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STUDY OF THE ELECTROSTATIC INTERACTIONS BETWEEN PEPTIDES AND LIPIDS AT THE MEMBRANE INTERFACE BY MOLECULAR DYNAMICS SIMULATION

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Charged peptides penetration into a membrane interface is analyzed using molecular dynamics simulation. Peptide having a single charge or two positive charges were presented to an uncharged membrane (a phosphatidyl-choline lipid, POPC) or to a negatively charged membrane (POPC plus a phosphatidyl-serine lipid, POPS). As expected equilibrium peptide penetration into the membrane interface critically depends on both the peptide and membrane charge. Penetration only occurs when the interface is negatively charged and is associated to a well defined transient change in the orientation of the dipole moment of the closest POPS polar heads emphasizing the importance of the very local electrostatic interactions when considering peptide-lipid interactions.

INTRODUCTION

The basic structure of the biological membrane is provided by a lipid bilayer. The lipid bilayer serves as a relatively impermeable barrier to the passage of most water-soluble molecules and it contains a diversity of lipids which firstly differ in nature of their polar headgroups. Transmembrane proteins, on the other hand, are dissolved in the lipid bilayer and mediate transport of specific molecules, function as enzymes, form structural links of plasma membrane to the cytoskeleton or other extracellular matrices, mediate energy conversion in the cell, and are important receptors for receiving and transducing chemical signals. Protein-lipid interactions are critical for the above mentioned functions of the membrane. However, there is relatively little detailed experimental information how proteins and lipids are interacting on a molecular level and relatively little is known about the specificity of these interactions, and the molecular forces involved. Nonspecific binding of proteins and peptides to charged membrane surface depends upon the combined contributions of hydrophobic and electrostatic free energies.

Electrostatic phenomena play a crucial role in many biological processes. For membranes they are significant because the electrostatic interactions can lead to an accumulation of charged species near the membrane surface which than acts as a catalytic site. It was experimentally proved that many peripheral proteins (cytochrom c, myelin basic protein, phospholipases, HIV matrix protein) use electrostatic interactions to bind to biological membranes. These proteins contain clusters of basic residues that interact with the acidic lipids in membranes and the binding of proteins depends only weakly on the chemical nature of either the basic residues or acidic lipids.[1] Another study[2] shows that anionic lipids stimulate Sec-independent insertion of a membrane protein and that mechanically driven patterning of proteins can electrostatically generate spatial patterns in the distribution of charged lipids. Ege and Lee[3] have also shown that electrostatics plays a major role in determining the level of insertion of Alzheimer's amyloid beta peptide into membranes.

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There are in the literature many models for the description of charged membrane behavior but the membrane surface is so complex that no universal theory of membrane electrostatics exists to date. Acceptable working models for different specific situations can be found, however under simplifying assumptions. One of these is the Gouy-Chapman (GC) model.[4] It considers that all structural membrane charges are confined to an infinitely narrow plane and that ion distributions are governed solely by Coulombic forces. There are in the literature some papers which reveal theoretical studies in the field of electrostatic interactions between peptides and cell membrane,[5-11] They use the GC model to treat the electrostatic interactions. They describe both peptide and membrane in atomic detail assuming that the peptides interact with a planar surface and that the structure of neither the peptide nor the lipid bilayer change upon binding. The peptide is placed at a given distance and orientation to the membrane. The electrostatic potential is determined by solving the Poisson-Boltzmann equation. The results are good from the qualitative point of view as far as peptide approach to the membrane surface is concerned but there is a discrepancy about 30% between the theoretical results and the experimental values of the binding constants and the Gibbs free energy of binding as binding implies peptide penetration into the membrane polar interface. There are many possible reasons for this discrepancy. When we study this kind of interactions we must consider the complexity of the membrane interface, the thermodynamics contributions of the bilayer itself and various electrostatics-based effects related to changes in peptide and lipids solvation and changes in dielectric constant.[11] Peptide-lipid interactions within the membrane interface must then be described from a microscopic stand point and molecular simulation is an essential tool to obtain such a description.

RESULTS AND DISCUSSION

In the present study we use simple dipeptides and charged or uncharged membrane interfaces to present a preliminary description of the phenomena involved in the penetration process of peptides into the membrane interface. In our studies we do fix neither the peptide nor the lipids and we determine the electrostatic energy of interaction between a peptide and bilayer using the simulation of the molecular dynamics technique. For this purpose we have build three different systems:

System I contains 25+25 palmitoyl-oleoyl-phosphatidyl-choline (POPC) bilayer hydrated with 2523 water molecules, the dipeptide GLUG-ARG (protonated glutamate and arginine) and a chloride ion for the neutrality of the system. The system contains 14341 atoms, a basic residue (arginine) and uncharged lipids.

System II contains 25 POPC lipids on the bottom layer, 21 POPC and 4 POPS (palmitoyl-oleoyl-phosphatidyl-serine) on the top layer, 2523 water molecules, the dipeptide GLUG-ARG and three sodium ions. The system contains 14230 atoms, 4 acidic lipids and a basic residue (arginine).

System III contains 25 POPC lipids on the bottom layer, 21 POPC and 4 POPS on the top layer, 2528 water molecules, the dipeptide arginine-arginine ARG-ARG and two sodium ions. The system contains 14211 atoms, 4 acidic lipids and two basic residues (two arginine residues).

For both systems I and II we use a protonated glutamate (GLUG) instead of GLU in order to keep the carboxylic group partially charged and susceptible to form hydrogen bonds. Indeed, in case of negatively charged interface the pH is between 1 and 3 units lower than the aqueous phase pH (it depends on the charge density and dielectric constant variation). So pH at interface may be lower than 4 which is the pK value of GLU carboxylic group and in these conditions the glutamate becomes protonated.

For each system under consideration we have determined the distance between the center of gravity of the peptide and the mean plane of lipid glycerol groups on the top layer. The center of gravity of peptide is obtained using the Cartesian coordinates of every atom within the peptide and its mass using the following equation:

$$\vec{r}_{MC} = \frac{\sum_i m_i \vec{r}_i}{\sum_i m_i} \tag{1}$$

where $\vec{r}_i$ is the position vector of each atom in the X ray coordinate system, m_i is the mass of the atom i and the sum is taken over all the atoms of the peptide.

The mean plane of lipid glycerol group is determined as the mean value of z coordinate of the C2 atoms of all lipids for a temporal configuration of the bilayer. The equation of the plan of C atom of glycerol for a lipid is:

$$Z = a.x + b.y + c \tag{2}$$

where a, b and c are the plane coefficients and they are given by the equations:

$$a = \frac{x.y}{\sqrt{(x.y)^2 + (y.z)^2 + 1}}$$
$$b = \frac{y.z}{\sqrt{(x.y)^2 + (y.z)^2 + 1}} \tag{3}$$
$$c = \frac{z}{\sqrt{(x.y)^2 + (y.z)^2 + 1}}$$

where x, y and z are the Cartesian coordinates of C2 atom from glycerol group of each lipid.

Then we compute the distance between the center of mass of peptide and this mean plane. All these computations are made using a program written in C++ language by the authors. The variation of this distance in time for each system is presented in the Fig. 1.

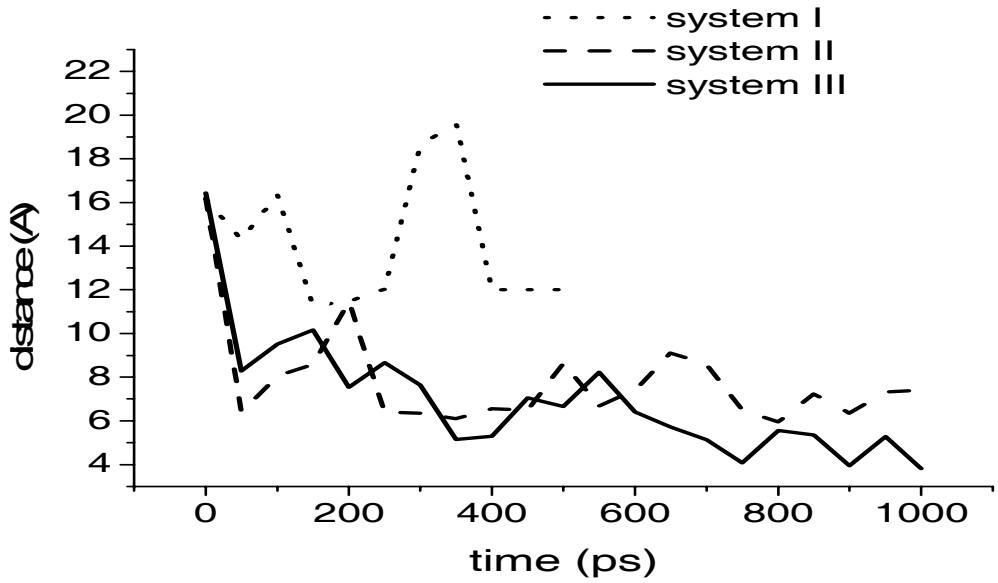

Fig. 1 – The variation in time of the distance between the center of gravity of the peptide and the mean plan of lipid glycerol groups on the top layer: dotted line corresponds to system I, dashed line corresponds to system II, solid line corresponds to system III

We notice that for system I, where we have only uncharged lipids, even if the peptide contains a basic residue, it moves away from the plan of lipids, there is not an evident electrostatic attraction between the peptide and the bilayer (dotted line in the Fig. 1). For system II we noticed that the peptide became closer to the bilayer, but it did not associate with it within 1 ns (the measured distance is always higher than 5 Å, dashed line in the Fig. 1). For system III, where we have two basic residues, the distance between the peptide and the bilayer is much smaller (around 3 Å after 800 ps) and the association is made (solid line in the Fig. 1). The association of peptide to bilayer in the system III is also proved by hydrogen bonds.

The energy of electrostatic interactions between the peptide and the bilayer versus distance is presented in the Fig. 2 for the systems II and III respectively. The system I is not considered here because the peptide remains far away from the bilayer. As it contains only one charged residue (ARG) and the bilayer does not contain charged lipids, the electrostatic energy is neglected. Anyway, in this case there is not an electrostatic contribution which contributes to the peptide insertion. From the Fig. 2 we notice that the energy of electrostatic attraction is higher for the system containing two basic residues and that the dependence of energy on distance is not linear.

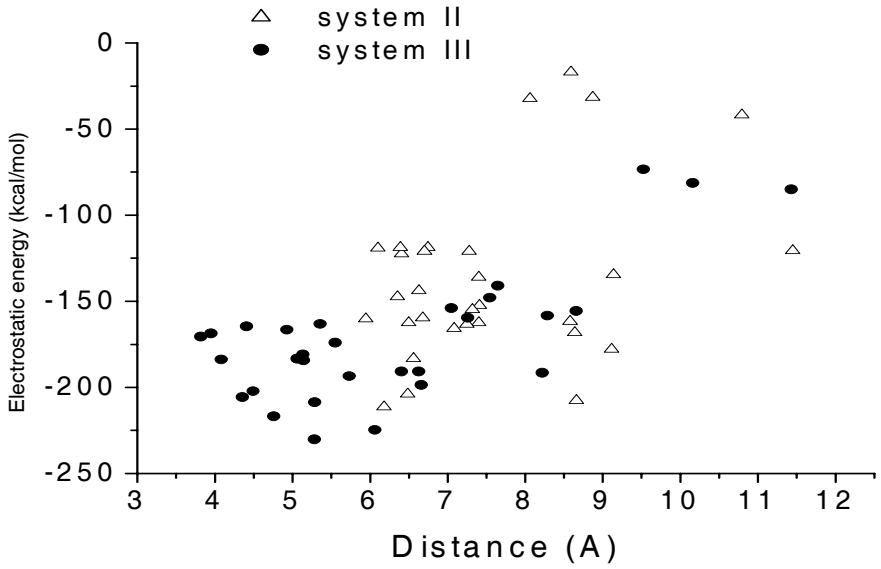

Fig. 2 – The energy of electrostatic interactions between the peptide and top layer versus distance

We have also checked the orientation of the P1N vectors (vector which binds the phosphor atom of the phosphatidyl group to the nitrogen atom of the choline or serine head group) relative to normal membrane surface during the simulation for the lipids considered to be involved in the interaction with peptide. By measuring the distance between the center of gravity of peptide and the center of gravity of lipid heads on top layer we have noticed that POPC 18 and POPS 4 could be involved in the interaction. The orientations of P1N vectors of POPC 18 and POPS 4 for both systems II and III are presented in the Fig. 3. We notice that for POPC 18 this vector orientation does not vary considerably during simulation for both systems (dashed and dotted lines in the Fig. 3).

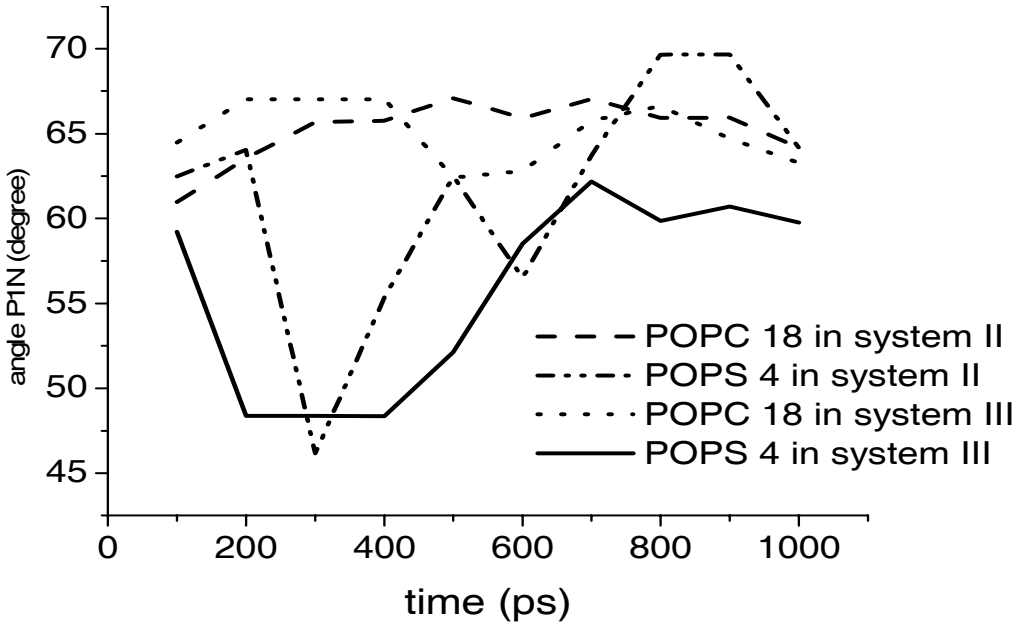

Fig. 3 – The P1N vector orientation relative to normal membrane surface during simulation for POPC 18 and POPS 4 in system II (dashed and dot dashed lines) and system III (dotted and solid lines)

In the case of POPS 4 we notice considerable variations of P1N vector orientation which means a reorientation of the acidic lipid head upon the interaction with the peptide (dot dashed and solid lines in the Fig. 3). The numerical correspondence of the P1N angle is the following:

A value of 0^o for this angle means a perpendicular P1N vector to the membrane surface that points to the exterior of membrane;

A value of 90° for this angle means a parallel P1N vector to the membrane surface;

A value of 180° for this angle means a perpendicular P1N vector to the membrane surface that points to the interior of membrane.

METHOD

We use in this study the molecular dynamics simulation technique.[12] The steps used for every system under consideration are the following:

After building, each system was minimized in 1000 steps of steepest descent of 1 ps each;

Each system was heated at 300 K at a rate of 10 K/ps and equilibrated in 100 steps of 1 ps each.

The 500 ps of molecular dynamics simulations for the system I and 1 ns for the systems II and III were performed.

Molecular dynamics simulations were carried out using periodic boundary conditions with constant volume and temperature and we have used CHARMM27b4 force field.[12] This force field considers the electrostatic attraction or repulsion between two charges described by Coulomb's law:

$$V_{ij} = \frac{q_i q_j}{4\pi\varepsilon_o \varepsilon_r r_{ij}} \tag{4}$$

where q_i and q_j are the atoms' partial charges, r_{ij} is the distance separating the atoms' centers, ε_0 is the permittivity of free space, and ε_r is the relative dielectric coefficient of the medium between the charges. Electrostatic potential is computed using the partial atomic charges provided in topology file of CHARMM. The distance between the atoms is computed from their Cartesian coordinates. Simulations were run with a 1-fs time step without SHAKE, the nonbonded cutoff was 14 Å using a force switching function for electrostatics and a potential shifting for van der Waals interactions and the dielectric constant was set to 1. At the beginning of every simulation, we have placed the peptide outside of the interface of the bilayer in the region of bulk-water density.

CONCLUSIONS

Peptide-membrane interactions are important for understanding the binding, partitioning and folding of membrane proteins. Detailed investigations of peptide-bilayer binding can provide insight into vital biological phenomena such as protein-membrane interactions, channel formation and nonspecific transport of molecules across cellular interfaces.

As we already have mentioned, the initial location of the peptide in every system was chosen as to obtain a spontaneous peptide-bilayer complex that was relatively independent of the starting conditions. So, at the beginning of simulation the distance between the center of gravity of peptide and the mean plane of lipids is high enough to allow the peptide to adopt a natural structure and orientation at the interface. The peptide fells a little influence from the bilayer initially, the measured distance being more than 10 Å (see Fig. 1) and the electrostatic energy of interactions is small (see Fig. 2). For the system I this situation persists for the entire simulation and peptide penetration is not expected to occur. On the contrary for the systems II and III penetration does occur. However the average penetration level is different: about 7 Å for the system II (one positive charge on the peptide) and less than 4 Å for the system III (two positive charges on the peptide). The peptide-interface interactions are essentially repulsive: peptide desolvation, electrostatic repulsion independent of the charge sign due to the dielectric constant transition, change in membrane surface due to peptide insertion, different additional entropic effects (see ref. 5-6). The only force that drives the peptide onto the membrane interface remains the charge-charge or charge dipole attraction between the positively charged peptide and the negatively charged lipid polar head. The hydrophobic effect that helps maintaining the peptide in the membrane negligibly acts in our case. The electrostatic effect is clearly observed in our simulation by comparing the peptide equilibrium insertion (Fig. 1) and electrostatic energy (Fig. 2) in the same interface for the singly charged peptide (system II) and the doubly charged peptide (system III).

It is also possible that this interaction depends on the chemical nature of the basic residue. This result is in good agreement with those revealed in other published papers.[9,10,16] Ben-Tal[9] and Aliste and coworkers[10] underline in their simulation studies the importance of electrostatic interactions for peptide insertion into lipid head interface. They show that the insertion depends on the number of charged residue. In the study made by Aliste and coworkers,[10] the bilayer is immobile and we consider of great importance to take into

account the mobility of bilayer for peptide insertion. In our study we notice that the insertion occurs after 400-600 ps. The noise is too large to detect a dynamical difference between the two peptides. The association of sandostatin to a POPC bilayer was noticed to occur much slower, in 2 ns[16] and the distance between the mean plane of peptide and the bilayer is smaller. The sandostatin peptide contains a positive charged residue as our peptides in the system I and II but this charged residue is lysine. It also contains a tryptophan and two phenylalanine residues which have hydrophobic interactions with bilayer[16] and the association is considered to be due to hydrophobic interactions not to electrostatic ones. We also must take into account the preference of various residues for the interface and that our peptide contains residues that strongly prefer the aqueous phase over the membrane interior. It would be interesting in our peptide to replace arginine residue by lysine to explore the difference between this two residues with regard to their ability to form hydrogen bond networks. It would also important to add a hydrophobic or aromatic residue to explore in more detail the influence of the hydrophobic effect.

It is now important to comment on the response of the lipid polar heads to the penetration of the peptide into the interface. This was followed by the orientational change in the P1N polar head dipoles (Fig. 3). There is obviously no response from the choline head group of POPC: the values of P1N angle remain in the same range, 62^0-67^0 and it means that the POPC head remains close to the membrane surface, its orientation is not affected by the peptide movement to the membrane surface. On the contrary there is a large amplitude (from 65° to 45°) and rapid (200-300 ps) change of the POPS head group orientation only in the close proximity of the arginine residue. The orientational is transient as it occurs only during the early step of the peptide penetration and relaxes after about 600 ps. This constitutes a very local and short range response of the interface that is usually not taken into account in macroscopic and phenomenological calculation. This result is also in good agreement with experimental data which show that the dipole moment of lipid head (PO_4^- - N^+) is very sensitive to changes in electric charges at membrane surface[15] and which reveals the important role of interfacial dipoles in peptide-lipid interactions.[16]

As the systems II and III contain also sodium ions in order to keep the neutrality of each system, during the simulations we have noticed a competition between the sodium ions and peptide to interact with POPS (data not shown). This result is also in good agreement with other simulation data which reveal that sodium ions penetrate deep into the ester of the water/lipid interface of a POPS bilayer where they interact with ester carbonyl oxygen atoms.[17]

The agreement between the simulation data reported here and the experimental data or other simulation data published in the literature provides proof that molecular dynamics simulation can reveal accurate, microscopic information on peptide-bilayer association. It could be of interest to perform molecular dynamics simulation studies of other systems containing other types of basic residues and/or with many basic residues and also with different numbers of acidic lipids in order to find a quantitative relationship between the number (and type) of charged residues and the number of charged lipids necessary for peptide association to membranes.

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