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Diffraction of X-rays by rippled phosphatidylcholine bilayers

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X-ray diffraction patterns have been obtained from the rippled phases of two pure synthetic phosphatidylcholines (dimyristoyl and dipalmitoyl) and mixtures of these phospholipids and cholesterol arranged in oriented multibilayer stacks. These show for the first time in an oriented specimen, a two-dimensionally resolved pattern near the meridian. For example, in pure dipalmitoylphosphatidylcholine the unit cell is two-dimensional and oblique. The ripples have a wavelength of 165.3 Å and are at least 1000 Å wide in the direction perpendicular to this, in the plane of the bilayer. The shape of the ripple is more complex than simply sinusoidal.

Although much of the work on X-ray diffraction by lipid systems has been done using unordered or partially ordered samples, some lipids can be organised into multibilayer samples which have a small mosaic spread [1] and are very suitable for X-ray studies. Until recently, however, highly ordered samples of this type have been studied under conditions where the lipid is far from fully hydrated (see, for example, Refs. 2 and 3), since multibilayers prepared with a lower water content may assume an ordered state more readily [2,4]. We consider that it may be important to work with lipid that is highly hydrated, if synthetic bilayer systems are to be used as models of biological membranes, and find that some samples remain highly ordered in the presence of an excess of water. Consequently, we have studied highly hydrated multibilayer samples of this type, prepared from either a pure phosphatidylcholine or a mixture of phosphatidylcholine and cholesterol.

The pure or mixed lipids (which were obtained from Fluka and contained no impurities that could be detected by thin-layer chromatography) were deposited on flat glass slides at a loading of approx. 0.3 mg/cm^2 by allowing a chloroform solution to evaporate. Traces of solvent were removed in 10 h at 0.01 torr. Samples were then hydrated for 3 h at 75°C in an atmosphere which was saturated with water vapour, and allowed to cool to 20°C within 1 h. During the diffraction experiment, the sample was housed in a specially constructed environmental cell, which permitted the temperature to be controlled closely by circulating water through pipes. The cell contained a boat with pure water to control the humidity.

In order to record the diffraction patterns satisfactorily, it was decided to use crystal monochromatised, point-focussed X-radiation, since this gave results which were much sharper and more well-defined than were obtained using either a collimated or line-focussed beam, or nickel-filtered CuK_α radiation. The X-ray camera was of the mirror monochromator type [5].

Since all the systems studied exhibited essentially similar behaviour, only one system will be discussed in detail. Fig. 1 shows the X-ray diffrac-

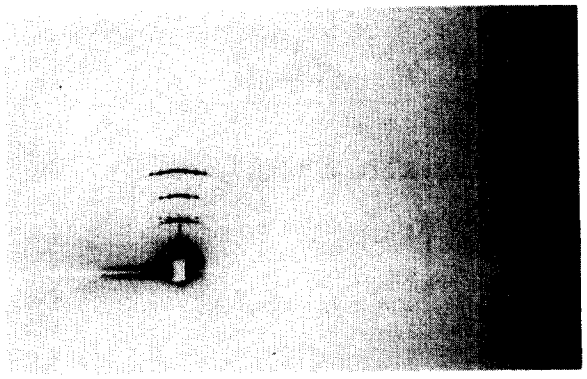

Fig. 1. Part of the X-ray diffraction pattern produced by ordered multibilayers of dipalmitoylphosphatidylcholine. The faint ring is an artefact produced by the windows of the sample holder. The sample temperature was 39.5°C.

tion pattern produced by an ordered sample of pure dipalmitoylphosphatidylcholine. This shows, for the first time from an oriented specimen, a two-dimensionally resolved pattern near the meridian. The pattern shows that the sample was predominantly in the P_{β}' phase, although a trace of a second phase (actually L_{β}') can be detected. At small angles ($d > 10$ Å), the diffraction pattern consists of a series of spots which are elongated into circular arcs by the mosaic spread of the sample, and are arranged symmetrically about the meridian. The symmetry could arise, for example, if the sample comprised a large number of plate-like domains, each of which is randomly oriented about the normal to the plane of the plates (i.e. normal to the plane of the supporting substrate). The sample thus behaves as a cylindrical powder. We can conclude from the observed sharpness of the X-ray reflections that the crystalline domains are at least 1000 Å wide.

With this assumption, the spots can be indexed (Table I) as diffraction from a two-dimensional oblique unit cell with sides of length $a = 61.0$ Å and $b = 165.3$ Å, and $\gamma = 96.17°$. The longer side is aligned parallel to the equator of Fig. 1 and the sample is disordered about the normal to this to produce the observed symmetry about the

TABLE I

COMPARISON OF THE POSITIONS OF THE OBSERVED DIFFRACTION SPOTS WITH THOSE CALCULATED ASSUMING AN OBLIQUE UNIT CELL ($a = 61.0$ Å, $b = 165.34$ Å, angle = 96.17°)

X and Y are the reciprocal space coordinates of the reflections (in units of $\sin 2\theta \times 10^4$), measured along the equator and the meridian, respectively. The column marked Int. gives the approximate relative intensities of the spots.

h	k	X_{obs}	X_{calc}	Y_{obs}	Y_{calc}	Int.
1	−1	−94	−93.1	243	243.9	475
1	0	0	0.0	254	253.9	16000
1	1	95	93.1	264	264.0	159
1	2	185	186.3	271	274.1	<10
2	−2	−188	−186.3	487	487.7	18
2	−1	−94	−93.1	498	497.8	403
2	0	0	0.0	507	507.9	495
2	1	94	93.1	517	517.9	154
2	2	188	186.3	527	528.0	21
2	3	280	279.4	539	538.1	<10
3	−2	−187	−186.3	741	741.7	71
3	−1	−92	−93.1	752	751.7	304
3	0	0	0.0	760	761.8	226
3	3	282	279.4	794	792.0	<10
3	4	373	372.6	802	802.1	<10
3	5	465	465.7	813	812.1	<10
4	−3	−278	−279.4	985	985.5	50
4	−2	−185	−186.3	997	995.6	278
4	−1	−85	−93.1	1008	1005.7	101
4	0	0	0.0	1014	1015.7	171

meridian. Unit cell dimensions of 65.6 Å and 140 Å have been observed previously in an unoriented aqueous dispersion of this lipid [6].

Table I also gives approximate estimates of the relative intensities of the spots, obtained with a scanning densitometer. When the observed reflections are plotted on a map of the reciprocal lattice (Fig. 2), it appears that the molecular transform that underlies the observed pattern is inclined at a small angle to the a^*-axis. This implies that the lipid molecules in the bilayer are tilted away from the direction normal to the b-axis. This conclusion is supported by the diffraction due to the intermolecular spacing (at 4.25 Å), which lies off the equator in Fig. 1 and shows that the lipid acyl sidechains are not perpendicular to the b-axis.

The pattern at larger angles ($d < 10$ Å) is due to diffraction by an array of lipid acyl sidechains, which are extended approximately parallel to each other. The diffraction is spread over a relatively large area, centred at approx. 4.25 Å. Given that the sidechains are tilted away from the normal to the b-axis, if we assume that the lateral packing of the chains is approximately hexagonal [6,7], we expect the hexagonal lattice in a sample of this type to give rise to twelve separate reflections, three of which should be visible in the quadrant shown in Fig. 1 [8]. It may be, then, that the 4.25 Å intensity maximum occupies a large area because it comprises three poorly resolved diffraction spots. The positions of the twelve reflections depend on the orientation of the tilt in the unit cell. For example, if the sidechains were tilted along one of the six equivalent radii of the hexagonal lattice, then a maximum of the diffraction intensity would fall on the equator, while a tilt in another direction would result in most of the intensity lying above and below the equator [8]. Referring again to Fig. 1, the diffraction at 4.25 Å appears to have no maximum of intensity on the equator. Control experiments with oriented samples of other lipids, in which the 4.25 Å reflections fall on the equator, showed that absorption of X-rays by the sample, which can be considerable near the equator of oriented samples, was not a problem in this case. Fig. 1 suggests, therefore, that the tilt direction is not along one of the radii of the hexagonal lattice.

In unoriented aqueous dispersion of lipids, a two-dimensional unit cell is usually ascribed to the formation of stacked bilayers which are distorted by a periodic ripple or pleat [7–9]. Since a simple sinusoidal ripple would give a rise to row-lines with $k = 0$ and $k = \pm 1$ only, the observation of several additional row-lines indicates that the shape of the ripple is more complex than sinusoidal. The actual form of the ripple has not been determined, however. The lack of three-dimensional (h,k,l) reflections and the observation of only two-dimensional sharp reflections indicate that the ripple is at least 1000 Å wide. A model consistent with these findings is illustrated in Fig. 3.

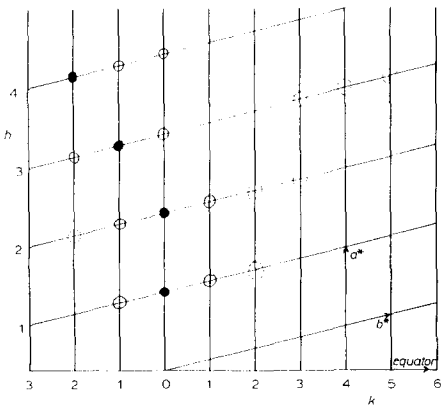

Fig. 2. The reciprocal lattice derived from the data in Fig. 1. The filled circles indicate the strongest reflection on each layer line. Solid circles indicate other strong reflections while broken circles represent weak diffractions. Most of the scattered intensity lies to the left of the a^*-axis, which indicates the direction of tilt of the molecular transform.

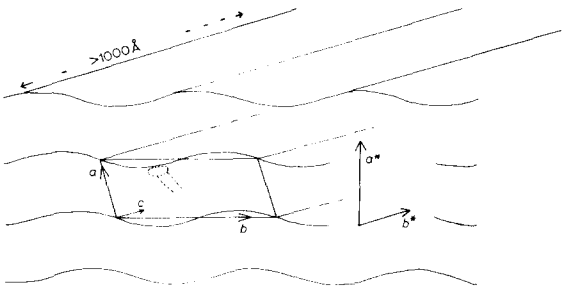

Fig. 3. A representation of the unit cell of the rippled phase of dipalmitoylphosphatidylcholine multibilayers. The insert shows the corresponding reciprocal vectors. The sinusoidal shape in the figure is for illustration only, since the precise shape and amplitude of the ripple have not been determined. A lipid molecule is shown (broken lines) to illustrate the direction of the molecular tilt.

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