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Lamellar packing of a chiral *N,N*-dimethylphosphatidylethanolamine: electron diffraction. Evidence for a lecithin-type headgroup conformation

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Lamellar electron diffraction intensity data from epitaxially crystallized 1,2-dipalmitoyl-*sn*-glycerophospho-*N,N*-dimethylethanolamine were used to determine the layer packing in order to compare the chiral structure to the crystal structure of a racemic homologue. After finding the chain orientation, the structure was determined by interpretation of the Patterson function, followed by independent crystallographic phase assignments with conventional direct methods (use of three phase structure invariants). The phase determination was verified by a translational search with a molecular model based on a similar lecithin structure. The final *R*-value is 0.29, and this is lowered to 0.18 after a correction is made for incoherent multiple electron scattering. The layer packing is found to be very much like that of a diacyl phosphatidylcholine with the *N,N*-dimethylethanolamine moiety parallel to the bilayer surface rather than the perpendicular arrangement of headgroups involved in an interdigitated layer, as seen for a racemic homolog.

Introduction

N-Methyl and *N,N*-dimethylethanolamine phosphoglycerides are important metabolic intermediates in the biosynthesis of lecithin from phosphatidylethanolamine [1]. Although the crystal structures of the two end products [2,3] have been shown to have similar molecular conformations in the solid state [4], the crystal structure of the (racemic) *N,N*-dimethyl intermediate [5] has a quite unusual headgroup conformation in that the substituted ethanolamine moiety packs in an interdigitated layer, effectively reducing the bilayer thickness for this lipid. In addition, the molecular geometry of the diacylglycerol moiety was also shown [5] to be quite different from that of lecithin or phosphatidylethanolamine, since conformational changes involving effective acyl chain rotations about their long axes can significantly affect the orientation of the glycerol moiety.

The unusual crystal structure of the dimethyl intermediate is quite puzzling when compared to published electron diffraction lamellar data from ester- and ether-linked C_{16} chain phospholipids in this series [6]. For unhydrated specimens, addition of the first methyl group to the ethanolamine causes a rather large increase of this layer spacing which changes only slightly for further methylations to the phosphatidylcholine. If the chain packing is untilted in all cases and the molecular conformations are similar (in contrast to the existing crystal structure), these data imply that the major influence for changing lamellar thickness is the bulk of the lipid headgroup.

In our electron diffraction structure analysis of an ether-linked *N*-methylphosphatidylethanolamine [6], we have already found that the molecular conformation and packing could be quite like that of the phosphatidylethanolamines and lecithins, at least for the polymorphic form of the material where the long chains are not interdigitated. In this paper, we establish that a second crystal form can also exist for the *N,N*-dimethylphosphatidylethanolamines in the solid state which resembles the other more typical packing schemes adopted by the other members of the series, thereby providing a facile explanation for the sequence of observed lamellar spacings.

Materials and Methods

Sample crystallization

1,2-Dipalmitoyl-*sn*-glycerophospho-*N,N*-dimethylethanolamine ($DPPEM_2$) was purchased from Calbiochem-Behring (La Jolla, CA) and stored at 1°C over

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desiccant. As described earlier [7], crystals were epitaxially nucleated on naphthalene to produce a projection onto the molecular axes, i.e., the lath-like microcrystals are formed with the molecular long chain axes parallel to the major crystal face. As will be described elsewhere with a phase diagram [8], this condition is established by ensuring that the phospholipid is dilute enough in the molten naphthalene solution to allow the aromatic hydrocarbon to crystallize first when the co-melt is cooled. However, there are local concentration gradients for which the phospholipid mole fraction lies at the other side of the eutectic point so that no specific interaction between the lipid and naphthalene takes place. In this case, the lipid will crystallize as if from solution, with chain axes now more or less perpendicular to the major crystal face. Both orthogonal molecular projections are important for our structure analysis.

Electron diffraction

Transmission selected area electron diffraction experiments were carried out at 100 kV with JEOL JEM-100B7 or JEM-100CXII electron microscopes. As usual, precautions were taken to protect the specimens from radiation damage by use of low-incident beam currents and a fast photographic emulsion (Kodak NS5T or DEF-5 X-ray films) for recording the diffraction patterns. Camera lengths for the diffraction experiments were calibrated with gold Debye-Scherrer patterns photographed at the same magnetic lens settings used to obtain the lipid diffraction patterns.

Lamellar intensity data in the electron diffraction patterns were evaluated by integrating peak areas after the films were scanned with a Joyce Loebl MkIIIC flat-bed microdensitometer. As is usual for these phospholipid electron diffraction patterns, the peaks were somewhat arced due to lamellar undulations [9]. Hence, an effective Lorentz correction was applied to the intensity data [10] to determine the observed structure factor magnitudes, i.e.,

$$|F_{00l}^{\text{obs}}| = k(I_{00l}^{\text{obs}} \cdot l)^{1/2}$$

where k is a scale factor and l the order of the lamellar reflection.

Crystal structure analysis

Four methods were combined to determine the lamellar packing of $DPPEM_2$:

(a) The chain orientation is found by the spacings and symmetry of the electron diffraction patterns from crystals where the molecular axes are oriented, more or less, along the incident beam direction. As has been frequently discussed [11], these patterns isolate the scattering component of the methylene subcell.

(b) One-dimensional Patterson functions $P(w) = \Sigma|F_{00l}|^2 \cos 2\pi lz$ are calculated from the observed data. As shown, e.g., by Khare and Worthington [12], this will provide information about the lipid headgroup conformation.

(c) A translational search is made with a conformational model based on a similar crystal structure. In our study we use a structure based on one of the lecithin conformers found in the crystal structure analysis of DMPC [3] for which the choline moiety is parallel to the lamellar bilayer surface. One of the two methyl groups on the choline with nearly the same z fractional coordinates is removed to construct the conformational model and the chains of DMPC are lengthened to the dipalmitoyl glycerol moiety size. One-dimensional structure factors are calculated by

$$F_{00l} = \sum_j f_j \exp 2\pi i(\vec{r}_j \cdot \vec{s}_{00l}) = 2\sum_j f_j \cos 2\pi(\vec{r}_j \cdot \vec{s}_{00l})$$

The implied centrosymmetry is due to the two-fold dyad axis parallel to the crystallographic b-axis. Here f_j is the Doyle Turner electron scattering factor [13] corrected for isotropic thermal motion. Following Hitchcock et al. [14], the temperature factor is approximated by:

$$B_j = (C + 4.0 + 108\, z_j^2)\,\text{Å}^2$$

where $C = 0.0$ Å^2 for molecular crystals. The calculated structure factors for translational shifts along c are compared to the observed values to seek a minimum in the crystallographic residual

$$R = \frac{\Sigma||F_{00l}^{\text{obs}}| - k|F_{00l}^{\text{calc}}||}{\Sigma|F_{00l}^{\text{obs}}|}$$

As shown by Hamilton [15], this figure of merit has a low precision when the number of refineable variables has the same order of magnitudes as the number of data.

(d) As is discussed in detail elsewhere [16,17], use of direct methods based on three-phase cosine structure invariants [18] can be used to phase a limited number of X-ray or electron diffraction lamellar data from phospholipids. The technique is particularly useful for relating low angle with wide angle phase values. For a centrosymmetric case, assuming space group $P\bar{1}$, the Σ_2-triples $\psi = \phi_{\mathbf{h}_1} + \phi_{\mathbf{h}_2} + \phi_{\mathbf{h}_3}$, where the Miller indices are constrained such that $\mathbf{h}_1 + \mathbf{h}_2 + \mathbf{h}_3 = 0$, can be evaluated based on the magnitude of the normalized structure factors $|E_{\mathbf{h}}|$. A hierarchy of most probable phase relationships is generated from these calculations according to the value of $A = g|E_{\mathbf{h}_1} \cdot E_{\mathbf{h}_2} \cdot E_{\mathbf{h}_3}|$ where g is a scaling term. Similarly Σ_1-triples, i.e.,

$$\psi = \phi_{\mathbf{h}_1} + \phi_{\mathbf{h}_1} + \phi_{-2\mathbf{h}_1}$$

can be generated and evaluated according to

$$A_1 = (|E_h|^2 - 1)|E_{2h}|/\sqrt{N}$$

where N is the number of atoms in the primitive unit cell. Triples where A_1 is a negative number are found to be the most reliable.

Results

A lamellar diffraction pattern from an epitaxially-oriented sample of $DPPEM_2$ is shown in Fig. 1 along with one from the orthogonal projection. The characteristic pattern from the hexagonal methylene, where $d_{100} = 4.15$ Å, indicates that the chains pack in uninclined layers in a rotationally disordered array, the so-called 'gel' phase of phospholipids [19].

A one-dimensional Patterson function calculated from observed intensities resembles those calculated with observed electron diffraction data for ether-linked lipids 1,2-dihexadecyl-*sn*-glycerophospho-*N*-methylethanolamine and 1,2-dihexadecyl-*sn*-glycerophosphocholine [6], as shown in Fig. 2. The positions of major inter-group peaks also match those found earlier.

When normalized observed structure factors are used for direct phasing of the diffraction data, 11 of 13 values are determined according to the protocols outlined in Table I. The resultant one-dimensional electrostatic potential map in Fig. 3 already has recognizable features which can be assigned to the headgroup and hydrocarbon chains.

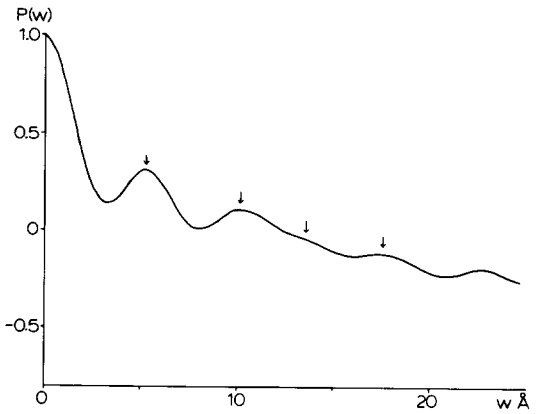

Fig. 2. One-dimensional Patterson function calculated from observed lamellar electron diffraction intensity data from $DPPEM_2$. Major peak positions occur at 5.3 Å, 10.0 Å, 13.5 Å (shoulder) and 17.6 Å, which can be compared to peak positions found at 5.5 Å, 10.8 Å and 17.2 Å for 1,2-dihexadecyl-*sn*-glycerophosphocholine [6].

TABLE I

Direct phasing for $DPPEM_2$

Origin definition: $\phi_{001} = 0$

Σ_2-triple invariants:

$\phi_{004} = \phi_{005} = \phi_{006} = \phi_{007} = \phi_{008} = \phi_{009} = \phi_{00,10} = \phi_{00,11} = \phi_{00,12} = \phi_{00,13} = a$

Σ_1-triple invariants (negative):

$\phi_{006} = \phi_{008} = \phi_{00,10} = \phi_{00,12} = \pi$

hence $a = \pi$

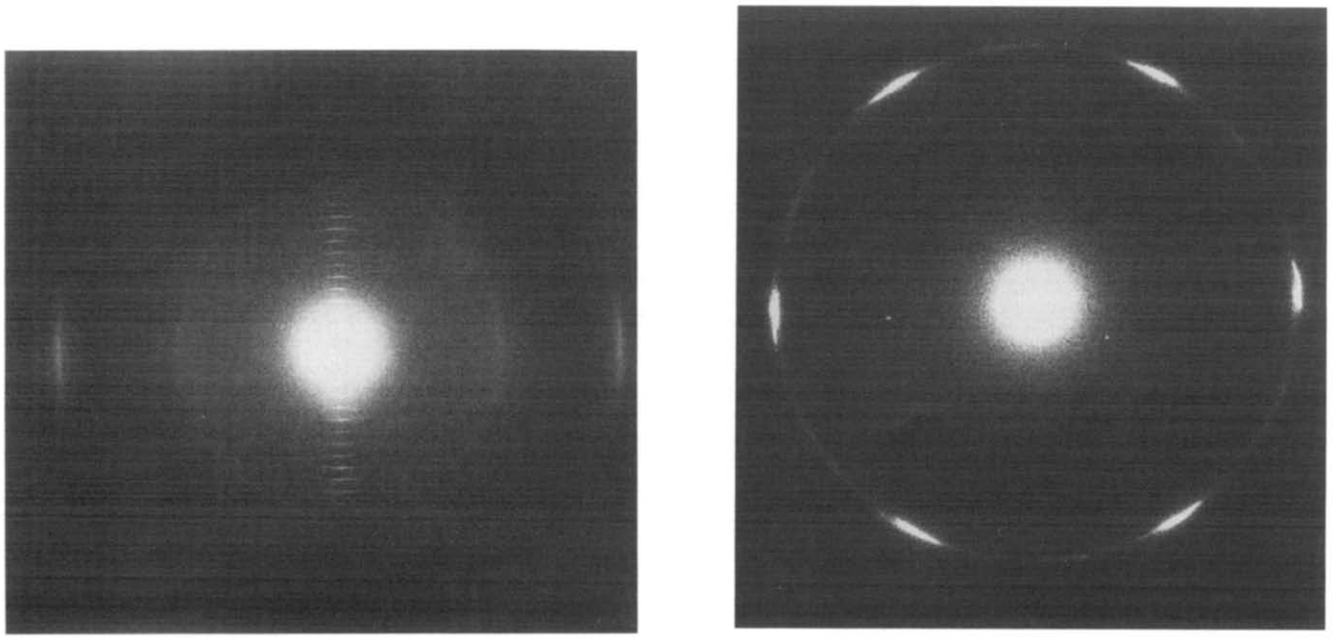

Fig. 1. Electron diffraction pattern from $DPPEM_2$. (a) Samples epitaxially crystallized on naphthalene; $d_{001} = 58.7 \pm 0.4$ Å. (Note that the inner order spots are obscured in this print by the gaussian inelastic background scatter from the carbon film support. The pattern is intended only to show the diffraction resolution, whereas structure factors of all reflections, measured from multiple exposures on several films, are listed in Table II.) (b) Non-epitaxial crystallization revealing characteristic 'gel phase' pattern from untilted acyl chains.

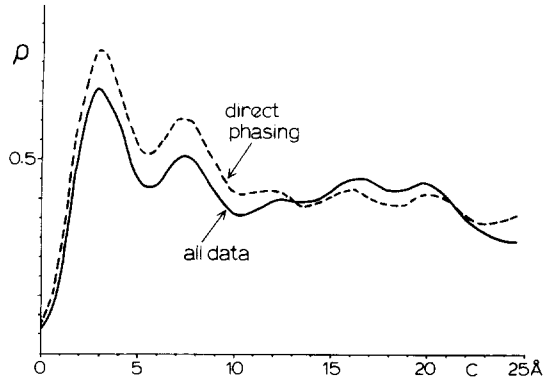

Fig. 3. One-dimensional electrostatic potential maps for $DPPEM_2$ calculated with observed structure factor magnitudes. Dashed line: partially phased data set obtained by direct methods; Solid line: totally phased data set obtained from model structure.

The model based on the lecithin structure with choline oriented parallel to the lamellar surface (as discussed above) was used for a translational search of the molecular packing based on finding a minimum of the crystallographic residual. A schematic of this search is presented in Fig. 4, showing that a minimum is found where $R = 0.29$. Yet another minimum is found where $R = 0.40$, and, although this difference is significant in terms of Hamilton's statistics, it is gratifying that the direct phasing analysis only agrees with the structure solution at the lower R-value, demonstrating the utility of this new phasing technique for limited diffraction data sets. Calculated and observed structure factors are compared in Table II, where the agreement of the phase assignments from this structure search with the previous direct phase determination is demonstrated. The electrostatic potential map computed with all 13 phases also is in close agreement with the map derived from direct phasing results (Fig. 3).

It is apparent from all our previous electron diffraction determinations of phospholipid lamellar packing

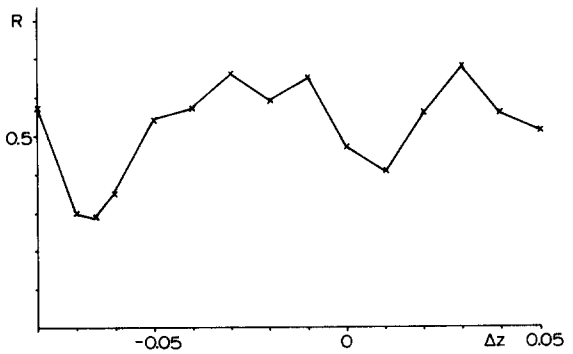

Fig. 4. Translational search in space group $P\bar{1}$ with molecular model based on the crystal structure of DMPC [3] with choline parallel to layer surface. At the beginning of the search, the outermost C_{16} chain carbon position is at a fractional coordinate $z = 0.5509$ and the headgroup phosphorus atom is at $z = 0.1278$. The lowest minimum is found when $\Delta z = -0.065$.

TABLE II

Observed and calculated structure factors for $DPPEM_2$ (absolute values based on model)

(00l)	$\lvert F_{calc} \rvert$	Direct phasing (Table I)	$\lvert F_{calc} \rvert$	$\lvert F_{calc} \rvert$ [a]	Model phases
1	0.344	0	0.379	0.313	0
2	0.205	–	0.109	0.143	π
3	0.149	–	0.062	0.118	0
4	0.166	π	0.257	0.218	π
5	0.149	π	0.114	0.158	π
6	0.144	π	0.207	0.184	π
7	0.114	π	0.153	0.154	π
8	0.120	π	0.128	0.139	π
9	0.127	π	0.181	0.161	π
10	0.166	π	0.099	0.120	π
11	0.153	π	0.141	0.132	π
12	0.123	π	0.120	0.123	π
13	0.122	π	0.133	0.125	π
			$R = 0.29$	0.18	

[a] Corrected for incoherent multiple scattering.

that some perturbation of the observed intensity data takes place (as also pointed out by a reviewer of one of our earlier papers). We have already established [10] that this perturbation is not n-beam dynamical scattering. The solution to this problem is evident after a careful analysis of electron diffraction data from epitaxially-oriented crystals of an n-paraffin [20] and polyethylene [21], where it was discovered that incoherent multiple scattering is the major factor here, consistent with the observed layer-like structure of the lath crystals [21]. As shown by Cowley et al. [22], the intensities needed for crystal structure analysis are distorted by a series of weighted n-fold convolutions ($*$) of the whole zone of excited reflections, i.e.,

$$I_l' = I_l + mI_l{}^*I_l + nI_l{}^*I_l{}^*I_l + \ldots$$

For these lamellar structures, even a correction for the first self-convolution term results in a dramatic lowering of the crystallographic residual to 0.18 (Table II).

Discussion

The analysis described above demonstrates that a second conformer of N,N-dimethylethanolamine-containing phospholipids can exist where the headgroup moiety is oriented parallel to the bilayer surface rather than being involved in an unusual interdigital packing across the lamellar interface. Given the match of Patterson vectors with those from other phospholipids of known structure [6,12] it is also probable that the diacyl glycerol conformation is the one usually found for other phospholipids. Pascher and Sundell [5] have stated that evidence also exists for the interconversions of the two glycerol conformations for aqueous dispersions of

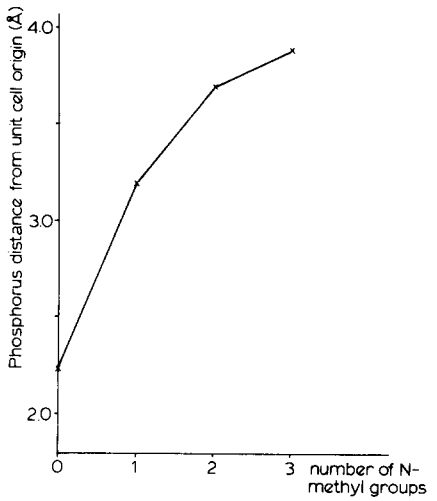

Fig. 5. Derived projected distance from phosphorus-to-unit cell origin for four successively methylated phospholipid structures studied by electron diffraction structure analysis. The greatest change in headgroup packing distance occurs when phosphatidylethanolamine is initially methylated.

ethanolamine- and choline-containing phospholipids, indicating that this behavior might be shared by all members of the series. Whether molecular chirality is involved in favoring one packing scheme over the other is presently unknown.

The projected phosphorus-to-origin distance for this *N,N*-dimethyl compound found in the structure analysis (3.69 Å) also fits a progression of such distances for examples of other headgroup classes in this series, even though the other members studied were ether-linked compounds (Fig. 5). This illustrates in another way that the increasing headgroup bulk is responsible for expanding the lamellar thickness, even for non-hydrated lipids.

Finally, it is interesting to note how well direct methods succeed in determining the phase values for most reflections in this diffraction pattern from an unknown structure. This is particularly remarkable since the intensity data are obviously affected somewhat by incoherent multiple scatter, but not to the extent where the main features of the molecular transform are lost (as evidenced also by the appearance of the Patterson function). The electrostatic potential map, which resembles electron density maps in X-ray structure analyses, is readily interpreted to locate the salient headgroup features and the hydrocarbon chain packing region. Difficulties in use of the direct phasing procedure for low angle data can be overcome by direct low-dose electron microscope images of the structure to independently check the phase assignments for this region of reciprocal space [16]. Nevertheless, the partial phase determination is shown here to reveal most structural features which are in agreement with an independent model structure determination based on a similar crystal structure.

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