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Effect of the Model -helical Peptides on the Structure of Lipid Bilayers Molecular Dynamics Simulations

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Abstract: The interaction of the model KALP -helical peptides K_2-A_{24}-K_2 (A_{24}), K_2-L_{24}-K_2 (L_{24}), K_2-(LA_{12})-K_2 (LA_{12}), K_2-I_{24}-K_2 (I_{24}), K_2-G-L_{24}-K_2-A (P_{24}) and K_2-V_{24}-K_2 (V_{24}) with lipid bilayers composed of dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC) both in a gel (T = 288 K [DMPC] / 296 K [DPPC]) and in a liquid-crystalline state (T = 310 K / 346 K) has been studied by the molecular dynamics simulation method. It has been shown that in a gel state the peptide causes a disordering effect on lipid bilayer but in the liquid-crystalline state only small change has been monitored. A peptide modifies (orders) lipids around itself to some level, which depends on the type of peptide. Peptides with linear shape of their sidechains (L_{24}, LA_{12}) prefer less ordered lipids than peptides with spherical sidechains (I_{24}, V_{24}).

Keywords: Molecular dynamic simulations, Helical peptides, Model lipid membranes, Phase transitions

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1. Introduction

Lipid-protein interactions are of a fundamental importance for understanding both structural integrity and functions of biological membranes. Among membrane proteins, the integral ones are of special importance due to the wide variety of function they perform in the cells, such are e.g. receptor activity, energy transduction or active transport. Despite extensive experimental and theoretical studies, the knowledge of the mechanisms of protein-lipid interaction is still incomplete (see [1 3] for recent reviews). In order to overcome the problem of the complicated structure of the integral proteins and their isolation and purification, chemically synthesized peptide models of specific regions of natural membrane proteins have been used in biophysical studies of the mechanisms of protein-lipid interactions. Among others the -helical peptide acetyl-K_2-L_{24}-K_2-amide (L_{24}) has been successfully utilized as a model of a hydrophobic transmembrane -helical segments of integral proteins [4]. This peptide contains a long sequence of hydrophobic leucine residues capped at both the N- and C-termini with two positively charged, relatively polar lysine residues. The central polyleucine region of these peptides was designed to form a maximally stable -helix which will partition strongly into the hydrophobic environment of the lipid core,

while the dilysine caps were designed to anchor the ends of these peptides to the polar surface of the bilayer lipid membrane (BLM) and to inhibit the lateral aggregation of these peptides.

Detailed biophysical studies of the interaction of P_{24} or L_{24} [4, 5] or WALP peptides [6] with BLM revealed the fact that the incorporation of these peptides into the phosphatidylcholine bilayers resulted in the decrease of the ordering of the bilayer in a gel state and the increase of the ordering in a liquid crystalline (LC) state. Our recent studies performed by means of precise densitometry and ultrasound velocimetry methods [7, 8] showed that L_{24} peptide induced complex effect on lipid bilayer of various thickness. Further information about the structure and dynamics of lipid bilayer as well as the molecular mechanisms of protein-lipid interactions can be obtained by use of molecular dynamics simulations (MD). MD method is widely used for the study of mechanisms of protein-lipid interactions [9 14]. In this work we have applied the MD method on the systems composed of A_{24}, L_{24}, LA_{12}, I_{24}, P_{24} and V_{24} peptides and phospholipid bilayer (DMPC, DPPC) both in the gel and in the liquid-crystalline state.

2. Method of molecular dynamics simulations

We have used molecular dynamics simulations for the determination of the changes of lipid bilayer caused by insertion of peptide. The presented simulations were done in periodic box using the Gromacs software [15] using the Gromos87 [16] forcefield with corrections [17,18]. The initial models of transmembrane -helix peptides have been generated by use of HyperChem [19]. Dr. Tieleman's equilibrated DMPC and DPPC bilayers with 128 molecules and 3655 molecules of water in L liquid-crystal state have been used [20] as the model bilayers. For simulations with membrane in L' gel state, we created the membranes on the basis of the experimental data (area per lipid and thickness) [3, 21]. Initial structures were solvated with SPC water (4764 molecules for DMPC and 4784 for DPPC) energetically minimized and run MD simulation for over 20 ns until the membrane were stabilized with parameters closely to the experimental values. A cylindrical hole has been created in a center of the bilayer by removing 4 lipids whose atoms occur within 0.23 nm of the central axis of the cylinder. The peptide was then inserted into the cavity. The resulting system (peptide, 124 PC molecules, 4 chlorine ions and water) has totally over 16 000 atoms for LC and over 20 000 for gel membrane. The system has been energetically minimized and equilibrated during the time of 0.5 ns while the peptides atoms were fixed. Then the molecular dynamics (MD) simulation took place at least for 40 at temperatures T = 288 K and 310 K (below and over the phase transition) for DMPC and 296 K and 346 K for DPPC bilayer. Molecular dynamics simulations were performed with constant the pressure of 1 bar, constant temperatures and with the time step of 2 fs. The LINCS algorithm has been used to constrain covalent bond lengths. The used conditions were similar to that reported by Berger et al. [22].

Trajectories were analyzed from the last 5 ns of the simulations by programs available from Gromacs package.

3. Results and discussion

The behavior of the peptides in DMPC bilayers has been studied in a gel and in a liquid-crystalline state. The hydrophobic length of pure gel bilayers at the end of the simulation was 3.6–3.62 nm for DPPC and 3.26–3.27 nm for DMPC. The area per lipids had a value of 0.4733 nm^2/lipid for DPPC and 0.4676 nm^2/lipid for DMPC membrane. These values are little lower than 0.479 nm^2/lipid (DPPC) and 0.472 nm^2/lipid (DMPC) [21, 23], but also in published work have the simulated membranes a lower area per lipid than experimental values [24, 25]. The hydrophobic length of bilayers in the LC state gained from Tieleman's web site are 2.97 nm for DPPC and 2.77 for DMPC and the area per lipid is 0.629 nm^2/lipid (DPPC) and 0.596 nm^2/lipid (DMPC).

Because the hydrophobic length of the peptide is approx. 3.6 nm (24 aminoacid residues and rise 0.15 nm per residue for ideal -helix), which differs from hydrophobic thickness of the bilayer in a gel (3.44 for DPPC and approx. 3.2 nm for DMPC) and in a liquid-crystalline state (2.85 nm for DPPC and 2.62 nm for DMPC) [5], we have analyzed the geometry of the systems in these two states.

The effect of the peptide on the bilayer has been traced by the evaluation of the following parameters for the lipids: deuterium order parameters, amount of dihedral angles in trans conformation, amount of transitions between trans and gauche states and thickness of the hydrophobic part. All of these parameters were evaluated separately in two slices around the peptide. The lipids were separated by the average distance between the middle C atom of lipids' chains and C of the peptide, each chain was averaged independently during the last 5 ns. The threshold values were 0.8 nm and 1.6 nm, which correspond to the 1st and 2nd layer around the peptide.

The changes of the order parameters are shown on the Fig. 1. In all gel cases the peptide produces a disordering effect on lipids which results in the decreasing of order parameters in the first slice. In case of LC the situation is more complicated. The temperature in DPPC/LC simulation is so high that the peptide stabilizes the membrane in its neighborhood. In the LC state of DMPC the average effect is very small. All of these values are comparable with the results obtained by Tieleman et al. [11] and those obtained in experiments [4–6]. These changes produce more or less the same configuration of the lipids around each type of peptide. The peptide with smaller sidechains (A_{24}) has a smaller effect on the lipids (the difference between values for the gel and LC state is bigger). There are also differences between residues with spheroid (I_{24}, V_{24}) and spike (L_{24}, LA_{12}) sidechains. The spike shape produces a more disordered environment (lower values of order parameters). The amount of the dihedral angles in the trans conformation (Tab. 1) depends on the state of the membrane (and temperature) – the lower value for LC and bigger in the gel state (72%). One can conclude that there is an increasing formation of kinks in the surroundings of the peptide, which shortens the hydrophobic chains. The smallest differences between slices occur in the case of V_{24} and I_{24}. Opposite character exhibits A_{24} with less hydrophobic character of its sidechains. Number of transitions between trans and gauche conformations shows that this parameter depends nearly only on the temperature – at higher temperature the higher number of changes take place. But there is small effect from the peptide - the effect of lowering the number of transitions for I_{24} and V_{24}. These correlate with the higher membrane ordering in presence of I_{24} and V_{24}.

Table 1. Amount of dihedral angles in trans conformation.

System	% of trans dihedral angles; 1st slice	% of trans dihedral angles; 2nd slice	System	% of trans dihedral angles; 1st slice	% of trans dihedral angles; 2nd slice
A_{24}&DMPC/gel	82.45 ± 3.51	86.04 ± 1.82	A_{24}&DPPC/gel	81.68 ± 4.18	88.64 ± 3.50
L_{24}&DMPC/gel	82.53 ± 1.07	84.10 ± 2.34	L_{24}&DPPC/gel	84.64 ± 2.83	90.61 ± 2.20
LA_{12}&DMPC/gel	80.58 ± 3.53	83.58 ± 3.33	LA_{12}&DPPC/gel	85.23 ± 1.90	90.73 ± 2.48
I_{24}&DMPC/gel	86.22 ± 2.73	85.72 ± 2.78	I_{24}&DPPC/gel	85.20 ± 1.98	87.15 ± 2.96
P_{24}&DMPC/gel	84.37 ± 4.71	87.48 ± 2.43	P_{24}&DPPC/gel	83.25 ± 2.57	88.57 ± 2.09
V_{24}&DMPC/gel	85.22 ± 2.48	87.17 ± 1.86	V_{24}&DPPC/gel	84.13 ± 2.01	88.58 ± 2.41
A_{24}&DMPC/LC	74.23 ± 2.48	75.61 ± 1.43	A_{24}&DPPC/LC	72.30 ± 0.82	72.54 ± 1.27
L_{24}&DMPC/LC	77.06 ± 2.01	77.81 ± 1.89	L_{24}&DPPC/LC	73.05 ± 2.27	72.61 ± 1.05
LA_{12}&DMPC/LC	75.20 ± 1.36	76.39 ± 2.31	LA_{12}&DPPC/LC	73.37 ± 1.13	72.08 ± 1.59
I_{24}&DMPC/LC	76.45 ± 2.67	76.01 ± 1.31	I_{24}&DPPC/LC	74.47 ± 2.18	73.76 ± 1.31
P_{24}&DMPC/LC	76.65 ± 1.80	76.53 ± 1.71	P_{24}&DPPC/LC	71.14 ± 1.08	72.65 ± 1.60
V_{24}&DMPC/LC	75.89 ± 2.02	75.97 ± 1.28	V_{24}&DPPC/LC	72.92 ± 1.25	72.86 ± 1.46

The thickness of membrane is the closest parameter to the effective length of the peptide hydrophobic part from the perspective of the "matching theory". The thickness of hydrophobic part of a pure membrane is: 3.6 for DPPC/gel, 3.2 nm for DMPC/gel, 3.0 nm for DPPC/LC and 2.6–2.7 nm for DMPC/LC. In all cases the values in the 2nd slice (compared with the 1st slice) are closer to the pure membrane – averages: 3.58 nm for DPPC/gel, 2.93 nm for DPPC/LC, 3.12 nm for DMPC/gel and 2.62 nm for DMPC/LC. The lipids closest to the peptide (1st slice) produce even thinner conformations – in most cases the membrane is by 0.3 nm thinner than effective length of peptide (only for DPPC/LC this difference is around 0.8–1.4 nm; with exception of LA_{12} – this peptide is characterized by different configuration). Because Lys sidechains are relatively long, it is possible to submerge nearly whole peptide into the membrane and the charged ends of these Lys sidechains can still be in the polar headgroups region. In this case the peptide tilt is bigger (according to the simulation results) and its effective length is smaller and therefore the membrane became thinner. But the average membrane thickness does not contain direct information on the orientation of individual lipid chains. Lipid chains can still be longer even in the LC state (higher order parameters, more trans positions of dihedral angles), because they can tilt in the same way like the peptide.

Tieleman et al. [20] performed 2 ns MD simulation of -helix with long hydrophobic segments (Flu_{26} and Flu_{34}) in POPC bilayers. They observed considerable extension of the membrane thickness around Flu_{26} peptide and declination by 10 . At the same time, they did not observe extension of the thickness for the peptide Flu_{34} with longer hydrophobic length, but the peptide molecules declined by 25 . As summarized by Killian [26] from experimental and simulation data, there is the change of the membrane thickness near the protein in systems with WALP protein and only a small tilt is created. However the KALP

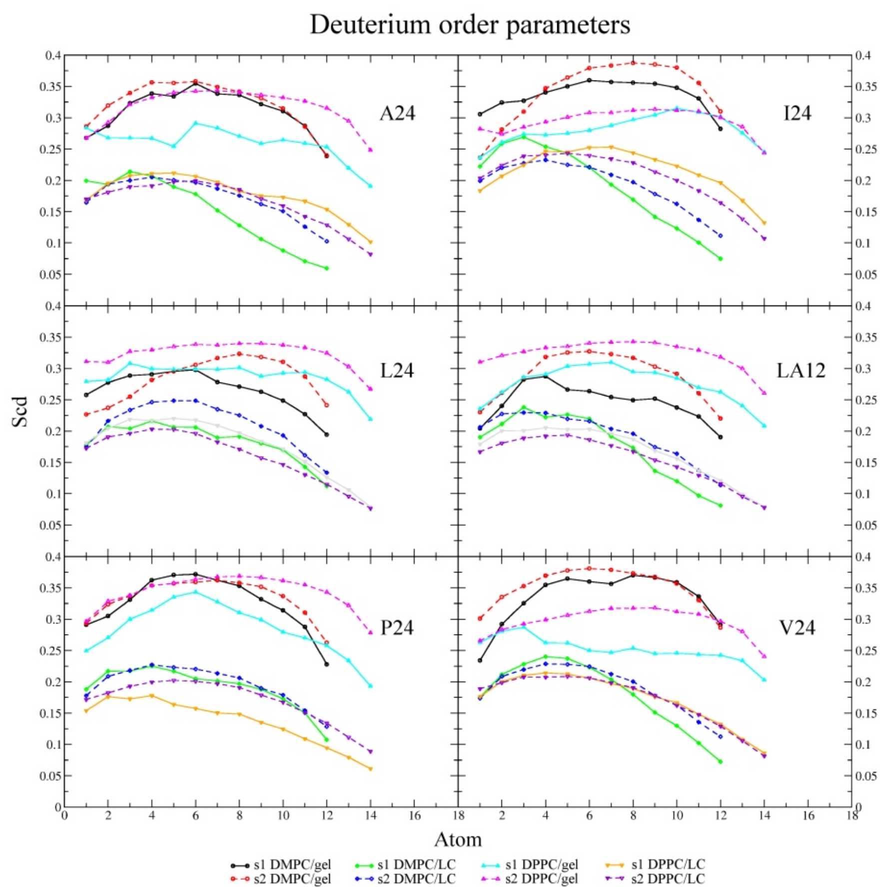

Fig. 1. Order parameters from the last 5 ns of the simulations of peptides and four types of lipids; cumulated by inserted peptide. Lipids are separated into 2 slices around the peptide: nearer to the peptide and more influenced *s1* and farther *s2*. Each peptide type prefers different lipid conformations in its surrounding - the more linear shape of the sidechain, the lower values of order parameters (more disordered lipids).

(like here presented L_{24}, LA_{12}, etc.) do not change the membrane thickness so extensively and rather increases the peptide's tilt. Petrache et al. [10] also discussed possible drawback of the molecular dynamics simulations. First, there are problems with the rather short time of the simulations restricted by several ns. At the same time, they received similar results with shorter – around 5 ns and longer – around 10 ns simulations. However, it should be noted that the characteristic time of relaxation of phospholipid dipole moments following disturbing of the membrane by voltage jump lies between micro- to mili- second scale. Longer time probably corresponds to the collective movement of lipid clusters [27]. Incorporation of the short peptide influences this relaxation time significantly [28]. We can therefore expect that the relaxation time of the short peptides, like L_{24}, should be comparable or even larger then that for phospholipids. Therefore, in order to receive equilibrium

state of the peptide in a membrane, the simulations should last in order of microseconds. However, this is beyond the possibilities of current computational technologies.

However, despite of large number of limitations the MD simulations represent a useful approach for the study of fast conformational movements of the peptide and phospholipids in a membrane. We are, however not sure whether the model system reached the equilibrium or not. But results obtained by MD simulation are consistent with the experimental results, in respect of inducing hydrophobic mismatch positive and disordering effect of peptide on the membrane in a gel state.

In the liquid-crystalline state the peptides induced ordering effect in DPPC bilayers (although the differences are small due to the high temperature), which agrees with the ^{2}H NMR studies of related systems [5, 6]. In the case of DMPC only small disordering effect occurs – probably due to the bigger hole for peptide created on the beginning. This idea is supported by the fact that the A_{24} peptide has the biggest disordering effect, which is, however still much smaller than in the case of a gel state.

We showed that short peptides depending on the amino acid composition have a different effect on the neighboring lipids and prefer some type of its orientation – different values of order parameters. Beside the size of the residue's sidechains (A_{24}) also the peptide shape – more spheroid (V_{24}, I_{24}) or linear (L_{24}, LA_{12}) plays a significant role. But due to limited data (only 5 types sidechains were simulated) more general conclusion requires further extensive studies.

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