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Phase characteristics of positional isomers of 1,2-di(heptacosadiynoyl)-*sn*-glycero-3-phosphocholine; tubule-forming phosphatidylcholines

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We have examined the phase behavior of positional isomers of a polymerizable diacetylenic phospholipid, 1,2-di(heptacosadiynoyl)-*sn*-glycero-3-phosphocholine which has the diacetylene in varying position along the acyl chains. Upon cooling multilamellar vesicles (MLVs) through the liquid-crystalline to gel phase transition, all isomers examined spontaneously formed hollow, cylindrical microstructures (or tubules). Differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) have been used to characterize positional isomers of this lipid in an effort to understand the effect of diacetylenic position on the molecular characteristics of tubule formation. Calorimetric results indicate that moving the position of the diacetylene along the acyl chain results in the alternation of the exotherm observed for the hydrated transition temperature associated with tubule formation, with higher transition temperatures (T_m) observed from isomers with an even number of methylenes between the diacetylene groups and the glycerol backbone. As the diacetylene is moved toward either end of the acyl chain, even with the observed alternation, the T_m was observed to increase. Calorimetric results of dry members of this series reveal an exotherm during cooling, the same temperature at which fully hydrated samples form tubules. This suggests that there is little difference in the phase behavior observed upon cooling the hydrated tubules and the dry diacetylenic material. FTIR results support the high degree of conformational order observed in tubules of this isomer series as a very strong CH_2 wagging progression is observed between 1375 and 1200 cm^{-1}. In addition, the C-H stretch region (3000 cm^{-1} to 2800 cm^{-1}) indicates tight acyl chain packing with many all-*trans* segments. These results provide further evidence that tubules are uniquely crystalline microstructures and that this inherent crystallinity, and the formation of tubules is not affected by diacetylenic position.

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Abbreviations: MLVs, multilamellar vesicles; SUVs, small unilamellar vesicles; $DC_{m,n}PC$, a positional isomer of 1,2-di(heptacosadiynoyl)-*sn*-glycero-3-phosphocholine; $DC_{8,9}PC$, 1,2-di(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine; DTPC, 1,2-ditricosanoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine; DSC, differential scanning calorimetry; FTIR, Fourier-transform infrared spectroscopy; T_m, liquid-crystalline to gel phase transition temperature.

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Introduction

The formation of phospholipid self-assemblies (e.g., monolayers, micelles, bilayers) is believed to be driven entropically by the energy gained during the association of hydrophobic regions of these amphiphiles as they are sequestered from the polar solvent water [1]. The driving force for higher order supramolecular assemblies of bilayers is less well understood. In these cases, the molecular characteristics of the phospholipid may influence the microstructure formed during assembly. For example, the formation of cochleate cylinders may be driven by ionic forces created by the interactions of the negative charge of the phosphatidylserine head group and divalent cations [2,3].

Acyl chain features of phosphatidylcholines may also be responsible for driving microstructure formation. Of recent interest has been the formation of tubular microstructures formed from phospholipid with diacetylenes in the acyl chains [4–10]. One such lipid which has been studied extensively is 1,2-di(tricosa-10,12-diynoyl)-*sn*-3-phosphocholine ($DC_{8,9}PC$), a lecithin which contains polymerizable diacetylenes in the mid-region of the acyl chains [4–12]. These novel microstructures (or 'tubules', see Ref. 4) differ from chochleate cylinders in that they have a hollow aqueous core, can be hundreds of microns long, and 0.5–1.0 μm in diameter. Tubule microstructures have been morphologically characterized extensively [6]. The ruggedness induced by polymerization, as well as the high aspect ratio of tubules makes them attractive microstructures for technological applications [8]. The acyl chain region of tubules examined thus far (principally $DC_{8,9}PC$) is very highly ordered as revealed by FTIR and Raman spectroscopies [4,5,10]. The degree of conformational order far exceeds that observed in the saturated analog of this lipid, ditricosanoylphosphatidylcholine (DTPC). In addition, the saturated analog does not form tubules, nor have they been observed in other saturated phosphatidylcholines [4,10].

The self-assembly of tubules has been observed by two distinctly different pathways. One method is the thermal formation of tubules which results as a consequence of cooling liquid-crystalline larger multilamellar vesicles or stacked bilayers sheets through a phase transition observed at approx. 39°C [4,10,13]. The molecular characteristics and the polymorphic phase behavior of this lipid during the thermal formation of tubules has been revealed by vibrational spectroscopy and differential scanning calorimetry [4,10–12]. Fluid phase large multilamellar vesicles will form tubules directly upon cooling through the phase transition at 39°C. Fluid phase small unilamellar vesicles supercool to 2°C at which temperature they undergo a transition to a polymorphic low-temperature phase, or stacked bilayer sheets [10,12]. The bilayer sheets are spectroscopically identical to the tubules [10,12]. The sheets from tubules when heated above the T_m and cooled back through the transition at 38°C. This indicates that one requirement for thermal formation of tubules may be formation of a precursor that has low degree of curvature. The formation of stacked bilayer sheets from highly strained small unilamellar vesicles fulfills this requirement. In addition, it has been suggested that the mechanism of thermal tubule formation occurs by the wrapping or rolling of large multilamellar vesicles [6]. This same mechanism could apply to the formation of tubules from stacked bilayer sheets [10].

Tubule formation has also been demonstrated by spontaneous formation in mixed solvent systems [13]. In this method, $DC_{8,9}PC$ is dissolved in ethanol and tubules are observed to form spontaneously upon the addition of water. The tubules formed from these two methods are morphologically similar in most respects (solvent grown tubules are longer and have fewer lamellae that comprise the tubule wall) and preliminary results on their molecular characteristics also indicate similarities [13].

In the present work, we have investigated the effect of changing the position of the diacetylenic group in the phase behavior of 1,2-di(heptacosadiynoyl)-*sn*-glycero-3-phosphocholine. This is similar to $DC_{8,9}PC$ except the acyl chain is 27 carbons in length. Fig. 1 shows the positional isomers that were studied. These represent isomers with diacetylenes close to the interfacial region and isomers with the diacetylenic group near the terminal methyl group. The nomenclature employed identifies the *m* portion of the alkyl chain, that is, the portion between the glycerol backbone

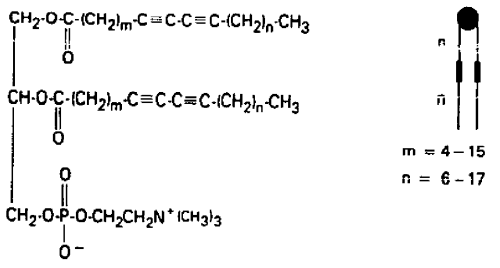

Fig. 1. Chemical formula of the (m,n) $DC_{27}PC$ isomer series.

and the diacetylenic group, and the n portion, between the diacetylene and the terminal methyl group. For instance, $DC_{4,17}PC$ is defined as the isomer that has 4 methylenes between the diacetylene and the glycerol backbone and 17 methylenes below the diacetylene.

A preliminary report on these lipids have demonstrated that these lipids will form tubules [12,14]. The polymerization efficiency of the isomers differed depending on position of the diacetylene [14]. In the present work, we have examined the molecular structure of these positional isomers in an effort to understand the effect of diacetylene position on the phase behavior of this lipid and of the tubule structures formed.

Materials and Methods

Synthesis of diacetylenic acids. The positional isomers of diacetylenic acids were synthesized by coupling the appropriate w-alkynoic acids with iodoalkynes using the procedure reported by Singh and Schnur [15]. The w-alkynoic acids were prepared by reacting the bromo analog of the acid with lithium acetylideethylenediamine complex at room temperature. The acids were purified by column chromatography on silica gel (chloroform as eluting solvent) followed by crystallization with hexanes. The synthesis and characterization of these acids has been described previously [15]. The acids were then converted into their anhydrides by reacting with 0.55 mole equivalent dicyclohexylcarbodiimide in methylene chloride, and stored in the dark at room temperature.

Synthesis of 1,2-di(heptacosadiynoyl)-sn-glycerol-3-phosphocholine. This method followed the procedure of Singh et al. [14,15]. $DC_{m,n}PC$'s were synthesized by reacting the appropriate diacetylenic acid anhydride with the D-α-glycerophosphocholine derived from egg phosphatidylcholine following the procedure by Gupta et al. [16]. The phospholipids were purified by column chromatography on silica gel followed by acetone precipitation. Their purity was monitored using a chloroform/methanol/water (65 : 25 : 4, v/v) solvent system with a sample loading of 1 mg of lipid in 30 μl of solvent. The molecular weight of the lipid was obtained by negative ion fast atom bombardment mass spectroscopy (FABMS) and was characterized using FTIR and ^{13}C-NMR. The lipid was stored as powder after filtering a methylene chloride solution through sintered glass.

Calorimetric characterization of $DC_{27}PC$. A Perkin-Elmer DSC-7 was used to generate all of the calorimetric scans presented. Transition temperatures of the dry polycrystalline material was generated by lyophilizing aliquots of $DC_{27}PC$ previously dried down from chloroform and stored in a vacuum desiccator. These samples were opened in a dry box purged with dry nitrogen and 2–5 mg was loaded into a stainless steel DSC pan. This procedure was used in the preparation of DPPC to qualitatively determine the amount of water present in these samples. Using this procedure, a transition temperature of 82°C was obtained for dry DPPC in good agreement with Chapman et al., for the transition temperature of DPPC dihydrate polycrystalline powder [17]. These samples were cycled on the DSC at 1 C°/min until consecutive scans were repeatable. Lipid weights were determined from the known weight of the pan before and after introduction of the sample.

For transition temperatures of hydrated material, a film of $DC_{m,n}PC$ was made in the DSC pan by loading small aliquots of lipid in chloroform at 60°C and drying over a stream of dry nitrogen. Residual solvent was removed by placing the pan in a vacuum dessicator for 24 h. The pan was weighed to determine the weight of the lipid which was usually between 2–4 mg. Triple distilled, deionized water was then added to the pan just before being hermetically sealed. The concentration of lipid in the pan was usually 50 and 100 mg/ml. The sample was held at 70°C for at least an hour to ensure good hydration. Each sample was then cooled slowly to 0°C at 1

C°/min. Slow cooling is necessary as more rapid cooling results in the formation of incompletely formed tubules. or shards [4]. After one cooling scan, the pan was opened at room temperature and the sample examined with light microscopy for the presence of tubules. Duplicate samples of each positional isomer were also run with repeated cycling to determine reproducible transition temperatures and enthalpies.

Preparation of tubules for FTIR spectroscopy. Tubules were prepared by hydrating polycrystalline powder (50 mg/ml) at 10°C above the hydrated transition temperature of the lipid (approx. 70°C) for at least one hour. This multilamellar suspension was then sonicated to clarity, cooled to 0°C at 1 C°/min, heated again above the transition temperature at the same rate and cooled back to room temperature. This cycle results in a very high conversion of lipid to tubules (via the formation of stacked bilayer sheets) and has been described in detail elsewhere [10,12]. These tubules have been shown to be morphologically and spectroscopically similar to the tubules formed from directly cooling multilamellar suspensions of this lipid [10,12]. After the cycle was completed, the sample was checked for the formation of tubules with light microscopy. A small aliquot was then loaded onto BaF_2 crystals with a teflon 0.25 μm spacer in a sealed demountable cell. This cell was temperature controlled by mounting it on an aluminum water jacket which was then placed in the infrared spectrometer. All spectra were taken with a Perkin-Elmer FTIR 1800 at 20°C. Spectra were collected with 2 cm^{-1} resolution and 500 scans co-added using triangular apodisation. Regions of interest were then copied and the absorbance expanded using a scale expansion software routine. This action expands the absorbance to a predetermined value keeping the relative ratios of all the bands constant.

Results and Discussion

Fig. 2 shows a typical calorimetric scan for a member of the positional series, $DC_{9,12}PC$. The cycle is begun with samples above their phase transition temperature. As slow cooling progresses, these samples undergo a liquid-crystalline to gel phase transition that results in tubule formation. In $DC_{9,12}PC$ the exothermic transition is observed at 52.3°C with an enthalpy of 25 kcal/mol. Subsequent heating of this sample reveals an endotherm at 55.9°C with the same enthalpy as the exothermic transition. Examination of the enthalpies of transition for each member of the isomer series shows that the enthalpy for an endotherm and exotherm are almost identical. In addition, there appears to be no apparent trend in the enthalpy of the transition as the diacetylene is moved down the acyl chain.

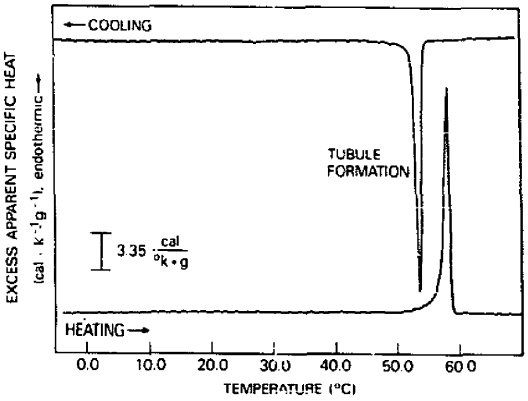

Fig. 2. Differential scanning calorimetric traces of the cooling of multilamellar vesicles of (9,12) $DC_{27}PC$ to form tubules and the subsequent heating of the tubule suspension. Scan rate was 1 C°/min for both the cooling and heating scan. Exact temperatures and enthalpies of the transitions can be found in the text and graphically in Fig. 3.

Fig. 3 shows the transition temperatures of the cooling scans (tubule formation) and heating scans of cycling multilamellar preparations of hydrated positional isomers of $DC_{m,n}PC$ as a function of length of m segment. These results indicate that there is a periodic alternation in both the endothermic and exothermic phase transition temperatures as the diacetylene is moved down the alkyl chain. For example, $DC_{5,16}PC$ has a gel to liquid-crystalline T_m at 55.4°C, while the T_m for $DC_{6,15}PC$ is 58.9°C. Moving the diacetylene one position further along the alkyl chain results in a shift of the T_m to 54.9°C, for $DC_{7,14}PC$. These results reveal that odd numbered m segments have consistently lower transition temperatures in both the heating and cooling scans. In addition, there is a constant hysteresis between the heating and cooling scans. The hysteresis observed in these

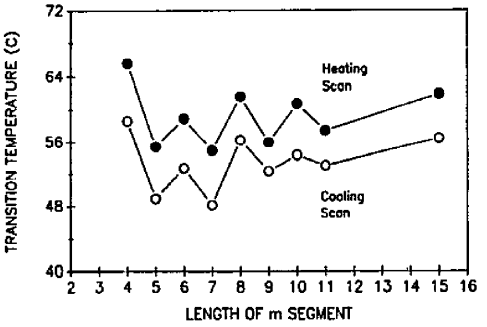

Fig. 3. The effect of length of *m* segment on the enothermic and exothermic transition temperatures of varying members of the $DC_{27}PC$ isomer series.

calorimetric results is repeatable, observed at slower scan rates, and has been reported previously for other diacetylenic lipids including $DC_{8,9}PC$. There was no observed trend in the enthalpies of these transition as the enthalpy of the endothermic transition varied between 26 and 29 kcal/mol.

The effect of unsaturation in the acyl chain region of fatty acids and their corresponding phosphatidylcholines on their phase transitions properties is well known [18]. In general, unsaturated fatty acids and phospholipids melt at lower temperatures than their corresponding saturated analogs. This result has been correlated to the increased disorder (*gauche* conformers) in the chain region induced by the presence of the unsaturated moiety. In this work, we have not examined the saturated analog of $DC_{m,n}PC$, but in previous studies, the saturated analog of $DC_{8,9}PC$, DTPC, melts at a temperature 32° C higher than its diacetylenic analog (unpublished results). The melting behavior of positional isomers of phosphatidylcholines with double bonds indicate that as the double bond is moved toward the middle of the chain, the transition temperature is lowered, rising again as the double bond is moved toward the terminal methyl [18]. In addition, the *cis* isomers of olefinic fatty acids and lipids display this effect with greater intensity than the *trans* isomer, presumably due to the increased *gauche* conformers that are dictated by the 120° angle introduced by the *cis*-double bond, and the maintenance of the acyl chain axis. Alternating melting behavior is also observed in both the *cis*- and *trans*-olefinic fatty acids, with the even position olefins having higher melting temperatures [19]. This odd-even effect is not observed in the corresponding phospholipids such as *cis*-unsaturated dioctadecenoylphosphatidylcholines [18,20].

In the present work, there is no marked overall trend (excluding the alternation) observed in the transition temperatures of the positional isomers of $DC_{m,n}PC$ as no significant decrease is observed in the transition temperature of $DC_{m,n}PC$ isomers with the diacetylenic groups in the middle of the alkyl chain (ΔT_m for the isomer series, 9 C°). The degree of disorder introduced by the diacetylene is not as great as in the *cis*-olefins because these groups introduce less deviation from the acyl chain axis. However, as the diacetylene is moved toward the head group, the transition temperature is raised slightly, in agreement with the behavior of acetylenic fatty acids [18,19]. It is interesting to note that the enthalpy of the endothermic and exothermic transitions are identical indicating that the enthalpy associated with the formation of tubules (wrapping of bilayers) may be negligible and that the transition represents chain melting and crystallization.

The persistence of odd-even effects in the $DC_{m,n}PC$ series may indicate that the chains are decoupled by the diacetylene and that the individual *m* and *n* segments are quite ordered. This may indicate that each acyl chains of $DC_{m,n}PC$ are behaving like two short independent segments of straight chain alkanes (*m* and *n*), as odd-even effects are observed as a function of chain length in saturated alkanes [18]. Previous Raman and infrared spectroscopic studies of $DC_{8,9}PC$ have suggested that the two portions of the chains separated by the diacetylene are highly ordered and vibrationally uncoupled [4,5,10,11]. Longitudinal acoustic modes have been observed in these lipids, which are not observed in saturated phosphatidylcholines. These modes have been tentatively attributed to *m* and *n* segments of the acyl chains [12,27].

The phase properties of hydrated and dry polycrystalline powder of $DC_{15,6}PC$ is presented in the calorimetric scans in Figs. 4A and 4B, respectively. Dry polycrystalline $DC_{15,6}PC$ material has an endothermic transition temperature of 63.7° C (enthalpy 29 kcal/mol) with an exothermic transi-

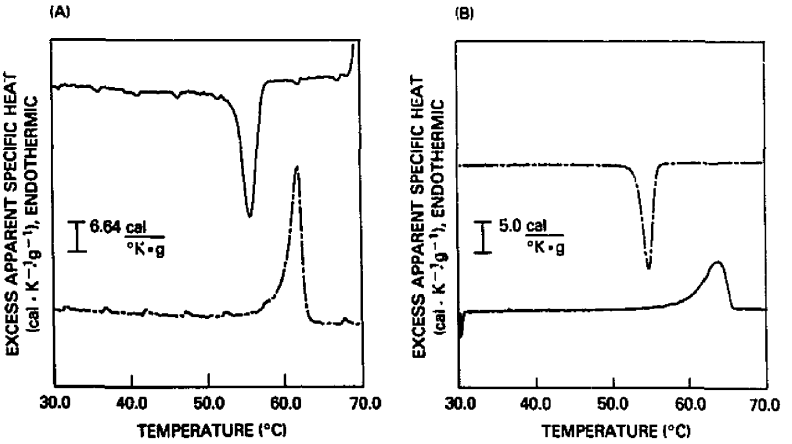

Fig. 4. (A) Calorimetric scans of cooling hydrated (15,6) $DC_{27}PC$ multilamellar vesicles to form tubules, with subsequent heating of the tubule suspension. (B) Calorimetric scans of the cooling and heating of polycrystalline (15,6) $DC_{27}PC$. All scan rates were 1 C°/min.

tion observed upon cooling at 54.7°C (enthalpy 26 kcal/mol). Dry $DC_{4,17}PC$ has an endothermic transition at 74.8°C (enthalpy 30 kcal/mol) with an exotherm observed at 61.5°C (enthalpy 28 kcal/mol) upon slow cooling (Fig. 5). The endothermic transition observed for hydrated $DC_{4,17}PC$ is 66.4°C and 58.9°C for the hydrated exothermic transition (see Fig. 3). The hysteresis observed in the hydrated scans of these lipids is also present in the dry phase behavior. In addition, the enthalpies of the dry transitions were slightly higher (1–2 kcal/mol) than the enthalpies of the corresponding hydrated transitions.

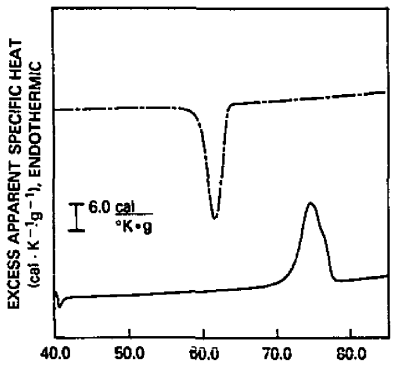

Fig. 5. Calorimetric scans of cooling (- - - -) and heating (———) of dry polycrystalline (4,17) $DC_{27}PC$. Scan rate was 1 C°/min for heating and cooling.

The behavior of the dry polycrystalline $DC_{m,n}PC$ reveals the unusual thermal properties (and perhaps crystalline nature) of tubules in fully hydrated systems. The difference in transition temperatures between other hydrated phosphatidylcholines and their polycrystalline powders is quite marked. For instance, fully hydrated DPPC has a melting endotherm at 41.6°C [18]. The polycrystalline dihydrate of DPPC melts at 75–80°C [16]. The difference in the melting temperature of the dry $DC_{m,n}PC$ phosphatidylcholines and the corresponding hydrated lipids is only 5–10°C (compare the transition temperature observed upon heating the hydrated tubules of $DC_{15,6}PC$ in Fig. 4A with the endotherm associated with melting the dry polycrystalline powder in Fig. 4B). This phenomenon is observed in $DC_{8,9}PC$ as well, which has a hydrated T_m at 43°C and a dry transition temperature of 52°C (unpublished data). The analog of $DC_{8,9}PC$, DTPC, which has fully saturated acyl chains, has a hydrated T_m at 73°C and a dry transition temperature of 112°C (unpublished data). The similarity of hydrated tubules and polycrystalline powder is revealed by examining the exotherm observed upon cooling the dry $DC_{m,n}PC$ polycrystalline material. The exotherm observed is very

close (1–2 C° higher) to the exotherm observed upon cooling the hydrated material as tubules form from multilamellar vesicles in excess water. This suggests that the hydrated low temperature phase of $DC_{m,n}PC$, which exists as tubules, may be very crystalline in nature with tightly ordered acyl chains and dehydrated head groups.

To more closely examine the molecular characteristics of the phase behavior of the positional isomers of $DC_{m,n}PC$, we have employed Fourier-transform infrared spectroscopy to explore the nature of chain packing and interfacial characteristics in the low-temperature tubule phase of these isomers. Fig. 6A reveals the fingerprint region (1400 cm^{-1} to 1000 cm^{-1}) of 4 members of the $DC_{m,n}PC$ series. A prominent CH_2 wagging progression between 1375 cm^{-1} and 1150 cm^{-1} is observed in all members of the series. This results from the wagging of *n*-coupled CH_2 oscillators in an all-*trans* conformation [21,22]. The frequency and intensity of the bands indicates the presence of many all-*trans* segments. The band intensity ratios in the progression are different however, indicating that the acyl chain packing of these diacetylenes are not identical. As the diacetylene is moved down the acyl chain toward the terminal methyl (as in $DC_{11,10}PC$, (a) in Fig. 6A) there is a narrowing of the asymmetric phosphate stretch at 1229 cm^{-1} and the bands in the CH_2 wagging progression become more prominent (Fig. 6A). This may indicate that the diacetylene introduces disorder by perturbing head group packing in the interfacial region as the diacetylene approaches the glycerol backbone (e.g., isomers with *m* segments 4–6). Alternatively, isomers with the diacetylene close to the interfacial regions such as $DC_{5,16}PC$ and $DC_{6,15}PC$, with longer *n* segments, may have increased *gauche* conformers in the longer methylene segments resulting in less well packed *n* segments and a decreased intensity of the CH_2 wagging progression. Spectral features of this region common to all isomers of the series are a broad C-C skeletal vibration at 1177 cm^{-1}, the symmetric PO_2^- stretch at 1087 cm^{-1} overlapped with the C-O-C stretch at 1068 cm^{-1}.

Fig. 6B shows the C-H stretching region (3100–2800 cm^{-1}) which reveal two major bands in all isomers of the series at 2920 cm^{-1} (CH_2 asymmetric stretch) and at 2850 cm^{-1} $9CH_2$ symmetric stretch). In addition, a band at 2937 cm^{-1} was observed in all isomers of $DC_{m,n}PC$ examined. In previous spectroscopic studies this band was tentatively ascribed to perturbations introduced by the diacetylene [4,10,12]. This perturbation might result from intrachain uncoupling (*m* and *n* segments) or local perturbations of methylenes adjacent to the diacetylenic groups. The ratio of intensity of this band to the asymmetric stretch at 2920 cm^{-1} was independent of chain segment length in all of the isomers indicating that

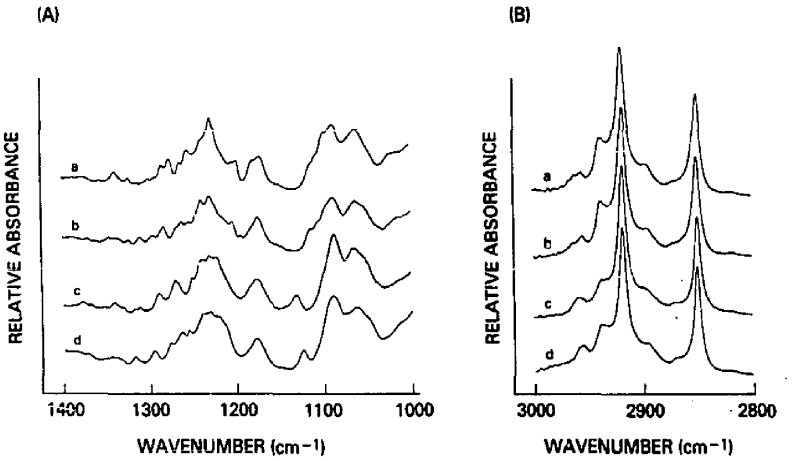

Fig. 6. FTIR spectra of four members of $DC_{27}PC$ isomer series. (a) (5,16), (b) (6,15), (c) (10,11), (d) (11,10). (A) 1400–1000 cm^{-1}. (B) 3000–2800 cm^{-1}.

local perturbation of the diacetylene in adjacent methylenes may occur. Alternatively, this may be good evidence that the *m* and *n* segments are vibrationally uncoupled and highly ordered as a broad band feature is observed at 2935 cm^{-1} in the spectra of short chain alkanes [25]. Isomers with the diacetylene in the middle of the acyl chain such as $DC_{10,11}PC$ and $DC_{11,10}PC$ also show splitting in the asymmetric CH_3 stretching band contour near 2954 cm^{-1}. This splitting occurs when the rotation of the methyl group is severely restricted and is observed in orthorhombic and triclinic crystals of alkanes and fatty acids [26].

Other spectroscopic features of all members of the $DC_{m,n}PC$ series in tubule form are a C=O stretch at 1718 cm^{-1} indicating partial dehydration and perhaps interfacial hydrogen bonding in the tubule low-temperature phase (data not shown). The observed C=O frequency observed and the bandwidth (21 cm^{-1}) indicates that it is very similar to the subgel phase of DPPC in which the water of hydration in the head group is reduced [10,24].

Overall, the spectroscopic features of the 27 carbon $DC_{m,n}PC$ series are very much like the features observed in $DC_{8,9}PC$. These features consist of tight acyl chain packing with a dehydrated interfacial region. A comparison of $DC_{8,9}PC$ with its saturated analog revealed that the diacetylenes have much narrower bands in the acyl chain regions indicating a higher degree of acyl chain packing [24]. Further examination of these modes and of other bands related to these diacetylenic phosphatidylcholine in deuterated analogs is currently underway to make more definitive assignments.

Conclusions

We have examined positional isomers of $DC_{m,n}PC$ to explore the effect of the diacetylene position on the formation of tubules and their molecular characteristics. The results presented indicate that all members of the $DC_{m,n}PC$ form tubules with similar molecular characteristics. Even though infrared spectroscopic evidence indicates slight differences in the packing of these isomers, all of the tubules formed from these isomers exhibit a high degree of conformational order. This is evidenced by the similar calorimetric properties of the hydrated and polycrystalline $DC_{m,n}PC$ and the spectroscopic features of hydrated tubules.

This body of data indicates that the tubule microstructures are uniquely crystalline which may have important bearing on the driving force for tubule formation. It may be that some inherent crystal parameter drives tubule growth as the phase transition occurs. However, the similarity in the phase behavior of the dry amorphous material and the hydrated tubules suggests that the acyl chain characteristics of the tubules alone are not sufficient to explain tubule formation. The dehydrated interfacial region of the low temperature phase of this lipid may indicate that hydration forces are important in the process of tubule formation. Further work will focus on examining deuterated analogs of these lipids, the role of the solvent in tubule formation, and examining the molecular characteristics of other tubule forming lipids, in an effort to determine the driving force for tubule formation.

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