DEUTERATED PHOSPHOLIPIDS AS NONPERTURBING COMPONENTS FOR RAMAN STUDIES OF BIOMEMBRANES

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ABSTRACT The deuterated phospholipid, 1,2-dipalmitoyl-d_{62}-phosphatidylcholine is shown by Raman spectroscopic measurements to be useful for obtaining information concerning phospholipid conformation in complex phospholipid and lipid-protein mixtures. The Raman bands of the deuterated phospholipid are assigned, and the sensitivity of these vibrational modes to conformational changes in the bilayer is demonstrated. Deuteration of the alkyl chains reveals the CH vibrations of the head group. A change in these bands is observed at the melting temperature and is assigned to alteration of the glycerol backbone conformation upon melting.

INTRODUCTION

Raman spectra of single component phospholipid systems can be interpreted to give highly detailed information about the structure of the component molecules (1–4). Multiple component systems, however, give spectra in which many of the structurally sensitive modes of different phospholipids or proteins may overlap. Other techniques can in theory resolve the behavior of individual types of molecules in multicomponent lipid mixtures, but only by introducing labeled molecules of one type or another. Unfortunately such probe molecules will tend to perturb the structure of the membrane (5). Even if the concentration of the probe is insufficient to substantially perturb the phase behavior of the bulk sample, the local environment of the probe—the only one "visible" in the experiment—may be perturbed.

Clearly, the best possible probe of normal phospholipid behavior is one that forms a thermodynamically ideal mixture with normal phospholipids and therefore does not perturb the structure of the bilayer. If these conditions are fulfilled, there is little distinction between "probe" and "host" except insofar as the spectroscopic observation is concerned. Deuterocarbons are isomorphous with hydrocarbons, but have different vibrational frequencies. Their use in Raman spectroscopy of phospholipid membranes was suggested as early as 1973 by Chapman (6), and studies of specifically deuterated stearic acids by Sunder et al. (7) have already shown such molecules to be

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useful in Raman Studies. It has been suggested from calorimetric studies (8)[1] that 1,2-dipalmitoyl-d_{62}-phosphatidylcholine is just the type of "ideal" component species required to study behavior of mixtures containing two classes of lipids—one deuterated and one undeuterated—maintaining phase behavior of the mixture identical to that present if both types of lipid were undeuterated. For Raman spectroscopy the advantage of such a system is that the vibrational frequencies of the two different types of lipids are now no longer overlapping. Furthermore, deuteration of the fatty acyl chains allows a spectroscopic examination of subtle structure changes in the nondeuterated head group.

METHODS

The sample of 1,2-dipalmitoyl-d_{62}-phosphatidylcholine (DPPC-d_{62}) was synthesized by Lipid Specialties, Inc. (Boston, Mass.), and dipalmitoyl phosphatidylcholine (DPPC) was obtained from Calbiochem (San Diego, Calif.). Routine purification of all phospholipids by chromatography in ethanol at 37°C on Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, N.J.) yielded dispersions with acceptable luminescence backgrounds. As indicated by differential scanning calorimetry, DPPC-d_{62} dispersions exhibit thermal behavior similar to that of DPPC: a small peak due to premelting at 28°C and a sharp, nearly symmetric melting transition at 37.5°C. These values agree with those obtained by Petersen et al. (8) by optical density measurements. A detailed discussion of the phase behavior of lipids will be reported elsewhere.[1]

Hexadecane was purchased from Applied Science Labs, Inc. (State College, Pa.); hexadecane-d_{34} and palmitic acid-d_{31} from Merck Sharp & Dohme (St. Louis, Mo.); palmitic acid, Cd^{++}-α-glycerophosphorylcholine (GPC), Ca^{++}-α-glycerophosphate, and phosphocholine from Sigma Chemical Co. (St. Louis, Mo.).

The differential adiabatic scanning microcalorimeter of the Privalov design (9) was model DASM-1M constructed by the Academy of Sciences U.S.S.R. Scientific Institute, Department of Biological Instrumentation (Poustchino, Moscow Region, U.S.S.R.).

A description of the preparation of phospholipid dispersions and the computerized Raman apparatus has been given previously (1). All spectra were collected at a digital resolution of 1 cm^{-1}. The spectra of solid samples were taken with 400 mW of 5,145 Å radiation at 150 μm slit width (3–4 cm^{-1} spectral split width), and averaged from six scans. Spectra of dispersions were taken with 600 mW at the same slit width and averaged from 10 scans. A scan set consisted of scanning each of the three relevant spectral regions in sequence once each, and then repeating the entire sequence 10 times. This minimized the effect of any time-dependent changes in the sample such as luminescence burn-off. All dispersions were allowed to equilibrate at low temperatures for several hours before scanning, and subsequent scans were taken in order of increasing temperature. It should be noted that none of the spectra presented has been normalized or smoothed, although base lines have been subtracted in all spectra by setting the lowest point in the spectrum equal to zero. Temperature corrections to account for laser heating of the sample and the true temperature of the sample-holding block have been applied in all cases (1).

The melting curves shown in Fig. 9 were produced as follows. The laser intensity was 400 mW, and slits were set at 150 μm. The sample was allowed to equilibrate for 3 h with the water bath at 10°C. The computer then increased the temperature of the water bath in approximately 0.6°C steps at intervals of 10 min. Between temperature steps, photoelectron

[1] Klump, H., B. P. Gaber, W. L. Peticolas, and P. Yager. Manuscript submitted for publication.

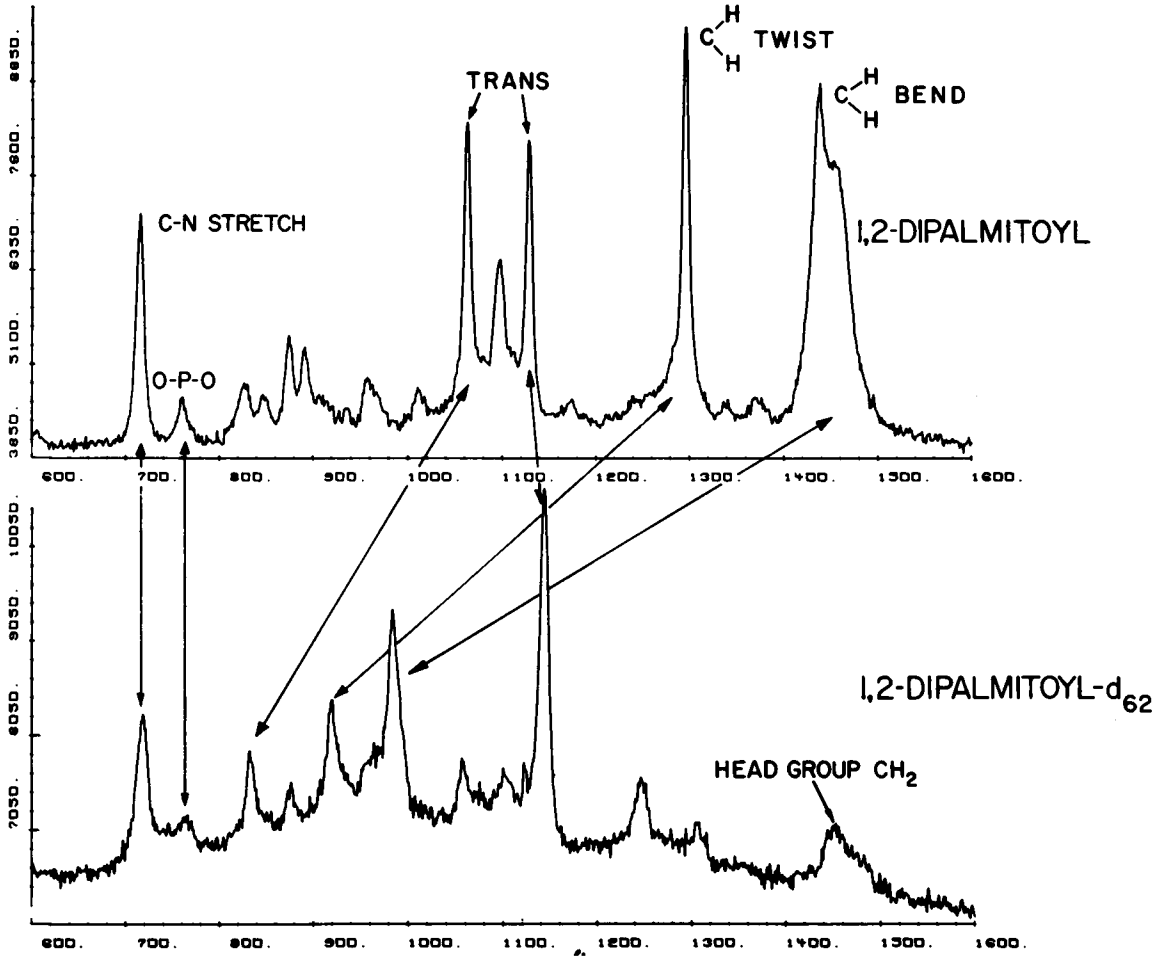

FIGURE 1 Raman spectra of solid samples of DPPC and DPPC-d_{62}, with arrows indicating correlations between some of the more important bands. Spectra were obtained with 400 mW of 5,145 Å at 150 μm slits, and represent averages of two scans. Note that vertical scales are not identical.

pulses were accumulated for 30 s at predetermined Raman shifts corresponding to maxima in the dispersion difference spectra, as well as at base-line points at 650, 2,000, and 2,700 cm^{-1}, and the peak of the C—N stretch at 716 cm^{-1}. The entire set of 66-point melting curves was collected over 11 h. The base-line intensities were subtracted from the peak curves, and all curves were normalized to slight fluctuations in scattering intensity by division of the peak values by the intensity of the C—N stretch. The resulting curves were plotted as 41-point melting curves with values interpolated by the computer from the original 66-point arrays. A temperature correction was applied to the abscissa of the melting curve displays to bring the observed main transition into correspondence with the calorimetrically observed main transition. The intensity axes of the melting curves are not to scale.

RESULTS

Spectral Features of DPPC-d_{62}

Figs. 1 and 2 compare the Raman spectrum of poly-crystalline DPPC-d_{62} with that of poly-crystalline DPPC. Arrows indicate how the major DPPC bands change in frequency upon deuteration. We have based our set of assignments for the deuterocarbon portion of the Raman spectra upon various theoretical normal coordinate calculations (10–13). Experimental results on model compounds by ourselves and others (7,14,15) have also been useful.

The Raman bands of DPPC-d_{62} in Figs. 1 and 2 arise from both the vibrational modes of the perdeuterated chains and modes of the undeuterated phospholipid head group. Assignment of the head group modes was facilitated by reference to the model compounds (glycerol, phosphocholine, α-glycerophosphate, and α-glycerophosphorylcholine). The full set of assignments is summarized in Table I.

The choline N—CH_3 stretch (716 cm^{-1}) is a useful intensity reference for DPPC spectra (1,2,16). In DPPC-d_{62}, however, a nearby CD_3 rocking mode adds intensity in this region; although the peak height is not highly conformation dependent, it must be noted that peak height ratios taken relative to 716 cm^{-1} for DPPC-d_{62} may not be directly comparable to similar ratios in DPPC. The O—P—O stretching mode near 1,100 cm^{-1} is observed in the DPPC-d_{62} spectrum. A comparison of the relative intensity at 1,100 cm^{-1} in DPPC-d_{62} and DPPC (Fig. 1) indicates that most of the intensity of this band in the DPPC spectrum is derived not from the weak O—P—O stretch but from a mode of the hydrocarbon chain near 1,100 cm^{-1}.

Deuteration of the acyl chains of a lecithin proves a window for observation of CH bands arising from the head group. In DPPC-d_{62} the head group methylene deformations occur at 1,451 cm^{-1}—a frequency significantly higher than that of the methylenes of a hydrocarbon chain.

A correlation of the C—H and C—D stretching modes is depicted in Fig. 2. The C—D stretching manifold falls in a spectral window free of bands from protonated lipids (or, for that matter, proteins). For assignments in this region we have used the work of Sunder et al. (6).

The C—H stretching modes of the phosphatidylcholine head group are revealed in the DPPC-d_{62} spectrum in the 2,700–3,100 cm^{-1} region (Fig. 2). The band contour is

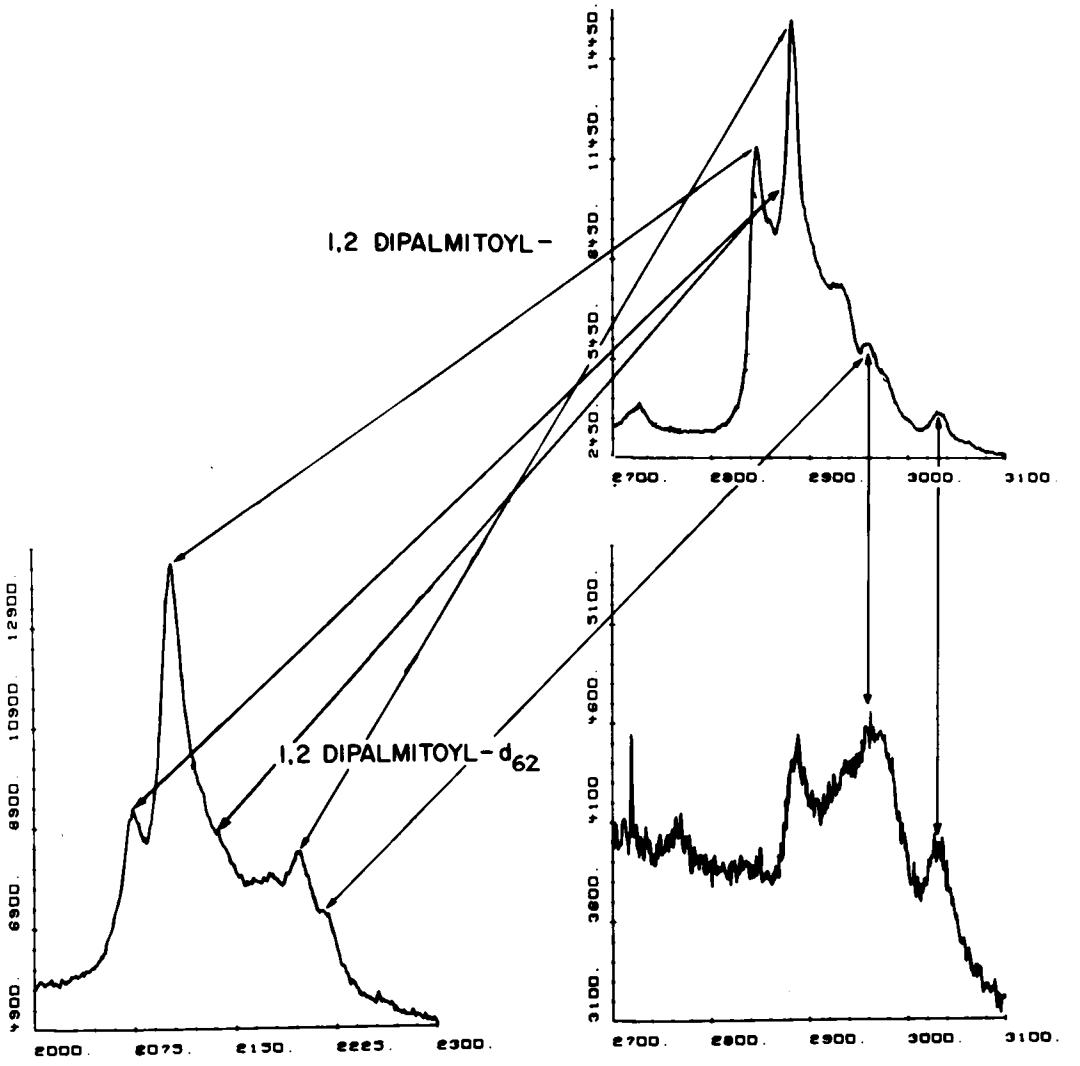

FIGURE 2 Raman spectra of solid samples of DPPC and DPPC-d_{62}, with arrows indicating correlations between some of the more important bands. Spectral parameters are identical to those in Figure 1; because the number of counts is provided on the *y*-axes, the intensities of the various scattering regions of each compound are comparable. Note that there are no peaks in the DPPC C—D stretching region.

totally different from that arising from alkyl chains and is nearly identical to that of solutions of α-glycerophosphorylcholine (see bottom trace, Fig. 7). We have obtained some variability in the shapes and intensities of these bands in solid samples, presumably due to differences in degree of hydration. Examination of several samples of deuterated hydrocarbons and fatty acids has indicated that the peak heights observed for the C—H stretches of DPPC-d_{62} (Fig. 2) are far greater than any possible contribution by random protons remaining on the perdeuterated chains.

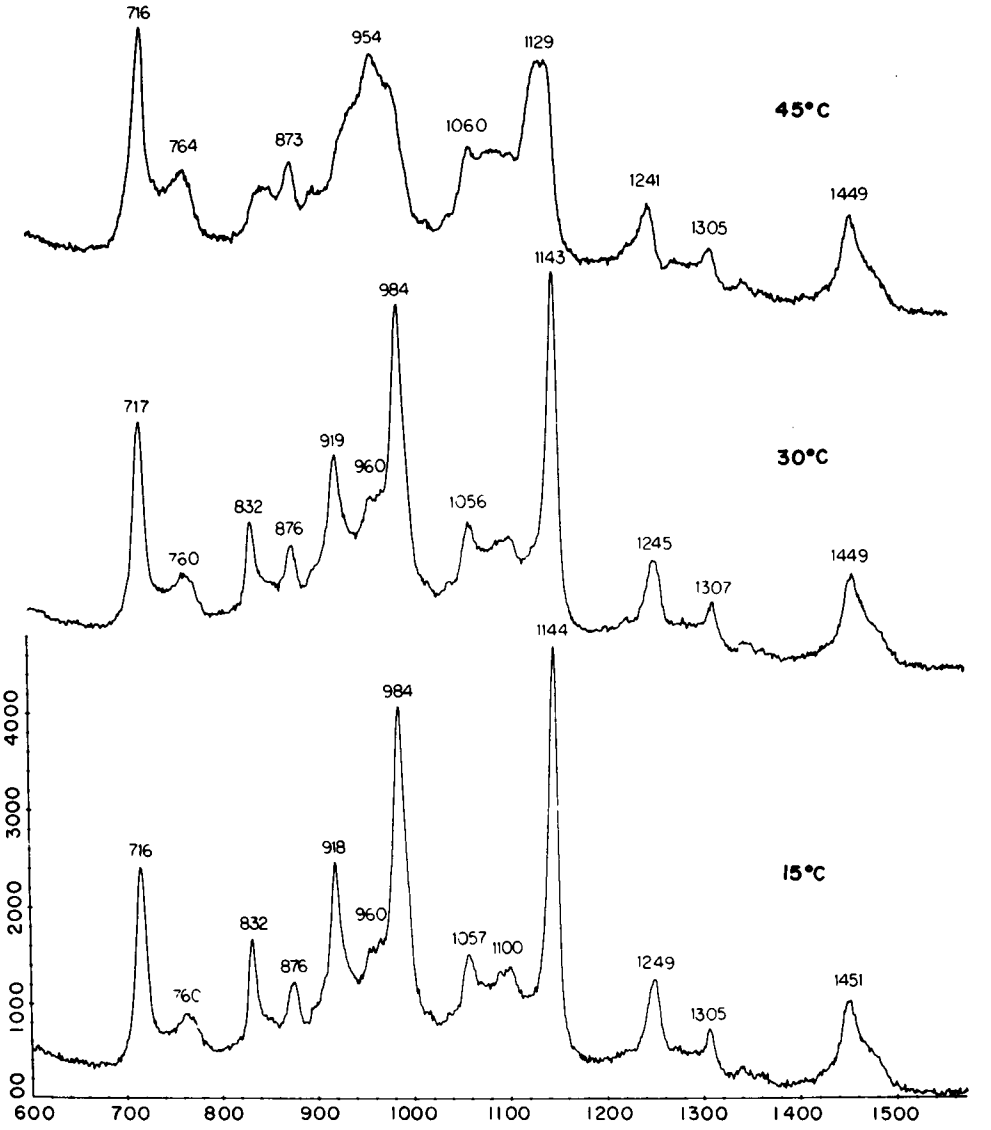

FIGURE 3 Raman spectra of a DPPC-d_{62} dispersion at 15°, 30°, and 45°C. All three spectra are on the same scale. Each spectrum represents an average of 10 scans with the laser at 600 mW of 5,145 Å, and the slits at 150 μm.

Conformational Sensitivity of Raman Spectra of DPPC-d_{62} Dispersions

Raman spectra of aqueous dispersions of DPPC are sensitive to changes in bilayer conformation occurring in the temperature region around the two endothermic phase transitions (1,2), and we have been able to assign specific changes in the DPPC spectrum to either the premelt or the melting transition. A comparable analysis may be

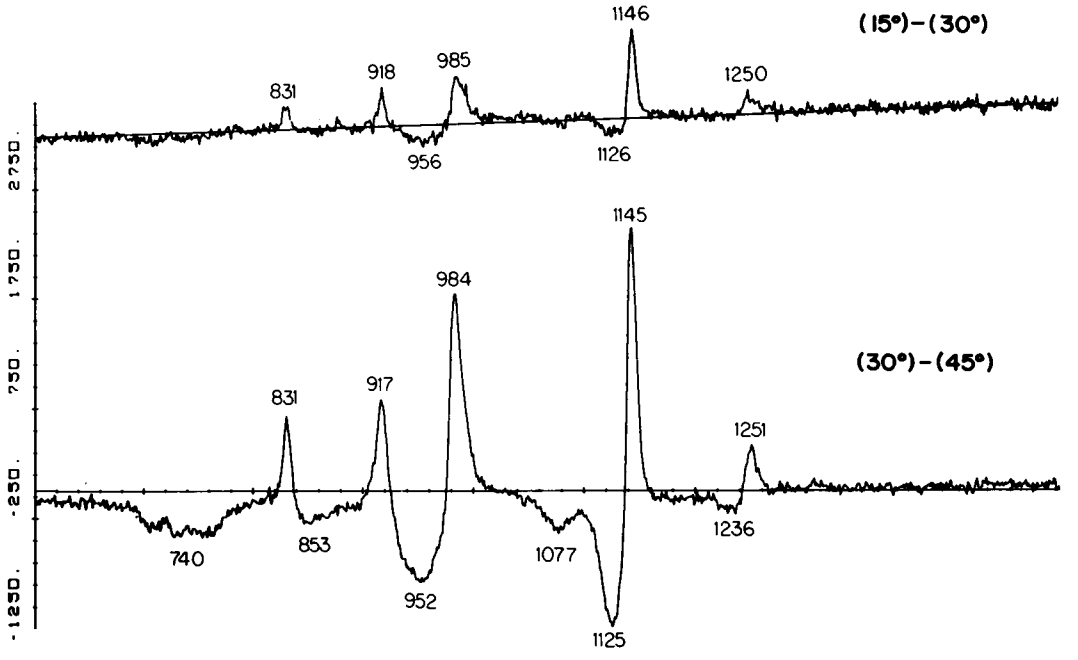

FIGURE 4 Difference spectra created by subtraction of the data shown in Fig. 3. Both spectra are on the same scale.

performed on spectra of DPPC-d_{62}, and those bands we have assigned as conformationally sensitive markers do indeed change with alterations in bilayer structure. The temperatures we have chosen to examine for absolute spectra of the DPPC-d_{62} dispersion correspond to $T < T_{pre}$, $T_{pre} < T < T_m$, and $T > T_m$.

That the spectra of the DPPC-d_{62} dispersion are sensitive to conformational changes is clear from the absolute spectra shown in Fig. 3. The exact nature of the changes can be more clearly shown by difference spectra shown in Fig. 4 generated by computer subtraction of the absolute spectra. Note that positive peaks reflect decreases in peak height upon melting, and that negative troughs reflect increases. Other types of band changes, such as peak shifts and changes in width, can also produce strong difference spectra.

Unlike the difference spectra published for DPPC (1), the 15°–30°C spectrum and the 30°–45°C spectrum in DPPC-d_{62} are qualitatively very similar. Thus, the difference spectra show no features that may be uniquely identified as "pre-melt markers" or "melting markers." As the data in both the 15°–30°C and the 30°–45°C spectrum are similar, the band changes seen upon melting will be discussed in terms of the latter spectrum only. These changes are summarized in Table I and discussed briefly below.

There is a broad increase centered at about 740 cm^{-1}, and inasmuch as the C—N stretch does not change, we assign the increase to the —CD_3 rocking modes that fall in this region. A similar increase is seen in these bands when hexadecane-d_{34} is melted. A skeletal mode at 831 cm^{-1} melts out, and appears to shift to higher values, resulting

TABLE I

ASSIGNMENTS OF DPPC-d_{62}

Δcm^{-1}	Change of band on melting	Assignment	Corresponding band in model compound
716		C—N stretch and CD_3 rock	715–716 in all choline-containing compounds
760	Increases	Phosphate symmetric diester stretch and CD_3 rock	772 in GPC solutions, weak at 760 in hexadecane-d_{34}
832	Decreases Broadens	CD* skeletal optical mode	828 in solid hexadecane-d_{34}
876		Head group and CD unassigned	877 in GPC, phosphocholine; weak in liquid hexadecane-d_{34}
918	Decreases Broadens Shifts to 940	CD_2 twist	918 in solid hexadecane-d_{34}, palmitic acid-d_{31}
954		CD unassigned	954 in hexadecane-d_{34}, palmitic acid-d_{31}
984	Decreases Broadens Shifts to 960	CD_2 scissoring	987 in solid palmitic acid-d_{31}, 991 in solid hexadecane-d_{34}
1,057		CD_3 symmetric bend	1,057 in hexadecane-d_{34}, palmitic acid-d_{31}
1,077	Increases	CD unassigned and head group	1,070 in GPC, 1076 in liquid hexadecane-d_{34}
1,100		O—P—O (–) symmetric stretch	1,089 in GPC
1,125	Increases	CD unassigned	1,129 in liquid hexadecane-d_{34}
1,144	Decreases	CD skeletal optical mode and deformation	1,145 in palmitic acid-d_{31}, 1,150 in hexadecane-d_{34}
1,249	Decreases Broadens Shifts to 1,241	CD_2 wag	1,251 in solid hexadecane-d_{34}, palmitic acid-d_{31} 1,245 in liquid hexadecane-d_{34}
1,305		CD unassigned	1,305 in hexadecane-d_{34}, palmitic acid-d_{31}
1,451		Choline CH_2 scissoring	1,450 in phosphocholine solution
1,470		Glycerol and choline CH_2 scissoring	1,470 in α-glycerophosphate, 1,480 in phosphocholine solution
2,075	Decreases	CD_3 symmetric stretch	2,073 in hexadecane-d_{34}
2,101	Decreases	CD_2 " "	2,101 in solid hexadecane-d_{34}, 2,107 in liquid
2,135	Decreases	CD_3 " "	2,135 in solid hexadecane-d_{34}
2,173	Increases slightly	CD_2 " "	2,173 in solid hexadecane-d_{34}
2,194	Decreases	CD_2 asymmetric stretch	2,198 in hexadecane-d_{34}
2,210		CD_3 " "	2,218 in hexadecane-d_{34} (sharp)
2,765		Unassigned	
2,885	Decreases Broadens Shifts to 2,900	Glycerol and unchanging contributions from lone protons on the deuterocarbon chains	2,889 in glycerol at 25°C, 2,902 in GPC solution
2,935		Glycerol and perturbed head group methylene	2,921 in glycerol at 25°C, 2,938 in phosphocholine solution
2,980		Perturbed head group methylenes and choline CH_3 symmetric stretch	2,988 in solutions of phosphocholine, GPC
3,041		Choline CH_3 asymmetric stretch	3,044 in phosphocholine solution 3,042 in GPC solution

*CD denotes modes of the deuterocarbon chain.

in the broad trough at 853 cm^{-1}. A band of moderate intensity at 876 cm^{-1} in the absolute spectrum remains unchanged in the difference spectra.

The CD_2 twist at 918 cm^{-1} decreases in intensity and, like the comparable band in hydrocarbons (1), shifts to higher frequencies and broadens upon melting. The broad increase centered at 952 cm^{-1} is also observed in melted hexadecane-d_{34}, and in that compound, the depolarization ratio changes across the width of the 952 cm^{-1} band

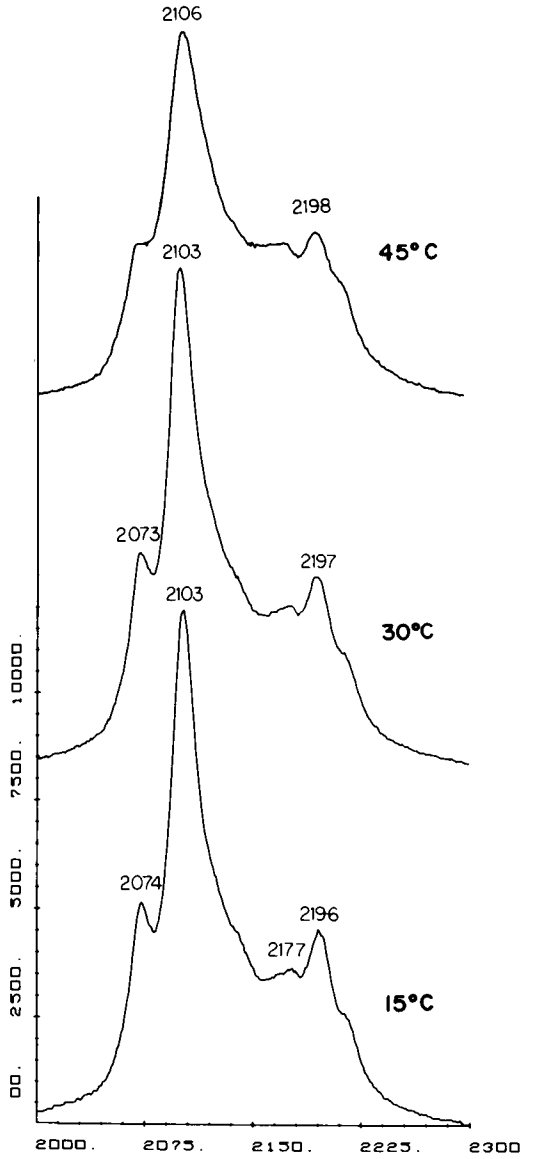

FIGURE 5 Raman spectra of the C—D stretching region of the same sample as shown in Fig. 3 taken at 15°, 30°, and 45°C. All three spectra are shown to the same scale.

in a continuous manner. There may be two or more broad bands arising from different modes in this region, one of which may be the frequency-shifted CD_2 twist noted above.

The rounded shape of the difference spectrum trough at 952 cm^{-1} indicates that the peak at 954 cm^{-1} in the absolute 45°C spectrum may arise from the same band seen at low temperatures as a shoulder at 960 cm^{-1}. If this is so, then the 954 cm^{-1} band does not change in any way upon melting. The upper portion of the 952 cm^{-1} difference trough is then accounted for by a decrease in the intensity of the 984 cm^{-1} CD_2 scissoring band, concomitant with a broadening and shift to lower Δcm^{-1}. Such a change per se is not observed in the analogous methylene scissors mode in hydrocarbons (1,440 cm^{-1}), but in hydrocarbons the scissors mode is also split by Fermi resonance with an infrared-active band at 722 cm^{-1}. Such a Fermi resonance does not appear to exist for this mode in deuterocarbons, and therefore exactly analogous band changes are not expected.

There is no change in the intensity of the 1,057 cm^{-1} symmetric CD_3 band throughout the temperature region explored, but there is an increase in a band at 1,077 cm^{-1}. We cannot exclusively assign the 1,077 cm^{-1} band to either the deuterocarbon skeleton or the headgroup. No change occurs in the O—P—O symmetric stretch at 1,100 cm^{-1}.

The behavior upon melting of the 1,144 cm^{-1} band (which is assigned primarily to the deuterocarbon symmetrical skeletal optical mode) is similar to that seen for the 1,127 cm^{-1} band of DPPC. That is, both the frequency of the band and the peak intensity decrease as the all-*trans* length and degree of packing decreases. The trough in the difference spectrum at 1,125 cm^{-1} and the peak at 1,145 cm^{-1} may arise from a downshift and broadening of one band. However, the 45°C absolute spectrum indicates that there may be two separate bands overlapping and centered at 1,129 cm^{-1}. This possibility is supported by the melting curves derived from these bands (see below).

It is notable that no changes are seen in the CH_2 scissors mode of the head group methylenes throughout the temperature range studied.

From the data in the C—D stretching region (Fig. 5), it can be seen that there is a systematic shift to higher frequency and a decrease in peak height of the symmetric CD_2 stretch with increasing chain disorder. The change is analogous to the behavior of the CH_2 symmetric stretching band in DPPC (1). The C—D stretching region of DPPC-d_{62}, even at 15°C, more closely resembles the spectrum of liquid hexadecane-d_{34} than that of the solid.[2]

As in the mid-frequency region, Fig. 6 shows that the primary changes in the spectrum occur between 30° and 45°C, and the changes that occur in both temperature ranges appear qualitatively similar. However, a pronounced trough occurs in the 15°–30°C difference spectrum at 2,117 cm^{-1} which is not evident in the main melting difference spectrum.

Upon melting there are decreases in the Fermi resonance split symmetric CD_3 stretches at 2,073 and 2,135 cm^{-1}, the antisymmetric CD_2 stretch at 2,195 cm^{-1}, and the symmetric CD_2 stretch at 2,101 cm^{-1}. There is a possible increase in the

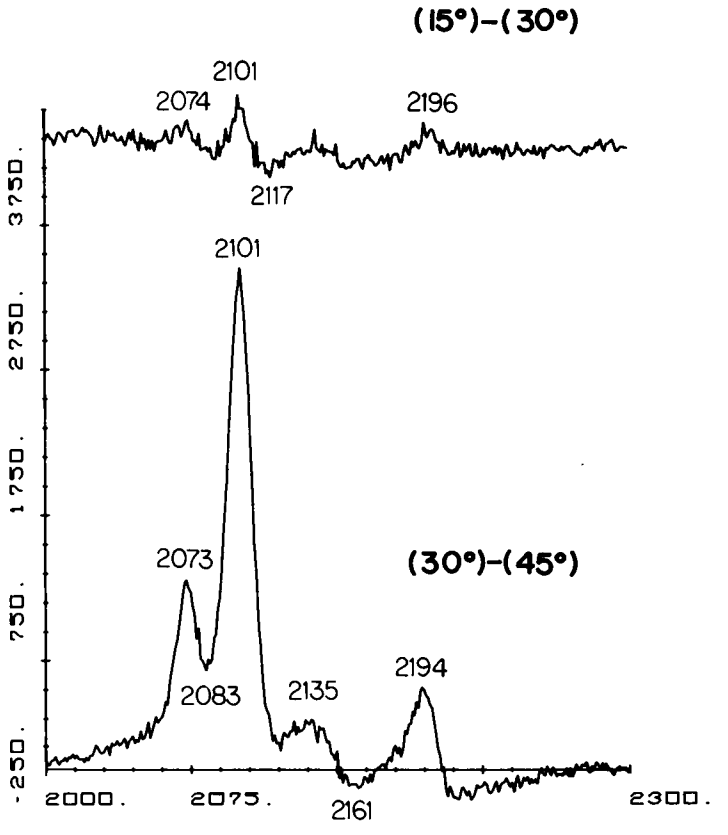

FIGURE 6 Difference spectra created by computer subtraction of the data shown in Fig. 5. Both spectra are on the same scale.

asymmetric CD_3 stretch at 2,220 cm^{-1}. The magnitudes of these changes are quite large, although not apparent in Fig. 5. The most striking difference between these difference spectra and those of the C—H stretching region of DPPC (1) is the much greater sensitivity of the methyl stretches in the deuterocarbons compared with those modes of the hydrocarbons.

Any changes seen in the C—H stretching region must be assigned to alterations in the structure of the head group because the 45°C spectrum is identical to that of a solution of α-glycerophosphorylcholine (see Fig. 7). A striking change appears in the head group difference spectrum (Fig. 8) as a decrease at 2,885 cm^{-1} (with a possible shift to higher Δ cm^{-1}; note slight trough at 2,900 cm^{-1}). Most significantly, the spectral change occurs at the melting temperature, and *not at the premelting temperature.* Studies of hexadecane-d_{34} indicate that lone protons (on the fatty acyl chains) give rise to an extremely weak band at about 2,888 cm^{-1}, but that this band is insensitive to the conformation of the carbon backbone. Spectra of head group model compounds indicate that the only possible assignment for the difference band at 2,888 cm^{-1} (and the shift of 13 cm^{-1} in the absolute spectrum) is a glycerol backbone C—H stretch.

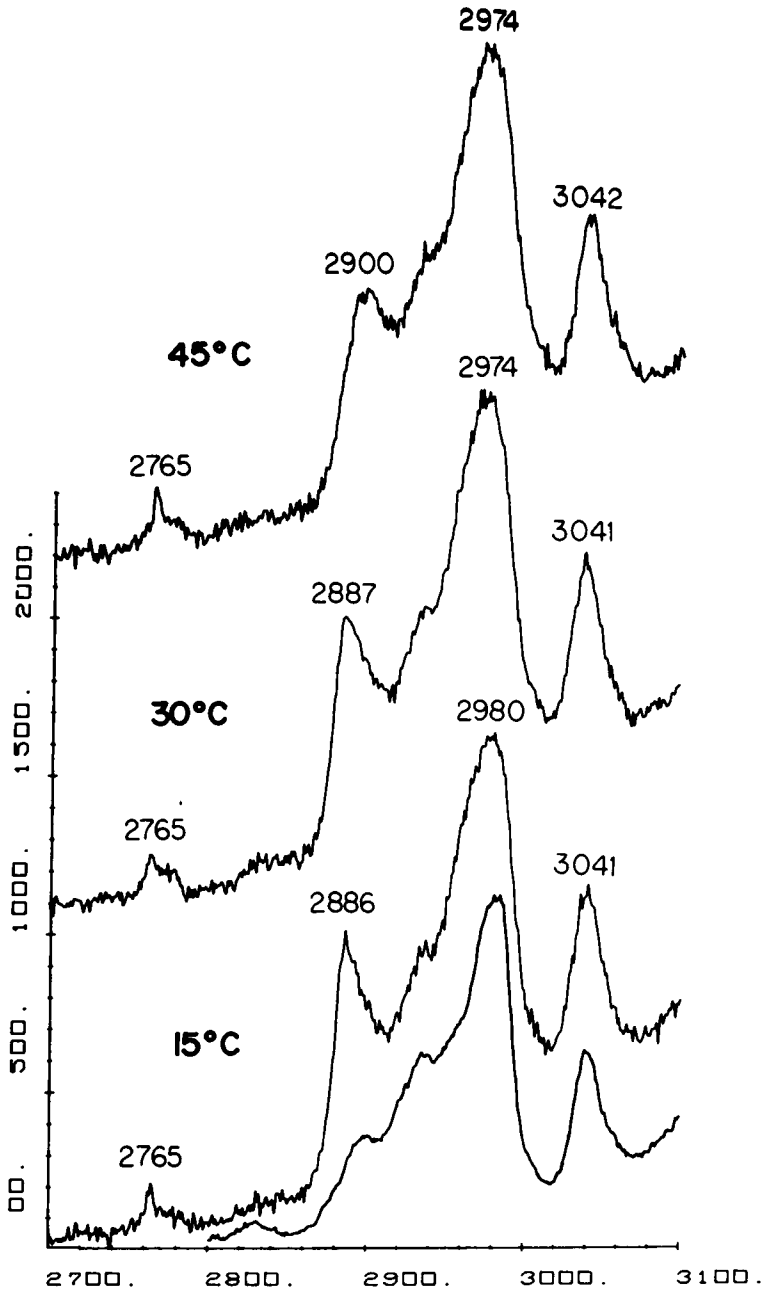

FIGURE 7 Raman spectra of the C—H stretching region of the same sample as shown in Fig. 3 taken at 15°, 30°, and 45°C; plus, at the bottom, a spectrum of a solution of Cd^{++} salt of α-glycerophosphorylcholine. The three DPPC-d_{62} spectra are shown to the same scale, and the fourth is arbitrarily scaled.

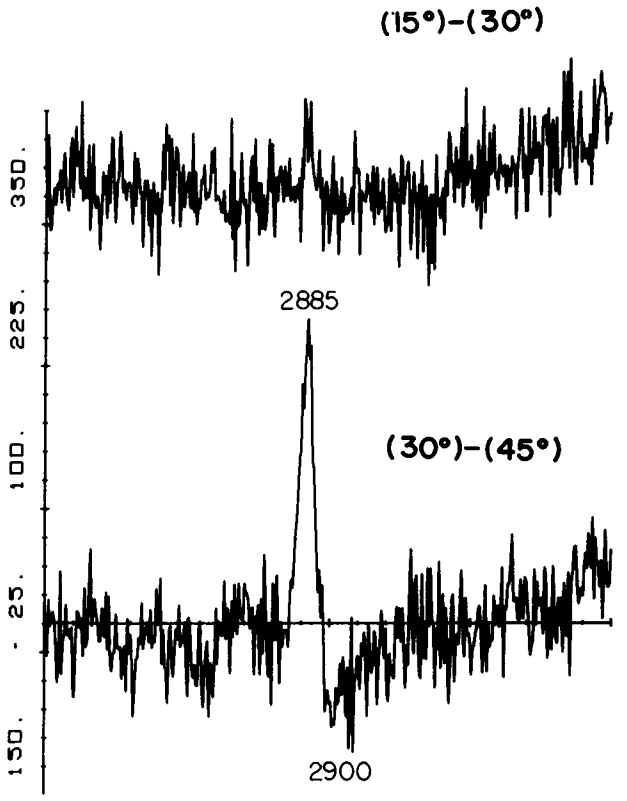

FIGURE 8 Difference spectra created by computer subtraction of the DPPC-d_{62} data shown in Fig. 7. Both spectra are on the same scale.

Melting curves derived from Raman band intensities of DPPC have been used to show that both hydrocarbon skeletal modes and C—H stretching modes are sensitive to changes in the bilayer structure (1). Presented in Fig. 9 are melting curves for eight structurally sensitive bands of DPPC-d_{62} separated into three general classes: (*a*) bands that are insensitive to the premelt and show an increase in intensity at T_m, (*b*) bands that show steep decreases in intensity at temperature below T_m, with abrupt changes in intensity at both T_m and T_{pre}, and (*c*) C—H and C—D stretches that show only minimal changes at the premelt, abrupt changes at T_m, and fairly flat slopes at temperatures below T_m.

The inclusion of the band at 1,125 cm^{-1} into the first group and the inclusion of the 1,145 cm^{-1} band into the second supports our suggestions (above) that the change in the 1,125 and 1,145 cm^{-1} bands are independent. Behavior similar to that of the 1,145 cm^{-1} band is observed for the 1,130 cm^{-1} skeletal optical mode band of DPPC (2), suggesting that the assignment of the 1,145 cm^{-1} band of DPPC-d_{62} to a skeletal optical mode is correct.

As we have proposed that the trough at 952 cm^{-1} in Fig. 4 is caused by at least two

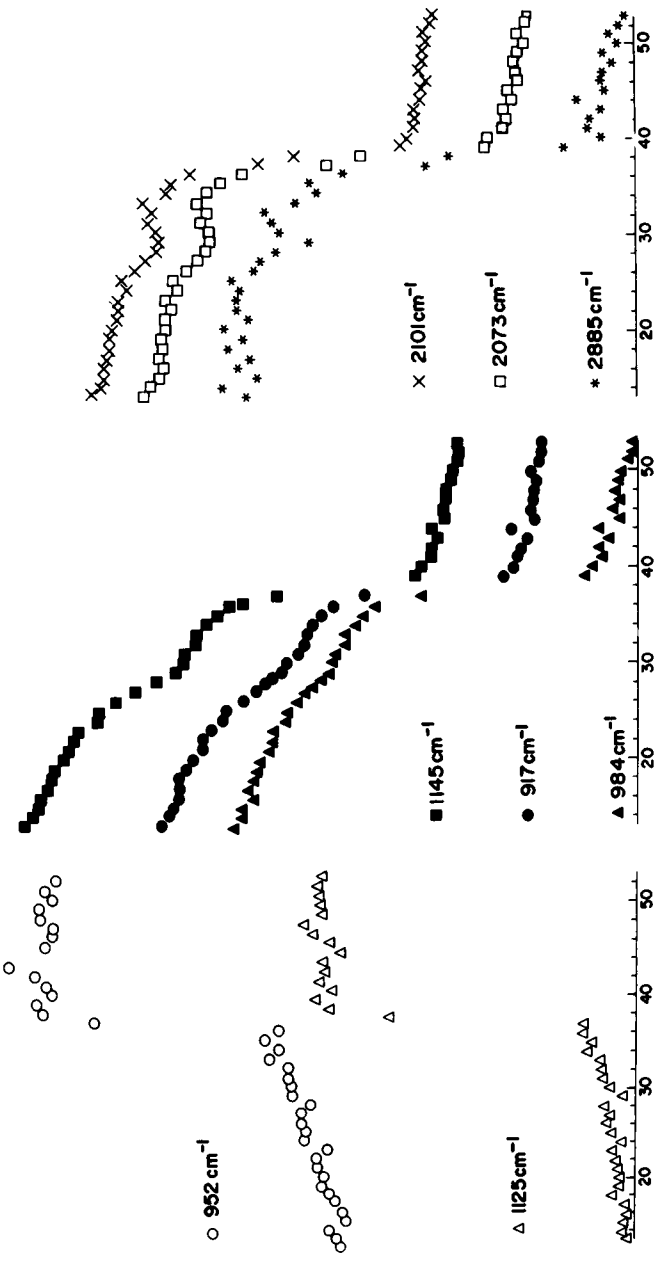

FIGURE 9 Melting curves of various structurally sensitive bands of DPPC-d_{62}. The temperature scales have been adjusted from the original water bath temperatures to account for laser heating of the sample and cooling of the sample-holding block. For a fuller description, see the text.

bands, the absence of an inflection at T_{pre} seems to indicate that neither of the contributing bands is sensitive to the premelt.

The melting curves of the CD_2 twist at 917 cm^{-1} and the CD_2 scissoring at 984 cm^{-1} are quite sensitive to the melting transition. Further, both curves show the steep slope below T_m seen in the skeletal optical mode band.

The three curves shown in the third panel of Fig. 9 are nearly identical. It is of interest that the symmetric CD_2 stretch at 2,101 cm^{-1} is relatively insensitive to the premelt as compared to the sensitivity of the analogous band in DPPC. There is a small dip at the premelting temperature but this may be an artifact. We have observed that the CD_2 stretch is relatively insensitive to crystal packing effects when hexadecane-d_{34} is isolated in a matrix of hexadecane.[2] Clearly, the pattern of Fermi resonance and crystal-field interactions at play in hydrocarbons (17) is different from that in deuterocarbons. The symmetric CD_3 stretch at 2,073 cm^{-1} is insensitive to premelting, but decreases sharply at T_m. The glycerol backbone band at about 2,885 cm^{-1} is included in the third panel of Fig. 9, again demonstrating that the conformational changes in the glycerol backbone in the lecithin molecule occur at the melting temperature, and not at the premelt as is widely believed.

DISCUSSION

The major goal of this work has been to develop a nonperturbing chemical component with which to monitor the behavior of complex lipid mixtures. The calorimetric data demonstrate that DPPC-d_{62} is a useful analogue of DPPC, and from the presence of the pretransition and sharp main transition, we conclude that the chain and head group conformations of the chain-deuterated lecithin are probably identical to those in DPPC. Furthermore, from the behavior of mixtures of DPPC and DPPC-d_{62}, it may be inferred that the two lipids mix nearly ideally (8).[1]

Although we have emphasized the similarities between DPPC and DPPC-d_{62}, differences in the patterns of the vibrational spectra of the two compounds give each a unique sensitivity to environmental effects in the bilayer. In DPPC the methylene stretches are sensitive to subtle alterations in chain packing (2,4) whereas in the deuterated compound the methylenes are not as sensitive, as the pattern of Fermi resonance overlaps of vibrational modes differs. However, for DPPC-d_{62} there exists an equally propitious set of Fermi resonances with the CD_3 stretches that makes the spectra of the deuterated compounds sensitive to the conformation and environment of the terminal methyl groups. Thus, the two sets of compounds together can provide detailed information about the total environment of the phospholipid in the bilayer not available from observation of the Raman scattering from either compound alone.

It is most important to note that the change that occurs in the 2,885 cm^{-1} band occurs at the melting transition, and not at the pretransition. If there is a change in the head group at the pretransition, Raman spectroscopy is not sensitive to it. Pri-

[2]Gaber, B. P., and P. Yager. Unpublished results.

marily it is the hydrocarbon chains that undergo conformational change upon premelting.

More than 10 other bands are sensitive to phase changes in DPPC-d_{62}. Many of these bands (and particularly those in the 2,000–2,300 cm^{-1} region) are situated in spectral regions that allow them to be cleanly separated from Raman bands arising from either nondeuterated lipids or proteins, thereby allowing one to monitor simultaneously the conformation of at least two species of molecules in the same sample or to monitor simultaneously the overall conformation of the two acyl chains of the DPPC molecule.[3]

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