J Mol Model (2010) 16:1693–1699
DOI 10.1007/s00894-010-0716-6

ORIGINAL PAPER

Theoretical study of structural changes caused by applying mechanical strain on peptide L_{24}

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Received: 9 November 2009 / Accepted: 27 February 2010 / Published online: 12 May 2010

Abstract The influence of the mechanical strain on the artificial protein L_{24} (acetyl-K_2-L_{24}-K_2-amide) has been studied at the molecular mechanics (MM) level of theory. The effect of the surrounding environment (DPPC molecules) has been observed during the stretching or compressing of the L_{24}. The calculations gave the view on the structural changes occurring during these processes. All calculations were done using the GROMACS code with the ffgmx forcefield enhanced with lipid-protein interaction potentials.

Keywords Ffgmx forcefield · GROMACS · Molecular mechanics · Nanoelasticity · Synthetic transmembrane peptides

Introduction

Proteins are the basic compounds from which life itself is formed. Understanding of elementary processes that take place at the nano-scale has a crucial role in the understanding of their capabilities, such as folding, synthesis or their function itself. Proteins are by no means random statistical molecules. Their function usually involves mechanical movement of several parts while preserving configuration of others. Considering these parts are individual secondary structure sequences, their mechanical properties play a fundamental role in the protein functionality.

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For the purpose of the study of their elasticity properties we have chosen the L_{24} artificial peptide. This peptide belongs to the group of membrane peptides where the lipid-protein interactions are of special importance due to the wide variety of the functions they perform in the cells, such as, *e.g.*, receptor activity, energy transduction or active transport. 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 [1–4]. Among others, the α-helical peptide acetyl-K_2-L_{24}-K_2-amide (L_{24}) (Fig. 1), has been successfully utilized as a model of the hydrophobic transmembrane α-helical segments of integral proteins [5]. 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 this peptide was designed to form an optimal stable α-helix which will incorporate 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 membrane (BLM) and to inhibit the lateral aggregation of these peptides. Detailed biophysical studies of the interaction of P_{24} or L_{24} [5, 6] or WALP peptides [7] with BLM revealed the fact that incorporation of these peptides into the phosphatidylcholine bilayers resulted in the decrease of the ordering of the bilayer in a gel state and increase of the ordering in a liquid crystalline state.

In our work we studied how the secondary structure of L_{24} would be resistant to applied force, what processes occur during the stretching (compressing) and how thesurrounding environment affects all of this. This kind of study

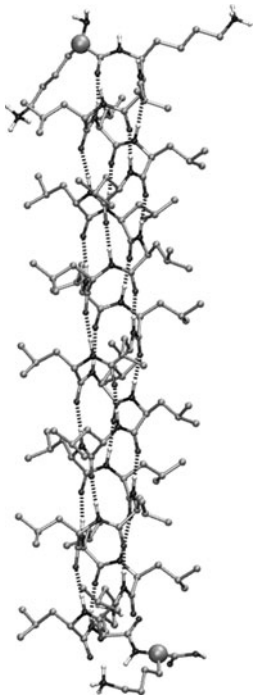

Fig. 1 L_{24} initial equilibrated geometry. Two emphasized carbons are those being fixed in simulations, which simulate applying force

may provide basic data for designing or validating parameters of the coarse grain approach to protein simulations, which would take secondary structure sequences as its fundamental elements. This may eventually lead to whole protein simulations on a sufficient timescale (microseconds-seconds). These simulations are required

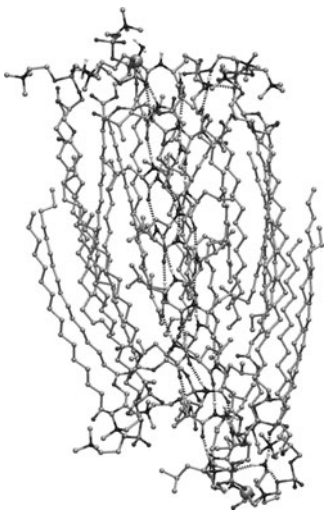

Fig. 2 L_{24} initial equilibrated geometry with surrounding 12 DPPC molecules. Two emphasized carbons are those being fixed in simulations, which simulate applying force. In simulations with DPPC fixed also DPPC atoms are fixed, but only in the direction of stretch (compression)-in Z direction. This should simulate integrity of membrane

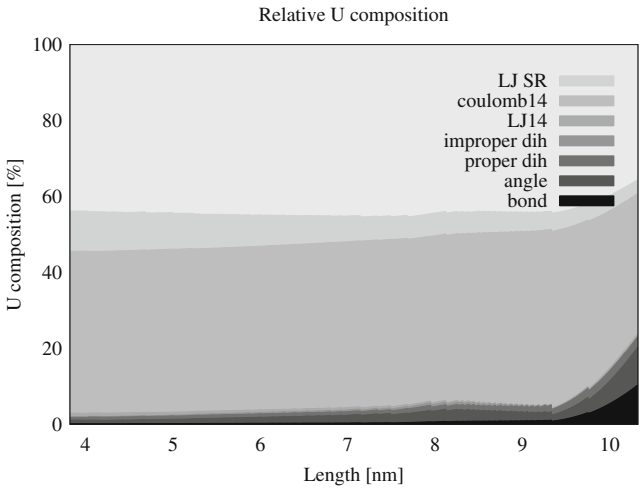

Fig. 3 Energy distribution among forcefield terms observed in simulation of stretching L_{24} in vacuum

for the insight to the protein function mechanisms and are presently unavailable due to the lack of computer power needed for using the current methodology.

Huge progress in the experimental nano techniques involving AFM microscopy make it also possible to verify theoretical results, since it is now possible to observe and manipulate individual molecules [8–14]. These type of experiments are ideal for supporting the theoretical models and for the testing of their predictions. Experimental works have been done to test several different theoretical approaches when studying elasticity. Good agreement was found with the theory of polymer entropic elasticity [15], when small force has been applied to single DNA molecule. Applying bigger force (~10 pN) however showed some differences between the theory and experiments, caused by inelastic deformation of the molecule skeleton [16]. These differences were even bigger when the force (~300 pN) was applied to molecule using AFM tip [8]. It was shown that in this case the difference occurred even between models that involved elasticity of skeleton [16, 17]. Based on these

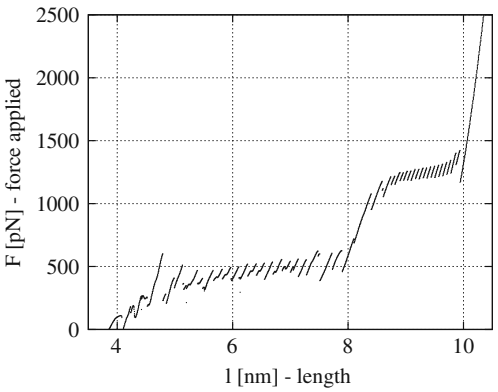

Fig. 4 Force required for stretching calculated from potential energy as observed in simulation of stretching L_{24} in vacuum

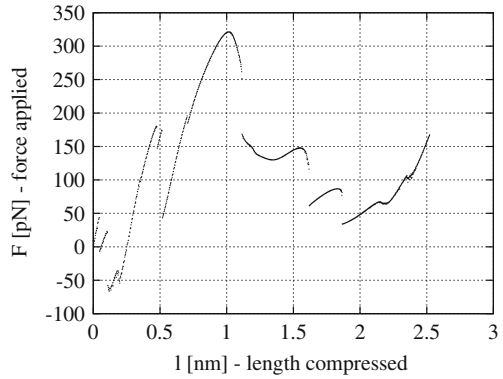

Fig. 5 Force required for compressing calculated from potential energy as observed in simulation of compressing L_{24} in vacuum

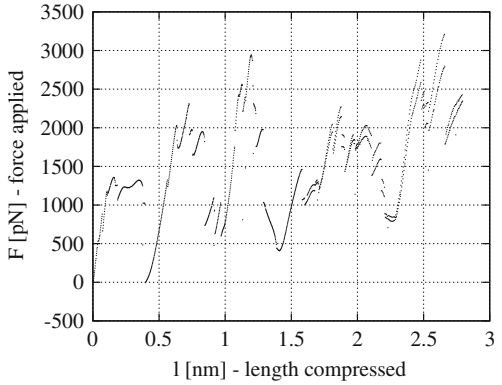

Fig. 7 Force required for compressing calculated from potential energy as observed in simulation of compressing L_{24} with surrounding DPPC fixed in Z direction

observations, new models involving equilibrium thermodynamics [18–20] and molecular dynamics simulation have been formed, trying to explain this phenomenon [21]. In these models, kinetic aspect was included, which explained additional extension of the skeleton with the conformation change. It has been shown that this model is able to explain visco-elastic properties of different types of polymers. There have been experiments for example with the molecule of titin (muscle protein) [9] and dextran (polysacharide) [8] where multi level conformational characteristics of molecules studied by Monte Carlo simulations have been compared with experimental values. The kinetic aspect of simulations and reversibility of the whole process has been also observed.

These types of studies and the fact that chemistry of primary structure of peptides is quite well known, suggest that molecular mechanics simulation based on intermolecular potentials could be a usefull tool to investigate peptide properties at reasonable computing speed with minimum restrictions.

Methodology

Due to the facts mentioned in the introduction we performed our simulations at the molecular mechanics level of theory. We used GROMACS code [22] with forcefield ffgmx as a simulation engine. This forcefield was originally designed for the calculations that include proteins only, therefore to compute contributions of environment (DPPC) to overall properties we had to provide additional interaction-potentials that would enable us to do such calculations. For this purpose we altered the forcefield based on study made by a group of authors [23], who were studying proteins integrated in phospholipid membranes and tested this approach.

Our method of implying force on the molecule consisted of 2 steps. At first we did homogeneous stretch (compress) of rescaled coordinates of all L_{24} atoms in Z direction (the direction of lateral axis of peptide). Then we equilibrated the system at zero temperature in vacuum with the ends of

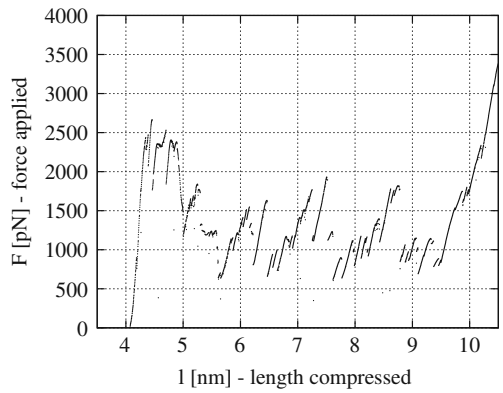

Fig. 6 Force required for stretching calculated from potential energy as observed in simulation of stretching L_{24} with surrounding DPPC fixed in Z direction

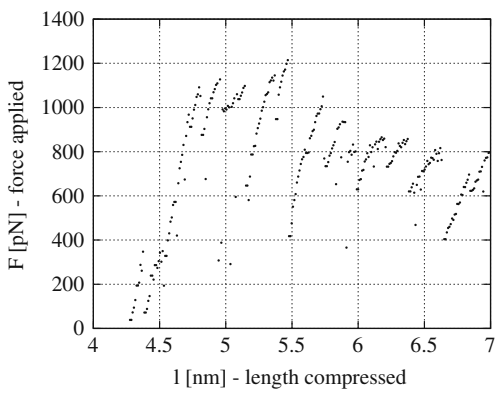

Fig. 8 Force required for stretching calculated from potential energy as observed in simulation of stretching L_{24} with surrounding DPPC free in all directions

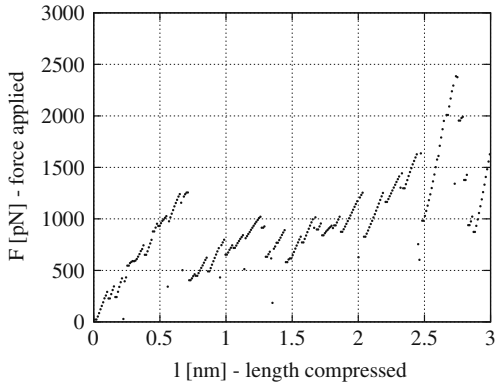

Fig. 9 Force required for compressing calculated from potential energy as observed in simulation of compressing L_{24} with surrounding DPPC free in all directions

the molecule fixed to its coordinates. The length of one stretch or compression step was 0.002 nm. Since we performed only a set of minimizations to investigate structural and energetic changes, there was no time parameter involved. We repeated the same procedure with free DPPC molecules organized to bilayer as environment and also with DPPC fixed in Z direction. Figures 1 and 2 present the images of our starting equilibrated geometries. Two highlighted atoms at the ends of peptide are those carbons which are being fixed during the simulations. In the simulation in vacuum the system consists only of L_{24} peptide (see Fig. 1) (547 atoms). In the simulation with DPPC environment system consists of L_{24} peptide and 12 DPPC molecules formed in two rings (see Fig. 2*)* (totally 2107 atoms). The system was simulated in cluster boundary conditions-no periodic box was present. Electrostatic charges were obtained from aminoacid database which is a part of the used forcefield. Charges in amino acids correspond to natural cell environment (pH~5.5) and were constant in all minimizations.

To test MM methodology and the conditions, we were observing the energy distribution among forcefield terms in our simulations. Doing this we found the limits of our approach. Original L_{24} was 3.86 nm long and during the simulation it was stretched up to 10.5 nm, however as Fig. 3 shows, the results beyond 10.0 nm are not realistic and normally a bond break would occur since too much energy is distributed to bond terms. After some testing runs, where we optimized the simulation step and convergence criteria we obtained the following results (Figs. 4, 5, 6, 7, 8, and 9). These graphs were obtained from analytic derivation of precisely computed potential energy.

Results and discussion

Figures 4, 5, 6, 7, 8, and 9 show that the effect of applied force is not continuous. Each jump on graphs is caused by

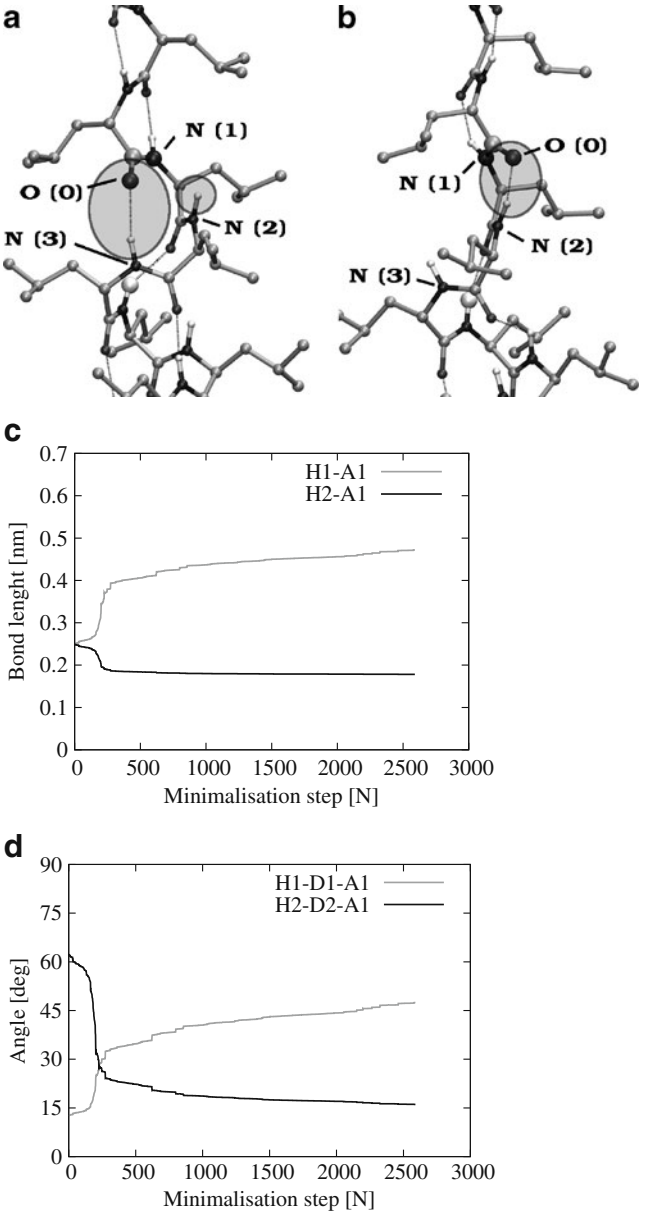

Fig. 10 **a**) Starting geometry of example of 1–3 to 1–2 H-bond jump. H-bond O(0) to H on N(3) will be broken and a new one will be formed with O(0) to H on N(2). This change is coupled with change of conformation, what results in sudden release of strain as observed in all force graphs. **b**) Final geometry of example of 1–3 to 1–2 H-bond jump. H-bond O(0) to H on N(3) was broken and a new one was formed with O(0) to H on N(2). **c**) Step 1034 minimization. Example of H-bond H1-A1, H2-A1 lengths change corresponding to Fig. 10a,b. A1 resembles oxygen in our case, H1, H2 are two different hydrogens on which the H-bond jump occurs. **d**) Step 1034 minimization. Example of H-bond HDA angle change corresponding to Fig. 10a,b. D1, D2, H1, H2 resembles two different nitrogens (hydrogens), A1 resembles oxygen on which the H-bond jump occurs

sudden conformation change caused by applied force. An example of such change observed in simulation of stretching without DPPC is presented in Fig. 10a,b. As we can see the highlighted H-bond, which stabilizes the molecule, is broken, a new one is formed and the system changes the

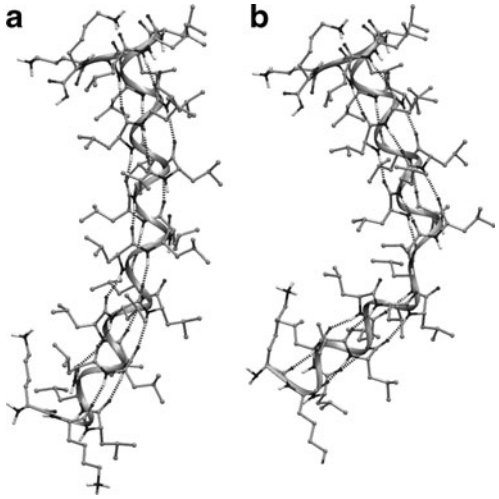

Fig. 11 **a**) Intermediate step of compressing of L_{24} in vacuum in range about 0.8 nm compressed. Bending is already apparent. **b**) Intermediate step of compressing of L_{24} in vacuum in range about 1.1 nm compressed. Helical structure is broken into two separated parts, but both of them keep their original helical structure

conformation. This change cannot be normally undone. After releasing ends of the helix, it stays in this new conformation. This is the prime reason why the stretching and compression is not elastic according to Hook's theorem and why there are so many irregular jumps on the graphs.

We could distinguish several stages of conformational changes, in which different types of H-bonds jumps occurred when we were observing L_{24} stretched alone. The type shown on pictures (Fig. 10a,b) responds to 1–3 to 1–2 jump. Better quantitative insight of this jump is given by graphs Fig. 10c,d. This type is responsible for periodical force jumps (Fig. 4) in the range of 5.5 nm–7.5 nm. Untwisting and H-bond jumping occurs from N-end of the peptide. This end is probably less stable due to inherent asymmetry of peptide chain of the molecule. According to Fig. 3, MM method is not suitable for stretching larger than 10.0 nm or as in our case for applying force larger than ~2500 pN, since the bonds are stretched to the extend, where the classical description in terms of forcefield looses its validity and normally covalent bond breaking would probably occur.

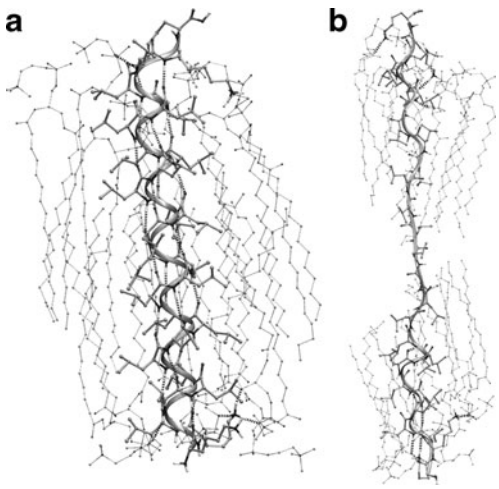

Fig. 12 **a**) L_{24} initial equilibrated geometry with surrounding 12 DPPC molecules (free in all directions) prepared for stretching. **b**) L_{24} geometry with surrounding 12 DPPC molecules (free in all directions) after applied stretching. Untwisting of L_{24} take place in middle part where no stabilizing effect of DPPC is present. This figure also demonstrates how Lysine anchors stick to polar heads of DPPC molecules, which in effect follow stretched molecule

When applying the compression to L_{24} alone, the molecule first undergoes an elastic bend. After being compressed to the size 2.8 nm (1 nm compress from its original size) it broke in the middle into two parts (see Fig. 5). Force required for compressing is much smaller than for stretching, this is due to the fact that the molecule was actually not compressed like a helical spring, but rather bent like a stick and then locally unfolded (Fig. 11a,b). H-bonds have stabilizing effect not only when the stretching is applied. They maintain a certain helix size and they make the molecule rigid rather than flexible.

Generally DPPC surroundings put a high degree of irregularity to the problem and gave L_{24} a higher level of stability. It discarded a step-wise look of the stretch process and different types of structure changes occur in apparent random order, depending on starting position of DPPC molecules. Simulation with fixed DPPC caused huge increase of force required for stretching (Fig. 6), this was due to the fact that big force has been required for the pulling of the polar ends out of membrane. When we repeat the simulation with free DPPC the increase is not that obvious (Fig. 8), but this behavior is rather strange, since the surrounding DPPC were attached to the ends of L_{24} and moved with them as L_{24} was stretching (Fig. 12a,b). In this case untwisting and H-bond breaking occurred form the middle of the peptide, where no DPPC was present.

The effect of the compression of L_{24} when DPPC was fixed in the Z direction also gave very interesting results. The environment again had a stabilizing effect, which can be seen in Fig. 7. The force which is required for compressing is almost five times bigger than without DPPC. L_{24} did not unfold into two rigid parts like in Fig. 5. The character of the compression has been completely changed-it resembles as if only the two atoms we kept fixed were affected by force-pushing them closer, despite the fact that all L_{24} atom coordinates were transformed before each minimization. The peptide completely unfolded from its N-end, *i.e.*, all stabilizing H-bonds have been disturbed in this end. The destabilized part behaved like free polymer thread, while the rest of the peptide remained in its helical form. This again demonstrates the size of the stabilizing effect of the surrounding DPPC on

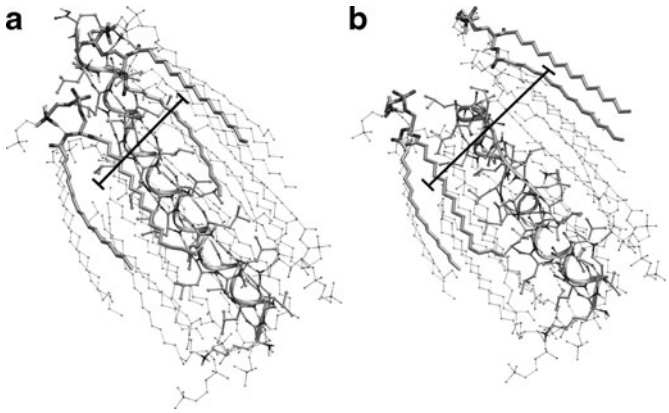

Fig. 13 **a**) L_{24} initial equilibrated geometry with surrounding 12 DPPC molecules (fixed in Z direction) prepared for compressing. Length marked shows starting distance between two DPPC molecules. **b**) L_{24} geometry with surrounding 12 DPPC molecules (fixed in Z direction) after applied compression. As can be seen two DPPC molecules are pushed away (their distance increases) and only one end of L_{24} is in motion. Untwisted part resembles rope, while the rest of the molecule keeps its original helical form. Force required for this behavior was quite high as can be seen in Fig. 7 which suggests high stabilizing effect of DPPC environment

the helical structure of the peptide. In the process of compression L_{24} peptide pushed away two DPPC molecules as can be seen in Fig. 13a and b. Anomalous behavior can be seen on Fig. 7 for the range 2.0–2.5 nm which shows numeric limits of our force calculating method. The compression with free DPPC caused all DPPC molecules to follow polar ends just like in the free stretching and big lipid globule around the bended L_{24} has been formed (Fig. 9).

These and similar results can be further used for better qualitative understanding of mechanical properties of the secondary structure fragments of the peptides. Quantitatively it can be used for designing or validating the appropriate coarse grained parameters.

Conclusions

The application of the MM method for the study of the model peptides can be a useful tool for the explanation of the force effect on molecules. We have shown that the inelastic change of the conformation of the peptides causes abrupt changes in the structure of the peptides. The influence of the force (stretching, compressing) is reduced by stabilizing effect of H-bond. This effect is quite high, causing the peptide to resemble a rather rigid stick more than elastic spring. Interaction with neighbor lipid molecules further stabilized the rod-like structure of this peptide. The lysine anchor groups effectively interact with polar heads of surrounding lipids, thus when embedded into the membrane the size mismatch can cause the local deformation of the membrane.

Acknowledgments This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0442-07

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