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Molecular dynamics simulation of interaction of Melittin and DMPC bilayer: Temperature dependence

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ABSTRACT

The interaction between proteins and membranes has an important role in biological pro-cesses. We have calculated energies of interaction between Melittin and **DMPC** bilayer in different temperatures. We have used the **CHARMM** software for MD simulation under the canonical (N, V, E) ensemble at different temperatures. The computations have shown that water molecules have more penetration into the bilayer around the transition temperature of DMPC bilayer.
Phosalone, malathion and diazinon were analyzed in corn oil using solid phase extraction (SPE) with lanthanum silicate as a new solid sorbent followed by gas chromatography with nitrogen the detector.

Keywords: Melittin, DMPC bilayer, Temperature stability, Molecular Dynamics Simulation

INTRODUCTION

Interaction between cell membranes and proteins is very important and interesting form molecular biology point of view. DMPC is one of the most important phospholipids in cell bilayers. Then, investigation of interaction between peptides and DMPC Bilayer is very important. Melittin which includes 26 residues (1):

Gly^{1}-Ile^{2}-Gly^{3}-Ala^{4}-Val^{5}-Leu^{6}-Lys^{7}-Val^{8}-Leu^{9}-Thr^{10}-Thr^{11}-Gly^{12}-Leu^{13}-Pro^{14}-Ala^{15}-Leu^{16}-Ile^{17}-Ser^{18}-Trp^{19}-Ile^{20}-Lys^{21}-Arg^{22}-Lys^{23}-Arg^{24}-Gln^{25}-Gln^{26}, is the major protein component of the bee venom (Apis mellifera), which is known to cause hemolysis (2-4).
Melittin's secondary structure is well established to be highly α-helical in its crystalline state (5) and may form some type of tetrameric aggregate in high ionic strength aqueous solutions (6-7). Each of peptides, in the core of tetrameric form, consists of an amphipathic curved helix which is included hydrophobic residues. It seems, position and orientation of melittin in phospholipids bilayer is depend on the experiment conditions. Whereas, the NMR structures were determined in detergent Micelles and in nonpolar solvent, in which the protein is present as a monomeric form (8-9). In accord with its amphipathic amino acid sequence, in α-helical form, the helix was oriented parallel to the membrane-solution interface such that the apolar residues are facing the hydrophobic core of the membrane and the polar residues are facing the water bulk phase (10-12). Other investigations, however, give a transbilayer (13-15) form and some of these give both of them (16-17).

In fact melittin, in α-helical form, has two segments: hydrophobic segment (from Gly^{1} to Leu^{13}) and amphiphilic segment (from residue Ala^{15} to Gln^{26}). The proline residue at position 14 is responsible for a bend separating two segments. This residue as well as the polar residues 23-26 at the C-terminus of melittin has been shown to be essential for the lysis activity (19-20, 23-25).

Orientation of melittin in lipidbilayer depends on **P**H, this presents N-terminus protonation condition and actually its interaction with membrane (18). In the present work we report the results of molecular dynamics trajectories for a fully hydrated DMPC bilayer in different temperatures.

METHODS

The microscopic system consist of one melittin monomer (N-terminus unprotonated), 41 DMPC (17 in the upper layer and 24 in the lower layer), and 1888 water molecules, for a total 10935 atoms. Each fully hydrated DMPC monomers were constructed by the HF level of theory and the basis-set, 6-31g (d). Because in fact, the average cross-sectional area of a single DMPC molecule is 64 $A^{\circ 2}$ (21), this corresponds to a total of 24 DMPC molecules (low layer), or to one melittin and 17 DMPC molecules (upper layer). To determine the initial position of each lipid, the DMPC polar heads were first represented by effective spheres with a cross-sectional area of 64 A°. Center of the system was assigned in Z=0 and the phospholipid head groups were constrained at Z=17 A° and Z=-17 A° for the upper and lower layers, respectively. Boundary conditions (cutoff, cuton, cutim) in all steps were constant. Dimensions of the system were chosen as: 48×32×71 $A^{\circ 3}$. Total charge of protein with unprotonated N-terminus was assigned +4e and normal of bilayer was put in Z direction. For illustrating an unlimited planer layer, periodic rectangular boundary conditions were applied. In simulation cell melittin is deeply inserted into the top layer and its axis was assumed roughly parallel to Y axis. The system was then fully hydrated by overlaying preequilibrated water box of the appropriate dimension in X and Y.

The Langevin dynamics was used for this study; in this manner following Langevin equation were applied. The Langevin equation is stochastic differential equation for a Brownian particle given by:

$$m\dot{v} + \partial\Phi(x, t)/\partial x = -\alpha v + \xi(t) \quad (1)$$

Where α is the friction coefficient and $\zeta(t)$ a randomly fluctuating with noise term, with $\langle\zeta(t)\rangle = 0$, and $\langle\zeta(t)\,\zeta(t')\rangle = 2\alpha k_B T\delta(t-t')$. The left-hand side in Eq.1 represents the deterministic, conservation part of particle dynamics, while the right-hand side accounts for the effects of thermal environment. The friction coefficient α and the thermal noise $\zeta(t)$ are connected through the Einstein relation

$$\boldsymbol{D} = k_B\boldsymbol{T}/\alpha \quad (2)$$

where $\boldsymbol{D}$ is the diffusion coefficient.

All of stages were mentioned above, repeated for the five temperatures (266, 276, 296, 316 and 336K). Before every dynamics step, the system was minimized for removing all of bad contacts. After that, 250 Ps was done to reach the system in equilibrium, then the system minimized in during 610 Ps dynamics until making a good correlation coefficient.

RESULTS AND DISCUSSION

According to the investigations before, degree of freedom of acyl chains of lipidbilayers are increased due to increasing in temperature (22). Results illustrate, because of this effect, many different kinds of conformation can be created to stabilize the lipidbilayer and the protein immersed (Fig. 1). In this manner, due to increasing in temperature, there are many possibilities for construction of several Vanderwalss bonds between hydrophobic segments of proteins and phospholipid acyl chains for optimizing of the system. The ensemble for this investigation was (N V E), for each of temperatures. The change of energy of the system goes down when the temperature approaches to 296K, the temperature of phase transition of DMPC bilayer, and Vanderwalss repulsive energy decreased, too (table 1). It is obvious, the system approaches to stability with increase in degree of freedom of acyl chains of each single DMPC molecules, when the system changes from gel phase to liquid crystal phase (Fig. 2).But with more increasing in temperature and degree of freedoms of acyl chains of lipidbilayer monomers, change in energy of the system increased whereas stability of the system decreased.

In this investigation it is obvious that perturbation in acyl chains of core of the lipidbilayer, due to increasing of temperature, increased. In this position, it is obvious from trajectories, penetration of water molecules into the lipidbilayer core, especially in region of Lys^{23}, is increased (Fig. 2). When temperature increased to T_t, perturbation decreased in core of lipidbilayer where hydrophobic segment of melittin is there, and after that increased.

In T_t, the hydrophobic segment approaches to normal of lipidbilayers and, the more approach, the energy of the system more minimized (Fig. 3). When temperature goes up from 266 K to 336 K, the curve passes through a minimum around 276 K (when acyl side chains of DMPC monomers achieve more degree of freedom and changes from totally trans form to partially gush).

Then as temperature increased the curve passes through another minimum about 296 K (when the system riches T_t). Since a biological system works better around normal temperature (298 K), this minimum has the most important role in this diagram. And then with increasing in temperature stability of the system decreased (Fig.3). These changes come from tow kind of energies, *Electrostatic* and *Vanderwalss*. As it is overused from (Fig.4), Electrostatic portion has the most important role in changes of stabilization energy of the system. While temperature increased form 266 K to 336 K, Electrostatic energy deeply increased (when polar end of melittin diffuse into polar head groups of DMPC monomers) and at the same time Vanderwalss repulsions between acyl chains of DMPCs increased. With more increase in temperature degree of freedom of acyl chains goes up and while temperature rich around 296 K, system access another stable state.

While amphiphilic segment of melittin diffuses in polar head groups of phospholipids, water molecules diffuse in lipidbilayer system, simultaneously (Fig. 2), and it is especially because of residue Lys^{23}, so at the same time tow water molecules diffuse into the nonpolar core of lipid bilayer.

In this time, increasing in electrostatic energy illustrates a potent evidence for this phenomenon (table 1). This is in an evidence for decreasing in Vanderwalss attractive forces between acyl chains of lipidbilayers in core and hydrophobic segment of melittin (table 1). Then it is obvious that the penetration of water molecules into the phospholipid bilayer system and melittin configuration are depend on temperature and position of residue Lys in position 23.

Interpretation of Ramachandran plots

It is assumed, melittin has a α-helical structure (5). For such structures, Phi and Psi have values about -60 and -40 degree respectively. As it is illustrated in (Fig.5) there are three areas for degrees of Phi and Psi with critical alterations. Some residues like Ala^{4}, Thr^{11}, Gly^{12}, $^{Arg}22$, Lys^{23} and Arg^{24} are in these areas. Perhaps, existence of these areas is depending on Gly and Thr and their inconstancy effect on α-helix structure. It is observed as well as relative increase in temperature the residues from Val^{5} to Thr^{11} and also from Pro^{14} to Arg^{22} which are depend on tow segments of melittin (hydrophobic segment and hydrophilic segment, respectively) have unchanged configurations, it means, these residues are in acceptable regions of Phi and Psi degrees. However, Conformation of melittin, with increasing in temperature, returns to roughly ordered configuration of α-helix, as it is observed in results of Ramachandran plots (Fig.5). While temperature increasing from 276K to 336K, relative percent of residues in acceptable regions of Phi and Psi is decreased (Table 2). There a significant relation, around 316 K, in increasing of attraction forces between penetrated water molecules and hydrophilic segment of melittin. In addition it is obvious that residue Lys^{23} when temperature increased from 276 K to 336 K, transfers from unaccepted region to accepted region of Phi and Psi. This illustrates, while increasing temperature to 296 K, fully helical structure formed around Lys^{23}, so that melittin access a more stable structure.

Table 1. Difference of energies of the system from initial states. All results are in terms of (kcal/mol)

Temperature (K)	ΔE	ΔVanderwalss	ΔElectrostatic
266	-30.83664	11.76545	207.76248
276	-6203.53489	178.6523	-4356.77173
296	-5518.42187	-30.6223	-3363.67711
316	-5029.76783	-37.43318	-2942.16116
336	-190.07075	70.41362	-521.37697

Table 2. Relative abundance of residues of melittin in acceptable regions of Ramachandran plots. Total results of energies are in term of (kcal/mol)

Temperature (K)	276	296	316	336
Rational percent of residues in acceptable regions	69.2	50	61.5	46.1

Fig.1. A sample conformation of Melittin in DMPC bilayer in 296 K.

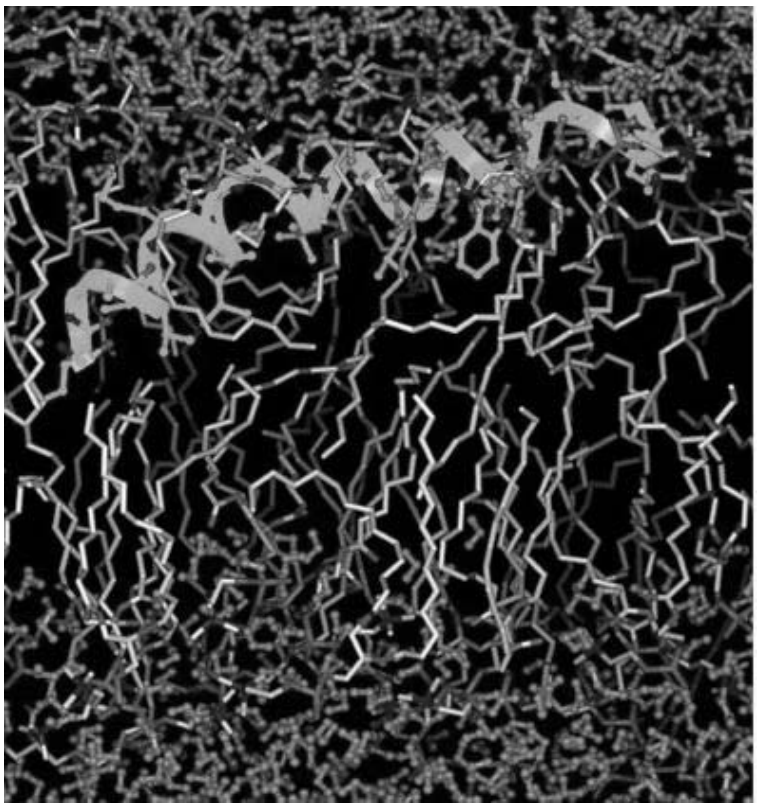


Fig.2. A sample conformation of the system of melittin immersed into DMPC bilayer in 296 K. In this picture melittin is into the top layer.

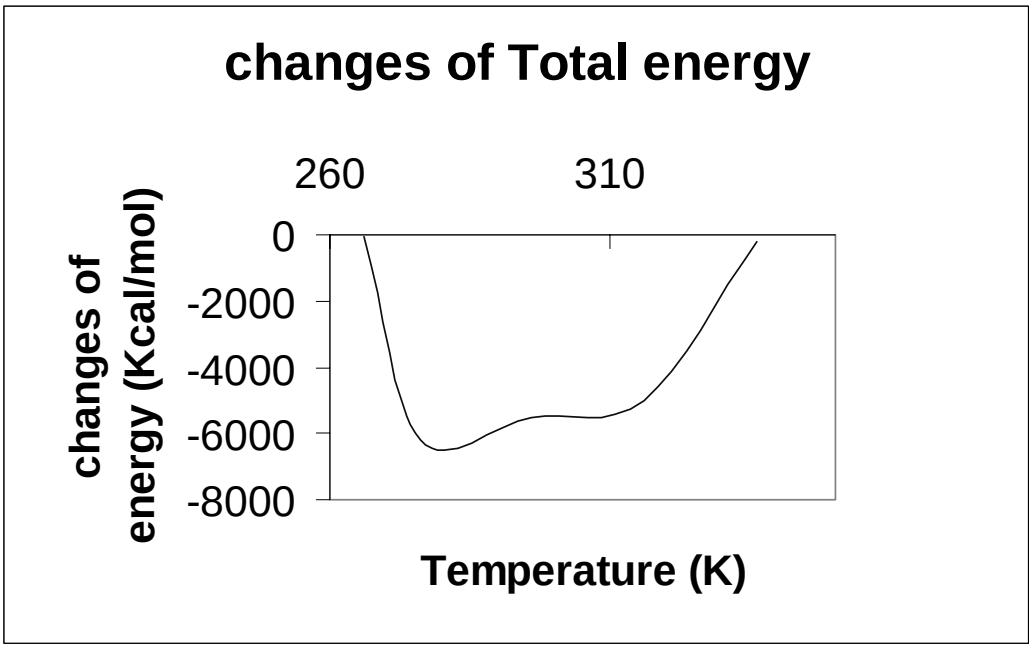


Fig. 3. Changes of total energy of the system of Melittin immersed in DMPC lipid bilayer in different temperatures, A to E is related to 266, 276, 296, 316 and 336 K respectively. Total results of energies are in terms of (kcal/mol).

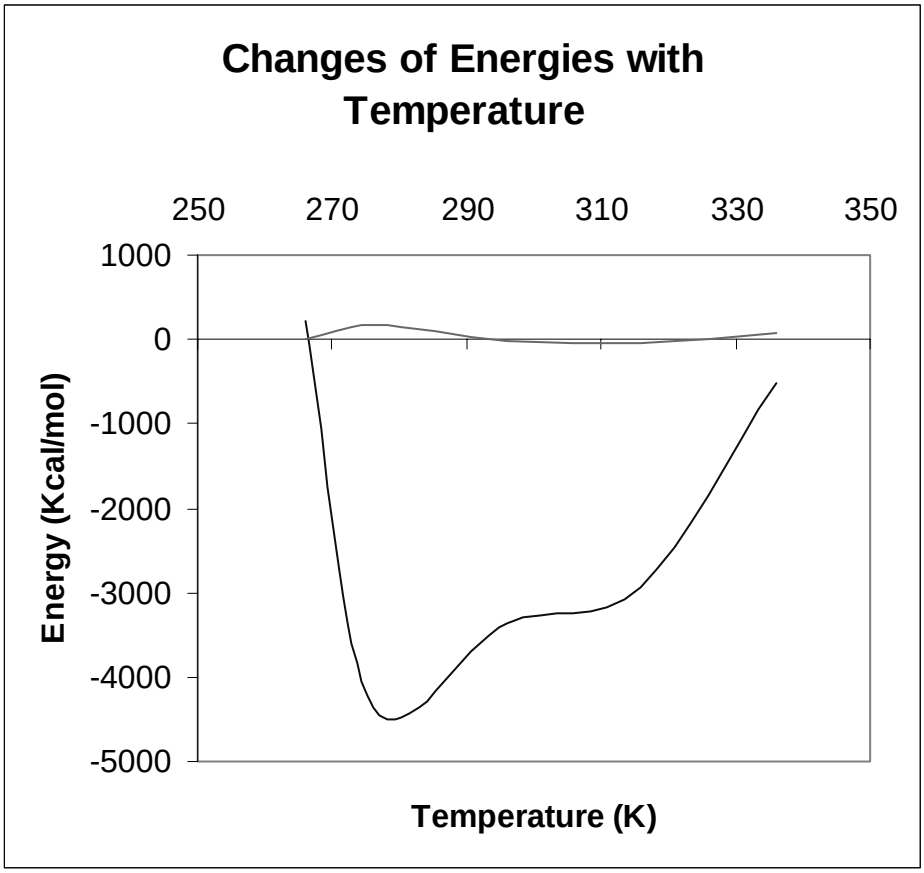
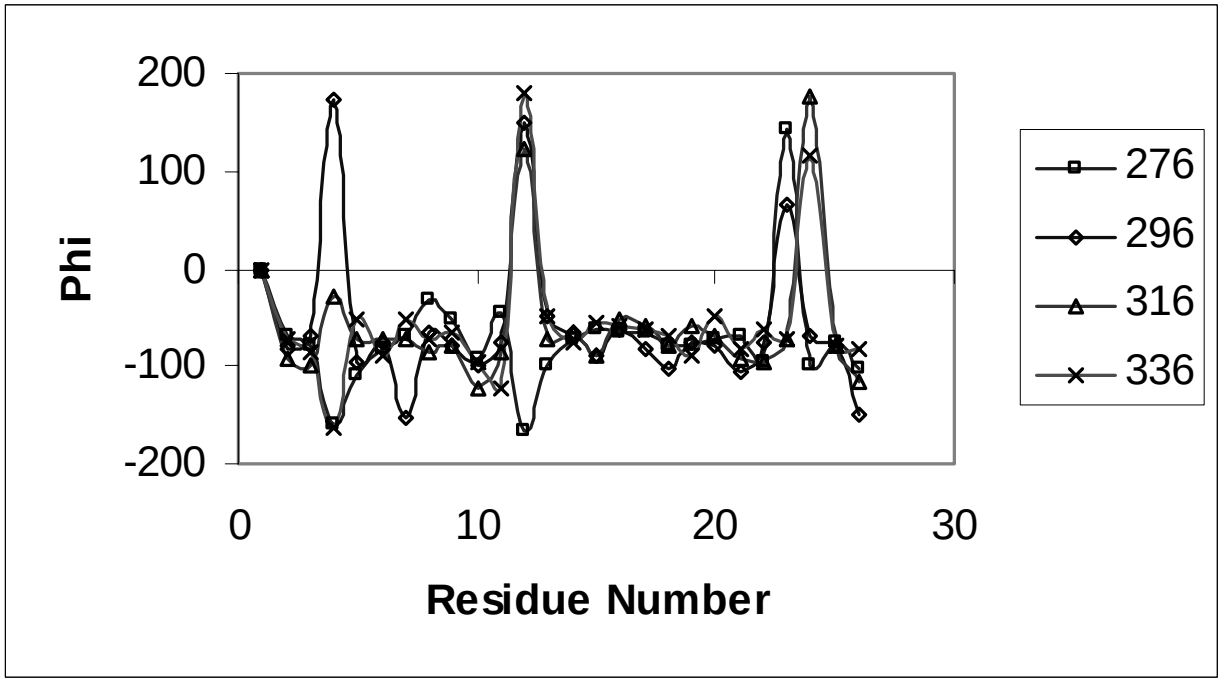


Fig.4. Electrostatic and Vanderwalss portion of energy of the system. Top curve is related to Vanderwalss and below curve is the Electrostatic portion. Total results of energies are in terms of (kcal/mol)



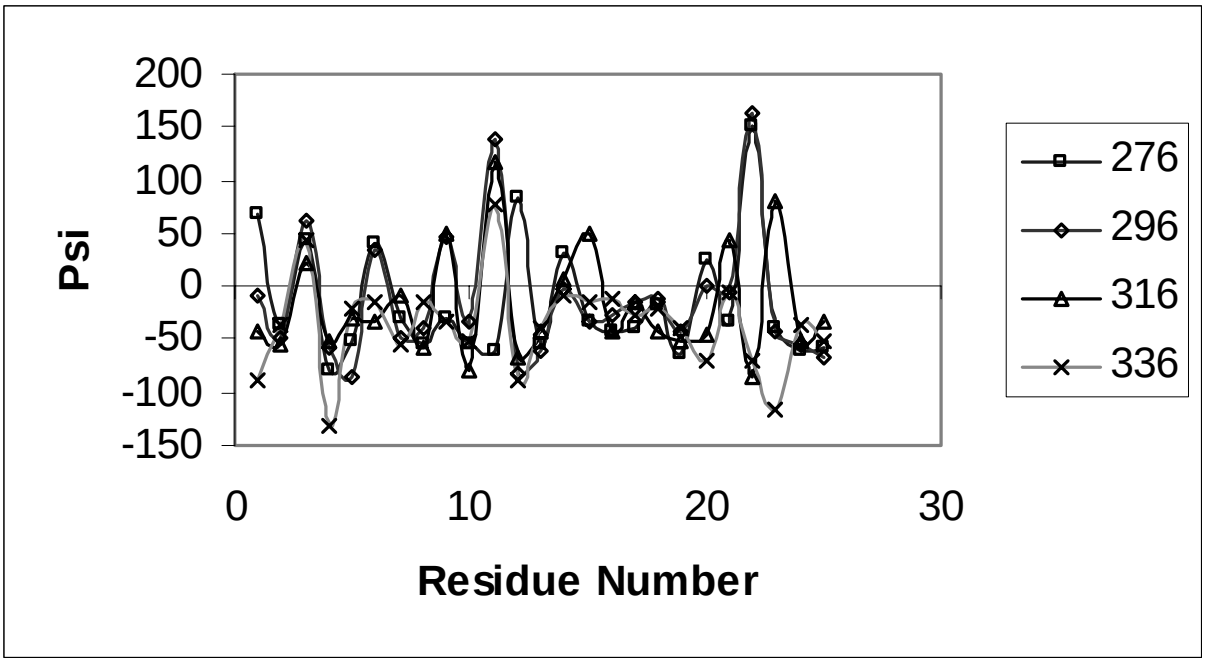


Fig.5. Fluctuations of Phi and Psi angles, accessed from Ramachandran Plots in different temperatures. (A) and (B) are related to Phi and Psi, respectively.

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