X-Ray Studies on Phospholipid Bilayers
I. Polymorphic Forms of Dimyristoyl Lecithin

M. Suwalsky * and J. Tapia **

Harvard Medical School, Boston, USA, and Departamento de Química, Universidad de Concepción, Chile

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A structural study of the synthetic phospholipid, L-α-dimyristoyl lecithin (DML), has been made by X-ray fiber diffraction methods. Three different types of oriented specimens were prepared. They were X-ray photographed under the same conditions, including temperature and relative humidity. Three different types of diffraction patterns, corresponding to different conformations and/or packing arrangements, were found. They are characterized by their unit cell dimensions, space groups, molecular conformations, and packing arrangements.

Introduction

The very important structural and functional roles played by phospholipids in cellular membranes have long been known. They, together with proteins, cholesterol and water, constitute the major components of such membranes. Lecithins are the phospholipids most commonly found in mamalian tissues and, therefore, it is not surprising that a wide range of X-ray structural studies have been performed on them. This subject has been reviewed by Shipley [1] and Luzzati [2]. Studies on both natural and synthetic lecithins indicate that they conform the bilayer structure which was proposed more than forty years ago. Nevertheless, scanty detailed structural information is available as there are very few single crystal studies on phospholipids. Most of the X-ray work has been done on powder samples [3–5] and partially oriented multilayers [6, 7] which, generally, did not show more than a single oriented reflection other than those coming from the bilayer repeat itself [7, 8]. This situation has forced investigators to work out the structures basing their calculations mostly reflections derived from the lamellar repeat [9, 10].

This paper reports different techniques used to produce suitable oriented samples of the synthetic phospholipid dimyristoyl lecithin (DML). These specimens were X-ray photographed at constant and similar conditions of temperature and relative humidity. Different types of X-ray patterns were observed which were only dependent on the method of preparation of the specimens. Each type of pattern is characterized in terms of unit cell dimensions and space groups. This information, together with model building studies, gives an approximation of the conformation and packing arrangements of DML molecules in the different bilayer structures.

Materials and Methods

Synthetic L-α-dimyristoyl lecithin from Sigma (Lots 65-C-8100 and 66-C-01881) and Calbiochem (Lot 810031) was used without further purification as it was determined by thin layer chromatography to be the only phospholipid present. Oriented molecular bilayers were prepared by three different techniques. In all of them DML was dissolved in chloroform or chloroform:methanol 1:1, and the solvent removed by slow evaporation at room temperature. The first type of oriented specimen, DML (I), was prepared by evaporating the solvent on a glass slide while stroking and rolling gently with a razor blade in a preferential direction. The samples thus prepared were cylindrically shaped. DML (II) was a single specimen, film-like, prepared by strongly compressing a sample of DML (I) between two glass slides. DML (III) was obtained by growing films on fine metallic rings. The specimens were kept at 21 °C ± 2 °C and 76% of relative humidity in glass capillaries or humidity cells con-

* Guggenheim Fellow. To whom correspondence should be addressed: Departamento de Química, Universidad de Concepción, Casilla 3-C, Concepción, Chile.
** Departamento de Química, Universidad de Concepción.

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nected to saturated sodium chloride solution, as described elsewhere [11, 12]. Photographs were taken with flat-plate cameras provided with fine glass collimators, using nickel-filtered Cu Kα radiation. The water content of the samples was estimated by gravimetry. Molecular models were built from CPK space-filling components (1.25 cm = 1 Å).

Results

X-ray photographs were taken at 76% r.h. and about 21 °C of the three types of oriented specimens of DML described in the previous section. An X-ray diagram produced by specimens of the type DML (I) is showed in Fig. 1. It presents approximately forty sharp and regularly oriented reflections of which about half are equatorial orders of a 54.7 Å reflection. The non-equatorial reflections are distributed along six layer lines. All the observed reflections could be satisfactorily indexed in terms of an orthorhombic unit cell with $a = 8.85$ Å, $b = 9.50$ Å, and $c = 54.70$ Å. These cell dimensions are highly suggestive of a bilayer structure.

A completely different pattern was obtained from DML (II), an oriented specimen prepared as DML (I) but which had been compressed between two glass slides. The diagram was obtained after keeping the specimen at 76% r.h. for about seven months. The X-ray photograph of DML (II) is presented in Fig. 2. It shows a large series of equatorially oriented reflections whose spacings are orders of a 63.2 Å repeat. Even a thirty-second order reflection was recorded. It also presents a meridional reflection of 4.37 Å and a number of non-equatorial reflections, distributed along four layer lines. The nearly fifty observed reflections were all indexed in terms of a monoclinic unit cell with $a = 5.11$ Å, $b = 7.00$ Å, $c = 63.20$ Å and $\gamma = 121.4°$. The cell dimensions suggest that DML molecules still form bilayers but their conformation and packing arrangements are different from the previous type.

The third type of specimen, DML (III), was the one that showed the highest cristallinity and orientation of the three of them as can be seen in Fig. 3. The indexing of the nearly twenty equatorially oriented reflections and the approximately fifty non-equatorial ones distributed along seven layer lines, indicates that the dimensions of its monoclinic unit cell are $a = 9.27$ Å, $b = 9.85$ Å, $c = 54.97$ Å, and $\gamma = 96.4°$.

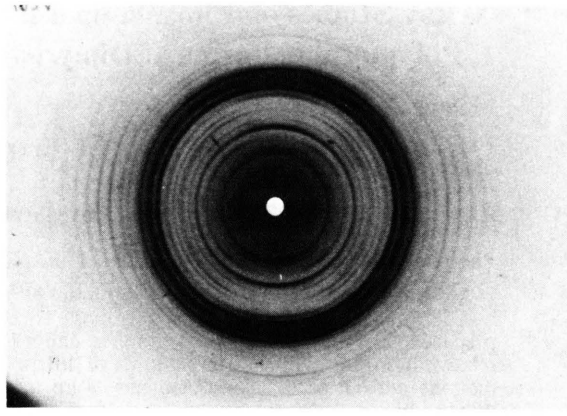

Fig. 1. X-ray diffraction photograph of an oriented specimen of type DML (I).

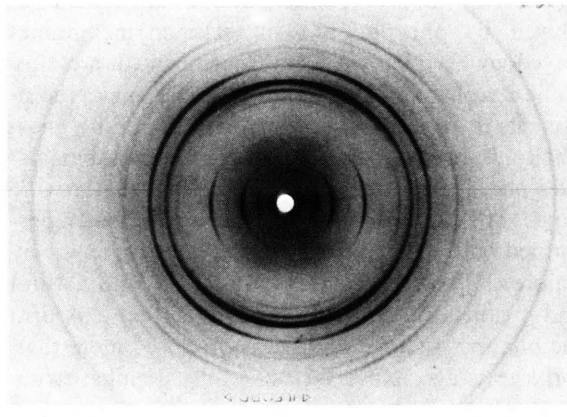

Fig. 2. X-ray diffraction photograph of an oriented specimen of type DML (II).

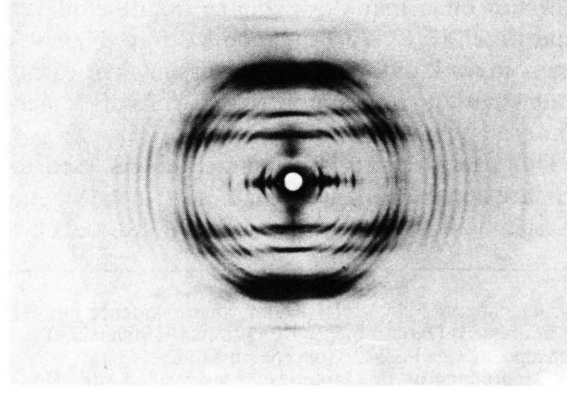

Fig. 3. X-ray diffraction photograph of an oriented specimen of type DML (III).

Discussion

It is evident that the three types of DML specimens produced significantly different X-ray patterns. The diagrams were obtained under the same experimental conditions, including temperature and relative humidity. It can safely be concluded, therefore, that only the different way of preparing these specimens determined, besides differences in their crystallinity and orientation of the reflections, different molecular conformations and/or packing arrangements.

No detailed crystal structure of a lecithin is yet available. From the molecular structure of L-α-glycerylphosphatydilcholine [13], by assuming 2.5 Å per pair of methylene groups for the fully extended hydrocarbon chains, and from molecular models, a length of about 32 Å for a fully-extended DML molecule seems reasonable. Therefore, if the DML molecules were completely extended and perpendicular to the bilayer plane, a bilayer width (or *c* axis) of about twice this value would have been expected. On the other hand, a close packing of fully extended DML molecules would result in an intermolecular distance, of *b* axis, of about 7 Å. Interestingly, DML (II) has a *c* axis of 63.2 Å and a *b* axis of 7.0 Å. This necessarily implies that in this form of DML the molecules are fully extended and closely packed parallel to each other (Fig. 4). The molecular structure of DML (II) will be discussed later.

On the other hand the forms (I) and (III) of DML have, instead, *c* and *b* axes of about 55 and 10 Å. Although it is not possible to definitely rule out other conformations, one model that seems particularly satisfactory for both forms is that in which DML molecules align parallel to each other perpendicularly to the bilayer plane and are equally spaced at about 10 Å. The molecules are not fully extended. Instead, the phosphorylcholine groups are bent almost perpendicularly with respect to the fully extended and parallel hydrocarbon chains and, therefore, lie in the bilayer plane (Fig. 5). The positively-charged choline end groups might interact, through a combination of electrostatic interactions and hydrogen bonds with the neighboring negative phosphate groups forming planar sheets parallel to the bc plane. Both halves of each sheet (monolayers) are in contact with each other and, in DML (I), are related by two-fold rotation axes parallel to *a*. X-ray [14], neutron diffraction [15] and NMR estudies on lecithins [16], also support this "bent" conformation of DML molecules. Indeed, potential energy calculations have shown that the flexibility of the phosphorylcholine group allows it to orient itself to optimize intermolecular interactions [17].

The length of the *a* axes, about 9 Å, is approximately twice the expected distance between close packed planar sheets of DML. On the other hand, no OKO reflections with odd values of K were observed. Therefore, it is possible to conclude that the sheets are piled up one on top of the other along the *a* axes in such a way that every alternate sheet is turned upside down with respect to its neighboring

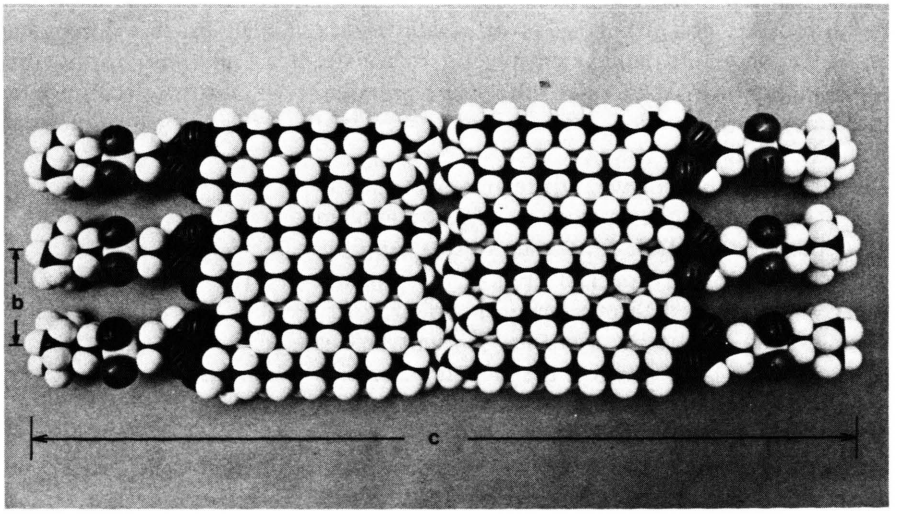

Fig. 4. Molecular model of DML (II). Only one planar bilayer sheet is shown.

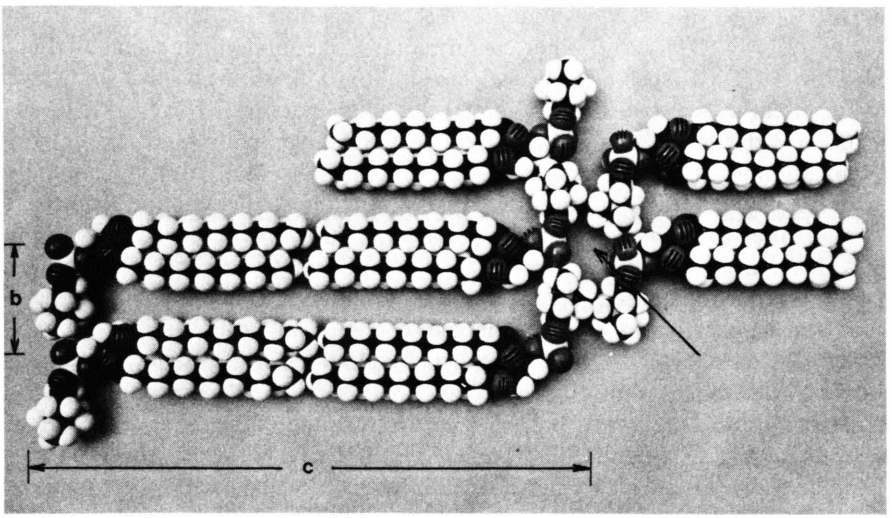

Fig. 5. Molecular model of DML types (I) and (III). Only one planar bilayer sheet is shown. Arrow points to an interbilayer space where water accomodates.

sheets, and shifted b/2 along the *b* axes. This arrangement allows further electrostatic interactions between oppositely charged-groups of adjacent sheets.

In DML (I), then, the molecules are related to each other by two-fold rotation axes along *a* and *c*, and by two-fold screw axes along *b*. The molecular arrangement of DML molecules, the symmetry elements present and the systematic absences are consistent with the space group $P22_12$. DML (III) has a similar molecular conformation. Nevertheless, while in DML (I) every third sheet is located exactly above the first ($\gamma = 90°$), in DML (III) they are slightly shifted along the *b* axis making $\gamma = 96.4°$. Because of the non-orthogonality of *a* with respect to *b*, the only remaining elements symmetry are two-fold rotation axes along *c*. Its space group is, therefore, P_2, first setting, with a pseudo C-centered monoclinic unit cell.

DML (II) has a somewhat different conformation and packing. In fact, as a result of pressure applied to a specimen of DML (I), the molecules are fully extended and closely packed (Fig. 4). The planar sheets are stabilized through hydrophobic interactions of the hydrocarbon chains. Its unit cell is monoclinically shaped, but no symmetry elements are present. The space group is, then, P_1 (triclinic). The asymmetric units consists of two DML molecules linearly aligned along their long axes, and which are almost hexagonally packed. In the other two forms of dimyristoyl lecithin there are four molecules of DML in their unit cells. They also contain about six molecules of water per molecule of phospholipid, except in form (II) in which it was not determined.

With the aid of molecular models it has been possible to show that all these molecular conformations, packing arrangements and cell dimensions are compatible with bilayer structures for dimyristoyl lecithin. In DML (I) and (III) the water molecules could easily be accomodated in the interbilayer spaces formed by the terminal hydrophilic groups (Fig. 5). All this information, together with the good agreement obtained for their observed and calculated spacings is consistent with the three described polymorphic forms of DML. This property of dimyristoyl lecithin can easily be understood on terms of the high molecular flexibility of lecithins.

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