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Spin-Labeled Alkylphospholipids in a Dipalmitoylphosphatidylcholine Bilayer: Molecular Dynamics Simulations

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Molecular dynamics simulation has been performed to investigate the structural properties of perifosine and its synthetic spin-labeled alkylphospholipid analogues. The conformations adopted by these compounds in water and in a dipalmitoylphosphatidylcholine bilayer as a function of the presence and position of the *N*-oxyl-4′,4′-dimethyloxazolidine ring (doxyl group) have been investigated by all-atom molecular dynamics. No predominant conformation was observed in water, but the molecules adopt specific orientations and conformations in the lipid bilayer. As is expected, alkyl chains tend to insert into the hydrophobic core, while charged groups stay at the lipid−water interface. A doxyl group in the middle of the alkyl chain moves up to the interface region, thus preventing adoption of the extended conformation. Compounds with a doxyl group close to the polar head group adopt conformations similar to that of unlabeled perifosine within the first nanoseconds of simulation. When the doxyl group is at the end of alkyl chain, the spin-labeled molecule needs more time to reach equilibrium. These results indicate a considerable effect of the doxyl position within the alkyl chain on its localization in the lipid bilayer and can be extended further to other similar spin probes used in the electron paramagnetic resonance spectroscopy of biological membranes.

Introduction

Nitroxides are widely used as spin labels and spin probes in electron paramagnetic resonance (EPR) spectroscopy. Recently, several interesting results concerning membrane structure and the structure of biomacromolecules have been published,[1,2] and various biologically active compounds have been spin-labeled and used for EPR investigations.[3−5] The influence on the chemical and biological properties of the nitroxide-containing heterocyclic rings commonly used in these studies is, however, poorly understood.

Many spin-labeled lipophilic and amphiphilic compounds have been used to study membrane properties by EPR, and, of these, a few spin-labeled fatty acids, their esters, and phospholipids have been used routinely.[1,6−10] Most have an *N*-oxyl-4′,4′-dimethyloxazolidine (doxyl) group close to the carboxyl group; those in which the doxyl group is closer to the terminal methyl group are used less frequently. Initially, it was assumed that the fatty acid spin probe in the lipid bilayer assumes an extended conformation and that the presence of low concentrations of a spin probe in the membrane sample would have a negligible perturbation effect.[1] The possible influence of the doxyl ring on the overall conformation of the molecules in the membrane was neglected.

Alkylphospholipids are metabolically stable analogues of lysolecithin and have broad biological activity. A member of this family, perifosine [octadecyl(1,1-dimethylpiperidinium-4-yl)phosphate, inner salt], a therapeutically important drug candidate currently in phase 2 clinical trials in Canada,[11] has a piperidine ring in place of the usual choline head group.[12,13] Synthetic spin-labeled analogues of perifosine have been used to trace this compound in the cell membrane.[3]

In recent studies of the physical and biological properties of spin-labeled alkylphospholipids (Sl-APLs, Chart 1), we have observed that the critical micellar concentrations (cmc's) and the biological activities are strongly dependent on the position of the doxyl ring within each specific alkyl chain.[3] It is clear that both the presence and the position in the alkyl chain of the doxyl ring influence the physical, structural, and, as a consequence, the biological properties of spin-labeled compounds.[5] For a clearer understanding of the influence of the doxyl ring on the interaction with the lipid bilayer, we have simulated the interaction of Sl-APLs with the lipid bilayer on a nanosecond time scale with perifosine and the three Sl-APLs shown in Chart 1.

Experimental

Each molecular dynamics (MD) simulation used a single molecule **1**−**4**, either in aqueous solution or in a bilayer (embedded into one of the monolayers with its extended chain parallel to the *z*-axis, or laid down on the lipid−water interface) constructed of 127 dipalmitoylphosphatidylcholine (DPPC) lipid

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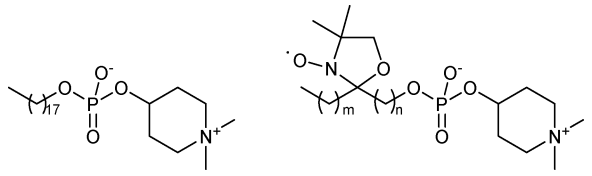
CHART 1. Perifosine and Its Spin-Labeled Analogues



1, Perifosine

2, m = 12, n = 4
3, m = 7, n = 9
4, m = 3, n = 13

molecules and ~4700 water molecules. The system was equilibrated, and production runs were carried out at 330 K (L_α phase) for 1−6 ns for the systems with the bilayer and 1 ns for those in pure water. Long-range electrostatic interactions were treated by the particle mesh Ewald method with a 12 Å cutoff. The integration time step used was 1 fs, and coordinates were saved every 0.1 ps for analysis. All simulations were performed with the CHARMM[14] program using parameters adapted from the CHARMM force field[15−18] or obtained by standard methods,[19] and time series for representative atoms were plotted along the *z*-direction (see Supporting Information).

Results and Discussion

Group distributions of **1**−**4** and DPPC were determined from the calculated trajectories. Figure 1 depicts these group distributions obtained by slicing along the *z*-direction (taken as a normal to the plane of the membrane) and counting the number of atoms in each functional group in each of 100 slices. Distributions were averaged over the last nanoseconds of simulations when equilibrium was reached and scaled to the number of DPPC molecules in the simulation cell.

Recognition of the influence of a doxyl group on the amphiphilic character and the prevalent conformation of the spin probe in phospholipid membranes can lead to advancement in the design and synthesis of new spin-labeled analogues as well as a more reliable interpretation of EPR data. It is important to correctly predict the surroundings of the nitroxide group. It is clear from the simulations that compounds **1**−**4** adopt prevailing conformations in the lipid bilayer. The time for reaching converged conformations changes with different initial conditions and depends significantly on the presence and position of the doxyl group.

In bulk water, molecules **1**−**4** can adopt many possible conformations, while molecules embedded in DPPC adopt more defined shapes. In general, the phosphate and piperidine moieties of the polar headgroups of **1**−**4** in DPPC reside in the phospholipid headgroup region of the DPPC bilayer.

Similarly, the group distributions for their hydrocarbon tails indicate that they are embedded in the hydrocarbon core region of the bilayer. The hydrocarbon side chain of **1** is predominantly in an extended conformation with the maximum density for the terminal methyl, like that for the terminal methyl of DPPC, at the bilayer center (see Figure 2, panel A). Compound **2** adopts a similar extended conformation with the doxyl group slightly under the DPPC glycerol (see Figure 2, panel B). In compound **3**, however, the doxyl group, which is in the middle of the alkyl chain, moves closer to the interface region within the first 600 ps of the simulation (see Figure 2, panel C). The part of the hydrocarbon chain that is between the polar head and the doxyl is twisted, and the remainder of the alkyl tail adopts a position in which the terminal methyl is slightly above the bilayer center. Compound **4** adopts an L-shaped conformation in which the doxyl group is very close to the bilayer center and the hydrocarbon chain is fully extended between the polar head

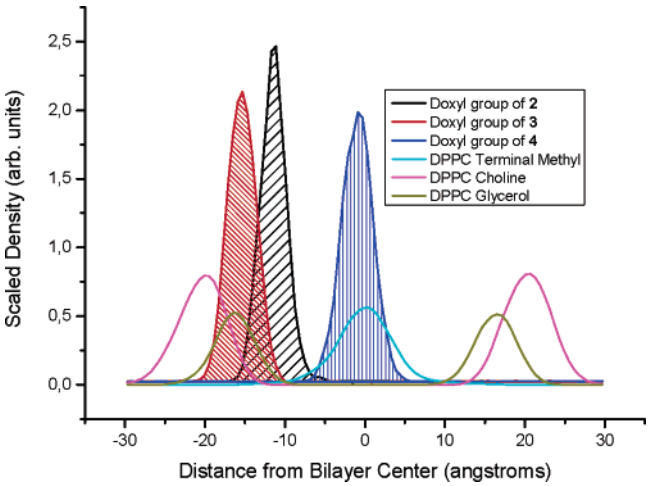


Figure 1. Doxyl group distributions for compounds **2**, **3**, and **4** embedded into the lower monolayer of DPPC compared to various DPPC moieties (choline, glycerol, and terminal methyl). Densities are scaled to the number of molecules and averaged over the last nanoseconds of the simulations (equilibrium).

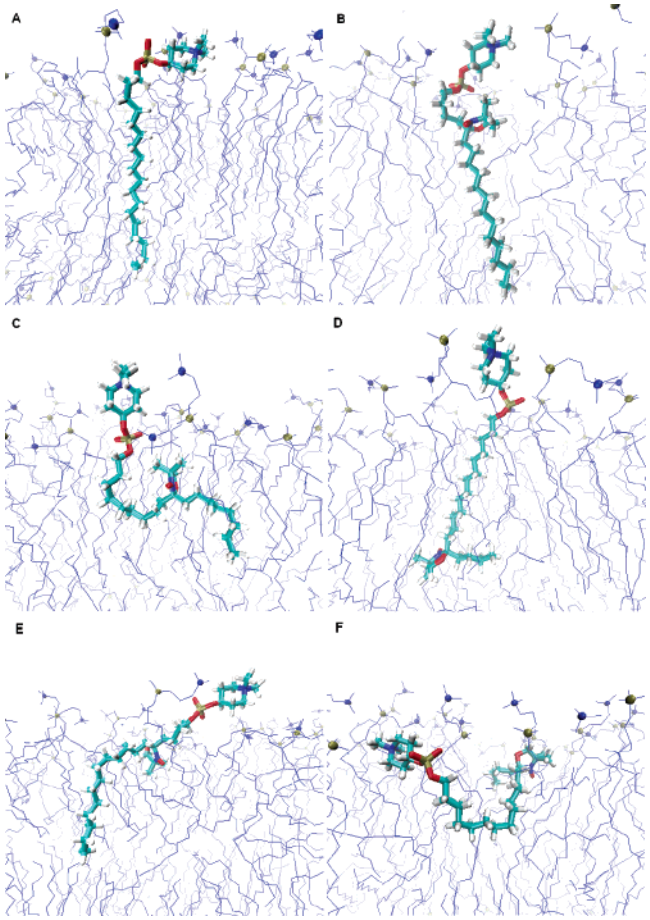


Figure 2. Snapshots from the trajectories for compounds (A) **1**, (B) **2**, (C) **3**, and (D) **4**, all inserted after 1 ns; (E) **2**, after 500 ps; and (F) **4** laid down on the lipid−water interface at the beginning of simulation.

group and the doxyl group (see Figure 2, panel D). The terminal butyl residue is perpendicular to the rest of the chain, which is bent at the carbon bearing the doxyl ring. Movement of the doxyl group in this compound to the bilayer surface was not observed, even after 6 ns of simulation.

In an experiment designed to estimate their ability to insert into the hydrophobic core, compounds **2**, **3**, and **4** were oriented along the lipid−water interface in a fully extended conformation

parallel to the bilayer surface. Compounds **2** and **3** adopt the same position within 600 and 1700 ps as if they were inserted into the bilayer. On the other hand compound **4** needs more than 4 ns to move out of the beginning U-shape (see Figure 2, panel F) and finally occupies the same position as if it were inserted into the lipid bilayer. To study the energetically most favorable orientation of compound **4**, additional experiments were performed starting the simulations from different initial positions. Compound **4** was inserted into the lipid bilayer in an extended conformation under the angles of 30, 45, and 60° relative to the membrane normal. Its polar head group was always in the phospholipid headgroup region. Trends of the movement of the alkyl chain with doxyl were observed in 3 ns simulations. In the 60° simulation, molecule **4** quickly adopts an orientation and conformation similar to what would be observed if it was inserted parallel to the *z*-axis. In the 30 and 45° simulations, molecule **4** tends to reach equilibrium by moving its doxyl group closer to the bilayer midplane. The terminal butyl residue is oriented forward and parallel to the *z*-axis. The hydrophobic effect of the alkyl chain, which is the primary driving force for insertion in the case of compound **2**, seems to be weaker in compounds **3** and **4**. Support for this conclusion is found in the 28-times-higher cmc of compound **3** and the 46-times-higher cmc of compound **4** compared to that of compound **2** determined in a previous study.[3]

In conclusion, MD simulations confirm that the predominant conformations of perifosine analogues in a DPPC bilayer strongly depend on the presence and position of the doxyl group. The position, size, and hydrophilicity of this group perturb the overall lipophilic nature of the compound as well as the anchoring properties of the alkyl chain. Thus, the doxyl group influences the membrane conformation of Sl-APL. Overall, these findings permit a better understanding of the behavior of APLs and their interaction with biological membranes as well as for more realistic EPR membrane spectra interpretation where doxyl spin probes are used. Moreover, they have also more general sense for all labeling techniques (fluorescence and spin-labeling) where a sterically large group bearing a fluorophoric or paramagnetic center is introduced into the parent molecule. In a forthcoming paper, we will compare the results of MD simulations with EPR data on real membrane systems.

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Supporting Information Available: Details of quantum mechanical calculations, empirical force field determination, MD simulations, and analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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