Radius of Gyration, Asymmetry, and Head-Group Orientation in Unperturbed Lecithins

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Abstract: Biologically interesting lipids derived from glycerol have available an enormous number ($>10^{20}$) of configurations if three states are assigned to each bond about which rotation occurs. Averaging over such a large number of configurations can be achieved using methods developed to treat branched polymers. Configuration-dependent properties for unperturbed lecithins were obtained via rotational isomeric state theory from a representative sample generated by Monte Carlo methods. Necessary a priori and conditional probabilities were obtained from a treatment which incorporates first- and second-order interactions. This procedure is a logical extension of an earlier successful treatment of triglycerides. Unperturbed root-mean-square radii of gyration are near 8 Å for those lecithins of greatest biological interest. This result is about 1 Å smaller than that for triglycerides derived from the same carboxylic acids. Asymmetry of the spatial distribution was assessed by examination of averaged principal moments ($\langle L_1^2 \rangle \geq \langle L_2^2 \rangle \geq \langle L_3^2 \rangle$) of the inertia tensor. Triglycerides and lecithins have nearly identical asymmetries when derived from saturated, straight-chain carboxylic acids having eight to nine carbon atoms. Lecithins, but not triglycerides, become more asymmetric as the size of the acyl groups increases. The average location of the phosphorylcholine with respect to the remainder of the lecithin is consistent with the head group *not* being oriented parallel to an axis normal to the plane of a lipid bilayer. Instead the head group would be tilted about 30° from the normal.

Conformational properties of lecithins are of current interest because these molecules are the dominant lipid in naturally occurring membranes. Two extreme models for polar head groups in a bilayer orient them extended normal or parallel to the membrane plane.[1] Recent experimental work finds that the head group is probably not extended parallel to the bilayer normal.[2,3] Instead the choline group has been described as executing rapid angular oscillation about an axis normal to the plane of the bilayer[4] while having a predominant orientation parallel to the bilayer plane.[2,3,5]

Lecithins in membranes are not unperturbed by long-range interactions, but understanding of their properties in the unperturbed state is pertinent nevertheless. It is important to inquire, for example, which properties of these lipids in membranes are those expected for a lecithin unperturbed by long-range interactions. If certain conformational properties of lecithins in membranes differ from those expected for the unperturbed molecule, it then becomes necessary to search for the long-range interactions responsible for the situation observed. The objective of the present communication is to present configuration-dependent properties for unperturbed lecithins. The results obtained are based on rotational isomeric state theory[6] for branched molecules[7-9] and its successful application to triglycerides.[10,11] From the model are calculated the mean square radius of gyration and the averaged principal moments of the inertia tensor. These properties are compared with those obtained for triglycerides derived from the same carboxylic acids. The model also yields the average angle between head group and tail. The result demonstrates that the head group would be tilted (by about 30°) away from a normal to the bilayer if the lecithin were unperturbed.

Computational Method

Structure. Carbon, nitrogen, oxygen, and phosphorus atoms in a small lecithin, diacetylphosphatidylcholine, are depicted in Figure 1. The molecule is comprised of three branches which meet at the β carbon atom of the glycerol moiety. Main-chain atoms are indexed by a presubscript which denotes the branch and by a postsubscript which denotes sequential position within a branch. Branch j contains n_j bonds in its main chain. Atoms in the carbonyl group are denoted by primes. Ester groups are maintained in the planar trans configuration. Bond lengths and angles are collected in Table I. Where applicable they are the average of values obtained for crystalline L-α-glycerylphosphorylcholine.[12] Remaining parameters are those used previously for triglycerides.[11]

Statistical Weight Matrices and Rotational States. The statistical weight matrix for bond i in branch j is denoted by ${}_j\mathbf{U}_i$. Statistical weight matrices for bonds in triacetin have been described by Mattice and Saiz.[10] When used in conjunction with appropriate generator matrices, they permit computation of a dipole moment, optical anisotropy, and molar Kerr constant in excellent agreement with experiment. Additional statistical weight matrices appropriate for treatment of larger triglycerides have also been described.[11] The foregoing statistical weight matrices are assumed to apply to those bonds rotation about which alters no first- or second-order interaction with $-PO_3CH_2CH_2N(CH_3)_3$. The only new statistical weight matrices required are those for bonds 2–6 in branch 3. Expressions used for these new statistical weight matrices at 25 °C are

$$ {}_3\mathbf{U}_2 = \begin{bmatrix} 1 & 0 & 1 \\ 1 & 1 & 0 \\ 1 & 0 & 0 \end{bmatrix} \tag{1} $$

$$ {}_3\mathbf{U}_3 = {}_3\mathbf{U}_5 = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{bmatrix} \tag{2} $$

$$ {}_3\mathbf{U}_4 = \begin{bmatrix} 0 & 0.07 & 0.07 \\ 0.07 & 1 & 0.05 \\ 0.07 & 0.05 & 1 \end{bmatrix} \tag{3} $$

$$ {}_3\mathbf{U}_6 = \begin{bmatrix} 1 & 2 & 2 \\ 1 & 10 & 0 \\ 1 & 0 & 10 \end{bmatrix} \tag{4} $$

Columns index the state of that bond, while rows index the state of the preceding bond. Order of indexing is trans, gauche$^+$, gauche$^-$ (dihedral angles 0, 120, 240°).

Null elements in ${}_3\mathbf{U}_2$ arise from the necessity to suppress states with a short internuclear distance (about 1.95 Å) between oxygen atoms in the phosphate group and either

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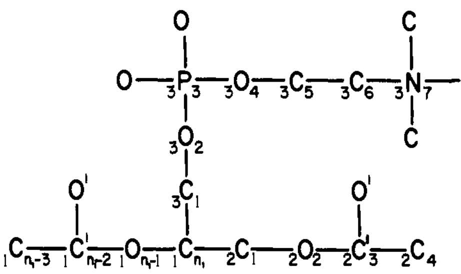

Figure 1. Nonhydrogen atoms in diacetylphosphorylcholine.

Table I. Structural Parameters

bond length, Å		bond angle, deg	
C—C	1.53	CCC	112
C—C′	1.50	CCC′	113.7
C═C	1.34	CC═C	125.0
C—N	1.51	CCN	115
C—O	1.43	CNC	109.5
C′—O	1.35	CCO	109
C′—O′	1.20	CC′O′	126.3
O—P	1.61	OC′O′	122.3
O═P	1.49	COC′	116.7
		COP	118
		OPO	103
		OP═O	108
		O═P═O	122

Table II. First-Order a Priori Probability for Population of Rotational States by Bonds 2-6 in Branch 3

bond	trans	gauche$^+$	gauche$^-$
2	0.51	0.08	0.41
3	0.06	0.47	0.47
4	0.06	0.47	0.47
5	0.18	0.41	0.41
6	0.11	0.44	0.44

$_1O_{n_1-1}$ or $_2C_1$. First-order interactions are different in t and g$^\pm$ states for bonds 2 and 5 in branch 3. For the fragment $CCOPO_3$, however, summed 6–12 interactions are only about 0.15 kcal mol^{-1} more negative at ±120° than at 0°. The net effect is small enough to be ignored.

Rotation about the third bond in branch 3 simply interchanges positions of oxygen atoms bonded to the phosphorus atom. First-order interactions can therefore be assumed approximately equivalent in all states. There are no other important interactions to be included in $_3U_3$. A similar situation obtains for $_3U_5$.

The next statistical weight matrix reflects interactions occurring in the segment $CH_2OPO_2OCH_2$. Energetics of this fragment have been considered previously by Newton[13] and Olson.[14] They find a strong preference for g$^\pm$g$^\pm$. This statistical weight matrix is formulated in accord with ab initio calculations reported by Olson.[14]

A gauche state at the sixth bond in branch 3 produces a first-order interaction between $_3O_4$ and $N(CH_3)_3$. Higher order interactions between $N(CH_3)_3$ and the remainder of the phosphate group are important if gauche states are occupied at both the fifth and sixth bonds in branch 3. These interactions are prohibitively repulsive (one O···C separation is less than 1 Å) if the gauche states are of opposite sign. Attractive electrostatic interaction of atoms in the quaternary ammonium and phosphate groups more than compensates for a repulsive 6–12 contribution to the first-order interactions in g$^\pm$ states and second-order interactions in g$^\pm$g$^\pm$ states. Statistical weights for these states are quite sensitive to the value of the local dielectric constant. The expression in eq 4 is obtained if the effective dielectric constant is near 3.5.

Configuration-Dependent Properties for Specified Configurations. The moment of inertia tensor, $\mathbf{S}_{\times 2}$, was evaluated for the N nonhydrogen atoms in a lecithin, ignoring differences in mass of carbon, nitrogen, oxygen, and phosphorus atoms. For a specified configuration this tensor is[15]

$$\mathbf{S}_{\times 2} = N^{-1} \sum_{i=1}^{N} \mathbf{r}_{0i}{}^{\times 2} - N^{-2} \left(\sum_{i=1}^{N} \mathbf{r}_{0i} \right)^{\times 2} \qquad (5)$$

where $\mathbf{r}_{0i}$ denotes the vector from atom $_1C_0$ to the ith nonhydrogen atom and $\times 2$ as a superscript denotes the self-direct product. This expression yields $\mathbf{S}_{\times 2}$ as a column comprised of the nine elements in reading order. Principal moments ($L_1{}^2 \geq L_2{}^2 \geq L_3{}^2$) are obtained by an appropriate similarity transform. Their sum is the squared radius of gyration, s^2. Principal moments and s^2 are conveniently expressed as a dimensionless ratio obtained through division by the sum of squared lengths for all bonds other than C-H. This sum will be denoted by $\sum l^2$. Its value for a lecithin prepared from glycerylphosphorylcholine and two molecules of a saturated carboxylic acid containing n carbon atoms each ($n > 1$) is

$$\sum l^2{}_{\text{lecithin}} = (35.61 + 4.68n) \text{ Å}^2 \qquad (6)$$

Triglycerides prepared from glycerol and three molecules of the same carboxylic acid have

$$\sum l^2{}_{\text{triglyceride}} = (13.27 + 7.02n) \text{ Å}^2 \qquad (7)$$

Monte Carlo Sampling of Configurations. Monte Carlo sampling is achieved using a random number generator in conjunction with a priori and conditional probabilities obtained from the configuration partition function, Z.

$$Z = {}_1\mathbf{U}_1{}^{(n_1)} ({}_2\mathbf{U}_1 \ominus {}_3\mathbf{U}_1)[({}_2\mathbf{U}_2)^{(n_2-1)} \otimes ({}_3\mathbf{U}_2)^{(n_3-1)}] \qquad (8)$$

Here ${}_1\mathbf{U}_1{}^{(n_1)}$ denotes the product of n_1 successive statistical weight matrices commencing with ${}_1\mathbf{U}_1$, ⊗ denotes the direct product, and ${}_2\mathbf{U}_1 \ominus {}_3\mathbf{U}_1$ is a rectangular matrix formulated by Mattice and Saiz.[11] The a priori and conditional probabilities are obtained from Z by procedures described previously in detail for a molecule which contains a trifunctional branch point.[7] The $L_i{}^2$ and s^2 were evaluated for each molecule in a sample. Corresponding moments were averaged to yield $\langle L_i{}^2 \rangle$, and the s^2 were averaged to yield $\langle s^2 \rangle_0$.

Five sets consisting of the larger of 200 or 100 ($n - 1$) configurations each were generated for every lecithin studied. Error bars denote standard deviations in the average for these five sets. In many cases the standard deviation is so small that an error bar is not apparent in the figures presented. Calculations were carried out for saturated lecithins with even n up to $n = 22$, as well as for $n = 1$ and 3, and also for unsaturated lecithins where the acyl groups were palmitoleyl or oleyl.

Results and Discussion

Rotational State Occupancies in the Phosphorylcholine Moiety. Table II presents a priori probabilities for population of rotational states in the phosphorylcholine moiety. Bonds 3 and 4 commonly occupy gauche states of the same sign.[16] This result is in accord with the probabilities collected in Table II. It is also in accord with second-order a priori probabilities, which yield $p_{g^+g^+} = p_{g^-g^-} = 0.42$ for these two bonds. Crystalline L-α-glycerylphosphorylcholine[12] has bonds 2–4 in states tg$^\pm$g$^\pm$, which is in accord with calculated first- and second-order a priori probabilities. Bonds 2 and 3 have $p_{tg^+} = p_{tg^-} = 0.24$. Experimentally, bond six has an overwhelming preference for the gauche states.[12,16-18] Entries in Table II are in accord with this result. In the crystal the fifth bond is close to a trans state.[12] This case is the only one where the state occupied by a bond in the crystal is not the most probable conformation in the unperturbed molecule. A similar situation exists for the states adopted by bonds 2–6 in branch 3 of crystalline 1,2-dilauroyl-DL-phosphatidylethanolamine.[19]

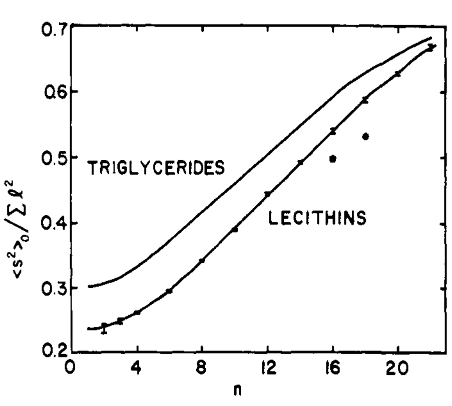

Figure 2. Characteristic ratios for lecithins and triglycerides containing saturated linear acyl groups. Each acyl group has n carbon atoms. Circles denote characteristic ratios for lecithins whose acyl groups are palmitoleyl or oleyl. Triglyceride data are from ref 11.

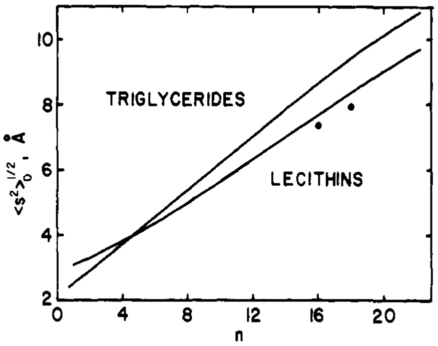

Figure 3. Root mean square unperturbed radii of gyration for lecithins and triglycerides containing saturated linear acyl groups. Each acyl group has n carbon atoms. Circles denote radii for lecithins whose acyl groups are palmitoleyl or oleyl. Triglyceride data are from ref 11.

Radius of Gyration. Figure 2 depicts $\langle s^2\rangle_0/\sum l^2$ as a function of the number, n, of carbon atoms, in a linear saturated acyl group. This figure also depicts the trend for previously reported[11] characteristic ratios of triglycerides. A consideration of the asymptotic behavior of polymethylene[20,21] ($(\langle s^2\rangle_0/\sum l^2)_\infty$ and $(\mathrm{d} \ln \langle s^2\rangle_0/\mathrm{d}T)_\infty$, as well as the effect of a single trifunctional branch point on the asymptotic behavior,[22] yields $(\langle s^2\rangle_0/\sum l^2)_\infty$ of 1.0 and 1.3 for triglycerides[11] and lecithins, respectively. Lecithins and triglycerides of biological interest clearly have characteristic ratios lying well below those obtained as $n \rightarrow \infty$. Furthermore, $\langle s^2\rangle_0/\sum l^2$ for biologically interesting lecithins is smaller than that for triglycerides derived from the same carboxylic acids, in contrast to the situation at the asymptotic limit. Characteristic ratios for these two classes of lipids cross when n is about 24.

Root mean square radii of gyration, $\langle s^2\rangle_0^{1/2}$, obtained from Figure 2 are depicted in Figure 3. The smallest member of each series, obtained when formic acid is the source of the acyl substituent, has a radius of 2.5 or 3.1 Å for the triglyceride or lecithin, respectively. Radii increase as acyl groups are lengthened. A more rapid increase is found for triglycerides, simply because each triglyceride contains three acyl substituents while the lecithin has only two. Lecithins have the smaller radii when n is larger than four.

The most prevalent acyl groups in lecithins obtained from natural sources are derived from C16 and C18 carboxylic acids, often with a cis double bond between carbon atoms 9 and 10. Filled circles denote characteristic ratios (Figure 2) and $\langle s^2\rangle_0^{1/2}$ (Figure 3) for lecithins with this unsaturation. A similar reduction in $\langle s^2\rangle_0^{1/2}$ is obtained by removal of a methylene group or introduction of a cis double bond between carbon atoms 9 and 10. Unperturbed radii near 8 Å can be expected for the most common lecithins. This result is about 1 Å smaller than that for triglycerides derived from the same carboxylic acids.[11]

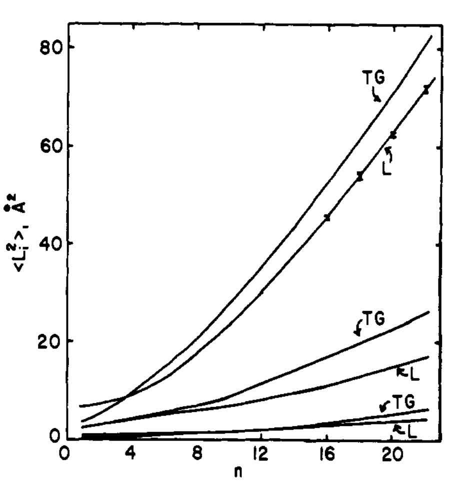

Figure 4. Averaged principal moments of the inertia tensor for lecithins (L) and triglycerides (TG) containing saturated linear acyl groups. Each acyl group has n carbon atoms. Triglyceride data are from ref 11.

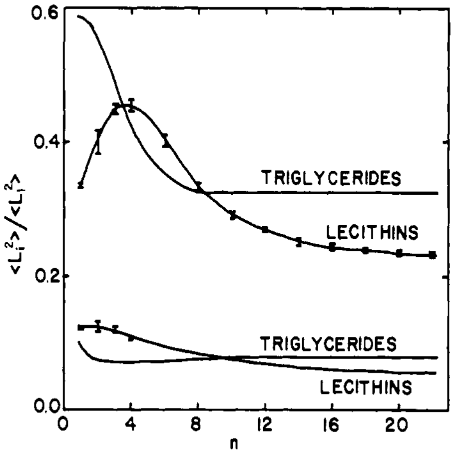

Figure 5. Ratios of the averaged principal moments of the inertia tensor for lecithins and triglycerides containing saturated linear acyl groups. Each acyl group has n carbon atoms. Triglyceride data are from ref 11.

Asymmetry of the Spatial Distribution. Figure 4 depicts behavior of averaged principal moments of the inertia tensor for lecithins. Principal moments for triglycerides are from ref 11. All principal moments increase with n. The value of $\langle L_i^2\rangle$ for a lecithin is less than that for the corresponding triglyceride when n is in the biologically interesting range. This effect is not equally manifest in each of the principal moments, however. When n is greater than 16, for example, the smallest percent reduction occurs in the largest principal moment. For this reason, $\langle L_2^2\rangle/\langle L_1^2\rangle$ and $\langle L_3^2\rangle/\langle L_1^2\rangle$ are smaller for the lecithin than for the corresponding triglyceride.

Ratios of the averaged principal moments are depicted in Figure 5. The value of $\langle L_3^2\rangle/\langle L_1^2\rangle$ for lecithins decreases continuously as n rises from 2 to 22. In contrast, $\langle L_2^2\rangle/\langle L_1^2\rangle$ rises when n goes from 1 to 4. Identical asymmetries are obtained for lecithins and triglycerides when n is 8 or 9. Asymmetry of triglycerides is unaffected when n increases above 10. Such is not the case of lecithins, however, owing to the decrease in $\langle L_2^2\rangle/\langle L_1^2\rangle$ and $\langle L_3^2\rangle/\langle L_1^2\rangle$. While unperturbed lecithins with n = 16 or 18 are not accurately described as prolate ellipsoids, they come closer to that description than do the corresponding triglycerides. Values of $\langle L_2^2\rangle/\langle L_1^2\rangle$ and $\langle L_3^2\rangle/\langle L_1^2\rangle$ for these lecithins are close to those computed for linear polymethylene chains of the same molecular

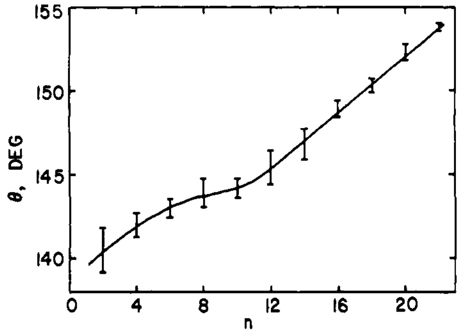

Figure 6. Average angle between head group and tail for lecithins containing saturated linear acyl groups. Each acyl group has *n* carbon atoms.

weight.[23] Asymmetry for dipalmitoleylphosphorylcholine and dioleylphosphorylcholine is virtually indistinguishable from that of the corresponding saturated lecithin.

Angle between Head and Tail. The head group will be defined as $-CH_2OPO_3CH_2CH_2N(CH_3)_3$ and the tail is $RCO_2CH_2CH(RCO_2)CH_2-$. These two portions of the molecule meet at the methylene unit common to both. Two vectors, $\mathbf{r}_{head}$ and $\mathbf{r}_{tail}$, are calculated for each configuration. These vectors extend from the carbon atom of the common methylene unit to the center of mass of the nonhydrogen atoms in the head group ($\mathbf{r}_{head}$) or tail ($\mathbf{r}_{tail}$). The angle between these vectors is denoted by θ. Our interest focuses on θ and θ^2 when they are averaged over all configurations accessible to a lecithin. Figure 6 depicts $\langle\theta\rangle$ for lecithins having even n running from 2 to 22. Its value increases from about 140° for $n = 2$ to 154° for $n = 22$. Dependence of $\langle\theta\rangle$ on n arises because the distribution for $\mathbf{r}_{tail}$ depends on the size of the acyl groups. Lecithins of greatest interest have $\langle\theta\rangle$ near 150°. Most configurations have θ near $\langle\theta\rangle$ ($\langle\theta^2\rangle/\langle\theta\rangle^2$ lies between 1.019 and 1.028 for those lecithins whose $\langle\theta\rangle$ is depicted in Figure 6).

Consider a two-dimensional array of unperturbed lecithins. Let them be ordered with their $\mathbf{r}_{tail}$ parallel to one another and oriented normal to the plane of the array. The foregoing demonstrates that the head groups would, on average, neither be extended along the normal nor oriented parallel to the surface of the array. The average angle between a head group and an axis normal to the plane of the bilayer would be about 30°. This result is obtained in the absence of any special intermolecular ordering effects. Clearly the model just presented is only a crude approximation for a membrane. Nevertheless, the model would predict that the head group is not extended parallel to the normal, in accord with recent deductions from experiment.[2,3] Furthermore, it indicates that a tilt of as much as 30° can be expected simply from a consideration of short-range intramolecular interactions. This tilt might be augmented by additional interactions not considered in a treatment of unperturbed molecules. A proposed interlocking set of intermolecular electrostatic associations of $N(CH_3)_3$ and phosphate serves as an example of an intermolecular interaction which may be present.[5] The tilt could further be modified if $\mathbf{r}_{tail}$ is not oriented precisely normal to the plane of the array.

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