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A Comparative Monomolecular Film Study of a Straight-Chain Phosphatidylcholine (Dipalmitoylphosphatidylcholine) with Three Isobranched-Chain Phosphatidylcholines (Diisoheptadecanoylphosphatidylcholine, Diisooctadecanoylphosphatidylcholine, and Diisoeicosanoylphosphatidylcholine)†

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ABSTRACT: The surface pressure vs. area per molecule isotherms for monomolecular films of a straight-chain lecithin [dipalmitoylphosphatidylcholine (DPPC)] and three isobranched-chain lecithins [diisoheptadecanoylphosphatidylcholine (DIHPC), diisooctadecanoylphosphatidylcholine (DIOPC), and diisoeicosanoylphosphatidylcholine (DIEPC)] are reported over a wide range of temperatures and surface pressures such that the full range of the liquid expanded/liquid condensed transition is documented in each case from the temperature at which a fully condensed film is observed to that at which a fully expanded film is found. A comparison of two lecithins having the same length for the primary alkane chain (DPPC and DIHPC), on either an absolute or reduced temperature basis, indicated that the isobranched lecithin differed primarily from the straight-chain lecithin in having a more expanded condensed state. This was attributed to impaired packing in the condensed state due to the methyl isobranch. The isobranched lecithin also differed in having a slightly more condensed expanded state. This was ascribed to reduced flexibility in the expanded state due to hindered rotation of the methyl isobranch. Similar conclusions were recently drawn for bimolecular assemblies of isobranched lecithins. A comparison of the three isobranched lecithins at similar reduced temperatures indicated that, while the condensed states are very similar, the expanded states occupied increasing areas per molecule with increasing chain length. Two points that may be of biological significance are that the earlier onset of a liquid expanded (or liquid-crystalline-like) state on insertion of an isobranch could provide a wider temperature range for membrane and cell survivability and that the branched-chain lecithins would appear to provide an improved alternative to unsaturated lecithins in terms of fluidity coupled with reduced oxidation susceptibility in various model membrane experiments.

Kaneda (1977) found that bacteria of the genus *Bacillus* contain isobranched and anteisobranched fatty acids as major fatty acid components of their membrane lipids. Methyl-isobranched and methyl-anteisobranched fatty acids are also found in eight other genera of Gram-positive and in four genera of Gram-negative eubacteria. These findings seem to have provided the impetus for a recent series of investigations (Lewis & McElhaney, 1985; Mantsch et al., 1985; McDonald et al., 1983; Kannenberg et al., 1983; McDonald et al., 1984; Church et al., 1986; C. P. Yang, M. C. Wiener, R. N. A. H. Lewis, R. N. McElhaney, and J. F. Nagle, unpublished results) into the physical properties of phosphatidylcholines (PCs)[1]

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[1] Abbreviations: PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DIHPC, diisoheptadecanoylphosphatidylcholine; DIOPC, diisooctadecanoylphosphatidylcholine; DIEPC, diisoeicosanoylphosphatidylcholine; π, surface pressure; A, molecular area; T_0, extrapolation of π_t vs. T to zero pressure; π_t, surface pressure at onset of LE/LC transition; T_c, monolayer critical temperature; LE, liquid expanded state; LC, liquid condensed state; T_m, main bilayer transition.

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containing branched fatty acid chains. Besides gaining insight into the structure, organization, and possible functional role of branched-chain lipids in these organisms, valuable information can be obtained about the fundamental effects of chain branching on chain packing. Of particular interest here is how such effects promulgate themselves in a lipid such as a PC, where the hydrocarbon chains within the molecule are postulated to extend to different vertical heights even though they contain the same number of carbon atoms.

In this paper, a monomolecular film study is presented for a series of PC films with each PC containing isobranched fatty acids. Monolayers of pure methyl-branched fatty acids have previously been examined and a methyl branch at the penultimate position was found to have the least perturbing effect. Weitzel et al. (1952) studied a series of monomethylstearic acids and observed that the close-packed molecular area is greatest when chain branching occurs near the center of the chain (C_7–C_8) and least when it occurs at either end of the chain. While isobranching thus produces a minimum perturbation, the close-packed molecular area of, for example, isostearic acid, is still, nevertheless, substantially greater (~25 Å^2/molecule) than is that of the corresponding straight-chain stearic acid (~19.5 Å^2/molecule). It is reasonable to expect that isobranched PCs will show similar behavior to their component fatty acids; however, the nonequivalence of the *sn*-1 and *sn*-2 chains in the PCs may well complicate matters. Therefore, in order to characterize the effect of the methyl branch in PCs, the behavior of a straight-chain PC will be compared to the behavior of the branched-chain PCs.

Materials and Methods

L-α-Dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma Chemical Co. (St. Louis, MO). The sample obtained was 99+% pure and was used as supplied. The presence of a relatively sharp transition in the monolayer experiment confirmed the stated purity of the sample. The synthesis and purification of L-α-diisoheptadecanoylphosphatidylcholine (DIHPC), L-α-diisooctadecanoylphosphatidylcholine (DIOPC), and L-α-diisoeicosanoylphosphatidylcholine (DIEPC) have been described elsewhere (Lewis & McElhaney, 1985); all three showed a single spot in thin-layer chromatographic development. It was necessary to employ a 9:1 (v/v) hexane/ethanol mixture as a spreading solvent due to the somewhat polar nature of the lipids. The ethanol (U.S. Industrial Chemicals Co., Division of National Distillers and Chemical Corp., New York, NY) was used as supplied; however, it was found necessary to purify the hexane by passage through a column of alumina and then by subjecting it to a final distillation. The water used as a substrate was particulate-free and deionized (SYBRON/Barnstead, Boston, MA) and subsequently quadrupally distilled, once from alkaline permaganate, once from H_2SO_4, and twice from itself.

The film balance system for measuring surface pressure (π) as a function of molecular area has been described in detail previously (Cadenhead, 1969). For ease of data collection and evaluation, the film balance was interfaced with a Data-General Micro-Nova computer.

Results

DPPC. π vs. A isotherms for DPPC are presented in Figure 1 for the temperature range 16.6–40.4 °C. Data for DPPC isotherm temperature dependency were already available in the literature (Albrecht et al., 1978; Phillips & Chapman, 1968); however, because of slight discrepancies and for improved comparison with the isobranched PC data, it was felt desirable to have all data obtained on the same film balance,

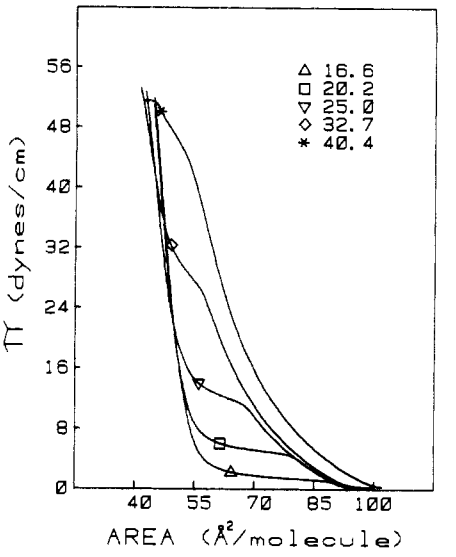

FIGURE 1: Surface pressure (π) vs. area per molecule isotherms for dipalmitoylphosphatidylcholine monolayers at the temperatures indicated (°C).

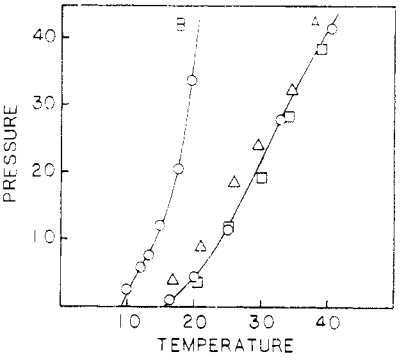

FIGURE 2: (A) Surface pressure at the compressional onset of the liquid expanded/liquid condensed phase transition (π_t) plotted as a function of temperature (°C) for the study reported in Figure 1 (○), by Albrecht et al. (1978) (□), and by Phillips and Chapman (1968) (△). The solid line constitutes a least-squares fit of the data reported in Figure 1 only. (b) Surface pressure at the compressional onset of the liquid expanded/liquid condensed phase transition (π_t) plotted as a function of temperature (°C) for films of diisoheptadecanoylphosphatidylcholine (○).

under similar conditions. The data in Figure 1 are in excellent agreement with those of Albrecht et al. (1978) but differ slightly from those of Phillips and Chapman (1968). The isotherms in Figure 1 all exhibit a liquid expanded/liquid condensed (LE/LC) transition with the onset of the transition (π_t) shifting to higher pressures with increasing temperature (see also Figure 2). The lowest temperature at which an isotherm exhibits such a transition, or the highest temperature at which a fully condensed isotherm can be obtained (T_0; Kellner et al., 1978), is 15.5 °C while the highest or critical temperature of the transition (T_c) is approximately 42.9 °C (Rice, 1986). At any given pressure, the areas per molecule found by the latter investigators were slightly lower than those of Albrecht et al. (1978) or those illustrated in Figure 1, while the expanded state at a given temperature persisted to slightly higher pressures. It would seem unlikely that differing trace impurities in the samples could explain these differences. It seems more likely that the slightly higher π_t at any given temperature (see Figure 2) may be explained by the fact that Phillips and Chapman used a 0.1 M NaCl substrate, since this has a well-known expanding effect on monomolecular films (Cadenhead et al., 1967). The apparently conflicting lower

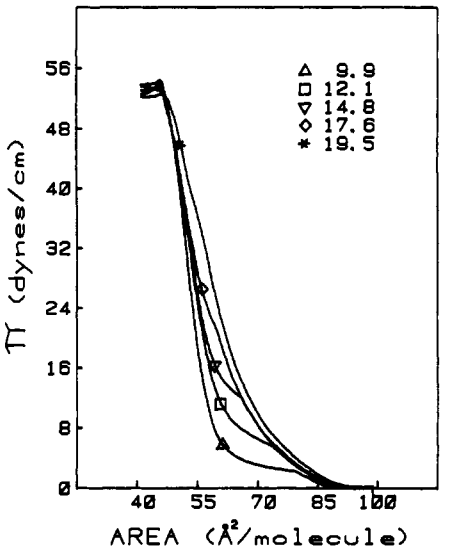

FIGURE 3: Surface pressure (π) vs. area per molecule isotherms for diisoheptadecanoylphosphatidylcholine monolayers at the temperatures indicated (°C).

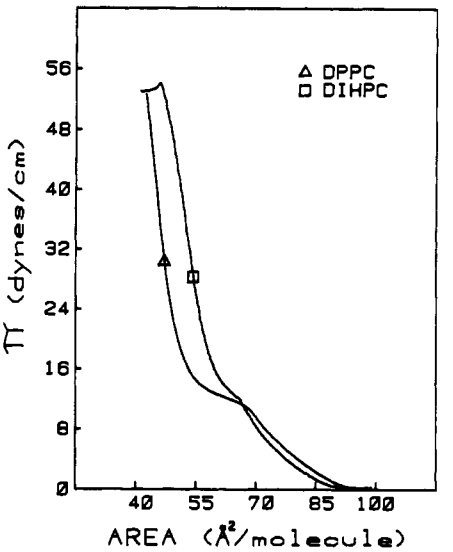

FIGURE 4: Surface pressure (π) vs. area per molecule isotherms for dipalmitoylphosphatidylcholine and diisoheptadecanoylphosphatidylcholine monolayers 25.0 and 14.8 °C, respectively.

areas per molecule of Phillips and Chapman may have arisen through a small error in the weight of DPPC employed due to the hygoscopic nature of lecithins.

Liftoff, or the onset of detectable surface pressure greater than 0.1 dyn/cm, occurs for DPPC around 96 Å^2/molecule, while the close-packed area is approximately 45 Å^2/molecule at 40 dyn/cm. In obtaining these measurements, the water level of the trough was adjusted to such a height that film leakage underneath the barriers was not a concern. However, this in turn resulted in the film being pushed over the edge of the trough before true film collapse occurred. The true collapse pressure of DPPC has been measured, and at 25 °C is near 72 dyn/cm (Hildebran et al., 1979). An LE/LC two-phase region was achieved over at least a 25 deg temperature range. The behavior of π_t as a function of temperature is shown in Figure 2. The best representation of the data was determined by a least-squares fit as being cubic. The data of Albrecht et al. (1978) and Phillips and Chapman (1968) were included for comparison; however, the data of these authors were not included in the fit (solid line). The T_0 value, as was already stated, was found to be 15.5 °C.

DIHPC. It is particularly interesting to compare the behavior of DPPC and DIHPC since these two lecithins have the same hydrocarbon main chain length (16 carbon atoms). The only difference is that DIHPC has a methyl group substituted for a hydrogen atom at the C_{15} position. π vs. A isotherms are shown in Figure 3 for DIHPC over the temperature range 9.9–19.5 °C. Comparing these curves to those for DPPC (Figure 1), it is immediately obvious that the intermediate state of DIHPC is much more restricted than that of DPPC. This smaller intermediate state could arise through differences in the condensed or expanded state or both. However, the temperature range over which such a transition is detectable is much smaller for DIHPC (~10 deg) than it is for DPPC (~25 deg). This is clearly shown in a plot of π_t vs. temperature (Figure 2). The T_0 value of DIHPC is 9.1 °C (6.4 deg lower than that of DPPC). It is also seen that the magnitude of the intermediate region of the isobranched chain (Figure 3) is much smaller than that found for DPPC (Figure 1). Because the transition temperature ranges are not centered around the same absolute temperature, it is not easy to make a comparison with absolute temperatures. At 19.5 °C the transition in DIHPC is considerably broadened and

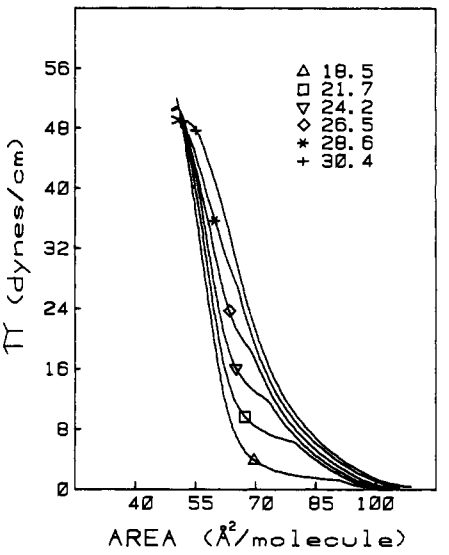

FIGURE 5: Surface pressure (π) vs. area per molecule isotherms for diisooctadecanoylphosphatidylcholine monolayers at the temperatures indicated (°C).

difficult to detect at about 34.7 dyn/cm, while at 20.2 °C DPPC has a well-defined transition at about 4.5 dyn/cm. On this basis the condensed state of DIHPC is significantly more expanded, while its expanded state appears slightly more condensed. As with DPPC, DIHPC exhibits a range of temperatures over which both an expanded and a condensed state are present. It seems better to compare DPPC at 25 °C with DIHPC at 14.8 °C, temperatures which differ substantially in an absolute sense but constitute almost the same relative temperature with respect to their T_0 values. The change in the magnitude of the intermediate region is again seen to result primarily from the condensed state of DIHPC being 7 Å^2/molecule more expanded than that of DPPC and the expanded state of DIHPC being about 2 Å^2/molecule more condensed. This similar reduced temperature occurs at a point at which the isotherms of DIHPC and DPPC have approximately the same fraction in expanded and condensed states (Figure 4). Each isotherm has approximately the same surface pressure at the onset of the LE/LC transition. Finally, DIHPC is significantly less effective than DPPC in reducing the surface tension of the water substrate, since DIHPC shows a definite

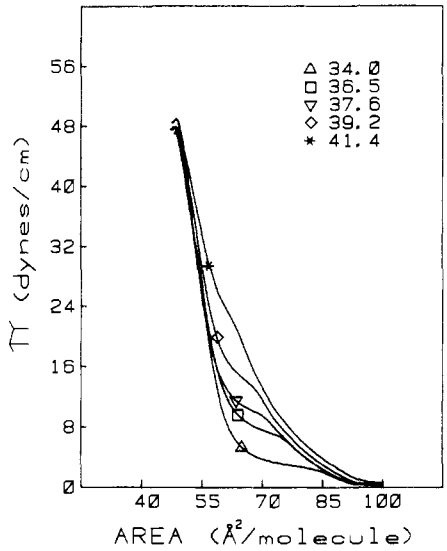

FIGURE 6: Surface pressure (π) vs. area per molecule isotherms for diisoeicosanoylphosphatidylcholine monolayers at the temperatures indicated (°C).

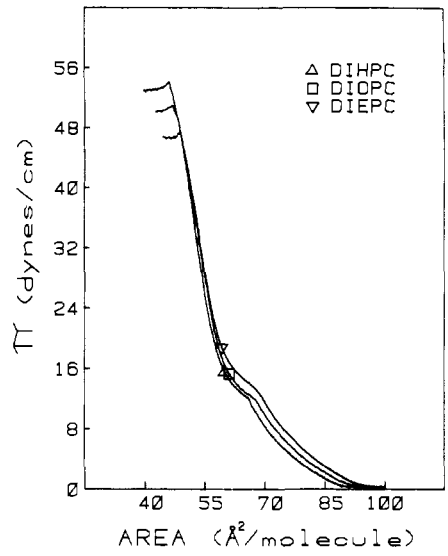

FIGURE 7: Comparison of surface pressure (π) vs. area per molecule isotherms of diisoheptadecanoylphosphatidylcholine at 14.8 °C, of diisooctadecanoylphosphatidylcholine at 24.2 °C, and of diisoeicosanoylphosphatidylcholine at 39.2 °C.

collapse at 52–54 dyn/cm irrespective of the temperature.

DIOPC and DIEPC. The π vs. A isotherms of DIOPC (Figure 5) and DIEPC (Figure 6) are very similar to those of DIHPC, except that the corresponding physical states are exhibited at higher temperatures. DIOPC shows a LE/LC transition over the temperature range 18.5–30.4 °C, with a T_0 of 17.2 °C. Isotherms of DIEPC could not be reproducibly measured above 4.14 °C due to problems with water evaporation within the thermostated environment. The T_0 of DIEPC was 32.2 °C. Isotherm data for DIEPC have been previously reported on a 0.1 M NaCl substrate (Suzuki & Cadenhead, 1985) where, due to the previously mentioned expansion effect of this substrate (Cadenhead et al., 1967), the T_0 value was slightly higher at 33 °C. For both DIOPC and DIEPC, the plot of π vs. T was nonlinear as shown by a least-squares analysis. The collapse pressure of the isobranched lecithins decreased with increasing chain length (DIHPC, 52–54 dyn/cm; DIOPC, 49–52 dyn/cm; DIEPC, 48–50 dyn/cm). Figure 7 shows a plot of the three members of the homologous series adjusted to approximately the same reduced temperature. The condensed areas are essentially identical; however, there is a slight expansion of the expanded areas as the chain length of the series is increased.

Discussion

As would be expected, the methyl branch interferes with the packing of the hydrocarbon chains in a close-packed arrangement. Figure 4 clearly shows the condensed area of DIHPC to be around 7 Å^2/molecule larger than that of DPPC. Kannenberg et al. (1983) measured monolayer isobars for DIHPC and DPPC. These authors also found that the DIHPC condensed state was more expanded than that of DPPC. Thus, both this study and that of Kannenberg et al. (1983) provide supporting evidence for the loosely packed gel state postulated by Lewis and McElhaney (1985) in DIHPC bilayers as a necessary intermediate between the low-temperature gel and liquid-crystalline states.

The loosely packed condensed state results from weaker van der Waals forces. Since the attractive forces are weaker, an expanded phase can be realized with less energy and thus will appear at a lower temperature. Because of this, the T_0 values of the branched-chain compounds are lower than those of the corresponding straight-chain compounds having the same main chain length. Similarly, Lewis and McElhaney (1985) observed a reduction of the chain melting transition (T_m) for bilayer vesicles of isobranched-chain PCs when compared to bilayer vesicles of straight-chain PCs that contain the same number of carbon atoms.

Church et al. (1985) have carried out X-ray diffraction studies of both DIHPC and DIEPC and found that the bilayer gel (LB) phases of both PCs have distorted pseudohexagonal lattices due to the rotationally disordered subchains. The liquid-crystalline phases appeared very similar to similar phases formed with straight-chain PCs. The major effect of perturbing the gel state was also observed in membranes of *Acholeplasma laidlawii* B by Macdonald et al. (1985), which were highly enriched in isopalmitic acid. A very recent study of Yang et al. (C. P. Yang, M. C. Wiener, R. N. A. H. Lewis, R. N. McElhaney, and J. F. Nagle, unpublished results) using dilatometry confirms and extends these findings. By combining the observations of Church et al. (1985) with their own, Yang et al. were able to deduce the volume per chain for a number of isobranched PCs as a function of temperature. Assuming that their findings translate semiquantitatively to related monolayer films, we would anticipate that, for either a series of isobranched films at one temperature or for one film at a series of temperatures, the condensed states would show only a slight increase in area per molecule, while the expanded states would show a clear, but still small, increase in area per molecule with increasing temperature.

The more expanded condensed state is primarily responsible for the reduction in the extent of the intermediate region of DIHPC over that of DPPC. However, as we have shown, the expanded phase of DIHPC appears slightly more condensed than that of DPPC (see Figure 4). This latter observation was not made by Kannenberg et al. (1983) and is indeed not evident from their monolayer isobars. Their data collection stopped before an expanded phase could be fully realized, and the somewhat less expanded state was presumably missed because of this. It is not surprising therefore that the liquid-crystalline state of the isobranched PC has been found to be more ordered than the liquid-crystalline state of the comparable straight-chain PC. The order parameters of *A. laidlawii* membranes grown on the appropriate straight- or branched-chain PC were determined by ^{19}F NMR of monofluorinated palmitic acids incorporated into these membranes

(Macdonald et al., 1983). *A. laidlawii* membranes grown on isohexadecanoic acid were seen to be more ordered than those grown on pentadecanoic acid when the two were compared at a temperature 15 deg above their T_m (i.e., the same reduced temperature). These authors suggested that the bulky methyl substituent hindered rotation about C–C bonds. The postulated reduction in conformation freedom would result in a localized ordering effect. Since there would be less gauche rotomers, the hydrocarbon chains could pack closer, resulting in the observed lower molecular areas for the liquid expanded state.

A direct consequence of the reduced extent of the intermediate region is that the branched-chain PC will tend to have a smaller enthalpy of transion (ΔH) than the straight-chain compound. From the two-dimensional Clapeyron equation

$$\Delta H = \left(\frac{d\pi_t}{dT} - \frac{d\gamma^\circ}{dT}\right)\Delta A \Delta T$$

ΔA, the area per molecule difference between expanded and condensed states, is reduced for the case of the branched-chain compound, but $d\pi_t/dT$ ranges from about the same value to a significantly higher value (see Figure 2), nevertheless. The correction for the temperature dependency of the surface tension of water ($d\gamma^\circ/dT$) is small. ΔH is always smaller for the isobranched PC. Lewis and McElhaney (1985), using differential scanning calorimetry, also observed the reduction in enthalpy of the main bilayer gel–liquid crystalline transition for the branched-chain PC compared to a straight-chain compound with the same total number of carbon atoms. The ΔH values for the two isotherms in Figure 4 (i.e., compared on a reduced temperature scale) are 9.0 kcal/mol for DPPC and 6.4 kcal/mol for DIHPC. In the evaluation of ΔH, the area per molecule in a condensed film was taken as the area of which a linear extension of the condensed state intersects a horizontal extension of the linear intermediate region beyond π_t. The area per molecule in an expanded film was taken as that at π_t.

When the three members of the homologous series DIHPC, DIOPC, and DIEPC are compared at the same reduced temperature, it can be seen that they have closely comparable condensed states. However, the areas of the expanded state increases slightly in the order DIHPC < DIOPC < DIEPC (see Figure 7). The longer hydrocarbon chain appears to form a liquid expanded state sooner (i.e., at larger areas) than a shorter hydrocarbon chain. Phillips and Chapman (1968) suggested that a homologous series should be identical in both expanded and condensed regions with the only difference being the extent of each region and the location and extent of the transition region. The results here indicate this is not true for an isobranched series and are in fact consistent with the bilayer findings of Yang et al. (C. P. Yang, M. C. Wiener, R. N. A. H. Lewis, R. N. McElhaney, and J. E. Nagle, unpublished results). We find that when DPPC and DIOPC are compared on a reduced temperature basis with their respective T_0 values, the LE states essentially match. It would seem that as the overall chain length increases the effect of chain branching diminishes and the transition or intermediate region increases in magnitude to approach that of a straight-chain PC. The additional two CH_2-groups in DIOPC would seem to compensate the effect of methyl isobranching in the lipid, at least in the LE state.

In understanding the collapse behavior of PC films it should be recognized that they are frequently metastable (Phillips & Hauser, 1974), but in the absence of a packing perturbation such as chain branching, highly stable condensed films can be achieved (e.g., DPPC with a collapse pressure of ~72 dyn/cm at 25 °C). With such a perturbation a substantial lowering in collapse pressure may be brought about, and Gaines (1966), in comparing straight- and branched-chain fatty acids, has pointed out that the latter always collapse at lower pressures. Similar findings have been made by D. A. Cadenhead and H. Matuo (unpublished results). The decrease in collapse pressure found with increasing branched-chain chain length is, at first, somewhat surprising. However, it may well relate to the equilibrium spreading pressure (ESP) of these films. On the basis of previous work (Kellner & Cadenhead, 1979), we would anticipate that the ESP would decrease with increasing chain length and that the longer branched-chain PCs would be less stable at equivalent pressures than shorter branched-chain PCs. It would appear that this degree of metastability is important once a perturbing branch is included in the chain.

The properties of these isobranched PCs should be of considerable interest to membranologists. Enzymes reconstituted into a membrane will function as they do in a native cellular membrane only when the lipids of the reconstituted system are in a liquid-crystalline state. Hence, an unsaturated PC, for example, egg PC, is most commonly employed in reconstitution studies. However, the use of unsaturated PCs leads to problems with oxidation of these lipids. In contrast, the use of branched-chain PCs in these studies would eliminate most of this problem, yet these branched-chain PCs can provide the same overall fluidity needed for normal enzyme function. Redwood et al. (1971) used a diphanatyl-PC to try to overcome such problems in forming bilayer lipid membranes (phytanic acid is a quadrupally methyl-branched fatty acid). Phytanic acid, however, was probably not the best choice due to its implied role in Refsum's disease, where the stearic restraints imposed by the multiple methyl branches have been postulated to give rise to the destruction of the myelin sheath (O'Brien, 1967). It would seem that the isobranched PCs reviewed here might have been a better choice. Additionally, with the ability to provide a liquid-crystalline phase to lower temperatures and to present a much more disordered gel state, such PCs might well prevent the macroscopic aggregation of transmembrane proteins taking place in membrane reconstitution studies.

Registry No. DPPC, 2644-64-6; DIHPC, 71368-21-3; DIOPC, 60683-79-6; DIEPC, 95799-75-0.

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Factors Affecting the C=N Stretching in Protonated Retinal Schiff Base: A Model Study for Bacteriorhodopsin and Visual Pigments†

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ABSTRACT: Factors affecting the C=N stretching frequency of protonated retinal Schiff base ($RSBH^+$) were studied with a series of synthetic chromophores and measured under different conditions. Interaction of $RSBH^+$ with nonconjugated positive charges in the vicinity of the ring moiety or a planar polyene conformation (in contrast to the twisted retinal conformation in solution) shifted the absorption maxima but did not affect the C=N stretching frequency. The latter, however, was affected by environmental perturbations in the vicinity of the Schiff base linkage. Diminished ion pairing (i.e., of the positively charged nitrogen to its anion) achieved either by substituting a more bulky counteranion or by designing models with a homoconjugation effect lowered the C=N stretch energy. Decreasing solvation of the positively charged nitrogen leads to a similar trend. These effects in the vicinity of the Schiff base linkage also perturb the deuterium isotope effect observed upon deuteriation of the Schiff base. The results are interpreted by considering the mixing of the C=N stretching and C=N—H bending vibration. The C=N mode is shifted due to electrostatic interaction with nonconjugated positive charges in the vicinity of the Schiff base linkage, an interaction that does not influence the isotope effect. Weak hydrogen bonding between the Schiff base linkage in bacteriorhodopsin (bR) and its counteranion or, alternatively, poor solvation of the positively charged Schiff base nitrogen can account for the C=N stretching frequency of 1640 cm^{-1} and the deuterium isotope effect of 17 cm^{-1} observed in this pigment. Conversion of bR to the photochemically induced intermediate K_{610} involves environmental perturbation in the vicinity of the C=N linkage, lowering the C=N stretch energy. The C=N stretching frequency (1660 cm^{-1}) observed for rhodopsin indicates very effective hydrogen bonding with the Schiff base counteranion and/or effective solvation by protein dipoles or residual water.

Both bacteriorhodopsin (bR)[1] [the protein pigment of the purple membrane of the halophilic microorganism *Halobacterium halobium* (Oesterhelt & Stoeckenius, 1971)] and visual rhodopsins consist of a retinal chromophore (all-trans in bR and 11-cis in visual pigments) that is covalently bound to a membrane protein (opsin) via a protonated Schiff base linkage at a lysine residue. Excitation of visual pigments leads to changes in the electrical potential of the photoreceptor cell membrane that are transmitted to the brain through appropriate synaptic processes. In bR, light energy is converted into a proton gradient across the membrane that is subsequently used via a chemiosmotic mechanism to synthesize ATP [see Ottolenghi (1980), Stoeckenius et al. (1979), and Birge (1981) for reviews].

Light absorption by visual pigments or bR is followed by a sequence of structural transformations involving both the retinal polyene and the protein. The primary event includes conversion to a red-shifted intermediate (K_{610} in bR and bathorhodopsin in visual pigment) followed by thermal processes (bathorhodopsin → lumirhodopsin → metarhodopsin I ↔ metarhodopsin II → metarhodopsin III → *all-trans*-retinal + opsin in rhodopsin and $K_{610} \rightarrow L_{550} \rightarrow M_{410} \rightarrow O_{640}$ in bR). Obtaining further information on the molecular mechanisms associated with the above transformations is a prerequisite for correlating these processes with the corresponding biological functions, i.e., release of the defusable transmitter in photoreceptor cells and pumping of H^+ ions in *H. halobium*.

It was demonstrated by several groups that resonance Raman spectroscopy [see Warshel (1977), Callender and Honig (1977), Mathies (1979), and Lewis (1982) for reviews] and Fourier-transform infrared (FTIR) spectroscopy (Bagley et

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[1] Abbreviations: bR, bacteriorhodopsin; FTIR, Fourier-transform infrared; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; $LiAlD_4$, lithium aluminum hydride (deuteriated); $RSBH^+$, protonated retinal Schiff base; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol.

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