Molecular Organization in Cholesterol–Lecithin Bilayers by X-ray and Electron Diffraction Measurements†

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ABSTRACT: The molecular organization in multilamellar vesicles and in free-standing bilayers of cholesterol–dimyristoylphosphatidylcholine (DMPC) was studied by X-ray and electron diffraction, respectively. Small-angle X-ray diffraction results showed that when 5% cholesterol was added to DMPC, the repeat spacings measured at temperatures less than 23 °C increased significantly, while those measured at temperatures higher than 23 °C remained unchanged. The difference between repeat spacings measured at different temperatures diminished at 20 mol % or higher portions of cholesterol. A sharp, wide-angle reflection was observed at low temperatures by both electron and X-ray diffraction of low cholesterol samples. The reflection was broadened when the temperature was raised above 23 °C. Azimuthal asymmetry in wide-angle electron diffraction patterns and radial asymmetry in the X-ray diffraction line vanished with 5% cholesterol. With increasing cholesterol contents, the sharp diffraction became gradually broadened and the peak spacings increased continuously. No coexistence of gel and fluid phases was observed. At 20 mol % or more cholesterol, abrupt thermotropic changes in the wide-angle diffraction peak width and peak spacing were no longer observed. The losses of diffraction intensity asymmetry and the increase in repeat spacing at 5% cholesterol signify the removal of the inherent acyl chain tilting in the gel-state DMPC bilayers following the addition of cholesterol. Gradual broadening and shifting of the wide-angle reflection with increasing cholesterol suggest that lecithin and cholesterol molecules are microscopically evenly distributed and that the 20% cholesterol marks the limit of cooperation between DMPC molecules. A 2-fold asymmetry in broad-band electron diffraction patterns indicates some degree of unidirectional orientation of molecules. We therefore favor a molecular arrangement model wherein lecithin and cholesterol molecules are freely miscible, but the cholesterol molecules are partically aligned, thereby forming unidirectionally ordered stripes.

Cholesterol and phospholipids are two major components of cell membranes. Their interaction has been the subject of extensive studies. Early X-ray diffraction (Engelman & Rothman, 1972; Lecuyer & Dervichian, 1969), transport (Tsong, 1975), nuclear magnetic resonance (Darke et al., 1972), and calorimetry studies (Hinz & Sturtevant, 1972; de Kruijff et al., 1974) indicated that there was an abrupt change of the physical properties of the membrane when the cholesterol mole percentage reaches 33% of the cholesterol–lecithin mixture. This finding led many investigators to believe that there existed a cholesterol–lecithin complex consisting of two lecithin molecules and one cholesterol molecule. Later studies, however, have shown that these abrupt changes of physical properties occurred at 20 mol % cholesterol rather than 33 mol %. These studies include differential scanning calorimetry (Estep et al., 1978; Mabrey et al., 1978), dilatometry (Melchior et al., 1980), fluorescence (Lentz et al., 1980), spin-label ESR (Recktenwald & McConnell, 1981; Presti & Chan, 1982), freeze–fracture electron microscopy (Copeland & McConnell, 1980; Kleemann & McConnell, 1976; Lentz et al., 1980), and ^{13}C nuclear magnetic resonance (NMR)[1] (Opella et al., 1976) studies. These findings have led many investigators to favor a 4:1 lecithin:cholesterol complex instead of a 2:1 complex. This complex is believed to coexist with pure lecithin at the composition range of less than 20% cholesterol; therefore, a region of two coexisting phases is proposed. The 20% cholesterol in lecithin marks a phase boundary in the phase diagram. Rubenstein et al. (1979) reported an abrupt change in the lateral diffusion coefficient at 20 mol % cholesterol. The change in lateral diffusion at 20 mol % cholesterol is similar to that which occurs when the lipid mixtures go through a phase transition from a solid to a liquid phase. Two explanations have been proposed for this observation. Copeland & McConnell (1980) as well as Presti et al. (1982) proposed that there existed ordered microscopic domains with ripples or strips of solid phases interspaced with more fluid domains of the cholesterol–lecithin complex, thereby forming barriers for a uniform lateral diffusion. Snyder & Freire (1980) proposed an alternative explanation. By using computer simulation, they found that at 20% cholesterol, the microdomains of the cholesterol–lecithin complex were large enough to merge into an interconnected network throughout the membrane plane, thereby abruptly altering the path of diffusion. Both of these explanations are based on the existence of microscopic domains of solid and fluid areas, differing only by the geometric interpretation. On the other hand, from monolayer studies, Muller-Landau & Cadenhead (1979) suggested that the changes at particular cholesterol levels were due to geometric fittings of lecithin molecules around cholesterol molecules. At a certain molar ratio, either one or more layers of lecithin molecules surrounded each cholesterol molecule, thereby reaching a new uniform molecular arrangement. A comparable model was also suggested by Martin & Yeagle (1978). By applying a molecular arrangement argument, they could explain physical abnormalities at certain molar ratios of cholesterol and lecithin without invoking phase boundaries. The cholesterol–lecithin membrane can therefore be regarded as a uniform, one-phase plane.

The questions of molecular organization and geometrical structures of the microdomain are best settled by diffraction and microscopic studies. Despite a great many physical studies of the lecithin–cholesterol systems, detailed diffraction studies have not been reported. Early X-ray diffraction studies by

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[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; T_c, transition temperature; NMR, nuclear magnetic resonance.

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Engelman & Rothman (1972) showed that two wide-angle diffraction lines were observed at temperatures below the transition temperatures of DPPC and at less than 33% cholesterol. The two lines, a sharp one at 4.2 Å and a diffuse one at 4.6 Å, were interpreted to represent pure lecithin in the gel state and the 1:2 cholesterol:lecithin complex in a fluid state. A small-angle diffraction study by Rand & Pangborn (1973) also favors a 2:1 lecithin:cholesterol complex. These findings are in agreement with the earlier Lecuyer & Dervichian (1969) findings. There has not been a report by diffraction studies on the recent finding of the abrupt change in structure at 20% cholesterol nor has there been any detailed diffraction study which offers a hint on the current controversial topic of molecular organization and microdomain geometry of the lecithin–cholesterol system. We report here our detailed studies of the lecithin–cholesterol mixtures by electron diffraction of hydrated single bilayers as well as by X-ray diffraction studies of multilamellar vesicles. Our results support the current observation of a change in structure at 20 mol % cholesterol. Moreover, from our diffraction spacing and line-width measurements, we provide evidence that there is no clear-cut phase separation of the pure lecithin domain and the domain of the lecithin–cholesterol complex as we commonly envisaged.

Materials and Methods

Dimyristoylphosphatidylcholine (DMPC) was purchased from Avanti Biochemical (Birmingham, AL). Cholesterol was purchased from Calbiochem (La Jolla, CA). The lipids were mixed at given molar ratios in chloroform solution. The chloroform solutions were stored at −70 °C until used.

The specimens for X-ray diffraction studies were prepared as follows: lipids in chloroform solution were vacuum dried in glass tubes and resuspended in aqueous medium by vortexing. The resulted multilamellar vesicles were concentrated by centrifugation at 6000*g*. The loose pellet was loaded in a specimen holder with mica windows. The specimen holder was mounted against a temperature-controlled plate. The fine-focus X-ray beam was generated by a Jarrel-Ash microfocus X-ray generator and focused by a Frank-type reflection camera. Both small-angle and wide-angle reflections were recorded by photographic films. The intensity of the diffraction pattern was measured with a Joyce-Loble microdensitometer.

Specimens for electron diffraction studies were made as follows: lipid mixtures in chloroform solution were spread on the air–water interphase on an Langmuir trough. The surface monolayer film was compressed to a surface pressure between 40 and 50 dyn/cm and was let standing at equilibrium for at least 10 min. A 1000 mesh electron microscope grid was dipped through the monolayer by using a hydraulic control arm. Free-standing bilayer was formed over the hole of the 1000 mesh grid by this dipping method (Hui et al., 1974; Hui, 1981). The whole operation was performed within an environmental control chamber in which the temperature and humidity could be fully regulated. The free-standing bilayer was stable only at the saturated water vapor environment. The grids carrying the bilayers were transferred via a transfer container to the environmental stage of the electron microscope. The description of the environmental stage has been given elsewhere (Hui et al., 1976). The temperature of the microscope stage was adjusted to be the same as that of the controlled chamber where the bilayer was formed. The bilayers usually remained intact after the transfer.

A fine-focus electron beam, typically 5 μm in diameter, was incident on the individual free-standing bilayer over a grid hole. Diffraction patterns were obtained by using a standard lens setting for electron diffraction (Hui et al., 1974; Hui, 1981).

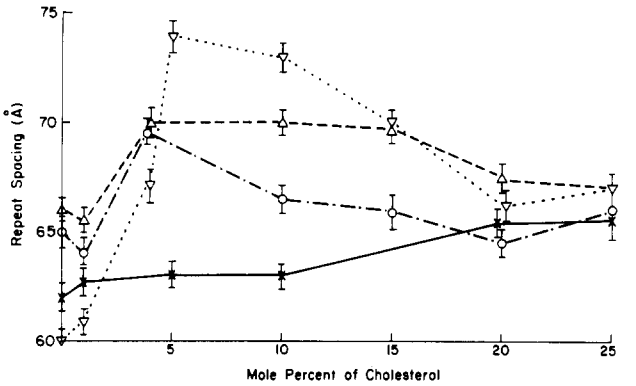

FIGURE 1: Repeat spacings measured by small-angle X-ray diffraction as functions of the mole percent of cholesterol in cholesterol–DMPC multilamellar vesicles: (▽) 5, (△) 15, (○) 23, and (×) 35 °C.

Only the center part of the free-standing bilayer was used for observation. The recording was made at an extremely low dose (less than 50 electrons/nm^2) over an exposure time of about 5 s. This condition ensured that the damage to the bilayer was within the detectable threshold (Hui, 1980) of lipid bilayers. Each recording was made with a previously unexposed bilayer. The bilayers were maintained fully hydrated at all experimental temperatures. The diffraction patterns were recorded on sensitive Kodak No-Screen X-ray film.

The diffraction intensities recorded on films were traced by microdensitometry. The peak position and the peak width were obtained by subtracting the densitometer tracing from a visually or mathematically fitted base line interpolated from the background on either side of the reflection peak. The peak widths were measured as the width of the peak at 0.707 of the peak intensity. The data were averaged from at least two but mostly three repeated experiments. The camera length was calibrated for each experiment by using an aluminum diffraction standard.

Results

Small-Angle X-ray Diffraction. Multilamellar vesicles with various molar ratios of cholesterol to lecithin were used for both wide-angle and small-angle X-ray diffraction experiments. Diffraction data were taken at 35 (greater than the T_c of DMPC), 23 (at the main transition of DMPC), and 15 and 5 °C (below the transitions of DMPC). Usually 3 orders of reflection were recorded. Figure 1 presents the small-angle repeat spacing as a function of the mole percent of cholesterol in the DMPC–cholesterol mixtures. Pure DMPC has a repeat spacing of 66 Å at 20 °C. At 35 °C, the repeat spacing was reduced to 61 Å. Both values agree in general with previously reported results (Janiak et al., 1976). When up to 5% cholesterol was added to DMPC, the repeat spacing measured at 5 °C increased from 60 to 74 Å, whereas at temperatures above the T_c of DMPC, e.g., 35 °C, the repeat spacings remained approximately the same as that of pure DMPC. As up to 20% cholesterol was added, the repeat spacings at high temperatures (greater than the T_c of DMPC) increased slightly while those at low temperatures (below the T_c of DMPC) decreased with the addition of cholesterol. Repeat spacings measured at high and low temperatures approached each other at about 20% cholesterol and became indistinguishable at higher cholesterol percentages. This trend is presented in Figure 1. An increase in repeat spacing at a small percentage of cholesterol was also depicted by Lecuyer & Dervichian (1969) and Janiak et al. (1974).

Wide-Angle X-ray Diffraction. Wide-angle X-ray diffractions of pure DMPC at 4 °C showed a radially asymmetric

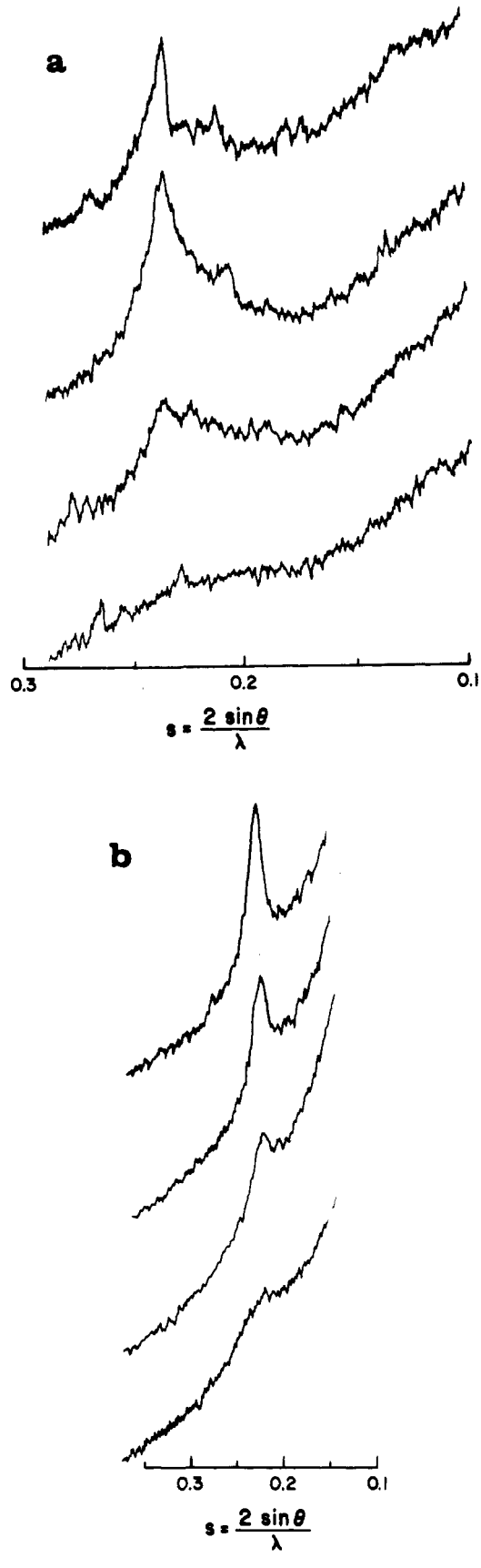

FIGURE 2: Densitometer tracings of (a) wide-angle X-ray diffraction of cholesterol–DMPC vesicles at 4 °C at the following cholesterol mole percentages (from top to bottom): 1%, 5%, 10%, and 25%. (b) Electron diffraction of free-standing bilayers of 7.5% cholesterol in DMPC recorded at the following temperatures (from top to bottom): 10, 20, 27, and 45 °C.

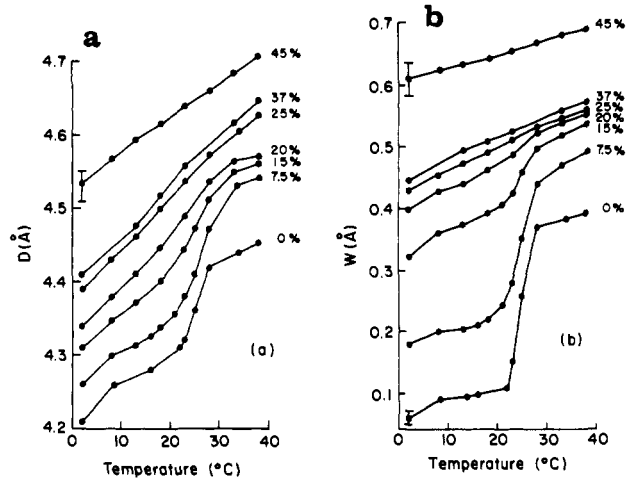

FIGURE 3: (a) Peak position (D) measured from electron diffraction patterns of cholesterol–DMPC bilayers at the indicated mole percent of cholesterol as a function of temperature. (b) Corresponding measurements of half peak width (W). Bars indicate typical errors in measurement.

reflection centered at 4.2 Å. This reflection became less asymmetric at temperatures approaching the transition temperature of 23.5 °C. Above this temperature, the sharp reflections then transformed into a broad diffraction band centered at 4.6 Å. This result is in agreement with previous findings (Tardieu et al., 1973; Janiak et al., 1976). With 4% or more cholesterol, the asymmetry in the low-temperature diffraction intensity was removed. A symmetric line centered at 4.2 Å was seen in all low-temperature data from 4% to 20% cholesterol, but the line width was continuously broadened with increasing cholesterol content (Figure 2a). At high temperature (35 °C), the broad diffraction band at 4.6 Å remained, and the spacing gradually increased with increasing cholesterol mole percentage. There was no abrupt change of line width with temperature in samples containing 20% or more cholesterol.

Wide-Angle Electron Diffraction. Single molecular bilayers with various cholesterol to DMPC molar ratios were used in the electron diffraction experiment. In these experiments, the electron beam was incident normal to the plane of the bilayer. Diffraction due to lamellar repeat was therefore undetectable, even if the specimens contain multilamellar structures. Only wide-angle diffractions were recorded. Some typical diffraction intensities are shown in Figure 2b. The bilayers were kept fully hydrated at all experimental temperatures, and data were taken at much closer temperature intervals than in X-ray diffraction experiments.

Both the peak positions (D) and the peak widths (W) were measured as functions of temperature (T) and percentage of cholesterol. Figure 3a presents the variation of peak position (spacings). The corresponding measurements of peak width are presented in Figure 3b. These experimental data were obtained from at least three repeating experiments, and both the peak spacing and the line-width measurements showed an obvious phase transition at a cholesterol percentage less than 20%. The midpoints of all phase transitions occurred at 23.5 °C, which was the midpoint also of the phase transition of pure DMPC. The width of the transition increased as more cholesterol was added to DMPC. The phase transition was still observable as a change of dW/dT and dD/dT until the percentage of cholesterol reached approximately 20%. At 20% or higher cholesterol, the change of both D and W as functions of T became gradual. This is best illustrated by plotting dW/dT or dD/dT as functions of the cholesterol percentage

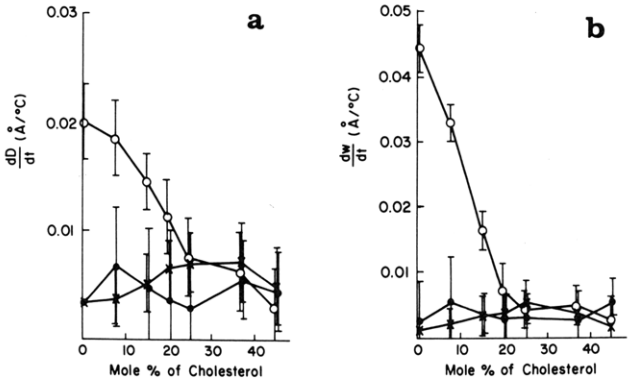

FIGURE 4: (a) Changes of peak position (D) with temperature (dD/dT) at 13 (×), 23 (○), and 33 (●) °C as functions of the cholesterol mole percentage. (b) Corresponding changes of half peak width (W) with temperature (dW/dT). Symbols same as in (a).

at a given temperature (T_i) as shown in panels b and a, respectively, of Figure 4. At temperatures far above or below the phase transition temperature, the change of dD/dT or dW/dT was approximately constant with cholesterol content. However, the slopes dD/dT and dW/dT near the midpoint of the phase transition (23 °C) varied drastically with cholesterol content. At approximately 20% cholesterol, the slopes dD/dT and dW/dT were reduced to the common value shared by off-transition measurements, indicating the disappearance of the phase transition at this particular cholesterol percentage.

The spacings measured by electron diffraction differ from those measured by X-ray diffraction at the low-temperature end and at low cholesterol content. The gel phase diffraction spacing measured by electron diffraction is consistently higher than those measured by X-ray diffraction by approximately 2%. At high cholesterol content, and/or at high temperature, the deviation is consistently lower by approximately 1%. The difference is systematic and reproducible, indicating there is a significant difference in the molecular organization as detected by these two methods. This difference will be discussed in the next section.

Azimuthal Asymmetry of Wide-Angle Electron Diffraction Patterns. The small observed area of electron diffraction enabled us to detect the local orientation of domains in the gel state. In electron diffraction patterns of pure DMPC and in some cases of DMPC–cholesterol mixtures containing 1% cholesterol, a unidirectional splitting of the wide-angle electron diffraction spacing was often observed (Figure 5a). This splitting invariably occurred in one direction only, indicating that the hexagonal lattice of acyl chains within a domain has been unidirectionally distorted. The samples which produced this pattern could be multilayered. This distortion has also been observed previously (Hui, 1976) and was interpreted as due to the tilting of the acyl chains with respect to the normal of the bilayer, resulting in an orthorhombic lattice rather than a hexagonal lattice on the plane of the membrane. This splitting vanished at higher cholesterol content.

At progressively higher cholesterol contents, a different type of asymmetry was observed. The increasingly broad diffraction ring tended to become two arcs rather than being circular as in all powder diffraction patterns. The axes of the pair of arcs were oriented randomly and were unrelated to the direction of dipping when the bilayers were formed from the monolayers (Figure 5b,c). However, the orientation of the arcs seemed to extend over a large part of the samples, ranging from 10 μm to hundreds of micrometers. The 2-fold symmetric diffraction patterns were observed only at cholesterol contents higher than 1% and were more often observed at cholesterol

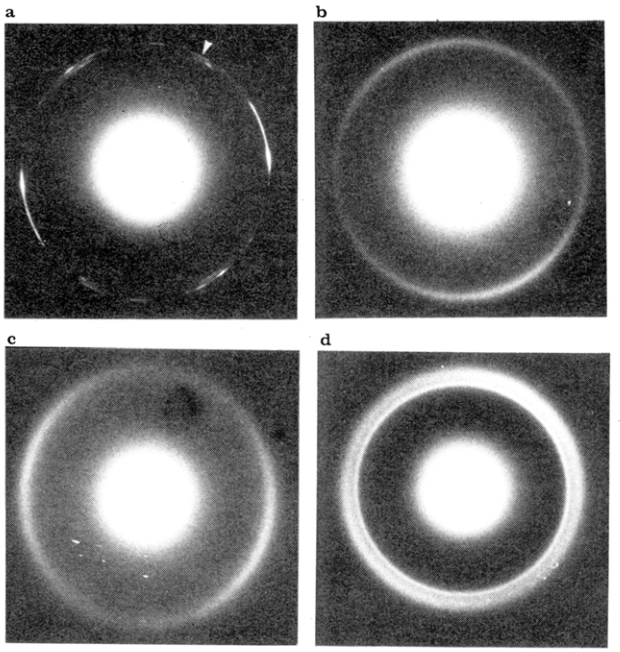

FIGURE 5: (a) Electron diffraction pattern of pure DMPC at 1 °C showing the unidirectional distortion (arrow) of the (100) reflection of a hexagonal lattice "powder" ring. The distortion diminishes with increasing temperature. (b and c) Electron diffraction patterns of 20% cholesterol (b) and 37% cholesterol (c) in DMPC at 4 °C showing a 2-fold symmetric pattern. (d) Electron diffraction pattern of 45% cholesterol in DMPC at 40 °C showing two sharper rings at 4.9 and 2.7 Å in addition to the diffuse ring at 4.7 Å.

contents higher than 20%. The 2-fold symmetric pattern indicates that the molecular arrangement of these bilayers has a preferred direction rather than a homogeneous two-dimensional structure.

At cholesterol contents greater than 20% and at temperatures higher than 23 °C, frequently but not consistently a smaller sharp ring was observed. This sharp ring, as shown in Figure 5d, has a spacing of 4.9 Å. A weaker ring with a spacing of 2.7 Å was sometimes observed. These rings were circularly symmetrical. Since these rings associated only with the broad, diffused diffraction band at 4.6 Å and at high cholesterol content, it could not be explained by the distortion of the hexagonal packing of acyl chains in the gel state of DMPC. These spacings coincide with the strong lines previously observed in electron diffraction of cholesterol crystals (Hui & Strozewski, 1979, and unpublished results). A reflection at 4.9 Å was also observed by X-ray diffraction of cholesterol monohydrate (Bognen & Larsson, 1963; Katz et al., 1976). It is likely, but by no means certain, that these additional rings arise from cholesterol–cholesterol spacing in high cholesterol specimens.

Discussion

Although the phase diagram of the cholesterol–lecithin mixture has been studied extensively by a number of physical techniques, comparable studies by diffraction have not been reported. Experiments in differential scanning calorimetry, in the partitions of the 2,2,6,6-tetramethylpiperidinyl-1-oxy (Tempo) electron spin probe, in dilatometry, and in fluorescence measurements all indicate a break in the 20% ratio of cholesterol. Dynamical studies by fluorescence photobleaching also showed an abrupt change at the 20% cholesterol level (references quoted in the introduction). Both our small-angle and wide-angle diffraction data show abrupt changes at about 20% cholesterol. At this percentage, the difference in long spacing due to the phase transition vanishes. Wide-angle

diffraction data also show the diminishing of the phase transition at 20% cholesterol content. From diffraction data alone, it seems that the changes form a so-called gel state to a so-called fluid state are no longer distinctive when the cholesterol content reaches 20%. This result is in agreement with recent measurements by other physical techniques. No obvious changes were detected at 33% cholesterol content as suggested by early calorimetry and diffraction measurements.

There is a small (1–2%) but consistent discrepancy between our wide-angle X-ray and electron diffraction spacings. The electron diffraction spacings are wider than those of X-ray diffraction at low temperature, but vice versa at high temperature. The only exception is pure DMPC at the gel state when results from both methods agree. Equivalently speaking, the phase transition observed by electron diffraction is broader than that by X-ray diffraction. The deviation is independent of the surface pressure of the precursor monolayer from which the free-standing bilayer is formed. Most free-standing bilayers have only one lamella, or at the most two to three lamellae. Unilamellar vesicles are known to have broader phase transitions (Van Dael et al., 1982). It is likely that the difference in electron and X-ray diffraction data represents the difference in the temperature ranges of the phase transition in the unilamellar and multilamellar systems. It is also possible that the boundary condition imposed by the closely spaced grid bars, and the thin layer of water overlaying the free-standing bilayer in electron diffraction samples, inhibits the change of the molecular packing of compressible bilayers during the phase transition, thereby imposing a temperature hysteresis. Nevertheless, the trend of cholesterol-induced effects holds for both methods of measurement.

Our diffraction results also differ from those previous reports (Engelman & Rothman, 1972) which showed the coexistence of both a sharp reflection at 4.2 Å and a broad reflection at 4.6 Å at a cholesterol content less than 33 mol %. Instead, we show by both X-ray and electron diffraction that only one wide-angle reflection is observed. The sharp reflection observed in pure DMPC [or dipalmitoylphosphatidylcholine (DPPC)] is gradually broadened; at the same time, its spacing is gradually widened with the addition of cholesterol. This gradual change, rather than a coexistence of two reflections, indicates that there is a continuous change of structure as the cholesterol content increases, rather than a coexistence of two phase-separated regions in the speciemen (Tsong, 1975). Our data were taken as soon as the samples were prepared, allowing no time for phase segregation to occur. The total phase segregation process may require a long time in many lipid systems. It could be that the freshly made samples were rather homogeneous, and the large, phase-separated domain had not had time to form. Since only 5 μm diameter area was observed at a time in our electron diffraction experiment, larger crystalline structures would give a single-crystal diffraction pattern similar to that observed previously in pure DPPC bilayers (Hui et al., 1974). In fact, gel-state DMPC bilayers in this experiment were often observed as single mosaic crystalline domains. As the cholesterol content increased, the single-crystalline diffraction was no longer observable. This observation indicates that as cholesterol is added to the specimen, larger crystalline domains no longer exist. Instead, the coherent length is reduced to much smaller than 5 μm. Large-scale phase separations of gel-phase domains of pure DMPC and of more fluid cholesterol-enriched areas were not observed. Even if complete phase segregation does eventually occur, the kinetics would be much slower than the formation of single mosaic crystalline domains of pure DMPC. Macroscopic phase segregation may be achieved more rapidly at higher temperatures due to a higher lateral diffusion rate and is probably responsible for the observation of pure cholesterol diffraction in high cholesterol, high temperature samples (Figure 5d). It may also lead to the electron diffraction contrast observation of domain stripes in high cholesterol samples (Hui & Parsons, 1975).

The continuous broadening of diffraction lines and the gradual increase of the diffraction spacing with increasing cholesterol imply (1) that the molecular arrangement becomes increasingly disordered and at the same time the separation between neighboring acyl chains is gradually widened and (2) that the domain of the gel-phase lipid becomes increasingly smaller, resulting in the broadening of the diffraction lines due to diminishing "crystal size". Since most of our wide-angle diffraction lines consist only of one order, it is very difficult to separate the disorder effect from the crystal size effect (Klug & Alexander, 1974). From the breadth of the diffraction line, it is more appropriate to regard the cholesterol–DMPC bilayer as a two-dimensional "very defective lattice" (Klug & Alexander, 1974). This theory assumes uniformly distributed defects, and the line broadening is determined by the defect concentration. The gradual change of line width strongly argues against phase separation into extensive cholesterol-rich or cholesterol-depleted domains as suggested by early X-ray diffraction results. Our data are more compatible with the domain model at the molecular scale (Presti et al., 1982) or the molecular mixing model (Martin & Yeagle, 1978; Cadenhead & Muller-Landau, 1979), wherein the coherent length among lecithin molecules is gradually reduced by the addition of cholesterol. According to the models, at approximately 20% cholesterol, the lecithin acyl chain lattice is reduced to one layer thick, and two-dimensional coherency, however small, is lost. Together with the loss of two-dimensional coherency is the disappearance of cooperative properties such as the phase transition. Moreover, our electron diffraction data show that whenever cholesterol is present there is a preferred orientation even after the disappearance of cooperative properties (Figure 5b,c). Such an observation can be explained by a model which depicts a preferential alignment of cholesterol molecules (Presti et al., 1982; Cadenhead & Muller-Landau, 1979; Rogers et al., 1979) so that even if the acyl chains of lecithin molecules are reduced to a single file along rows of cholesterol molecules, a one-dimensional repeat order is still retained. The idea of cholesterol alignment is justified by the unique interaction between cholesterol and phospholipids (Huang, 1976). If there are excess lecithin molecules, they are likely to accumulate along the stripes. These stripes may extend hundreds of micrometers as indicated by electron diffraction patterns. These stripes may be equivalent to the unidirectional structures seen in freeze–fracture electron microscopy (S. W. Hui and N.-B. He, unpublished results; Copeland & McConnell, 1980) and the unidirectional movements detected by fluorescence photobleaching (Rubenstein et al., 1979). This model of longitudinal order may or may not imply the formation of the cholesterol–lecithin complex but requires approximate cholesterol–cholesterol alignment in cholesterol-rich stripes. These stripes evenly interrupt the cooperativity of the lecithin at the microscopic scale, and no macroscopic phase separation domains exist in freshly formed bilayers. For this reason, we did not observe diffraction patterns due to the macroscopic phase separation.

There is a significant change of small-angle spacing in the gel state when only 4% cholesterol is added to DMPC. Under the same comdition, the seemingly orthorhombic diffraction

arcs in the wide-angle electron diffraction patterns and the radial asymmetry in the wide-angle X-ray diffraction pattern vanish. These results indicate that there is a change of molecular packing when a small amount of cholesterol is added to the pure DMPC bilayer. Both the loss of asymmetry and the loss of the orthorhombic pattern are interpreted as a reduction of distortion of the hexagonal packing due to acyl chain tilting (Janiak et al., 1976; Hui, 1976; Ranck et al., 1974). Additional cholesterol alters the tilting of the chains such that the orientation of the acyl chains is almost normal to the plane of the bilayer. This change of chain tilting results in an increase in the bilayer thickness which manifests as an increase in the repeat spacing in the small-angle diffraction. Chain tilting requires the formation of large domains in which all chains tilt in the same direction. The disorder introduced by the addition of cholesterol (which prefers to form stripe domains) renders this long-range interaction unfavorable. The condition where chain tilting occurs coincides with that of the one-phase zone at low cholesterol content (Janiak et al., 1974; Darke et al., 1972; Lentz et al., 1980). This interpretation is compatible with the model presented by Presti et al. (1982).

In conclusion, our results indicate that up to a 1:1 molar ratio, the lecithin–cholesterol mixture is a macroscopically miscible system. However, due to some degree of alignment between cholesterol molecules, the structure in the bilayer phase has an imperfect one-dimensional order. The cholesterol "stripes" disrupt the order of the lecithin packing and result in a gradual reduction in cooperativity at the microscopic scale. The information we obtained about the properties of the cholesterol–lecithin mixture would help us to understand the functions of cholesterol in biological membranes.

Registry No. DMPC, 13699-48-4; cholesterol, 57-88-5.

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