The frequencies and relative intensities of these two bands are generally similar to those exhibited by most of the liquid-crystalline phospholipid bilayers studied so far. This indicates that in the liquid-crystalline phase, the organization of the CO ester interface of these lipids is indistinguishable by infrared spectroscopy from those of other liquid-crystalline phospholipid bilayers so far studied. In the gel states, however, there is evidence that the organization of the ester interfaces of the odd- and even-numbered homologues are different, and that both differ from those of many of the phospholipids that have been characterized. With the even-numbered homologues, the band contour resolves into two bands at 1,723 and 1,742 cm^{-1}, of which the lower frequency is the more intense. Thus for these homologues, the transition to the liquid-crystalline state is accompanied by a decrease in the relative intensity of the low-frequency component (versus the high-frequency component) and its shift to a higher frequency (1,728 cm^{-1}). The frequency of the low-frequency component (1,723 cm^{-1}) found in the gel state of these even-numbered homologues is unusual, because with most lipids the CO stretching band contour resolves into two bands near 1,728 cm^{-1} and 1,742 cm^{-1} at all temperatures, and the typical L_β/L_α transition (see reference 31) or the L_c/L_β transitions of odd-numbered isoacyl PCs and of DPPC (9, 36) merely involve changes in the relative intensities of the two components. The relatively low frequency of the sn-2 CO ester stretching band observed with the *t*-butyl PCs is probably indicative of the formation of L_c-like phases in which the conformation of the glycerol backbone is different from that normally found in most phospholipids, or of the formation of L_c-like phases with weakly hydrogen bonded sn-2 ester carbonyls.

The deconvolved CO band contours of the odd-numbered ω-tertiary butyl PCs differ from those of their even-numbered counterparts in that apart from the CO ester bands at 1,742 cm^{-1} and 1,728 cm^{-1}, there is an additional sharp and very intense band at frequencies below 1,720 cm^{-1}. This additional low-frequency band is considerably more intense than the other two bands and overlaps with and often masks the presence of the band at 1,728 cm^{-1}. The latter can sometimes only be demonstrated by a very high degree of resolution enhancement (see, for example, with 16,16-dm-17:0 PC). A band below 1,720 cm^{-1} has previously been observed in CO stretching band contours of the L_c phases formed by even-numbered isoacyl PCs (9), by the odd-numbered anteiso- and ω-cyclohexyl acyl PCs (15, 19), by a thio analogue of DPPC (40), as well as by crystalline hydrates of DPPC (41).

This additional sharp CO stretching band may be the result of the formation of ordered L_c-like phases with interfacial regions that are partially dehydrated and contain strongly bound water(s) of crystallization hydrogen bonded to one of the ester carbonyl groups (42).

An examination of the methyl deformation bands between 1,360 and 1,400 cm^{-1} reveals some additional points pertinent to the organization of the *t*-butyl groups in the middle of the lipid bilayer. With these lipids the *t*-butyl groups give rise to two distinct methyl deformation bands (the so-called methyl umbrella modes near 1,390 and 1,365 cm^{-1}, see Fig. 6), of which the low-frequency component is the more intense (43, 44). These bands are observed at 1,392 and 1,364 cm^{-1} in the liquid-crystalline phases of both the odd- and even-numbered homologues of this class of PCs, and upon cooling to their respective L_c-like gel phases, there is a small decrease in the frequencies of both bands (to ~1,390 and 1,363 cm^{-1}, respectively). However, Fourier self-deconvolution of these bands (see Fig. 6) indicates that the gel state properties of the odd- and even-numbered homologues are different. In contrast to the gel phases of the even-numbered homologues, Fourier self-deconvolution of the spectra exhibited by the odd-numbered homologues reveals that the band contours of both the high- and low-frequency components of the umbrella vibrational mode are composites of two bands with a 4 cm^{-1} separation of their maxima. The result thus shows that in marked contrast to their even-numbered counterparts, there is factor group splitting of the methyl umbrella bands in the gel state of the odd-numbered PCs. Such factor group splitting arises as a result of the organization of the hydrocarbon chains into ordered solid-like structures in which there are coupled interactions between the vibrating groups (45). Thus, it is clear that in the gel state of the odd-numbered PCs there must be strong interactions between the terminal *t*-butyl groups of the acyl chains. In the case of the hydrocarbon chain packing of the even-numbered lipids, the absence of such-factor group splitting indicates that such end group interactions are either prohibited by geometric constraint or are considerably weaker.

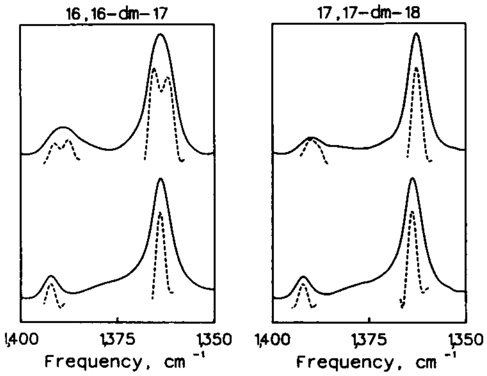

FIGURE 6 Infrared absorption band contours of the CH3 umbrella deformation mode of representative odd- and even-numbered omega *t*-butyl PCs. The spectra shown were acquired in the gel phase (*top panel*) and liquid-crystalline phase (*bottom panel*) of 16,16-dm-17 PC (*odd-numbered*) and 17,17-dm-18 PC (*even-numbered*). In all cases the spectra are shown before (*solid line*) and after (*dashed line*) deconvolution leading to a decrease in the band width of 1.8.

DISCUSSION

These studies have provided another striking example of the so-called odd-even effect, a property commonly exhibited by many long chain paraffinic compounds and lipid bilayers (3, 8, 9, 13–15, 17, 19, 27, 46–49, and references sited therein). Here the odd-even discontinuities in the transition temperatures, the transition enthalpies and the kinetics of the gel-state transformations of these lipids clearly suggest that there must be significant structural differences in the gel states formed by these lipids. Interestingly, however, though structural differences between the odd- and even-numbered homologues were detected by the infrared spectroscopic studies, the data suggest that as far as the acyl chain packing modes are concerned, such differences are subtle, and not the major structural differences one would have inferred from the magnitude of the odd-even discontinuities in the calorimetric data. Instead, the infrared spectroscopic data suggest that with all of these lipids the significant structural differences occur at the *t*-butyl and ester carbonyl end groups of the odd- and even-numbered homologues. In this respect these particular lipid bilayers are unusual, because with the other omega-substituted lipids so far studied (i.e., the isoacyl, anteisoacyl, and ω-cyclohexyl PCs), odd-even discontinuities in physical properties are often manifest by radically different acyl chain packing modes in the gel state (9, 13, 15, 19). The possibility that there may be some overall similarities between the packing of the polymethylene chain segments of the odd- and even-numbered homologues raises other questions about the physical basis of the pronounced odd-even discontinuities in the other physical properties of these lipids. The currently held opinion is that odd/even discontinuities in the solid-state properties of long-chain paraffinic compounds arises naturally from the differences in the possible end group interactions which can occur in a crystalline (or quasi-crystalline) state in which the long polymethylene chains are tilted to the end group planes (48). However, in the 1,2 diacyl glycerolipid bilayers, tilting of the acyl chains may not be a necessary precondition for such odd-even discontinuities in physical properties. From the single-crystal x-ray studies on dimyristoyl phosphatidylcholine (DMPC) dihydrate, it is expected that ω-end groups on the sn-1 and sn-2 chains are inequivalent with respect to their penetration of the bilayer (49) and as a result, the interactions between the two acyl chains in the regions near their ω-end groups would be inequivalent. Thus, if the ω-end groups are large, and if the orientation of the acyl chains relative to each other approximates that observed in DMPC dihydrate (see reference 49), then the nature of the interaction between the sn-1 and sn-2 chains of a given lipid molecule would depend on whether the chain has an odd-and even-number of carbon atoms. Using molecular models it can be easily demonstrated that if, for example, the end group on the sn-2 chains of the even-numbered homologues is directed towards the methylene segments on the sn-1 chain (of the same molecule), then with the odd-numbered homologues the sn-2 end group would be directed away from the sn-1 chain. The net effect of this is that for one set of homologues interactions between the sn-2 end group and methylene segments on sn-1 acyl chains would be intramolecular while for the other set of homologues such interactions would be intermolecular. This type of odd-even alternation would be independent of whether there is any acyl chain tilt with respect to the bilayer normal. In fact, if the acyl chains are aligned along the bilayer normal, then it would be possible for both odd- and even-numbered homologues to adopt chain packing modes that may be crystallographically (and hence spectroscopically) similar but be markedly different as far as the energetics are concerned. Such considerations may be especially applicable to these omega *t*-butyl PCs on account of their relatively large, bulky and three dimensionally branched end groups.

In considering some of the unusual properties of these ω *t*-butyl PCs, it should be noted that, unlike the other omega-substituted lipid bilayers so far studied, the all-*trans* carbon skeleton of these lipids project in three dimensions relative to the mean line defined by the zig-zag of the main polymethylene chain. In this respect the other omega-substituted lipid bilayers studied so far can be considered as having two-dimensionally branched carbon skeletons. In the instances where the branched hydrocarbon chains are two-dimensional, it can be demonstrated with molecular models that the bulk of the substituent can be easily accommodated within the normal hydrocarbon chain packing mode and still have good van der Waals contacts by the tilting of the all-*trans* acyl chain in the direction of the branch. Indeed, this has been observed in single crystal x-ray studies of fatty acids with single methyl branches (51–55), and inferred from x-ray powder studies of ω-cyclohexyl alcohols and fatty acids (46, 47). However, with three dimensionally branched hydrocarbon chains such as those with an ω-*t*-butyl group, it is readily shown with molecular models that the usual chain packing modes would not enable good van der Waals contacts between the main chains or the end groups even with strongly tilted chains. Evidently the optimal packing requirements of the ω-*t*-butyl group must be very specialized. We suspect that in meeting some of the specialized packing requirements of the ω-*t*-butyl group, some structural distortions must occur in other parts of the lipid bilayer. The spectroscopic data presented here suggest that one of the sites at which such structural distortions occur is the polar/apolar carbonyl ester interface of the lipid bilayer.

It is also clear from these studies and previously published work (3, 8, 9, 13–15, 17, 19) that the presence of a bulky group at, or near to, the end of a hydrocarbon chain can dramatically alter the physical properties of a lipid bilayer. With this particular class of lipids, it evident that such substitutions can affect properties of their gel and liquid-crystalline states as well as their gel/liquid-crystalline phase transition temperatures. That lipids with large substituents have lower gel/liquid-crystalline phase transition temperatures than do comparable counterparts with unsubstituted chains is not surprising, because the bulkiness of the substituent groups should destabilize the gel state by perturbing the packing of the hydrocarbon chains. Moreover, the observation that the bulky terminal *t*-butyl group reduces the conformational disorder of the hydrocarbon chains of the liquid-crystalline phases of these lipid bilayers was also expected, because the same has been inferred from studies with other model and biological membranes containing omega-substituted hydrocarbon chains (9, 15, 18–21, 25, 56). In addition, it is also clear that the omega *t*-butyl substituent also promotes the formation of ordered L_c-like gel phases, and as has also been observed with a number of other omega substituted diacyl PCs (2, 8, 9, 13–15, 17, 19), although L_c phases formed are usually not as ordered as those formed from systems with unsubstituted acyl chains. Apparently as far as the formation of L_c-like structures is concerned, the dominant effect of the presence of the bulky omega end groups is kinetic, because when large bulky omega end groups are present the formation of L_c-like gel phases should be made thermodynamically less favorable on account of their disruption of interchain interactions.

The fact that the presence of a *t*-butyl group at the end of an acyl chain disrupts gel-state packing of a lipid bilayer is not unexpected. Indeed, the disruption of gel-state packing has so far been the common theme of all studies of lipid bilayers with substituted acyl chains. In agreement with previously published monolayer studies of fatty acids (57), the data presented here also suggests that the *t*-butyl group is more disruptive than similarly substituted isopropyl groups (methyl iso-branched compounds) or even cyclohexyl groups, but is not as perturbing as an isobutyl group (methyl anteiso-branched compounds). That a *t*-butyl groups is more disruptive of gel state packing than an isopropyl group should not be surprising, because it seems logical that two methyl groups substituted to the penultimate carbon of an acyl chain should be more perturbing of gel state packing than one. However, arguments based on the number of carbon atoms forming the substituent cannot rationalize the fact that a *t*-butyl group is more disruptive than a cyclohexyl group, despite the fact that the latter involves more carbon atoms and forms an intrinsically more rigid structure. Evidently the effects of a substituent on the overall shape of the terminal portion of the all-*trans* hydrocarbon chain is another factor to be considered. From the observations reported here, it seems that a terminal three-dimensionally branched substituent can pose more packing problems than a somewhat larger and more rigid two dimensionally branched substituent. However, arguments based on size and shape or rigidity cannot explain why the two methyl groups at the penultimate carbon of the acyl chain (ω-tertiary butyl) is not as perturbing as the single methyl branch of methyl anteiso-branched compounds. Evidently the position of the substituent is also a very important factor which determines the extent to which it can disrupt the packing of all-*trans* hydrocarbon chains. Finally, the fact that the presence of a large group on the hydrocarbon chain of a lipid molecule can so dramatically alter the properties of a lipid bilayer has some important implications concerning the use of lipid probe molecules in spectroscopic studies of lipid bilayers. Given the data obtained here and the fact that many of the lipid molecules used as spectroscopic probes have large reporter groups on their hydrocarbon chains, we suggest that great care should be exercised in the interpretation of data obtained in studies using such molecules.

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