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Structural Chemistry of 1,2 Dilauroyl-DL-phosphatidylethanolamine: Molecular Conformation and Intermolecular Packing of Phospholipids

(lipid bilayers/hydrocarbon chain packing/lipid–protein interactions/membrane structure)

PETER B. HITCHCOCK*, RONALD MASON*, K. MARK THOMAS*, AND G. GRAHAM SHIPLEY†‡

* School of Molecular Sciences, University of Sussex, Brighton, U.K.; and † Biophysics Division, Unilever Research Laboratory, The Frythe, Welwyn, Hertfordshire, U.K.

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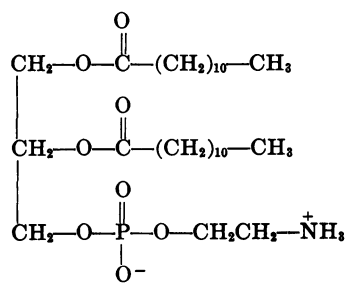
ABSTRACT **Crystals of 1,2 dilauroyl-DL-phosphatidylethanolamine:acetic acid are monoclinic with a = 46.2, b = 7.77, c = 9.95 Å, β = 92.0°; space group $P2_1/c$. The structural analysis, based on the visual estimates of 1467 reflection intensities, was achieved by direct methods, and least squares analysis convergence was to R_1 = 0.28. There are marked differences between the observed molecular conformation and those that have been predicted theoretically. The mean planes containing the lipid chains are essentially parallel to one another; the phosphodiester moiety has a double *gauche* conformation, while intermolecular hydrogen bonding modifies the conformation that could be anticipated for an isolated phosphatidylethanolamine molecule. The intermolecular packing produces the classical lipid bilayer structure, adjacent lipid bilayers being separated by acetic acid molecules of crystallization. The hydrocarbon chain packing can be considered either as a quasi-hexagonal type or as a complex orthorhombic subcell arrangement. One-dimensional electron density profiles across the lipid bilayer at increasing resolution clearly demonstrate the origin of features present on the low resolution profiles of both model and natural membranes.**

Phospholipids, glycolipids, and glycosphingolipids play a key structural and functional role in animal, plant, and bacterial cell membranes (1). Furthermore, phospholipids are important molecular components of the serum lipoproteins responsible for fat transport in the body (2) and of bile secreted by the liver where, together with bile salts, they solubilize cholesterol in a complex, aqueous, mixed micellar system (3).

Although much information is available on the behavior of phospholipids in water (4, 5), the mutual interactions of different lipid classes, particularly phospholipid–cholesterol (6), and phospholipid–protein interactions [these systems in turn being used as models for the more complex membranes and natural lipoproteins], no detailed structural information exists for phospholipids. In particular, no single crystal studies have been reported on the complex lipids themselves, although this type of structural information is available for their components. For example, crystal structures of fatty acids (for a review, see ref. 7), glycerol (8), and sphingosine derivatives (9) have been reported. Also various substituted aminophosphates (10–12), including glycerylphosphorylethanolamine (13) and ethanolamine phosphate (14), and sugars (15), which determine the different lipid classes, have been described. From this structural information, conformational analyses of different phospholipids have been made and, on this basis, the most likely structures have been predicted (12, 16, 17).

In this paper we describe a single-crystal analysis of a phospholipid, the synthetic compound 1,2 dilauroyl-DL-phosphatidylethanolamine.



A knowledge of the molecular conformation and intermolecular packing of phosphatidylethanolamine and other phospholipids will allow a more detailed interpretation of the x-ray diffraction data derived from lipid–water, lipid–protein, and natural membrane systems.

Experimental

Attempts to grow single crystals of the phosphatidylethanolamine suitable for single-crystal analysis from usual lipid solvents were unsuccessful. Finally, the phosphatidylethanolamine was dissolved in glacial acetic acid and thin, transparent crystals of plate habit were obtained by slow, controlled evaporation of the solvent. Crystal data—$C_{29}H_{58}O_8NP$: ($C_2H_4O_2$), F. W. 639.9; a = 46.2(3), b = 7.77(3), c = 9.95(3) Å, β = 92.0(2)°, U = 3569.6 Å^3, Z = 4, D_c = 1.19 g cm^{-3}. Space group $P2_1/c$ (C^5_{2h}, no. 14) from the systematic absences $h0l$ for l odd and $0k0$ for k odd. Cu–K_α radiation, λ = 1.5418 Å, μ(Cu–K_α) = 11.1 cm^{-1}.

By ordinary crystallographic standards, the crystals were of mediocre quality, and the mosaic spread of the reflections on the Weissenberg photographs was very large. The observed intensities were based on multiple film-multiple exposure equi-inclination Weissenberg methods, the $h0l \rightarrow h5l$ and $hk0 \rightarrow hk2$ zones of reflections being sampled. The 1467 independent reflections given nonzero intensities by visual methods and having $\sin\theta/\lambda \leqslant 0.57$ contain, without doubt, considerable random and systematic errors due to difficulties

Abbreviation: Phosphatidylethanolamine, 1,2 dilauroyl-DL-phosphatidylethanolamine.

Requests for reprints should be addressed to G. G. Shipley‡.
‡ Present address: Biophysics Division, Department of Medicine, Boston University School of Medicine, Boston, Mass. 02118.

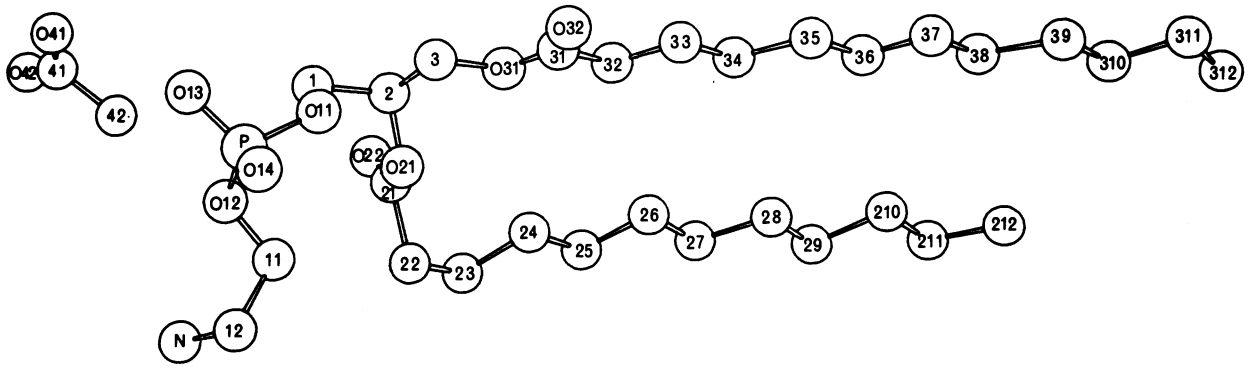


FIG. 1. The molecular conformation of 1,2 dilauroyl-DL-phosphatidylethanolamine: acetic acid.

of integration. No absorption corrections were made, and the poor quality of the intensity data is undoubtedly reflected in the large, final discrepancy index (see below) and contributed to the considerable difficulties met in the structure determination.

Structural analysis

The hydrocarbon chain directions were obvious from the three-dimensional Patterson function, and coordinates for the phosphorus atom could be assigned from the Harker section (18). However, superposition and Fourier methods did not extend these interpretations. Initial attempts at a structural determination by direct methods were unsuccessful. The analysis eventually proceeded smoothly by direct methods routines written by P.B.H. Normalized structure factors, $|E_H|$, were calculated by standard methods, using an overall Debye–Waller factor of 4.0 Å^2; a correction was made to the $|E_{hk0}|$ values to allow for the effects of an approximate mirror plane perpendicular to *c*. Symbolic addition methods (19), using all reflections with $|E_H| \geq 1.2$, provided several phase solutions, the best of which had a consistency index of 0.89 and a Karle reliability value of 0.25. The Fourier synthesis based on this set of signs showed a peak consistent with a phosphorus atom (as suggested earlier by the Patterson function) and unresolved strings of electron density, which could be assigned to the lipid chains. However, the map lacked sufficient clarity for any obvious progress to be made by conventional phase refinement, techniques, and the remaining electron density syntheses derived by symbolic addition methods were not chemically promising. A new phase determination was now set up by a multiple start method (20). An initial set of nine reflections was chosen by convergence mapping (21), three having their signs fixed (arbitrarily) to define the unit cell origin. Each of the 64 starting sets were then used to generate signs for as many reflections as possible having $|E_H| > 1.55$, the four best sets having very similar values for their consistency indexes and Karle reliability values.

The optimal set was then extended to predict, by standard methods, the phases of 484 reflections having $|E_H| > 0.8$. The corresponding Fourier synthesis provided a poorly defined view of the molecule, and this was not improved for the three remaining phase sets. However, from these syntheses the assignment of coordinates for 23 atoms could be made with some confidence. Their contributions to the structure factors corresponded to $R_1[\Sigma(|Fo| - |Fc|)/\Sigma|Fo|] = 0.48$, and subsequent Fourier syntheses showed all the remaining atoms with the exception of two carbon atoms. Furthermore, an acetic acid molecule of crystallization became evident at this point. Four cycles of unit weight-full matrix least squares analysis converged *R* from 0.51 to 0.29, and a difference Fourier synthesis confirmed all the atomic positions with the exception of O32, for which new coordinates were derived. The remaining two carbon atoms, C311 and C312, were also indicated as very weak peaks (ρ max ~ 0.8 e·Å^{-3}).

Four further cycles of refinement converged to $R_1 = 0.28$, with only carbon atoms C311 and C312 providing any difficulties of convergence. These atoms have very high temperature factors, probably reflecting static disordering of their positions as much as genuine thermal vibrations. The packing of the molecules in the crystal is such that these atoms have no short intermolecular contacts and they project out into the gap at the center of the bilayer (see below). A final examination of intra- and intermolecular bond lengths and angles allowed the distinction of carbon and oxygen atoms in the acetic acid molecule, and four final cycles of least squares analysis converged to $R_1 = 0.28$. The final indicated shifts of the atomic coordinates did not exceed 0.2 σ, the average positional shift being 0.02 σ. A difference electron density synthesis contains peaks up to 1.5 (4) e·Å^{-3}, which are consistent with some general disorder in the crystal, but no totally convincing scheme for disordering can be provided.

Molecular structure

A general view of the phosphatidylethanolamine molecule and the acetic acid molecule of crystallization is shown in Fig. 1; the atom labeling corresponds to the parameters of Table 1. (A listing of observed and calculated structure factor amplitudes and the bond length/angle data is available from the authors.) The standard deviations of the bond lengths and bond angles are relatively high and preclude any detailed discussion of these results. Rather, the interest attaches to the molecular conformation and particularly its relation to those structures of phospholipids that have been predicted by simple considerations of nonvalence interactions. For consistency, the conformational notations of Sundaralingam (12) and of McAlister *et al.* (16) are followed (Fig. 2).

The conformation of the glycerol fragment is defined by the torsion angles ($\beta_{1'}$, θ_3), which have the values (214°, 182°). With the torsion angles $\gamma_1 = \gamma_2 = \beta_2$ fixed as 180° (see the observed values of 190, 181, and 179°, respectively), McAlister *et al.* (16) calculated six minima in the potential energy as a function of θ_3 and $\beta_{1'}$; of these, the deepest minimum is at $\beta_{1'} = 270°$ and $\theta_3 = 60°$. Although the observed structure is at a secondary minimum in the potential energy profile, about

TABLE 1. *1,2 Dilauroyl-DL-phosphatidylethanolamine:acetic acid–atomic coordinates and isotropic thermal parameters*

Atom	x	y	z	μ (Å^2 × 10^{-2})
P	0.0775(2)	0.9596(16)	−0.1385(13)	0.0
N	0.0550(8)	1.4761(51)	−0.0334(31)	3.2
O11	0.1088(5)	0.8764(34)	−0.0881(31)	1.4
O12	0.0721(5)	1.1133(35)	−0.0389(31)	1.8
O13	0.0550(5)	0.8231(38)	−0.1069(30)	1.8
O14	0.0804(4)	0.9846(30)	−0.2665(26)	0.0
C11	0.0928(8)	1.2621(56)	−0.0380(46)	1.7
C12	0.0751(10)	1.4134(63)	−0.1287(55)	3.3
C1	0.1099(9)	0.8372(66)	0.0574(54)	3.2
C2	0.1436(9)	0.8629(63)	0.1060(50)	2.4
C3	0.1599(9)	0.7575(58)	0.0002(48)	2.3
O31	0.1911(9)	0.7880(57)	0.0662(45)	8.0
O32	0.2086(10)	0.6584(67)	−0.1199(56)	10.0
C31	0.2093(11)	0.7317(76)	−0.0190(68)	4.8
C32	0.2383(12)	0.7852(78)	0.0925(62)	6.1
C33	0.2623(11)	0.7174(73)	0.0176(59)	5.2
C34	0.2919(15)	0.7688(92)	0.1046(74)	9.1
C35	0.3162(14)	0.6986(94)	0.0130(72)	8.8
C36	0.3455(19)	0.7669(115)	0.1039(91)	13.1
C37	0.3690(19)	0.6989(118)	0.0278(92)	13.0
C38	0.3964(19)	0.7685(117)	0.1264(94)	13.7
C39	0.4216(26)	0.7156(153)	0.0138(118)	19.5
C310	0.4499(35)	0.7600(196)	0.1266(159)	25.7
C311	0.4758(49)	0.6903(306)	0.0391(223)	41.7
C312	0.4993(47)	0.8042(276)	0.1624(190)	38.5
O21	0.1489(5)	1.0514(44)	0.0837(32)	2.6
O22	0.1433(7)	1.0665(49)	0.3097(40)	4.9
C21	0.1493(7)	1.1201(51)	0.2113(48)	0.1
C22	0.1560(10)	1.3234(73)	0.1655(56)	4.7
C23	0.1821(13)	1.3732(83)	0.2760(68)	7.5
C24	0.2098(10)	1.2574(65)	0.2071(54)	3.8
C25	0.2378(15)	1.3228(101)	0.3092(77)	9.9
C26	0.2631(14)	1.2092(88)	0.2574(68)	7.7
C27	0.2875(20)	1.3031(131)	0.3414(97)	14.9
C28	0.3171(17)	1.2194(106)	0.2569(83)	11.3
C29	0.3414(16)	1.3207(107)	0.3513(80)	10.8
C210	0.3702(18)	1.2151(111)	0.2838(87)	11.8
C211	0.3914(20)	1.3127(131)	0.3743(98)	14.8
C212	0.4224(25)	1.2417(149)	0.2904(117)	19.6
C41	0.0042(10)	0.8043(76)	0.1245(59)	4.2
C42	0.0287(7)	0.9224(49)	0.1215(41)	0.1
O41	0.0004(7)	0.6886(50)	0.0429(35)	4.2
O42	−0.0082(11)	0.8250(75)	0.2667(59)	12.6

0.5 kcal mole^{-1} more in energy, this must not be taken to reflect on the quality of the assumptions of the nonbonded interatomic potential functions (16). The three minima in the energy profiles at each of the $\beta_{1'}$ values of 200° and 270° are established by "hard sphere" interactions (corresponding to the three staggered positions of O31 with respect to C1), and the two $\beta_{1'}$ values derive from similar considerations. It seems clear that a distinction of conformers differing only by 0.5–1.0 kcal mole^{-1} will require much more sophisticated and precise potential energy calculations than is presently achieved by the atom–atom approach.

The conformation of the acyl ester groups is established by (θ_3, $\beta_{1'}$, γ_1) = (182, 214, 190)°. The calculations (16) provide an overall minimum at (60, 270, 180)° for (β_2, γ_2) = (180, 180)°, while for θ_3 = 180°, $\beta_{1'}$ = 200°, the calculated minimum is at γ_1 = 180°, β_3 = 270°, values that are quite close to the observed results. Although the conformation of the primary acyl group is not precisely *trans*, it is sufficiently so to discuss the way in which the β chain folds to meet the γ chain and hence establish the bilayer, "tuning-fork" arrangement. The calculations established two likely conformations for the stacking between the β and γ chains; in the first, the dihedral angle between the chain planes is 72°, while for the second it is 57°. The observed situation is obviously quite different from prediction: the dihedral angle between the planes defined, respectively, by C32–C312 and C22–C212 is 8°, only slightly different from strict parallelness. The observed geometry appears to be very similar to that labeled as (c) by McAlister *et al.* (16). The major discrepancies occur not in the γ chain conformations, but follow from the lack of theoretical definition of the β chain conformation. Thus, while for the calculated conformation of the phospholipid θ_{3calc} = 180°, θ_{4calc} = 60° (observed values of 182° and 73°), the observed and calculated β_n values are 214° and 270° ($\beta_{1'}$), 227° and 240° (β_3), and 74° and 290° (β_4). McAlister *et al.* (16) do suggest that the conformation (β_4, γ_1) = (60°, 180°) should become increasingly important with increase in hydrocarbon chain-length, but it seems also that their potential for the hydrogen nonbonded interactions is too hard.

The angles α_2 and α_3 (51°, 64°) for the phosphodiester moiety correspond essentially to the double *gauche* conformations ((g+, g+) = (60°, 60°)). For the isolated phosphorylcholine moiety, based on a double *gauche* conformation of the phosphodiester group, the calculations predict values for α_4, α_5, and α_6 of 180°, 300°, and 180°, respectively. The observed results for α_4 and α_5 are 101° and 77°, and without doubt reflect the formation of the intermolecular N—H...O bond.

Bilayer formation and intermolecular bonding

A major rationale for the structural analyses of phospholipids is that they may be of considerable value in model building and in the interpretation of diffraction patterns from both model and natural membranes. This expectation is borne out since the intermolecular packing of the phosphatidylethanolamine molecules *is exactly in the form of a classical lipid bilayer* (Fig. 3). The lipid chain axes are essentially parallel to one another and to the *a* axis. The centrosymmetrically related methylene and methyl groups towards the end of each lipid chain have few and long intermolecular contacts and also little "anchoring" from intramolecular bonding. These factors provide the chain flexibility, which is reflected in large static or dynamic disorder. The separation of the centers of gravity of the polar groups, within one section of bilayer, is 39.0 Å.

The architecture of the bilayer is established, apart from the intramolecular chain interactions and normal dispersion interactions, by an N—H...O13 hydrogen bond system with a bond length of 2.79 Å. The different sections of the bilayer are built up through the glide plane and translation symmetry operations shown in Figs. 3 and 4, and the molecular interactions are obviously dispersion and weak electrostatic forces. The bilayers are insulated from one another by a layer comprised of the acetic acid molecules of crystallization. The glide plane related acetic acid molecules are hydrogen bonded together (O41...O42′ = 2.77 Å) and make only a very weak hydrogen bond (O41 ..N = 3.13 Å) to the polar ends of the phospholipid molecules (Fig. 4). It seems probable that the acetic acid molecules have little effect on the molecular con-

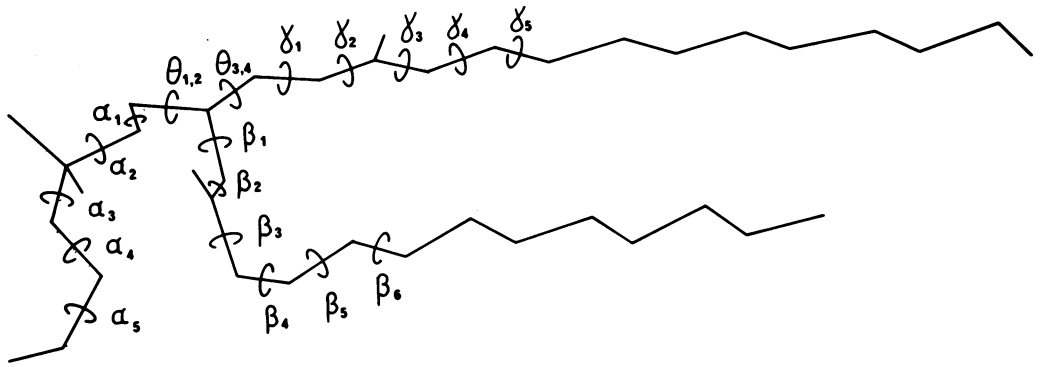


FIG. 2. Definition of torsion angles for phosphatidylethanolamine. Torsion angles (degrees):

θ_1	θ_2	θ_3	θ_4	α_1	α_2	α_3	α_4	α_5
310	64	182	73	211	51	64	101	77
β_1	β_2	β_3	β_4	γ_1	γ_2	γ_3	γ_4	$\beta_{1'}$
105	179	227	74	190	181	176	176	214

formation and the intermolecular packing of the phospholipid. The packing of the hydrocarbon chains in phosphatidylethanolamine shown in Fig. 4 can be considered either as a quasi-hexagonal arrangement, with a chain–chain separation of about 4.6 Å, or as a centered orthorhombic subcell arrangement (22).

Several comments are now justified in connection with recent structural results on model and natural membranes. Fig. 5*a* shows the electron density synthesis constructed from the (calculated) 100, 200, 300, and 400 structure factors (d_{min} = 11 Å), which corresponds closely to the resolution obtained from the model systems and natural membranes (23–25); Fig. 5*b* shows the profile derived from the 100 to 800 structure factors, whilst Fig. 5*c* shows the curve corresponding to higher resolution data, d_{min} = 1 Å. The low resolution synthesis shows striking similarities to those calculated for the synthetic membranes even though the phases of the four reflections (+, +, +, −) are different from those assumed for the lecithin bilayers (+, +, −, +), and the relative intensities of the reflections are not closely related. The assumption that the polar headgroup contributes maximum electron density and that the chain region has a low density is borne out by the syntheses at progressively higher resolution. In addition, the structural origin of shoulders on the main peaks observed at intermediate resulution may be derived, and the half-widths of the peaks may be compared with corresponding peaks of membrane electron density profiles at similar resolution. For example, at a resolution $d_{min} \sim 10$ Å, the half-width of the low electron density trough in the middle of the myelin membrane bilayer is 12–15 Å (25) compared with about 6 Å found in the low-resolution series for phosphatidylethanolamine, and it seems probable that the increased half-width may indeed be due to the inclusion of steroid and/or protein in the membrane bilayer.

Finally, there is evidence to suggest that in certain synthetic and natural membranes the hydrocarbon chains are relatively disordered and undergo large molecular motion (4, 5, 26–30). This chain disorder has been studied extensively by spectroscopic methods, notably spin-label electron spin resonance and nuclear magnetic resonance, in both lipids and membranes (for reviews, see refs. 31–33). For example, spin-label electron spin resonance experiments show that for dipalmitoyl lecithin in both the gel (ordered hydrocarbon) and liquid crystal (disordered hydrocarbon), the amount of disorder, as measured by the electron spin resonance order parameter S, increased on progressing along the hydrocarbon chain from the carboxyl group to the terminal methyl group (34). In addition, ^{13}C nuclear magnetic resonance studies of aqueous dispersions of lecithins show that the relaxation times of individual carbon atoms along the hydrocarbon chain increase from the carboxyl group towards the terminal

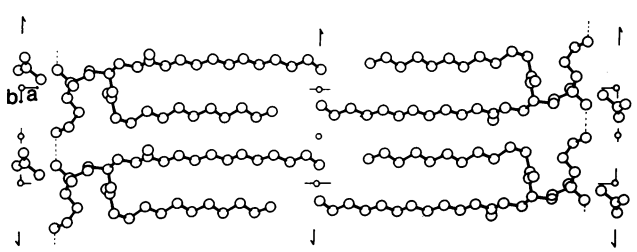


FIG. 3. Molecular packing of phosphatidylethanolamine: acetic acid projected onto the *ab* plane.

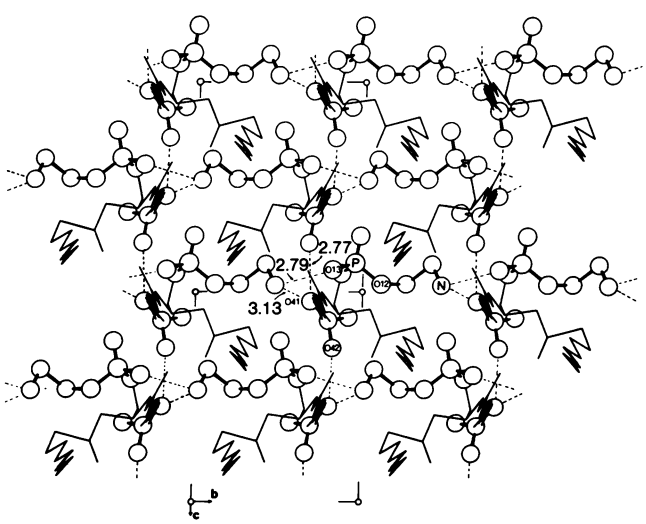


FIG. 4. Molecular arrangement of phosphatidylethanolamine: acetic acid projected onto the *bc* plane, showing the intermolecular hydrogen bonding systems and the hydrocarbon chain packing arrangement.

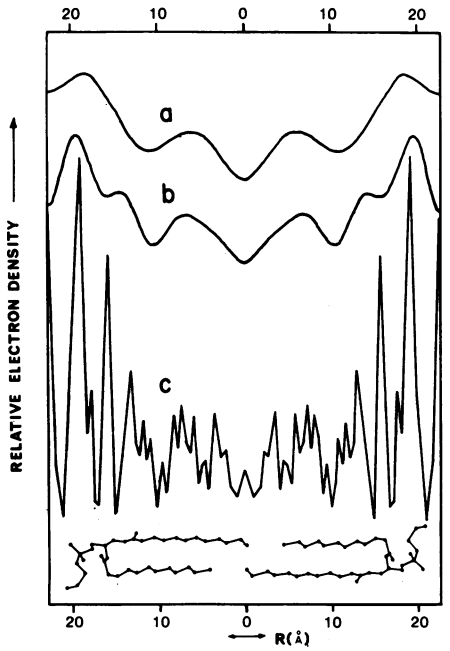


FIG. 5. One-dimensional electron density profiles calculated from $h00$ reflections. (*a*) h = 1–4; (*b*) h = 1–8; (*c*) h = 1–44.

methyl group (33, 35). The variation of the Debye thermal factors of the chain carbon atoms is again consistent with a progressive increase in the thermal motion along the hydrocarbon chains even in the crystalline state (Fig. 6). Although these parameters are subject to considerable systematic errors due to difficulties in estimating the integrated reflection intensities, e.g., the Debye factor of the phosphorus atom is physically unreasonable, the general trend in Fig. 6 illustrates static and/or dynamic disordering in the lipid chains, and this correlates well with the weakness of the nonvalence forces at or around the center of the bilayer. Thus, in the crystalline state we are probably observing the precursor molecular events that eventually lead to the temperature- or solvent-induced cooperative melting of the hydrocarbon chains.

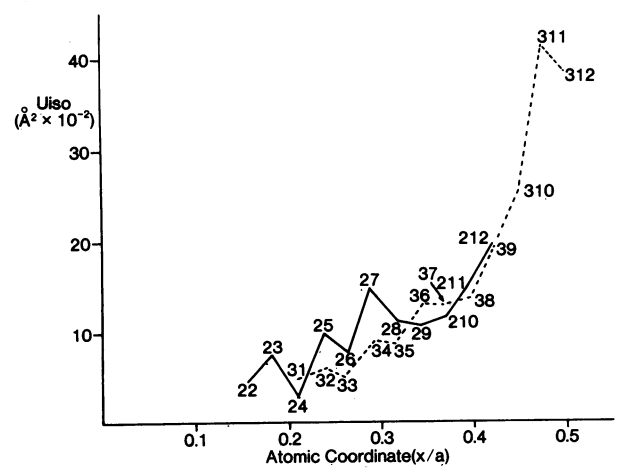


FIG. 6. Plot of the Debye thermal factor (U_{iso}) of chain carbon atoms as a function of their position along the *a* axis: — β chain, - - - - γ chain (atomic numbering, as in Table 1 and Fig. 1).

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