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Thermodynamic and Conformational Studies on *sn*-2-Phosphatidylcholines in Monolayers and Bilayers[†]

Joachim Seelig,* Ruud Dijkman, and Gerard H. de Haas

ABSTRACT: 1,3-Dipalmitoyl-*sn*-glycero-2-phosphocholine has been spread at an air–water interface. The area–pressure isotherms of the *monolayer* have been recorded by using the Wilhelmy plate method and are compared with those of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. At a given surface pressure the *sn*-2-phosphatidylcholine is always found to be more expanded than the *sn*-3-phosphatidylcholine. The thermodynamic behavior of *bilayers* composed of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine was investigated by means of differential scanning calorimetry. The lipid undergoes an endothermic pretransition at 17–23 °C with a transition enthalpy of ΔH = 1.7–4.2 kcal/mol and an endothermic gel to liquid-crystal transition at 37 to 38 °C with ΔH = 9.4 kcal/mol. The pretransition is strongly dependent on the prehistory of the sample. The conformational properties of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine in liquid-crystalline bilayer membranes were studied by deuterating the lipid at various chain and head group segments and measuring the deuterium and phosphorus-31 nuclear magnetic resonance spectra of the corresponding bilayer phases. The ^{2}H and ^{31}P NMR parameters of the phosphocholine head group are very similar for *sn*-2- and *sn*-3-phosphatidylcholines, suggesting almost identical head group structures for the two lipids. On the other hand, distinct conformational differences are observed for the hydrocarbon region. In bilayers of *sn*-2-phosphatidylcholine both fatty acyl chains begin parallel to the bilayer surface and are bent perpendicular to it after the C-2′ segment. In corresponding bilayers of *sn*-3-phosphatidylcholines only the *sn*-2 chain is bent, whereas the *sn*-1 chain is perpendicular to the membrane surface at all segments. As a consequence of the unusual conformation at the chain beginnings, the hydrocarbon interior of bilayers of *sn*-2-phosphatidylcholines appears to be more disordered than that of the *sn*-3 analogue.

sn-2-Phosphatidylcholines (β-lecithins), though not naturally occurring, have been shown to be substrates for phospholipase A$_2$, and the enzyme specifically cleaves the ester bond at the C-1 position (de Haas & van Deenen, 1963, 1964). In the case of naturally occurring *sn*-3-phosphoglycerides phospholipase A$_2$ catalyzes the hydrolysis of the C-2 ester bond. The enzyme acts highly stereospecifically, and only *sn*-3-phospholipids are degraded whereas the *sn*-1-phospholipids are competitive inhibitors characterized by the same binding constant as the *sn*-3 stereoisomers (Bonsen et al., 1972a,b). The stereospecificity of phospholipase A$_2$ action originates from the size, the shape, and the chemical nature of the active site, and the question can be raised of how the conformation of *sn*-2-phosphatidylcholines compares with that of the naturally occurring *sn*-3 analogues.

In the present study we have investigated this problem with deuterium and phosphorus-31 nuclear magnetic resonance (Seelig, 1977, 1978). For this purpose 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine was selectively deuterated at the C-2′ segment of the *sn*-3 chain and at segments 2′, 3′, 4′, and 8′ of the *sn*-1 chain. The hydrocarbon chain ordering in the presence and absence of cholesterol was deduced from the residual deuterium quadrupole splittings. Analogously, the conformational properties of the head group were studied by selective deuteration of the two choline methylene segments, whereas the motion of the phosphate segments was studied with phosphorus-31 nuclear magnetic resonance. In addition, the thermodynamic properties of 1,3- and 1,2-dipalmitoyl-*sn*-glycerophosphocholine were compared by means of differential scanning calorimetry and monolayer experiments.

Materials and Methods

Chemical Synthesis. Selectively deuterated fatty acids (labeled at carbon atoms no. 2, 3, 4, and 8) were synthesized as described previously (Seelig & Seelig, 1974). 1,3-Bis([2′,2′-^{2}H$_2$]palmitoyl)-*sn*-glycero-2-phosphocholine was prepared from the corresponding deuterated 1,3-dipalmitin according to Hirt & Berchtold (1958). Phospholipase A$_2$ degradation led to the lyso compound 3-[2′,2′-^{2}H$_2$]palmitoyl-*sn*-glycero-2-phosphocholine which was reacylated with C-3-, C-4-, and C-8-deuterated palmitic acid (de Haas & van Deenen, 1963).

[1-^{2}H$_2$]Choline iodide, $(CH_3)_3NCD_2CH_2OH^+I^-$, and [2-^{2}H$_2$]choline iodide, $(CH_3)_3NCH_2CD_2OH^+I^-$, were synthesized as described by Gally et al. (1975). 1,3-Dipalmitin was made from *rac*-2-benzylglycerol as described by Bonsen et al. (1972a,b) and was reacted with POCl$_3$ and deuterated cholines following the method of Eibl (1978). The head group deuterated 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholines are abbreviated as $^+NCD_2CH_2O$-DPPC and $^+NCH_2CD_2O$-DPPC.

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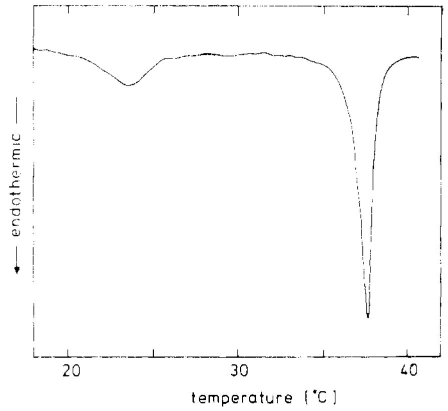

FIGURE 1: Differential scanning calorimetric tracing of dispersions of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine. The lipid was dispersed in water (30 wt % lipid/70 wt % water) and mixed thoroughly by vortexing. Measurements were started at 10 °C and finished at 50 °C.

Deuterium and Phosphorus-31 Nuclear Magnetic Resonance Measurements. Random lipid bilayers were prepared by thoroughly mixing the phospholipid (~50 wt %) and water (~50 wt %) and carefully heating the mixture in a sealed ampule. Approximately 100–200 mg of liquid-crystalline phase was used for measuring powder-type spectra. For the NMR experiments the ampule was placed inside a 10-mm NMR tube.

Mixtures of phospholipid and cholesterol were prepared by dissolving the appropriate amounts of cholesterol and phospholipid in chloroform–methanol. The solutions were transferred to glass ampules, and the solvent was removed by blowing a stream of nitrogen over the samples followed by at least 24 h under high vacuum.

^{2}H NMR measurements were performed at 13.8 and 61.4 MHz on a Bruker-Spectrospin HX-90 or WH-400 FT spectrometer. From the quadrupole splitting, $\Delta\nu_Q$, of the powder-type spectra the deuterium order parameter, S_{CD}, can be calculated according to Seelig (1977)

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD}$$

where e^2qQ/h (=170 kHz) is the static quadrupole coupling constant. ^{31}P NMR spectra were recorded at 36.4 MHz with broad-band proton decoupling. The experimental conditions are the same as those described in more detail in previous publications (Seelig & Seelig, 1974; Gally et al., 1975).

Differential Scanning Calorimetry and Monolayer Experiments. Following the NMR measurements, the thermodynamic properties of the lipid–water systems were studied by means of differential scanning calorimetry using a Mettler TA 2000 instrument. Additional measurements were performed by Dr. P. W. M. van Dijck with a Beckmann DSC-2 instrument. The agreement between the two sets of experiments was very good. Monolayer experiments were performed as described earlier (Demel, 1974).

Results

The thermodynamic properties of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine are similar to those of the 1,2 analogue. As is illustrated in Figure 1, the phase behavior of the lipid–water mixture is characterized by an endothermic pretransition (transition temperature 17–23 °C, transition enthalpy $\Delta H \approx 1.7$–4.2 kcal/mol) and an endothermic gel to liquid-crystal transition (transition temperature 37 to 38 °C, $\Delta H = 9.4$ kcal/mol). The pretransition is sensitive to such factors as the water content and the prehistory of the sample.

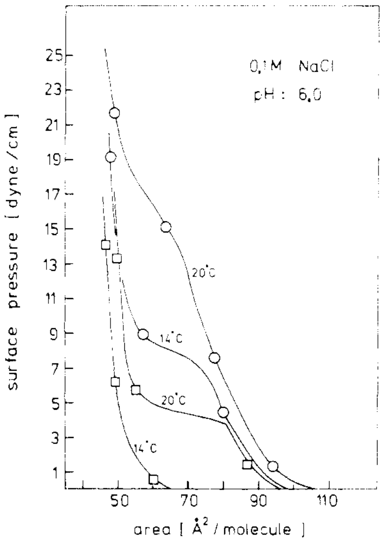

FIGURE 2: Area–pressure characteristics of monolayers of *sn*-3- and *sn*-2-phosphatidylcholines spread on 0.1 M NaCl, pH 6: (□) 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; (○) 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine.

The largest ΔH for the pretransition is generally observed in the first calorimeter scan; subsequent scans tend to decrease the transition enthalpy and also shift the pretransition to lower temperatures. The nature of the pretransition was not further investigated in this study. The main transition shows fairly constant properties, and the thermodynamic parameters are independent of the water content (at least for a water content of >50 wt %), the prehistory of the sample, and the number of calorimeter scans. Compared to 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine which has a transition temperature and transition enthalpy of 41 °C and 8.7 kcal/mol (Chapman et al., 1967), respectively, the main transition of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine occurs at slightly lower temperature but has an increased heat content. Both lipids were spread at an air–water interface, and the area–pressure diagrams were recorded for two different temperatures (Figure 2). At 14 °C 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine is in the liquid-condensed state at all surface pressures whereas 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine is considerably more expanded and exhibits a liquid-expanded → liquid-condensed transition. At 20 °C both lipids undergo a liquid-expanded → liquid-condensed transition, but again the area requirement of the 1,3-lipid is clearly larger than that of the 1,2-lipid at all surface pressures. This result is in good agreement with a related monolayer study on 1,2-distearoyl-*sn*-glycero-3-phosphocholine and 1,3-distearoyl-*sn*-glycero-2-phosphocholine spread at an oil–water interface (Llerenas & Mingins, 1976).

We now turn to a comparison of the conformational properties of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine as revealed by deuterium and phosphorus-31 nuclear magnetic resonance. In earlier studies we have shown that the two hydrocarbon chains of 1,2-glycerophospholipids are motionally inequivalent and assume two different conformations in the membrane (Seelig & Seelig, 1975; Seelig & Browning, 1978; Büldt et al., 1978). The difference between the *sn*-1 and *sn*-2 chains manifests itself most strikingly if both chains are deuterated at the C-2′ segment, i.e., adjacent to the carbonyl groups. As is shown in Figure 3A, the ^{2}H NMR spectra of bilayers of 1,2-bis([2′,2′-^{2}H$_2$]palmitoyl)-*sn*-glycero-3-phosphocholine are composed of three quadrupole splittings. The large quadrupole splitting, $\Delta\nu_1$, can be assigned to the C-2′ segment of the *sn*-1

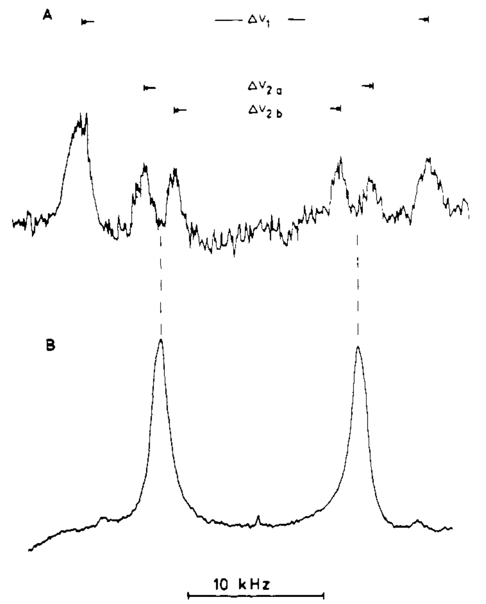

FIGURE 3: Deuterium magnetic resonance spectra of bilayers composed of *sn*-2- or *sn*-3-phosphatidylcholines deuterated at C-2′ of both fatty acyl chains (50 wt % lipid/50 wt % H_2O). Measuring temperature was 50 °C. (A) 1,2-Bis([2′,2′-$^2H_2$]palmitoyl)-*sn*-glycero-3-phosphocholine. (This spectrum was measured at 13.8 MHz and is characterized by a rather low signal-to-noise ratio.) (B) 1,3-Bis-([2′,2′-$^2H_2$]palmitoyl)-*sn*-glycero-2-phosphocholine. (The spectrum was measured at 61.4 MHz and demonstrates the dramatic improvement in sensitivity at high magnetic field strength.)

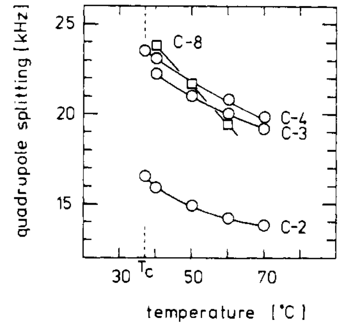

FIGURE 4: Temperature dependence of selectively deuterated 1-[n',n'-$^2H_2$]palmitoyl-3-[2′,2′-$^2H_2$]palmitoyl-*sn*-glycero-2-phosphocholine (50 wt % lipid/50 wt % H_2O).

chain, whereas the two smaller splittings, $\Delta\nu_{2a}$ and $\Delta\nu_{2b}$, are due to the corresponding segment of the *sn*-2 chain (Seelig & Seelig, 1975). The two deuterons in the *sn*-1 chain are motionally equivalent and yield the same quadrupole splitting while those in the *sn*-2 chain are inequivalent and give rise to two separate splittings (Seelig & Seelig, 1975; Browning & Seelig, 1980). A much simpler spectral pattern is observed if the same experiment is performed with 1,3-bis([2′,2′-$^2H_2$]palmitoyl)-*sn*-glycero-2-phosphocholine. Figure 3B demonstrates that both chains and all four deuterons are characterized by the same quadrupole splitting. The size of the quadrupole splitting of the 1,3-lecithin is identical with the average, $(1/2)(\Delta\nu_{2a} + \Delta\nu_{2b})$, of the *sn*-2 chain of 1,2-lecithin.

Figure 4 summarizes the temperature dependence of the quadrupole splittings of the various deuterated 1-[n',n'-$^2H_2$]palmitoyl-3-[2′,2′-$^2H_2$]palmitoyl-*sn*-glycero-2-phosphocholines (n' = 2, 3, 4, and 8). Increasing temperature leads to a reduction of the quadrupole splitting due to a more random chain motion. The extent of reduction varies with the position of the labeled segment and becomes more pronounced if the deuterated segment is moved toward the terminal methyl group (cf. C-8 in Figure 4). The gel to liquid phase transition is reflected in a loss of the ^2H NMR signal at temperatures below 37 °C.

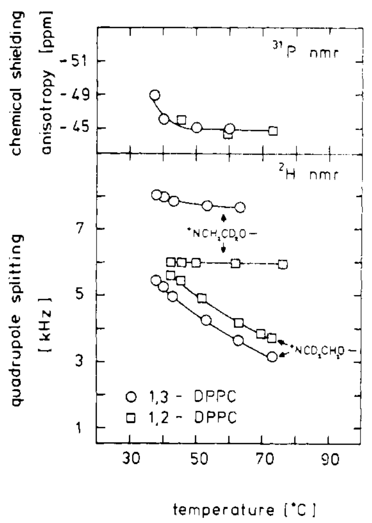

FIGURE 5: Comparison of the phosphocholine head group of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (□) and 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (○) (50 wt % lipid/50 wt % H_2O).

A similar ^2H NMR analysis has been performed for the choline head group of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine, and the results are compared in Figure 5 with those previously obtained for the 1,2 analogue (Gally et al., 1975). Qualitatively, corresponding segments of both lipids exhibit the same dependence on temperature; in particular, the quadrupole splittings of the $^+NCH_2CD_2O$ segment in both lipids remain constant from 37 to 70 °C, whereas those of the adjacent $^+NCD_2CH_2O$ segment decrease in a parallel fashion by ~40% in this temperature interval. Quantitatively, it should be noted that the quadrupole splitting of the $^+NCH_2CD_2O$ segment is larger for the 1,3-lipid than for the 1,2-lipid, whereas the situation is just reversed for the $^+NCD_2CH_2O$ segment. The differences between the two lipids can therefore not be accounted for by assuming a more random motion of one of the head groups. If this were the case, *both* quadrupole splittings of one of the head groups should be found to be smaller.

The motion of the phosphate segment in bilayers of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine has been investigated by means of ^{31}P NMR. The spectra are virtually identical with those reported earlier for the 1,2 analogue (Gally et al., 1975). The motional restrictions experienced by the phosphate group are expressed quantitatively in terms of the chemical shielding anisotropy (Seelig, 1978), and the temperature dependence of this parameter for both the 1,3- and 1,2-dipalmitoyl-*sn*-glycerophosphocholines is also included in Figure 5.

Finally, we have prepared mixtures of deuterated *sn*-2-phosphocholines with cholesterol in order to study the influence of the steroid molecule on the hydrocarbon chain ordering (Figure 6). The experiments show that increasing amounts of cholesterol lead to a concomitant increase of the quadrupole splitting, indicating a stiffening of the hydrocarbon chains. At a concentration of 50 mol % cholesterol the quadrupole splittings of the three segments investigated (C-2′, C-3′, and C-4′) have almost doubled their values compared to the cholesterol-free bilayer (cf. Figures 6 and 4). The effect of cholesterol is further to eliminate the phase transition, at least for cholesterol concentrations of $\gtrsim$30 mol %. Figure 6 demonstrates that upon addition of 50 mol % cholesterol it is relatively easy to record ^2H NMR spectra well below the

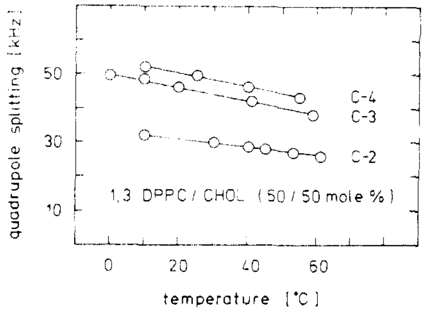

FIGURE 6: Deuterium quadrupole splittings of mixtures of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine and cholesterol (50 wt % lipid/50 wt % H_2O).

transition temperature without any discontinuity in the deuterium quadrupole splitting at $T_c = 37$ °C.

Discussion

In many respects the molecular properties of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine are remarkably similar. Both lipids show a pretransition at around 20 °C which in the case of the 1,2-lipid has been identified as a transition from a smooth to a rippled bilayer structure (Janiak et al., 1976). Both lipids undergo a gel to liquid-crystal transition at approximately the same temperature and with about the same transition enthalpy. This transition is eliminated upon addition of cholesterol (Gally et al., 1976; Haberkorn et al., 1977; Oldfield et al., 1978). Moreover, ^{31}P and ^{2}H NMR parameters of the phosphocholine head group have a strong resemblance, suggesting closely related head group structures for the *sn*-2- and *sn*-3-phosphatidylcholines.

By contrast, distinct conformational differences exist between the two lipids with regard to the hydrocarbon region as is obvious from inspection of Figure 3. The comparison of the two spectra in Figure 3 allows the following conclusions.

(1) In bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine the *sn*-1 chain and *sn*-2 chain are characterized by different conformations because different quadrupole splittings are observed for the two side chains (Figure 3A). The quantitative analysis of the data shows that the *sn*-2 chain begins parallel to the membrane surface and is bent perpendicular to it after the C-2′ segment. By contrast, the *sn*-1 chain is perpendicular to the bilayer surface at all segments (Seelig & Seelig, 1975). This conformation has been confirmed by neutron diffraction studies on selectively deuterated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (Büldt et al., 1978; Zaccai et al., 1979) and is found in the gel state as well as in the liquid-crystalline state. It is independent of the nature of the polar group (Browning & Seelig, 1980), the degree of unsaturation (Büldt et al., 1978; Seelig & Waespe-Sarcevic, 1978), the cholesterol content of the membrane (Haberkorn et al., 1977), and the presence of membranous proteins (Gally et al., 1979). The constancy of the phospholipid conformation under a variety of physical and chemical conditions suggests that this conformation is not a consequence of the packing constraints within the lipid bilayer but represents the minimum of the intramolecular energy of the individual molecule. It is therefore not unreasonable to assume that phospholipid molecules in monomeric solution will adopt a similar average conformation. ^{1}H NMR spectra of molecularly dispersed lecithins support this idea even though no detailed molecular picture can be derived from such studies (Roberts et al., 1978).

(2) In bilayers of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine both fatty acyl chains have the same average conformation at the C-2′ segment, characterized by identical quadrupole splittings (Figure 3B). Since the size of this quadrupole splitting corresponds to that of the *sn*-2 chain of the 1,2 analogue, it must be concluded that *both* chains of the 1,3-phospholipid assume a *bent* conformation in the lipid membrane.

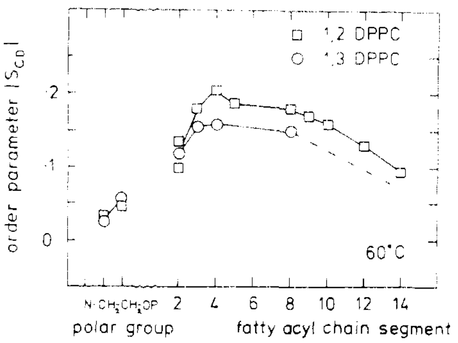

FIGURE 7: Comparison of the deuterium order parameter of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine and 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine (50 wt % lipid/50 wt % H_2O).

(3) The spectrum of conformations available to the lipid molecules appears to be larger for 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine than for the 1,2-lipid. This is suggested by the observation that in the latter compound the two deuterons of the *sn*-2 chain have different quadrupole splittings whereas this difference is motionally averaged out for the 1,3-phospholipid.

As we noted above, the area per molecule of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine spread at an air–water interface is larger than that of the 1,2-lipid measured under the same conditions. This is consistent with the above conclusion of two bent fatty acyl chains in the 1,3 compound, since the surface area requirement of such a molecule is naturally larger than that of the 1,2-lipid with only one bent chain.

The barrier properties of a lipid bilayer can only be maintained as long as the hydrocarbon chains form a densely packed hydrophobic layer. If the surface area is expanded due to a bending of both fatty acyl chains, the rest of the chains must assume a more disordered conformation in order to cover the enlarged surface area. This is indeed borne out by the experimental results. Figure 7 contains a comparison of the deuterium order profiles of 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine and of the *sn*-2 chain of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (Seelig & Seelig, 1974). The figure demonstrates that the bilayer interior (segments C-3′, C-4′, and C-8′) of the 1,3-lipid is more disordered compared to the 1,2-lipid. By use of a simple model (Seelig & Seelig, 1974), it can be estimated that the reduction in the deuterium order parameter corresponds to a contraction of the average chain length by ~10% (not including the additional shortening caused by the bend at the C-2′ segment). The figure also demonstrates the constancy of the polar head group region, since the deuterium order parameters of the choline segments are much less changed compared to those of the fatty acyl region.

Work is in progress to investigate whether there exists any connection between the established data on stereospecificity and substrate binding to phospholipase A_2 (Slotboom et al., 1976) and the molecular conformations derived for 1,3-dipalmitoyl-*sn*-glycero-2-phosphocholine.

Acknowledgments

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Apolipoprotein B of Avian Very Low Density Lipoprotein: Characteristics of Its Regulation in Nonstimulated and Estrogen-Stimulated Rooster[†]

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ABSTRACT: The high molecular weight apolipoproteins of very low density lipoprotein (VLDL) have been examined in VLDL fractions from nonstimulated and estrogen-stimulated roosters. Apolipoprotein B (apo VLDL-B) as previously described [Williams, D. L. (1979) *Biochemistry 18*, 1056] was detected in nonstimulated as well as estrogen-stimulated animals. Comparison of apo VLDL-B species from nonstimulated and estrogen-stimulated animals showed no differences, suggesting that the same, or a very similar, apolipoprotein gene is expressed in the presence and absence of estrogens. Plasma apo VLDL-B was observed to accumulate 500-fold in response to a single treatment with diethylstilbestrol. A specific and quantitative assay has been developed to measure hepatic apo VLDL-B synthesis. This assay has been used to measure apo VLDL-B synthesis in liver slices from nonstimulated and estrogen-stimulated roosters as well as animals challenged with the antiestrogenic drug, tamoxifen. The results of these studies show that basal apo VLDL-B synthesis is 2–2.5% of liver protein synthesis in nonstimulated roosters. This level of synthesis is not effected by tamoxifen, indicating that basal apo VLDL-B synthesis is not due to low levels of endogenous estrogens. In response to estrogen treatment, apo VLDL-B synthesis increased sharply after a lag period of 1.5 h, reached a maximum at 15–24 h, and returned to the control level. At the response maximum apo VLDL-B synthesis was 12–15% of total liver protein synthesis. This response was completely blocked by tamoxifen. Calculation of the absolute increase in apo VLDL-B synthesis following estrogen treatment showed that increased hepatic synthesis alone cannot account for the 500-fold accumulation of apo VLDL-B in rooster plasma.

The association between hyperlipoproteinemic states and the development of cardiovascular disease has focused considerable attention on the structure and metabolic regulation of VLDL[1] (Goldstein & Brown, 1977; Schaefer et al., 1977; Osborne & Brewer, 1977). The importance of estrogenic hormones in human VLDL metabolism is evidenced by studies which have shown plasma triglycerides and VLDL to increase during therapy with estrogen-containing contraceptives (Wynn et al., 1969; Wallace et al., 1979). The increased incidence of cardiovascular complications associated with estrogen therapy (Kaplan, 1978; Shapiro et al., 1979) clearly emphasizes the need for a detailed understanding of VLDL metabolism and the alterations which occur in response to estrogens. The chicken has frequently been used for studies of VLDL since massive increases in plasma VLDL occur in response to exogenous estrogens in the rooster or with the onset of egg laying in the hen (Schjeide, 1954; Hillyard et al., 1956). A variety

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[1] Abbreviations used: VLDL, very low density lipoprotein; apo VLDL-B, apolipoprotein B of VLDL; apo VLDL-II, apolipoprotein II of VLDL; DES, *trans*-α,α'-diethyl-4,4′-stilbenediol; tamoxifen, *trans*-1-(*p*-dimethylaminoethoxyphenyl)-1,2-*trans*-diphenylbut-1-ene.

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