

Effect of Cholesterol on the Properties of Phospholipid Membranes. 3. Local Lateral Structure

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The local lateral structure of dimyristoylphosphatidylcholine (DMPC)–cholesterol mixed membranes of different compositions has been investigated on the basis of computer simulation results. For this purpose, the centers of mass of the molecules of each simulated membrane layer have been projected to the plane of the membrane, and the 2D Voronoi tessellation of the resulting projections has been determined. Various characteristics of the Voronoi polygons (VP) have been determined and compared for cholesterol, their nearest DMPC neighbors, and DMPC molecules having no near cholesterol neighbors. It has been found that there is a strong, specific interaction between cholesterol molecules and their nearest DMPC neighbors, whereas when lacking a sufficient number of cholesterol a different kind of specific interaction occurs between some pairs of neighboring DMPC molecules. These interactions often involve direct cholesterol–DMPC hydrogen bonding and charge pairing between oppositely charged segments of the headgroups of two neighboring DMPCs, respectively. In addition, the DMPC–cholesterol nearest-neighbor interaction involves the ordering effect of the rigid cholesterol ring system on the nearby lipid tails, which helps to keep the hydrocarbon tails and thus the center of mass of the DMPC molecule close to the cholesterol. The analysis of the VP area distributions has revealed that the lateral condensation of the membrane upon adding cholesterol to it can be explained solely by the formation of strongly interacting, often hydrogen-bonded DMPC–cholesterol pairs.

Introduction

The microscopic structure of various phospholipid membranes has been intensively studied in the past decade by computer simulation methods.¹ The importance of such studies stems from the fact that they helped us to understand the molecular-level origin of many properties of such membranes. Thus, among others, the role of the presence of unsaturated bonds,^{2–6} branches,⁷ or F atoms⁸ in the apolar part of the membrane, the effect of the solvent⁹ and headgroup type on the membrane properties,^{10–12} the existence of water wires across the apolar part of the membrane,¹³ and free-energy profiles of small molecules across such membranes^{14–17} have been investigated in detail. Such studies are essential to the understanding of interactions between living cells and their environment because phospholipid molecules are the main constituents of the membranes separating living cells from the outside environment.

Besides the phospholipid molecules, cholesterol is another important component of the plasma membranes of eukaryotic cells. Its concentration can be as high as 50 mol % in some

cases.¹⁸ Cholesterol and phospholipids are not perfectly miscible with each other.^{18,19} For instance, at 37 °C and atmospheric pressure the region of immiscibility of dimyristoylphosphatidylcholine (DMPC) and cholesterol covers approximately the cholesterol mole fraction range of 0.1–0.3.¹⁸ Because the average concentration of cholesterol in the membranes of living cells falls in this range, the separation of domains of high and low cholesterol concentration occurs in the cell membrane. Cholesterol influences the properties of phospholipid membranes in many ways.¹⁹ Thus, among others, adding cholesterol to a liquid-crystal phase phospholipid bilayer leads to lateral condensation,^{20,21} an increase in mechanical strength^{22,23} and bending elasticity,²⁴ and a reduction of the passive permeability^{25–27} of the membrane.

Phospholipid/cholesterol mixed membranes have also been studied by computer simulation.^{17,28–35} These studies provided a great help in understanding the molecular-level origin of the above effects. Recently, Pasenkiewicz-Gierula et al. have analyzed the possible interactions (e.g., direct hydrogen bonding, hydrogen bonding through a bridging water molecule, charge pairs) acting between the headgroup of cholesterol and DMPC molecules in the membrane.³³ In a previous study,³⁵ we have shown that the interaction between cholesterol and their nearest

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DMPC neighbors is responsible for many of the cholesterol-induced changes of the membrane structure. Several properties (e.g., conformation of the hydrocarbon chains, orientation of the C–C bonds, deuterium order parameter) have been found to be markedly different for DMPC molecules located next to a cholesterol compared to those obtained for DMPCs surrounded solely by other DMPC molecules.³⁵ These findings indicate that the presence of a near-lateral (possibly hydrogen-bonded) cholesterol neighbor should considerably modify the local environment of a DMPC molecule, and these changes in the local environment might be responsible for some of the changes of the phospholipid membrane properties occurring when adding cholesterol to the system. Therefore, a detailed study of the local lateral environment of the molecules in DMPC/cholesterol mixed membranes seems to be an important step toward a deep understanding of the structure-forming role played by cholesterol in the membranes of living cells.

For a set of points in three dimensions, the Voronoi polyhedra³⁶ are the convex regions around each point where every point is closer to its central particle than to any other one (i.e., the equivalent of the Wigner–Seitz cells of a crystal).^{36,37} Voronoi polyhedra were found to be a very efficient tool for the characterization of the local environment of particles in a disordered system.³⁸ The Voronoi polygon (VP) is the 2D analogue of the Voronoi polyhedron. Thus, the VPs generated by a set of points (particles) in a plane provide a tessellation of the plane: the VPs fill the plane completely, and the plane is divided unequivocally into cells allocated to certain particles. The area of a VP is related to the free area available to its central particle. Conversely, the reciprocal of this area is a measure of the local surface density around the central particle. A VP edge is the assembly of those points that are equally far from two particles (the VPs of which share this edge) and are farther from any other particle than from these two. This property of the VP edges allows a purely geometrical definition of the neighbors: two particles are neighbors if their VPs share a common edge. Thus, the number of edges of a VP gives the number of neighbors surrounding the central particle, whereas the length of an edge is related to the distance of the corresponding neighbor (i.e., longer VP edges indicate closer neighbors). Hence, the shape of the VP characterizes the local arrangement of the particles around each other. The analysis of the properties of Voronoi polyhedra or polygons has frequently been used in the investigation of various problems in the field of molecular biophysics^{39–45} as well as in various other areas of science such as condensed-matter physics,^{46–53} astrophysics,^{54,55} or physiology.^{56–57}

In this paper, we present a detailed analysis of the local lateral environment of the DMPC and cholesterol molecules in their mixed, fully hydrated membranes. For this purpose, we project the center of every molecule onto the plane of the membrane, treating the two membrane layers independently from each other, and construct the 2D Voronoi tessellation of these projections. This approach is similar to what has been used by Shinoda and Okazaki in analyzing lipid area fluctuations in a dipalmitoyl-phosphatidylcholine (DPPC) bilayer.⁴⁰ In the analysis, we distinguish the DMPC molecules located next to a cholesterol from those having no near cholesterol neighbors and compare the properties of their VPs with those of cholesterol. For comparison, the entire analysis is also performed on a pure DMPC membrane as a reference system.

Calculation Details

Computer Simulations. The Voronoi analyses have been performed on two simulated DMPC–cholesterol mixed bilayers

of different compositions and on a pure DMPC bilayer as a reference system. The compositions of the two mixed bilayer systems have been chosen from the two sides of the immiscibility region of DMPC and cholesterol: the mole fraction of cholesterol has been set equal to 0.08 and 0.40 in the two systems. The structures of the bilayers have been compared and analyzed in detail in our previous paper.³⁵

The simulations that we performed have been described in detail in our previous paper,³⁵ so just a brief summary of the calculations is given here. The bilayers have been simulated by the Monte Carlo method using the program MMC⁵⁸ on the isothermal–isobaric (N , p , T) ensemble under physiological conditions (i.e., 37 °C and 1 atm) in hexagonal prism-shaped basic simulation cells. Both of the membrane layers contained 25 molecules, among which 2 and 10 have been cholesterol in the two mixed systems, respectively. The bilayers have been hydrated by 2033 water molecules, described by the TIP3P potential.⁵⁹ The DMPC and cholesterol molecules have been modeled by the CHARMM22 force field.⁶⁰

In the Monte Carlo procedure, water molecules located closer to the membrane have been selected to move with higher probabilities. Solute molecules (i.e., DMPC or cholesterol) have been chosen for displacement in shuffled cyclic order,⁶¹ whereas the torsional angles to be changed have been selected in sequential order going from the end of the chain toward the middle of the molecule but have been subject to a probability filter allowing more frequent changes of the torsions located farther from the end of the chains.⁶² Overall solute rotations as well as torsional changes have been performed using the novel extension biased scheme (i.e., the maximum angle of rotation has been set to be inversely proportional to the square root of the distance of the farthest rotated atom from the axis of rotation⁶²). Volume-change steps have alternated between changing the cross section of the system and the length of the membrane's normal axis⁶³ to equilibrate the total density of the system and the surface density of the membrane independently from each other. The analysis of each system has been based on 1000 sample configurations separated by 10⁵ Monte Carlo steps each.

Voronoi Analysis. To construct the 2D Voronoi diagrams of the simulated membrane configurations, each DMPC and cholesterol molecule has to be represented by a single point in the plane of the membrane. For this purpose, the center of mass of each molecule has been projected onto the plane of the basic hexagon of the simulation cell. The two layers of the membrane bilayer have been treated as independent samples, hence for each system 2000 sample configurations, containing the 25 projections of the DMPC and cholesterol centers of mass for each, have been analyzed.

The Voronoi tessellation of a sample has been determined using a 2D analogue of the procedure described by Ruocco, Sampoli, and Vallauri in 3 dimensions.⁴⁷ Thus, the Voronoi cell of each particle has been determined independently, without using the information obtained in the determination of the Voronoi cells of the other particles of the system. The procedure of determining the Voronoi cell of a given particle starts from an initial tentative cell, which is large enough to fully contain the real (and yet unknown) VP. In the present analysis, the basic hexagon of the simulation box, centered on the considered particle, has been chosen as the starting tentative polygon. Then the rest of the particles have been sorted according to their distance from the central one, and the following procedure has been repeated for each particle, starting with the closest one.

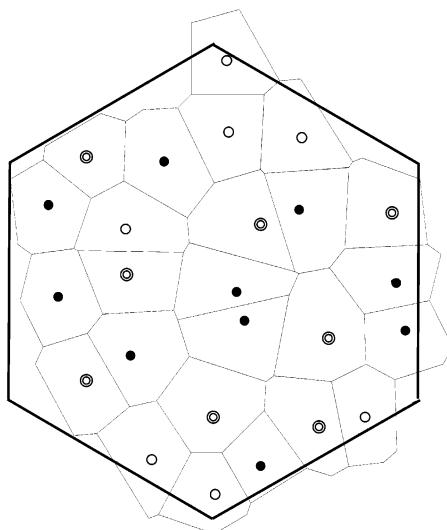


Figure 1. Voronoi tessellation of a snapshot of the projected DMPC and cholesterol centers of mass of a membrane layer, as picked up from the simulation of the system containing 40% cholesterol. Centers of cholesterol, DMPCs having a near cholesterol neighbor, and DMPCs having no near cholesterol neighbors are marked by solid circles, double open circles, and open circles, respectively.

The orthogonal bisector of the segment joining the central particle and its next neighbor considered is determined. If this bisector cuts the tentative Voronoi cell into two polygons, then the new tentative polygon is the part of the original tentative cell that is cut down by the bisector and contains the central particle. If the tentative polygon is not intersected by the bisector, then it is kept unchanged in this step. The procedure stops when the next particle to be checked is at least twice as far from the central particle as the farthest vertex of the tentative polygon. This procedure provides an exact determination of the Voronoi tessellation of the system, given that the particles are in a general position (i.e., three of them are never lying along the same line, and four of them are never located along the same circle). However, in a disordered system, such as the membranes studied here, these special arrangements of the molecules have vanishingly small probabilities. To test the consistency of the determination of the Voronoi tessellation, we have compared the sum of the area of the VPs in each sample configuration with the area of the basic hexagon of the corresponding simulation cell. These two values have always agreed within the numerical accuracy of the calculation. The Voronoi tessellation in a snapshot of the projected DMPC and cholesterol centers of mass is shown in Figure 1 for the cholesterol-rich system.

To determine the area of a VP, its vertices (or edges) have to be sorted according to their geometric sequence along the circumference of the polygon. Thus, for each edge the list of the two particles sharing this edge and for each vertex the list of the three particles sharing this vertex have also been determined. (It should be noted that, because of the general position of the molecules, a VP vertex has never been shared by more than three particles.) Two edges follow each other in the sequence (i.e., their intersection represents a real vertex) if the union of their lists of sharing particles is equivalent to the list corresponding to a vertex. Conversely, two vertices follow each other in the sequence (i.e., represent the two endpoints of an edge) if their lists contain a common particle besides the central one. Once the sequence of N_v vertices $\{r_i|i = 1, \dots, N_v\}$, is determined, the area of the VP, A , can be calculated from

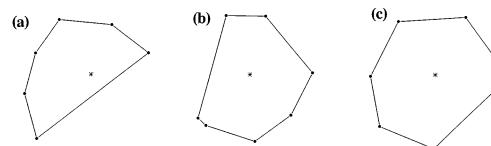


Figure 2. Typical Voronoi polygon corresponding (a) to a cholesterol molecule, (b) to a DMPC molecule having a near cholesterol neighbor, and (c) to a DMPC molecule without near cholesterol neighbors, as picked up from an instantaneous configuration. The projected centers of mass of the central particles are marked by asterisks.

the areas of the triangles formed by $\{r_1, r_i, r_{i+1}|i = 2, \dots, N_v - 1\}$:

$$A = \frac{1}{2} \sum_{i=2}^{N_v-1} |(r_i - r_1) \times (r_{i+1} - r_1)| \quad (1)$$

The circumference length of the polygon, L , is simply the sum of the edge lengths $\{l_i|i = 1, \dots, N_v\}$:

$$L = \sum_{i=1}^{N_v} l_i \quad (2)$$

The shape of the VP is characterized here by the acircularity parameter, ϕ , defined in the analogy of the asphericity parameter η in 3 dimensions,⁴⁷ as

$$\phi = \frac{1}{4\pi} \frac{L^2}{A} \quad (3)$$

As seen, the value of ϕ is exactly 1 for a circle, and it becomes larger for less-circular objects. For instance, the ϕ values of a perfect hexagon, square, and equilateral triangle are 1.103, 1.273, and 1.654, respectively.

DMPCs Having and Not Having a Near Cholesterol Neighbor. In a previous study, we have found that the local structure of the DMPC molecules located in the vicinity of a cholesterol is different from that of DMPCs that are far from cholesterol.³⁵ This finding outlines the importance of investigating the influence of a nearby cholesterol on the local lateral environment of the DMPC molecules, leading us to distinguish between DMPC molecules having and not having a near cholesterol neighbor. The mixed membranes are thus regarded as containing three different membrane constituents (i.e., cholesterol, their nearest DMPC neighbors, and DMPCs having no near cholesterol neighbors). The VP properties of the three membrane constituents are determined separately and are compared with each other in the mixed systems.

To determine which DMPC molecules can be considered to be near to a cholesterol, we have calculated the 2D pair correlation function of the projections of the DMPC and cholesterol centers of mass in the plane of the membrane. The integration of the resulting pair correlation function has revealed that the lateral DMPC–cholesterol center-of-mass coordination number is 1 at 6.05 Å. Thus, DMPC molecules whose projected center of mass is within 6.05 Å of a projected cholesterol center of mass are regarded as DMPCs having a near cholesterol neighbor, whereas the other DMPC molecules are regarded as DMPCs having no near cholesterol neighbors in the following analyses. Typical VPs corresponding to a cholesterol molecule, a DMPC molecule having a near cholesterol neighbor, and a DMPC molecule without near cholesterol neighbors are shown in Figure 2, as shown by an instantaneous simulated configuration.

Results and Discussion

The mean values and standard deviations of the VP properties investigated here (i.e., polygon area A , circumference length L ,

TABLE 1: Mean Values and Standard Deviations of the Voronoi Polygon Properties of the Different Membrane Constituents as Obtained from the Simulation of the Three Different Systems

	15 DMPC-10 chol.	23 DMPC-2 chol.	25 DMPC
$\langle A \rangle / \text{\AA}^2$			
all molecules	53.2 ± 8.6	56.7 ± 8.8	58.3 ± 8.7
cholesterols	50.9 ± 8.5	52.3 ± 11.9	
DMPCs next to a chol.	50.9 ± 7.5	52.6 ± 11.1	
DMPCs far from chols.	59.1 ± 6.8	57.4 ± 7.9	58.3 ± 8.7
$\langle L \rangle / \text{\AA}$			
all molecules	28.8 ± 1.9	29.7 ± 1.7	30.0 ± 1.5
cholesterols	28.3 ± 1.9	28.6 ± 2.2	
DMPCs next to a chol.	28.5 ± 2.0	28.8 ± 2.4	
DMPCs far from chols.	29.9 ± 1.3	29.9 ± 1.4	30.0 ± 1.5
$\langle \phi \rangle$			
all molecules	1.256 ± 0.086	1.250 ± 0.088	1.245 ± 0.098
cholesterols	1.269 ± 0.089	1.278 ± 0.139	
DMPCs next to a chol.	1.281 ± 0.088	1.279 ± 0.057	
DMPCs far from chols.	1.207 ± 0.055	1.245 ± 0.082	1.245 ± 0.098
$\langle N_v \rangle$			
all molecules	6.00 ± 0.99	6.00 ± 0.88	6.00 ± 0.83
cholesterols	5.90 ± 0.95	5.87 ± 1.19	
DMPCs next to a chol.	5.79 ± 0.83	5.77 ± 0.81	
DMPCs far from chols.	6.38 ± 1.10	6.03 ± 0.85	6.00 ± 0.83

acircularity parameter ϕ , and number of vertices N_v) are summarized in Table 1 for the pure DMPC and both mixed membranes. The values obtained solely for the VPs of the cholesterol molecules, for the DMPCs located next to a cholesterol, and for DMPCs having no near cholesterol neighbors are also given in the Table. The probability density functions of these quantities are shown and compared in the following Figures for the three different membrane constituents distinguished here, as obtained in the system containing 15 DMPCs and 10 cholesterol per layer. No such comparisons are shown for the simulated cholesterol-poor membrane because in this system each layer contains only two cholesterol, and, on average, two DMPCs having a near cholesterol neighbor, which is clearly not enough to provide sufficient statistics for calculating the distributions of their VP properties. The distributions corresponding to DMPCs having no near cholesterol neighbors are compared in the insets of the Figures, which resulted from the three systems of different compositions.

The area distributions of the Voronoi polygons of the different membrane constituents are shown in Figure 3 for the cholesterol-rich system. The distribution corresponding to the DMPC molecules without near cholesterol neighbors is clearly shifted toward larger values with respect to the other two distributions. However, the distributions corresponding to the cholesterol and to their nearest DMPC neighbors are rather similar to each other; although the cholesterol distribution is somewhat narrower, their peaks are located at the same position of about $48-50 \text{ \AA}^2$. This is confirmed by the comparison of the mean values of these distributions, as listed in Table 1. A similar relation of the mean VP area values of the different membrane constituents is found in the cholesterol-poor mixed system (Table 1). This clear difference between the average free area available for DMPCs without near cholesterol neighbors and for the other two membrane constituents reflects the fact that, on average, there is considerably closer contact between cholesterol and their nearest DMPC neighbors than between any other pairs of neighboring molecules. Such unusually close contact indicates a specific attractive interaction between nearest DMPC-

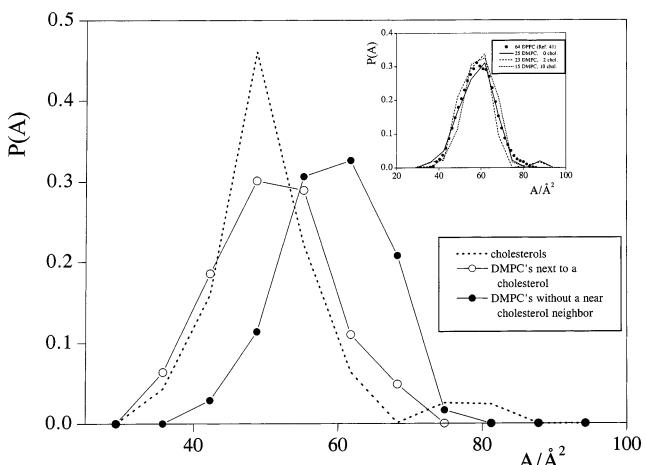


Figure 3. Comparison of the Voronoi polygon area distribution of the cholesterol molecules (dashed line), DMPC molecules located next to a cholesterol (open circles), and DMPCs having no near cholesterol neighbors (solid circles) in the membrane containing 40 mol % cholesterol. The inset shows the comparison of the distributions of DMPCs having no near cholesterol neighbors in the pure DMPC membrane (solid line) and in the mixed membranes containing 8 mol % (dashed line) and 40 mol % (dotted line) cholesterol. For reference, results obtained by Shinoda and Okazaki for a pure DPPC membrane⁴⁰ are also shown (solid circles).

cholesterol pairs. This interaction is certainly rather complex. For some of the DMPC–cholesterol pairs, it involves direct hydrogen bonding, which can act only between cholesterol molecules, as H donors, and their nearest neighbors. To demonstrate this, we have calculated the pair correlation function of the cholesterol oxygens and DMPC ($P=O$ and $C-O(-C)$ oxygen atoms. (Here, $(P=O)$ and $(C-O(-C))$ denote the double bonded, nonester phosphate oxygens and the ester oxygen atoms connecting the two lipid tails to the glycerol backbone, respectively. These atoms are marked as 21 and 22 and as 30 and 39 in Figure 1 of ref 35, respectively.) Both of the obtained pair correlation functions, shown in Figure 4, exhibit a sharp peak at low distances (i.e., at about 2.7 and 3.1 \AA for the pair correlation functions corresponding to the $(P=O)$ and $(C-O(-C))$ oxygen atoms, respectively). These peaks are due to the O atom pairs connected by a direct hydrogen bond through the cholesterol H atom. The fact that the hydrogen bonding peak of the pair correlation function corresponding to the $(P=O)$ atoms is sharper and appears at smaller distances than that of the $(C-O(-C))$ atoms indicates that the hydrogen bonds accepted by the phosphate oxygens are, in general, stronger than those accepted by the tail ester oxygens. The integration of these peaks up to the following minima reveals that about 13% of the cholesterol are connected to a neighboring DMPC molecule through a hydrogen bond. It should be noted that the pair correlation function of the cholesterol O atoms with any other DMPC oxygen does not show such a hydrogen bonding peak. To demonstrate that the formation of a direct hydrogen bond between a DMPC and a cholesterol molecule is always accompanied by the sufficiently close contact of the centers of mass of the molecules, we have also determined the contributions coming from cholesterol and their nearest DMPC neighbors (considering only the closest $(P=O)$ and $(C-O(-C))$ oxygen atoms of the DMPC molecule to the cholesterol oxygen) to these pair correlation functions. Nearest DMPC neighbors have been defined here in the same way as DMPCs having a near cholesterol neighbor (i.e., when the lateral distance of the DMPC and cholesterol centers of mass is smaller than 6.05 \AA). The nearest-neighbor contributions obtained are shown

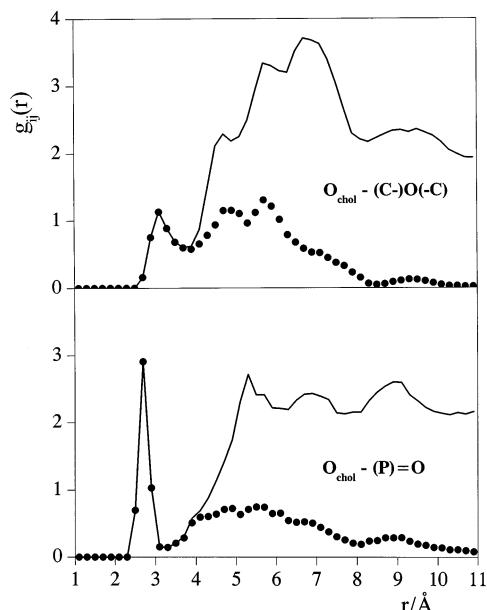


Figure 4. Partial pair correlation function of the cholesterol O and DMPC tail ester O atoms (top) and cholesterol O and DMPC nonester phosphate O atoms (bottom), as calculated from the simulation of the system containing 40 mol % cholesterol. Solid lines: full pair correlation functions; circles: contribution of the closer of the two relevant atom pairs of the nearest DMPC–cholesterol lateral center-of-mass–center-of-mass neighbors to the full pair correlation function.

and compared to the full pair correlation functions in Figure 4. As seen, the hydrogen bonding peak of both functions is fully accounted for by these contributions, and hence hydrogen bonding can occur only between DMPC–cholesterol pairs, the centers of mass of which are also close enough to each other.

Besides direct hydrogen bonding, which occurs between the polar headgroups of some of the neighboring DMPC–cholesterol pairs, the specific near-neighbor DMPC–cholesterol interaction also has to account for the close approach of the long apolar tails of the molecules. It has been shown several times that the vicinity of a rigid cholesterol ring has a considerable ordering effect on the tails of the DMPC or DPPC molecules. Hydrocarbon tails located next to a cholesterol contain fewer gauche dihedrals and hence extend less in lateral directions and are more likely to point straight toward the middle of the bilayer than those located far from cholesterol.^{29,32,35} Therefore, the ordering effect of a nearby cholesterol on the tail conformation of the DMPC molecules also helps to keep the centers of mass of the neighboring, often hydrogen-bonded DMPC–cholesterol pairs close. The number of gauche dihedrals along the lipid tails is known to correlate consistently with the area of the VP of the molecules (i.e., fewer gauche dihedrals correspond, on average, to a smaller VP area and hence to closer contact with the neighbors⁴⁰).

The inset of Figure 3 shows that there is no noticeable difference between the VP area distributions of DMPCs having no near cholesterol neighbors in the membranes of different compositions. (It should be noted that these $P(A)$ distributions also agree very well with the one obtained by Shinoda and Okazaki for the pure DPPC membrane using a different potential model.⁴⁰ This distribution is also shown in the inset of Figure 3 for comparison.) The independence of the $P(A)$ distribution of the DMPCs without near cholesterol neighbors from the cholesterol concentration indicates that the lateral condensation of the membrane observed upon adding cholesterol to the system³⁵ (reflected also in the $\langle A \rangle$ values averaged over all of the 25 molecules of the membrane layers, see Table 1) is solely

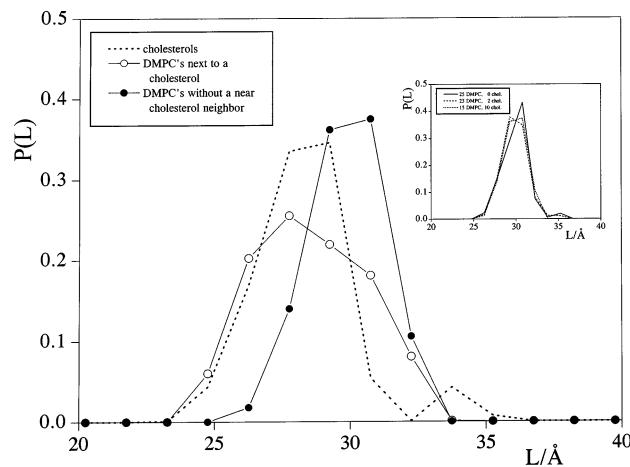


Figure 5. Comparison of the Voronoi polygon circumference length distribution of the cholesterol molecules (dashed line), DMPC molecules located next to a cholesterol (open circles), and DMPCs having no near cholesterol neighbors (solid circles) in the membrane containing 40 mol % cholesterol. The inset shows the comparison of the distributions of DMPCs having no near cholesterol neighbors in the pure DMPC membrane (solid line) and in the mixed membranes containing 8 mol % (dashed line) and 40 mol % (dotted line) cholesterol.

due to the specific interaction between cholesterol and their nearest DMPC neighbors.

Similar conclusions can be drawn from the comparison of the VP circumference length distributions $P(L)$ of the different membrane constituents, shown in Figure 5 as obtained in the cholesterol-rich mixed system. The distribution corresponding to the DMPCs having no near cholesterol neighbors is again shifted to larger values than the other two distributions, and it is again independent from the overall composition of the system, as seen from the inset. The $P(L)$ distributions of cholesterol and of their nearest DMPC neighbors are centered on similar L values. Consistently, the average L values agree well for these two types of membrane constituents in both mixed systems (Table 1). Substituting the A and L values of the main peak of the $P(A)$ and $P(L)$ distributions of cholesterol (i.e., $A = 49 \text{ \AA}^2$ and $L = 29 \text{ \AA}$) into eq 3 yields a ϕ value of 1.37. This value is considerably higher than the average ϕ value obtained for cholesterol of $\langle \phi \rangle = 1.269$ (Table 1). These two ϕ values are marked by arrows in Figure 6, showing the $P(\phi)$ distributions of the different membrane constituents.

As seen from Figure 6, the $P(\phi)$ distribution of the cholesterol molecules has a rather broad shoulder on the large- ϕ side of its main peak. The ϕ value estimated from the peak position of the $P(A)$ and $P(L)$ distributions falls in the region of this shoulder. The $P(\phi)$ distribution of the DMPC molecules located next to a cholesterol is rather similar to that of the cholesterol, with the only difference being that here the shoulder is developed into a separate, split peak. The position of the main peak of both distributions is at $\phi = 1.18$, somewhat lower than the main peak position of the distribution corresponding to the DMPCs having no near cholesterol neighbors. The integration of the cholesterol distributions up to $\phi = 1.37$ (i.e., the ϕ value estimated from the position of the $P(A)$ and $P(L)$ distribution peaks) reveals that about 85% of the cholesterol give rise to its main peak, whereas the large ϕ part results from 15% of the molecules. Considering also our finding above that about 13% of the cholesterol form direct hydrogen bonds with their nearest DMPC neighbors, this observation suggests that the large- ϕ side shoulder of the obtained cholesterol $P(\phi)$ distribution is due to the molecules connected to their nearest DMPC neighbor by a hydrogen bond. Such a hydrogen bond brings the participating

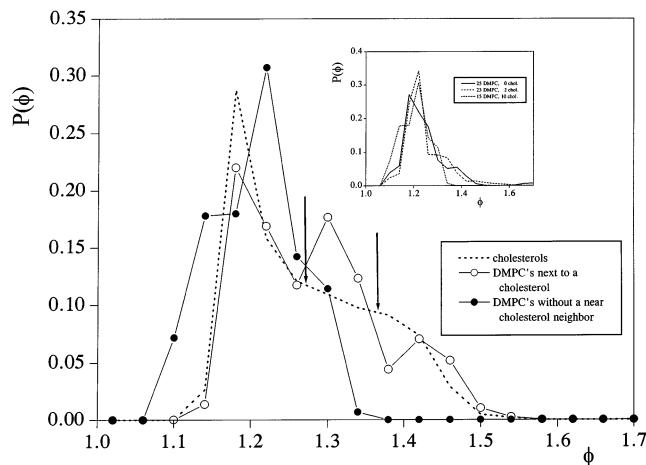


Figure 6. Comparison of the distributions of the acircularity parameter ϕ of the Voronoi polygon of the cholesterol molecules (dashed line), DMPC molecules located next to a cholesterol (open circles), and DMPCs having no near cholesterol neighbors (solid circles) in the membrane containing 40 mol % cholesterol. The arrows indicate the average ϕ value of 1.269 of the cholesterol distribution, and the ϕ value of 1.37 obtained by substituting the peak position values of the $P(A)$ and $P(L)$ distributions into eq 3 for cholesterol. The inset shows the comparison of the distributions of DMPCs having no near cholesterol neighbors in the pure DMPC membrane (solid line) and in the mixed membranes containing 8 mol % (dashed line) and 40 mol % (dotted line) cholesterol.

molecules closer to each other and hence distorts the isotropy of their local lateral environment to a larger extent, which is manifested in larger values of the acircularity parameter ϕ .

The comparison of the $P(\phi)$ distributions of the DMPC molecules without a near cholesterol neighbor in the different systems (inset of Figure 6) shows that in the cholesterol-poor and pure DMPC systems the distributions of these molecules also exhibit a clear shoulder on the large- ϕ side of the main peak. This observation indicates that in the absence of cholesterol a particularly strong interaction occurs between some of the neighboring DMPC pairs, distorting the symmetry of the local lateral environment of these molecules. However, this strong interaction between two DMPC neighbors is not preferred with respect to the interaction of the nearest DMPC–cholesterol neighbors, as indicated by the fact that in the cholesterol-rich system the distribution of DMPCs without a near cholesterol neighbor does not show such a shoulder; instead it drops to zero after its main peak at about $\phi = 1.35$.

The comparison of the different $P(N_v)$ distributions (shown in Figure 7) leads to similar conclusions. The cholesterol molecules and their nearest DMPC neighbors have rather similar $P(N_v)$ distributions. The mean values of these distributions also agree well in the cholesterol-poor system (Table 1). However, the $P(N_v)$ function of the other DMPC molecules is shifted toward larger values, indicating that the VPs of these DMPC molecules have noticeably more edges and vertices than those of the cholesterol and of their nearest DMPC neighbors. This result is consistent with our finding above that the VPs of the DMPCs having no near cholesterol neighbors are, on average, considerably more circular than the VPs of the other membrane constituents. Both observations reflect the fact that the strong, specific interaction between cholesterol and their nearest DMPC neighbors distorts the symmetrical local lateral environment of these molecules because they approach each other considerably more closely than other neighboring molecule pairs.

The comparison of the $P(N_v)$ distributions of the DMPCs having no near cholesterol neighbors (inset of Figure 7) shows

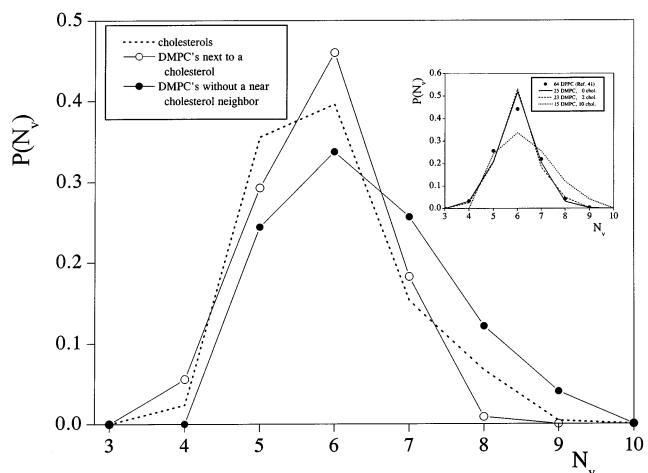


Figure 7. Comparison of the distributions of the number of vertices pertaining to the Voronoi polygon of the cholesterol molecules (dashed line), DMPC molecules located next to a cholesterol (open circles), and DMPCs having no near cholesterol neighbors (full circles) in the membrane containing 40 mol % cholesterol. The inset shows the comparison of the distributions of DMPCs having no near cholesterol neighbors in the pure DMPC membrane (solid line) and in the mixed membranes containing 8 mol % (dashed line) and 40 mol % (dotted line) cholesterol. For reference, results obtained by Shinoda and Okazaki for a pure DPPC membrane⁴⁰ are also shown (solid circles).

that the VP topologies of these molecules are very similar in the cholesterol-poor and pure DMPC systems. These distributions also agree well with the one obtained by Shinoda and Okazaki for a pure DPPC bilayer using a potential model different from the one used here (shown also in the inset of Figure 7 for comparison).⁴⁰ However, in the cholesterol-rich system the tail of the distribution is extended to larger N_v values, indicating that in the presence of enough cholesterol the VPs of the DMPCs having no near cholesterol neighbors have, on average, more edges and vertices (i.e., more geometric neighbors, indicating a more symmetrical local lateral environment) than they do in systems lacking a considerable amount of cholesterol. This observation again stresses that in the absence of cholesterol a strong specific interaction is acting between some pairs of neighboring DMPC molecules and this interaction distorts somewhat the symmetry of their local lateral environment. This distortion indicates that a DMPC molecule can interact with only a few (or possibly just one) nearest neighbors in this way. In the presence of a sufficient amount of cholesterol, this specific DMPC–DMPC interaction becomes less important, as seen from the extension of the $P(N_v)$ function toward larger values (and also from the lack of the large- ϕ -side shoulder of the main peak of $P(\phi)$, see Figure 6) in the cholesterol-rich system. The likely reason for this is that the specific nearest-neighbor DMPC–cholesterol interaction, which includes in some cases direct hydrogen bonding between the molecules, is favored with respect to this specific DMPC–DMPC interaction, which can therefore appear only between two DMPC molecules, none of which has a near cholesterol neighbor. Obviously, this specific DMPC–DMPC interaction, which is similar to the interaction of the nearest DMPC–cholesterol neighbors, should also be rather complex and cannot be specified solely from the analysis of the VP properties of the molecules. However, the above findings suggest that this interaction likely involves charge pairing between the headgroups of neighboring DMPC molecules, as described by Pasenkiewicz-Gierula et al.⁶⁴

It is seen from Table 1 that the mean value of N_v is exactly 6.00 in all three systems when averaged over all molecules present in the membrane layer. Although it would be tempting

to interpret this finding as a sign of the overall hexagonal packing of the molecules in the membrane layers, it should be interpreted with care. To investigate the significance of this result, we have determined the mean value and standard deviation of N_v of randomly arranged planar points. The resulting mean value of $\langle N_v \rangle = 5.98 \pm 1.31$ agrees very well with the $\langle N_v \rangle$ value of 6.00 obtained for all three membranes, indicating that this mean N_v value can be a universal feature of the Voronoi tessellation of disordered planar systems.

Summary and Conclusions

In this paper, we have demonstrated the usefulness of the Voronoi analysis in the investigation of the lateral structure of mixed planar layers by analyzing DMPC–cholesterol mixed membranes. In particular, the appearance of specific, strong interactions between molecules of given types has been identified. Thus, the majority of cholesterol molecules have been found to form strongly bound pairs with their nearest DMPC neighbors, which distorts the symmetry of the local lateral environment of both molecules. Because this interaction appears only between a cholesterol and one of its neighbors, we have concluded that in many cases it involves direct hydrogen bonding in which cholesterol is the H-donor molecule. We have confirmed this conclusion by calculating pair correlation functions of the cholesterol oxygen and the H-bond-accepting DMPC oxygen atoms. The integration of the first, hydrogen-bonding peaks of the obtained pair correlation functions has revealed that about 13% of the cholesterol molecules are forming hydrogen bonds with their nearest DMPC neighbor. Besides hydrogen bonding, which keeps the polar headgroups of the participating molecules close to each other, the ordering effect of the cholesterol ring system on the nearby lipid tails^{29,32,35} is also responsible for the observed close lateral approach of the centers of mass of the nearest DMPC–cholesterol pairs.

The detailed investigation of the properties of the VPs corresponding to the cholesterol molecules, in particular, the analysis of their acircularity parameter distribution, has revealed that this specific interaction appears in at least two different forms in the membrane. The first of these two forms appears more frequently and distorts the isotropy of the local lateral environment of the participating molecules to a considerably smaller extent than the second, less frequent form. It has been found that the fraction of cholesterol molecules forming a hydrogen bond with their nearest DMPC neighbor agrees well with the fraction of cholesterol having an acircularity parameter that is larger than the value estimated from the peak positions of the VP area and circumference length distributions. Considering this finding and also that for hydrogen-bonded molecule pairs a smaller separation of their centers of mass and hence less-circular VPs can be expected, we have concluded that the two forms of the specific nearest-neighbor DMPC–cholesterol interaction differ in whether they involve a direct hydrogen bond or not.

The comparison of the Voronoi properties of the DMPC molecules having no near cholesterol neighbors in the membranes of different compositions has revealed that another specific, strong interaction occurs between several DMPC pairs lacking a sufficient amount of cholesterol. However, in the case of the cholesterol-rich system no sign of such specific DMPC–DMPC interaction has been found. This finding indicates that the DMPC molecules prefer the nearest-neighbor DMPC–cholesterol interaction, which often involves hydrogen bonding, rather than this interaction with another DMPC molecule. This suggests that this specific DMPC–DMPC interaction probably

involves charge pairing between oppositely charged parts of the headgroups of the two participating molecules, as described by Pasenkiewicz-Gierula et al.⁶⁴

Finally, the comparison of the VP area distribution of the different membrane constituents in the different systems has revealed that the average VP area of a DMPC molecule without near cholesterol neighbors is independent of the membrane composition and is considerably larger than that of cholesterol and their nearest DMPC neighbors. Therefore, the results of the present study suggest that the lateral condensation of the phospholipid membranes occurring upon adding cholesterol to the system^{20,21,35} is solely due to the observed specific interaction occurring between neighboring DMPC–cholesterol pairs.

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