

Influence of Highly Polyunsaturated Lipid Acyl Chains of Biomembranes on the NMR Order Parameters

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Abstract: We investigate the effect of specific conformations of double-bond segments in highly polyunsaturated acyl chains on the deuterium ^2H NMR order parameters of a fully hydrated 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (SDPC, 18:0/22:6 PC) lipid bilayer. The system is analyzed by performing a molecular dynamics simulation study at ambient conditions in the fluid lamellar phase. By separately calculating the different partial contributions to the total order parameter profiles measurable experimentally, we are able to get insights into the molecular origin of earlier experimental and theoretical observations. The effect of the position of the different conformations of double-bond segments along the polyunsaturated acyl chain is also examined. As in experiments performed in a series of lipid bilayers with an increasing number of cis double bonds per lipid molecule [Holte, L. L., et al. *Biophys. J.* **1995**, *68*, 2396], we find that unsaturations influence mainly the order of the bottom half of the saturated chain. Specific conformations of the polyunsaturated chain close to the lipid headgroups have a distinct effect on the order of the bottom half of the saturated chain and on the top half of the polyunsaturated chain. Our results indicate that for SDPC the conformation of the region of the polyunsaturated chain located between the first three cis double bonds is responsible for the major effects on the orientational order of both the saturated and the polyunsaturated chains.

Introduction

Fatty acids with multiple unsaturations (double bonds) are rather abundant in cerebral and retinal tissues, and in the olfactory bulb.¹ These unsaturations are known to affect a number of biophysical properties of membranes constituted by phospholipids with one or both acyl chains containing cis double bonds. Examples of these effects are the low main order–disorder phase transition temperatures,^{2,3} the enhanced permeability to small organic solutes and water,^{4,5} the enhanced elasticity or decrease in area compressibility modulus,^{6–8} etc. However, the importance of polyunsaturated lipids seems not to be limited to a mere structural role. In some situations polyunsaturated lipids are needed for the proper function of membrane-embedded proteins. This is the case, for instance, of the G-protein coupled visual receptor rhodopsin,⁹ which is found in the rod cells of the retina. Polyunsaturated fatty acids,

especially the docosahexaenoic fatty acid (DHA), are present in large amounts in these rod membranes. By modifying the stability of MetarhodopsinII (MII) versus MetarhodopsinI (MI), which are intermediate molecular forms of rhodopsin in the cascade that follows the absorption of a photon, the DHA content influences the proper function of rhodopsin.^{10–12}

A particularly interesting situation is the case of membranes constituted by phospholipids with mixed (saturated/polyunsaturated) chains. For these lipids, the biologically relevant fluid lamellar phase can be achieved at room temperature for unicomponent lipid bilayers. Because polyunsaturated acyl chains decrease the temperature for the main (gel-to-liquid-crystalline) phase transition,¹³ T_m , these model membranes with mixed acyl chains are thus fluid under physiological conditions, in contrast to lipids with saturated chains of similar lengths.^{3,14} For the 1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (SDPC, 18:0/22:6 PC) lipid, in particular, the main order–disorder transition temperature in multilamellar dispersions containing 50 wt % H_2O was measured by Nuclear Magnetic Resonance (NMR) spectroscopy and found to be $T_m \sim -6\text{ }^\circ\text{C}$.³

Besides their importance as constituents of biomembranes, polyunsaturated fatty acids can serve as precursors for the biosynthesis of prostaglandins and thromboxanes¹⁵ and leuko-

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trienes.¹⁶ In addition, the DHA is known to be an inhibitor of prostaglandin biosynthesis but not of thromboxanes.¹⁷ Polyunsaturated fatty acids can also modify the activity of receptors acting as ligands. For instance, the DHA has been recently identified as a ligand for the retinoid X receptor (RXR) in mouse brain, indicating that DHA may influence neural function through the activation of an RXR signaling pathway.¹⁸

The disorder of the acyl chains of lipid bilayers in the fluid lamellar phase is habitually measured experimentally by deuterium ²H NMR spectroscopy. This technique is used to determine the orientational order parameter profiles by deuterating the different hydrogen atoms along the acyl chains and measuring the spectra. Thus, the orientational order parameters are available as a function of the position of the carbon atoms along the chains. The effective chain lengths and areas per lipid¹⁹ are usually derived from these measurements and the temperatures of the main phase transition³ are determined from the first moment of the deuterium spectra, M_1 . Although both the average chain conformation of the acyl chains and their motions are reflected in the orientational order parameters, it is out of the reach of experiment to separate the different contributions because the microscopic details are averaged out in experiment. Some trends can be derived, however, from extensive comparative experimental studies on different systems and at different thermodynamic states. Experimentally, it was thus observed that the presence of polyunsaturated chains in membranes constituted by phosphatidylcholines (PCs) with mixed chains affects the orientational order of the saturated chains.^{2,20,21} Specifically, the saturated chain exhibits an increase in disorder at the bottom half of the chain (close to the membrane center) as unsaturation is increased in a series of PCs with mixed chains, while the effect of increasing temperature on the chain order consisted of an overall decrease in order specially noticeable at the top half of the chain (close to the water–lipid interface).²¹ Therefore, the inclusion of unsaturation in the sn2 chains produced an inhomogeneous disordering along the saturated (sn1) chains, which was associated with a change in the molecular shape for PCs containing a polyunsaturated (three or more cis double bonds) chain toward a more wedge-shaped form.²¹

Computer simulations provide a suitable tool to analyze the membrane properties from an atomic level, and offer a direct connection between the microscopic details of the system and the macroscopic properties of experimental interest.^{22–25} Although classical molecular dynamics (MD) simulation studies have been extensively used in the past decade to investigate model membranes, earlier studies of water–lipid phosphatidylcholine systems in the lamellar phase were restricted to disaturated lipids (see for instance, ref 26) or lipids with a low

degree of unsaturation (see for instance, ref 27). In a recent MD simulation study²⁸ of an SDPC lipid bilayer in the liquid crystalline phase at ambient conditions, we demonstrated the ability of current force fields to reproduce reasonably well the experimental structural properties of this membrane with highly polyunsaturated acyl chains.

In the present work, we use MD simulation of SDPC as a tool to evaluate the effect of the molecular conformation of the polyunsaturated (DHA) chain on the orientational order of both the saturated sn1 (C₁₈) and the polyunsaturated sn2 (C₂₂) chains of this fully hydrated mixed-chain lipid bilayer. To gain more insight into the molecular origin of the features of the experimentally available quantities, the different contributions of the individual lipids to the total experimentally measurable NMR orientational order parameters are extracted. These different contributions are gathered for lipids with particular conformations of the molecular fragments comprising three consecutive cis double bonds of the polyunsaturated chain. This methodology, used here to evaluate the effect of individual conformations of chain segments on the overall NMR orientational order parameters, can be applied to a wide variety of chemical systems to get more insight into the particular microscopic characteristics associated with an experimental property.

Computational Details

The MD simulation was performed at constant temperature, $T = 303$ K, and pressure, $P = 1$ atm, on the model membrane and had similar characteristics to those reported elsewhere.²⁸ The present lipid bilayer consisted of 64 lipid molecules and about 27.5 water molecules per lipid, which corresponded to a fully hydrated lipid bilayer with an area per lipid, A , of 61.4 Å² and a lamellar spacing, d , of 70.0 Å. The simulation was carried out at constant pressure under NPT conditions. We used the Nosé-Hoover thermostat chain extended system isothermal–isobaric dynamics method with an orthorhombic simulation cell, as implemented in the program PINY_MD.²⁹ The reversible multiple time step algorithm³⁰ permitted the use of a time step of 5 fs. The different properties were evaluated over a production run of 1 ns after an equilibration period of almost 2 ns. At the end of this initial period, energies and cell dimensions converged to their equilibrium values and the dihedral angles of both acyl chains displayed symmetric distributions, which is an indication of the equilibrium state reached by the system.

The molecular and potential model used for the lipid molecules was the recent version of the all-atom CHARMM force field (CHARMM27) for lipids,³¹ whereas we used a rigid TIP3P model³² for water. All the motions involving hydrogen atoms were frozen, which allowed us to use a time step of 1 fs in the integration of Newton's (classical) equations of motion. The intermolecular parts of the force fields are pairwise additive functions, which consist of simple Lennard-Jones plus Coulomb terms.

We used periodic boundary conditions and the constraints in our system were handled by means of the SHAKE/ROLL and RATTLE/ROLL methods.³⁰ The short-range forces were computed using a cutoff of about 10 Å and the minimum image convention, and the long-range

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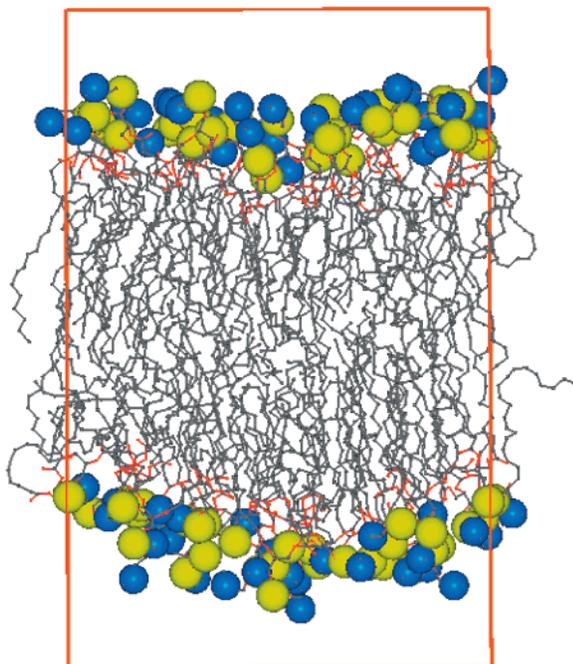


Figure 1. Instantaneous configuration of the SDPC lipid bilayer. For the sake of clarity, water molecules, which are located between the headgroups of two opposite leaflets, i.e., the void region, are not shown. The headgroup region is indicated by the positions of the nitrogen atoms (colored blue) and the phosphorus atoms (colored yellow). The rest of the lipid atoms are displayed as balls and sticks. By the use of periodic boundary conditions, images of the simulation cell are replicated at every face of the orthorhombic central cell (colored red).

forces were taken into account by means of the Particle Mesh Ewald (PME) technique.³³

In Figure 1, we show an instantaneous configuration of the SDPC lipid bilayer. The water molecules, which solvate the lipid headgroups, are not drawn. The disorder displayed by the lipid chains, located in the middle of the cell, indicates the fluid lamellar phase, L_a , of the membrane. The most polar part of the membrane, the zwitterionic PC headgroup region, is indicated by the position of the nitrogen atom (colored blue) and the phosphorus atom (colored yellow).

Orientational Order and Molecular Conformation: Methodology

The behavior of acyl chains in lipid bilayers is usually studied through the orientational order parameter, S_{CD} , which can be obtained experimentally by NMR spectroscopy from the directly measured quadrupolar splitting, $\Delta\nu_Q$, using the relationship $\Delta\nu_Q = 3/4(e^2qQ/h)S_{CD}$, where e^2qQ/h is the quadrupolar coupling constant. By site directed deuteration of the different hydrogen atoms in the acyl chains, the experimental order parameter profile, $S_{CD}(n)$, can be derived for each position, n , along the deuterated chain. In the MD simulations, the orientational order parameter profile can be obtained directly and is given by,

$$S_{CD}(n) = \frac{1}{2} \langle 3 \cos^2 \beta_n - 1 \rangle \quad (1)$$

where β_n is the angle between the orientation of the vector along a C–H bond of the n -th carbon atom of the saturated (sn1) and/or the polyunsaturated (sn2) chains and the bilayer normal. Here, the brackets indicate averages over time and lipid molecules. Since S_{CD} takes a value of 1 when the reference vectors are parallel, a value of -0.5 when the reference vectors

are perpendicular, and a value close to zero for random orientations, the $-S_{CD}(n)$ values for lipid bilayers are usually in the range $0-0.5$.

In previous computer simulation studies, it was observed that the presence of cis double bonds leads to orientational order parameter profiles for the unsaturated chains that are quantitatively and qualitatively different from those characteristic of saturated acyl chains (see for instance, refs 27 and 28). Deviations of the order parameter profiles from the features typical of saturated chains also have been observed in lipids with branched acyl chains.³⁶ In the SDPC lipid bilayer, in particular, due to the different molecular structure and dynamics of the polyunsaturated chain, the $-S_{CD}(n)$ values obtained from MD simulations are significantly lower for the polyunsaturated chain than for the saturated chain,²⁸ in good agreement with experiment.³⁴ Furthermore, the orientational order parameters for the DHA chain were found to be strongly correlated with the position of the C–H bond along the hydrocarbon chain, especially at the first (between the fourth and the fifth carbon atoms; termed C4=C5, where “=” indicates the position of the double bond) and second (C7=C8) cis double bonds and to a lesser degree at the third one (C10=C11). In contrast, the $-S_{CD}(n)$ curves presented a smoother profile for the C–H bonds located at the bottom half of the chain. The former suggested a strong dependence of the order parameters on the chain conformation of the top half of the chain,²⁸ while the latter indicated that the addition of more cis double bonds after the first three, when the unsaturations begin close to the headgroup, does not have an additional effect on the order of the polyunsaturated chain, which is consistent with earlier theoretical⁸ and computer modeling³⁵ calculations.

However, the influence of unsaturations on the orientational order of hydrocarbon chains in biomembranes seems not to be limited to the unsaturated chains. It is known from experiment that the presence of polyunsaturated acyl chains affects the order parameters of the saturated chains.^{2,20,21} Specifically, the saturated chain exhibited an inhomogeneous decrease in order at the bottom half of the chain as the degree of unsaturation is increased in PCs with mixed chains.²¹

To gain more insight into the molecular origin of these experimental and theoretical observations, it would be desirable to separate the contributions to the total order parameter profiles from the different lipids with particular characteristics. The advantage of MD simulations is that it allows one to compute individually the properties of molecules with, for instance, different intramolecular conformations. Accordingly, in this work, we calculated the $S_{CD}(n)$ functions for lipid molecules with different intramolecular structures of their polyunsaturated chains. The total order parameter profiles for both the saturated and the polyunsaturated chains are thus given in terms of the partial components by

$$S_{CD}(n) = \sum_i f_i S_{CD}^{(i)}(n) \quad (2)$$

where $S_{CD}^{(i)}(n)$ is given by eq 1 but here the brackets indicate averages over time and those lipid molecules with a conformation of type i , f_i is the fraction of lipid molecules with a conformation of type i , and the sum runs over all the possible conformation types. The different lipids were classified by the

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typical structures of the DHA chain, i.e., helical, angle-iron, and hairpin. The rest of conformations were classified as *other*. These structures are defined by the conformations of the dihedral angles between three consecutive cis double bonds. Therefore, the angle-iron structure consists of the ($=skew^{\pm}skew^{\mp}==skew^{\mp}skew^{\pm}==$) conformation, whereas the helical conformation corresponds to ($=skew^{\pm}skew^{\pm}==skew^{\pm}skew^{\pm}==$). The $skew^+$ ($skew^-$) conformations of the dihedral angles are given by the ranges 60° – 180° (180° – 300°). Helical and angle-iron shapes were predicted by a molecular modeling approach³⁵ to be relevant for biological membranes. Since these two structures have almost straight chain axes, one expects rather tight intermolecular packing arrangements.

In Figure 2, we show an instantaneous configuration of a helical and an angle-iron structure viewed along the main axis and perpendicular to it. It was shown previously²⁸ that, on average, helical and angle-iron conformations represented 37% and 29% of the groups of dihedral angles, respectively, which together constituted 66% of the total population and were indeed quite stable, representing the two most abundant conformations for the studied conditions. For the rest of the DHA chains (34%), a tight packing of the chains is not possible. In particular, 2% of the dihedral angles adopt the hairpin conformation ($=skew^{\pm}skew^{\mp}==skew^{\mp}skew^{\pm}==$). Although it presents a rather looped shape (Figure 2), the hairpin structure is the conformation with the lowest energies in the gas phase. In Figure 2, we show a hairpin conformation and an example of a typical instantaneous configuration of a chain which does not correspond to a helical, an angle-iron, or a hairpin structure. These nonlinear hairpin-like structures are also expected to have lower energies than the angle-iron and helical structures in the gas phase.

Since the DHA chain has six cis double bonds and only three consecutive cis double bonds are needed to define the different conformations, we also studied the effect of the position of the conformations of three cis double-bond segments along the DHA chain. Therefore, all the $S_{CD}^{(i)}(n)$ curves were also computed for the conformations represented by the four dihedral angles located between three consecutive cis double bonds as a function of its position along the polyunsaturated chain, m (with $m = 1, 2, 3,$ and 4). Here, $m = 1$ and 4 correspond to the conformations of the DHA segments closer to the beginning (headgroups) and to the end of the polyunsaturated chains, respectively.

Results

Oriental Order of the Saturated Chains. The effects of the specific conformations of the molecular fragments of the polyunsaturated chain on the order of the saturated chain are evidenced in Figure 3a,b, where we plot the partial order parameter profiles, $S_{CD}^{(i)}(n)$, for the saturated chain. Figure 3 clearly shows that different polyunsaturated conformations have a distinct effect on the orientational order of their covalently bonded (saturated) chains. Individual conformations of the dihedral angles close to the lipid headgroup produce differences in the lower region of the saturated chain (Figure 3a), in agreement with experimental observations.²¹ Specifically, the major effects are found around the carbon atom C12. In this region, the straightest structures (angle-iron and helical) at the beginning of the unsaturated chain increase the order, whereas the less straight conformations (hairpin and *other*) decrease the order of the saturated chains. In contrast to what one would intuitively expect, the order parameters for the top part of the saturated chain change only slightly (for instance at the carbon atom C2) as a function of the structure of the top part of the polyunsaturated chain. This counter intuitive behavior can be

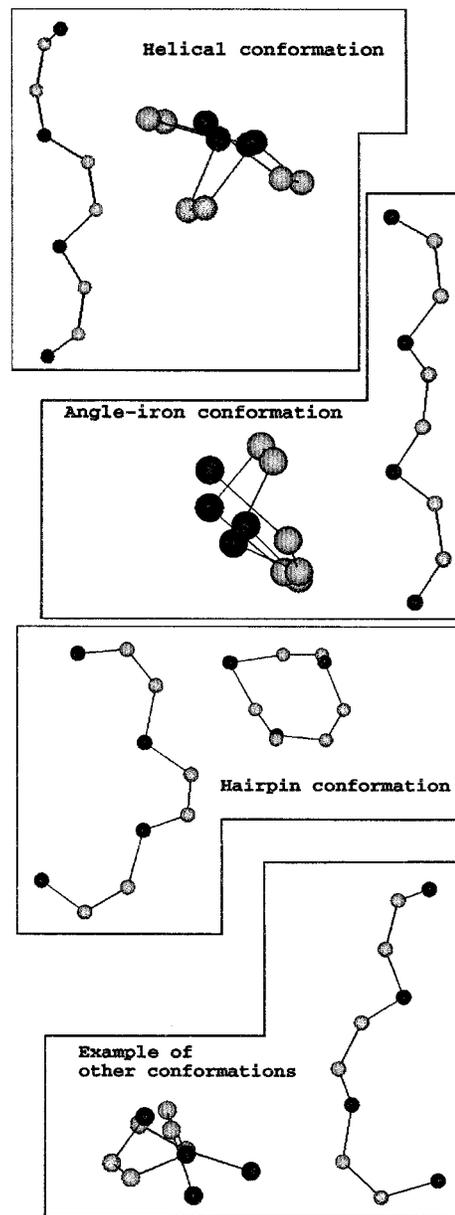


Figure 2. Molecular structure of the polyunsaturated chains corresponding to instantaneous configurations of the most extended conformations (top), helical and angle-iron, and those corresponding to the less straight conformations of the polyunsaturated chains (bottom), hairpin and another hairpin-like nonlinear conformations. The structures are shown as viewed along the main axis and perpendicular to it. The chain segments are represented as balls and sticks with light and dark spheres corresponding to the CH and CH_2 atomic groups, respectively. For clarity, the hydrogen atoms are not shown.

rationalized in the following way. Close to the membrane interface, the atomic density is high and, even though there is a tendency for the nonlinear (linear) type of lipids to arrange in “disordered” (“ordered”) lateral domains, most of the lipids are located at the domain interfaces. Here the environment is rich enough to produce an average result in the region close to the membrane surface, rather independent of the particular conformation of the covalently bonded polyunsaturated chain. The behavior of the lower part of the saturated chain, however, is determined by the local free volume available which, in turn, is directly connected with the conformation of the covalently bonded polyunsaturated chain in the region close to the interface. The straightest polyunsaturated conformations will hinder the

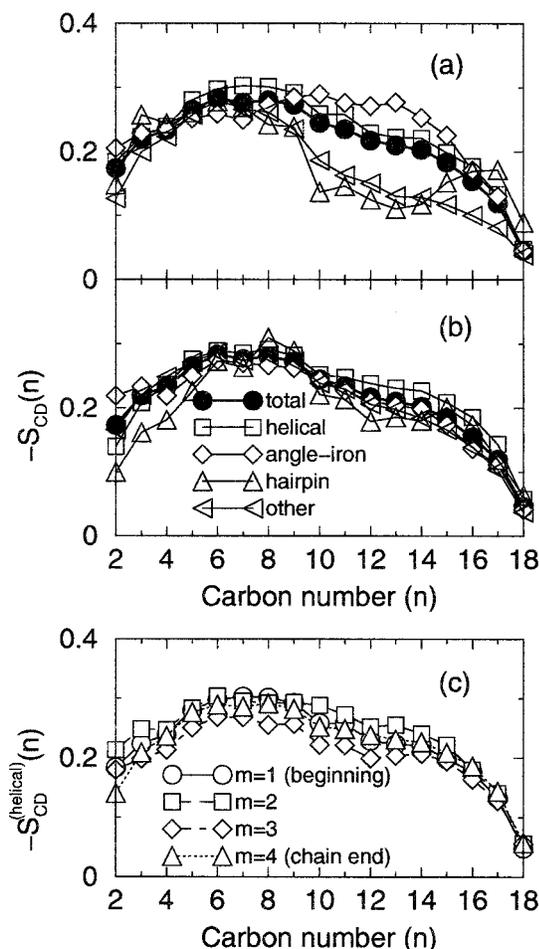


Figure 3. Orientational order parameter profiles of the SDPC saturated chains obtained by averaging over different conformations of the region of the polyunsaturated chains (a) close to the headgroups ($m = 1$) and (b) close to the chain ends ($m = 4$). The lines are visual guides. Results for the different conformations are compared with the total $S_{CD}(n)$, obtained after averaging over all the lipids independently of their conformations.²⁸ (c) Orientational order parameter profiles of the saturated chains obtained by averaging only over the helical conformation of the molecular segments, as defined in the text. The curves represent the different positions of the helical conformations along the polyunsaturated chain ($m = 1, 2, 3$, and 4). Note that by conformation we mean the intramolecular conformation of the molecular fragments of the polyunsaturated chain comprised between three consecutive cis double bonds, as defined in the text.

mobility of the saturated chains and/or induce favorable chain–chain intramolecular interactions. The less straight polyunsaturated conformations will increase the number of gauche defects by unfavorably interacting with their covalently bonded chains and/or will remain at the interface or visit farthest regions of the membrane and, thus, produce a local increase of free space available for the saturated chain. For the sake of completeness, we also show the results for the hairpin conformations, even though they represent only 2% of the population. Note that, in general, the helical conformation gives an order parameter quite close to the total value, obtained after averaging over all the lipids independently of their conformations. This is reflected in the quite similar results obtained for the curves corresponding to the helical conformations of the molecular segments as a function of their position along the chain, $m = 1, 2, 3$, and 4 (Figure 3c).

When lipids are classified by their conformation at the end of the DHA chain ($m = 4$), the results involve averaged

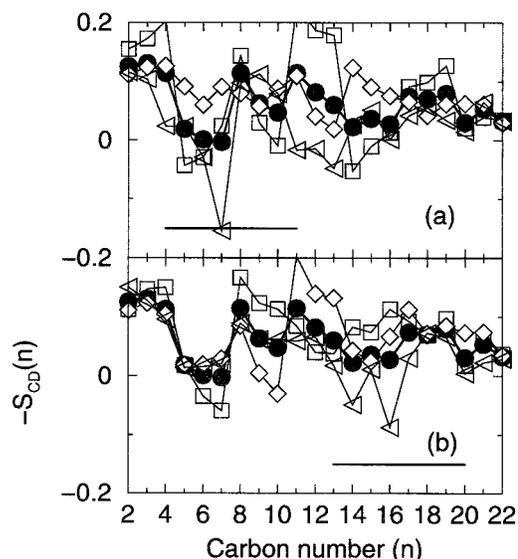


Figure 4. Orientational order parameter profiles of the polyunsaturated chains obtained by averaging over different conformations (a) close to the headgroups ($m = 1$) and (b) close to the chain ends ($m = 4$), as defined in the text. The lines are visual guides. Symbols are as in Figure 3a,b. The bold horizontal lines represent the position of the carbon atoms involved in the definition of the distinct conformations for $m = 1$ (a) and 4 (b). Results for the different conformations are compared with the total $S_{CD}(n)$, obtained after averaging over all the lipids independently of their conformations.²⁸

conformations at the beginning of the lipid chains. This averaging leads to results that are quite similar for the different structures and quite close to the total $S_{CD}(n)$ curves (Figure 3b). This phenomenon agrees with several experiments and theoretical predictions for which the results obtained are dependent on the number and position of the unsaturations. In those cases, the introduction of more double bonds after the first three did not produce a further effect when the unsaturations began close to the headgroups. This behavior is reasonable since the center of the bilayer is the most fluid part of the membrane, so the conformations in this region would have a very minor effect.

In summary, our results clearly indicate that the conformation of the region of the unsaturated chain located between the first three cis double bonds is responsible for the major effects on the orientational order profiles of the saturated chains.

Orientational Order of the Polyunsaturated Chains. The order parameter profiles of the polyunsaturated chains are shown in Figure 4a,b for the different conformations of the double-bond region located at the beginning ($m = 1$) and at the end ($m = 4$) of the DHA chains, respectively. As a general trend, the $-S_{CD}^{(i)}(n)$ curves corresponding to the polyunsaturated chains present lower values than those of the saturated chains, which is fairly independent of the position and type of the conformation. The particular shape of the curves of the polyunsaturated chains varies with the specific DHA conformation. In general, however, the polyunsaturated partial $S_{CD}^{(i)}(n)$ curves display a correlation with the cis double bond position. It should be remembered that by DHA conformation we mean the intramolecular conformation of the molecular fragments of the DHA chain comprised between three consecutive cis double bonds, as defined in the previous section.

When molecules are classified by their conformation at the beginning of the polyunsaturated chains ($m = 1$), clear differences appear in this region, close to the headgroups. Those molecules with a helical conformation show a strong correlation of the orientational order parameter at the first three double

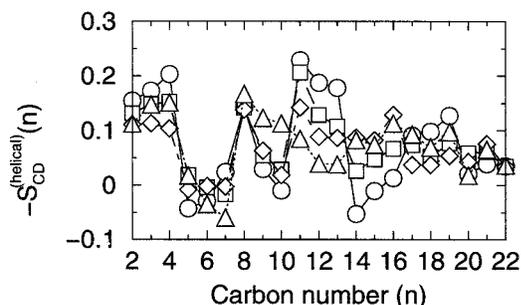


Figure 5. Orientational order parameter profiles of the polyunsaturated chains due to averaging over the helical conformations of the molecular segments. The curves represent the different positions of the helical conformations along the polyunsaturated chain ($m = 1, 2, 3,$ and 4). The lines are visual guides. Symbols are as in Figure 3c.

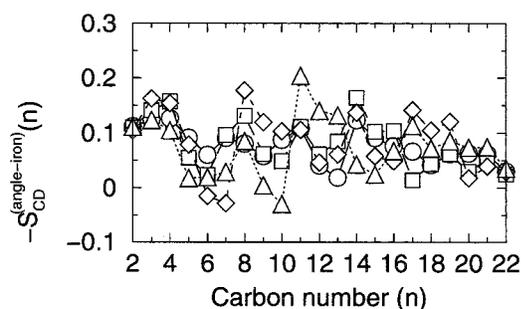


Figure 6. The same as in Figure 5 but for the angle-iron conformation of the molecular segments. Symbols are as in Figure 3c.

bonds, which is quite similar to the behavior of the total $S_{CD}(n)$ curve. The angle-iron conformation, however, displays the least correlated behavior of the $-S_{CD}^{(i)}(n)$ functions in this region, even though it is also characterized by a parallel orientation of consecutive cis double bonds. It is worth pointing out, however, that the rest of the conformations of the molecular fragments (*other*) also display, on average, a correlation in this region.

When molecules are classified by their conformations near the chain ends ($m = 4$), the results obtained for the first two double bonds are similar and close to the mean value, while the results are only qualitatively similar in the region between the second and the third cis double bond. This is again reasonable since the bottom part of the chains are more probably located at the center of the bilayer, where the overall fluidity is higher than near the lipid–water interface.

In Figures 5 and 6, we plot the orientational order parameter profiles for the helical and angle-iron conformations of the polyunsaturated molecular fragments, respectively, as a function of the positions of these molecular segments along the DHA chain, i.e., for $m = 1$ (close to the headgroup), 2, 3, and 4 (close to the chain end). For the helical conformation, the effect of considering different molecular segments along the chain can only be noticed after the eighth carbon atom (C8) and the correlation at the third cis double bond is only lost when all the conformational degrees of freedom are included except for the conformation of the region closest to the chain ends. The angle-iron conformation, however, displays in general a smoother dependence with the position of the C–H bonds, n . Our results indicate that the region between the first two double bonds is quite well defined for our system. In this zone, which corresponds to a region of about 5 Å wide located 5 Å below the water–lipid interface (data not shown), in general there is a steep decrease and increase of molecular order correlated with the position of the unsaturations (cis double bonds).

The main differences between the most linear conformations (helical and angle-iron) are found in the region closer to the headgroups. The $-S_{CD}(n)$ curves for the angle-iron conformation experience a smooth decrease of order with the carbon position, decaying from about 0.15 to about 0.05. In contrast, the results for the helical conformation are strongly correlated with the double bonds in the region between the first three cis double bonds, similar to the results obtained for the less extended conformations in the same region.

Conclusion

The effect of highly polyunsaturated fatty acid chains on the orientational order, as measured in NMR experiments, has been investigated by MD simulations at ambient conditions in a model biomembrane constituted by phospholipids with mixed (saturated/polyunsaturated) acyl chains. By separately calculating the different contributions to the order parameter profiles from the individual lipids with specific conformations of the molecular segments comprised between three consecutive cis double bonds of the polyunsaturated chains and at different locations along the chain, we are able to get insights into the molecular origin of the different theoretical and experimental findings on these complex organized systems. In particular, we show the results obtained using this methodology for the case of a fully hydrated SDPC (1-stearoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine) lipid bilayer at constant temperature and pressure in the biologically relevant fluid lamellar phase, L_{α} .

In agreement with experiment, we found that unsaturations (cis double bonds) influence the order of the bottom half of the saturated chain. As a general trend, the unsaturations affect the order of the bottom half of the saturated chain and significantly decrease the order of the polyunsaturated chain compared to the order parameters for the saturated one. The order parameter for the top part of the saturated chain, however, do not change as a function of the structure of the top part of the polyunsaturated chain. The most linear conformations (helical and angle-iron) at the beginning of the polyunsaturated chain increase the order of the bottom half of the saturated chain, whereas the less linear conformations decrease the order of this region of the saturated chain. The order parameter profiles are rather independent of the polyunsaturated chain structure at the chain ends.

Helical and angle-iron conformations of the molecular segments comprised between three consecutive double bonds have a distinct influence on the order parameters of the region of the polyunsaturated chain close to the headgroups, specifically, between the first two cis double bonds and, in less degree, between the first three cis double bonds. While helical conformations produce a strong correlation of the order with the unsaturations in the polyunsaturated chain, and $-S_{CD}$ decreases from about ~ 0.15 to almost 0, the angle-iron conformation is not correlated with the unsaturation positions and the order smoothly decreases in this region. The rest of the conformations, which present the least linear shaped segments, on average, also show a strong correlation in this region. As a general feature, they increase the disorder in both chains, as expected.

The inclusion of membrane proteins, such as rhodopsin, in similar model membranes has been shown to produce an effect on the order parameters of polyunsaturated lipids,^{34,37} suggesting a change in the average conformation of the polyunsaturated (DHA) chain and/or some preference of the protein to interact with the polyunsaturated lipid. Accordingly, our results indicate

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that a change of conformation of the region of the polyunsaturated chain close to the lipid–water interface (the first three double bonds) will produce a measurable effect on the order parameter profiles of both the saturated and the unsaturated chains.

In summary, we have found that in SDPC lipid bilayers the conformation of the region of the unsaturated chain located between the first three *cis* double bonds, which corresponds to a zone of about 7 Å wide located approximately 5 Å below the water–lipid interface, is responsible for the major effects on the orientational order parameter profiles of both the saturated and the polyunsaturated chains. A methodological aspect to be emphasized, which can be applied to other molecular systems,

is that calculating a property separately for different molecular conformations, or molecular characteristics, can help us to understand the microscopic origin of macroscopic quantities of experimental interest.

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