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Research Article

Interaction of zervamicin IIB with lipid bilayers. Molecular dynamics study

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ABSTRACT

In this work we have studied the interaction of zervamicin IIB (ZrvIIB) with the model membranes of eukaryotes and prokaryotes using all-atom molecular dynamics. In all our simulations zervamicin molecule interacted only with lipid headgroups but did not penetrate the hydrophobic core of the bilayers. During the interaction with the prokaryotic membrane zervamicin placed by its N-termini towards the lipids and rotated at an angle of 40° relatively to the bilayer surface. In the case of eukaryotic membrane zervamicin stayed in the water and located parallel to the membrane surface. We compared hydrogen bonds between peptide and lipids and concluded that interactions of ZrvIIB with prokaryotic membrane are stronger than those with eukaryotic one. Also it was shown that two zervamicin molecules formed dimer and penetrated deeper in the area of lipid headgroups.

1. Introduction

Zervamicin IIB is an antimicrobial peptide that interacts with the cell membrane and increases ion permeability. ZrvIIB, isolated from cultures of *Emericellopsis salmosynnemata*, is a member of the antibiotics peptaibol family. Peptaibols usually have activity against Gram-positive bacteria and lack of toxicity towards eucariotic cells (Argoudelis et al., 1974). Also they are known to be potentially useful for chemotherapeutic applications in oncology (Oh et al., 2002). Zervamicin IIA and zervamicin IIB appear to inhibit the locomotors activity of test mice, probably via their effect on the brain. These effects of zervamicin IIA become apparent at lower dosages (0.05–2.0 mg/kg) as compared to zervamicin IIB (0.5–12.0 mg/kg). ZrvIIB consists of 16 amino acid residues and, like other peptaibols, contains a high proportion of helix-promoting α,α-dialkylated amino acids. The N-terminus of the peptide forms an alpha-helix, whereas C-terminus has 3(10)-helical structure (Shenkarev et al., 2004). ZrvIIB, as opposed to long peptaibols such as alamethicin, possesses helical structure in mixed solvents of different polarity ranging from $CDCl_3/CD_3OH$ (9:1, v/v) to CD_3OH/H_2O (1:1, v/v) (Balashova et al., 2000). In planar lipid bilayer ZrvIIB forms voltage-dependent ion channels with multilevel conductance state (Balaram et al., 1992). The exact channel structure is still unclear. But the conventional model for

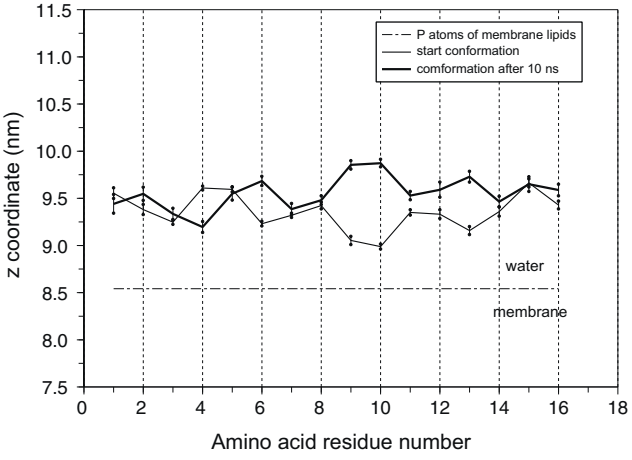

Fig. 1. Position of Ca-atoms relatively to the membrane surface in the starting conformation and after 10 ns of dynamics (POPC lipid bilayer).

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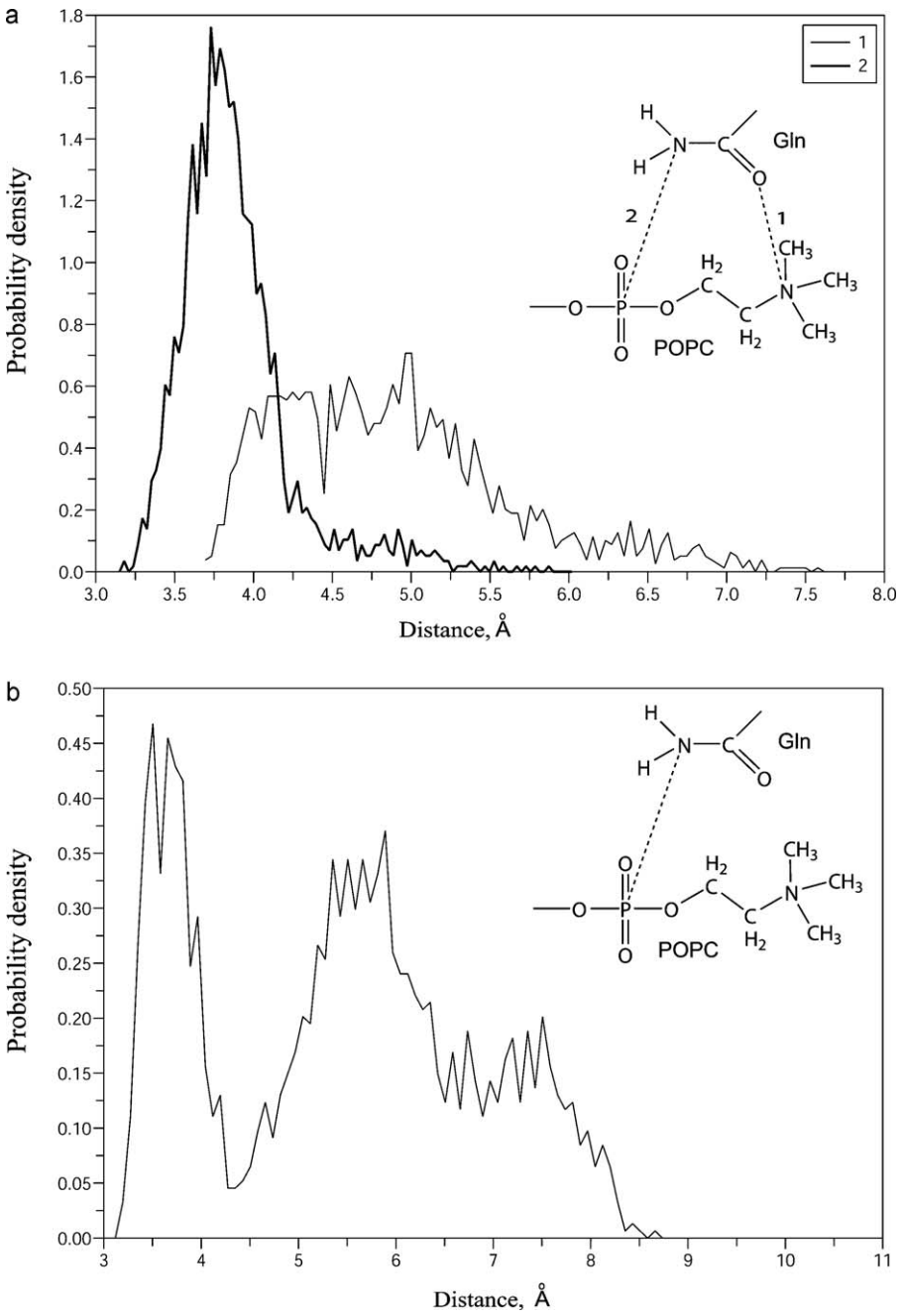

Fig. 2. (A) Distance between carboxyl oxygen of Gln11 side chain and nitrogen atom of POPC (1), between nitrogen atom of Gln3 side chain and phosphorus atom of POPC (2). (B) Distance between nitrogen of Gln3 side chain and phosphorus atom of POPC.

voltage-gated peptaibol channel action involves the formation of the water-filled pore by a bundle of parallel helices (Laver, 1994). Different conductance levels are thought to correspond to different numbers of helices in a bundle (Agarwalla et al., 1992). According to barrel-stave model (BS-model) of peptaibol action, peptaibol adsorbs on the membrane surface and embeds into the lipid bilayer under the transmembrane potential. In this article we have studied the interaction of zervamicin IIB with the model membranes of eukaryotes and prokaryotes using all-atom molecular dynamics. Also the ability of zervamicin molecules to aggregate with each other on the membrane surface has been investigated.

2. Methods

POPC lipid bilayer was used as model of eukaryotic membrane and POPE/POPG (in a proportion of 4:1) bilayer as prokaryotic one. Each monolayer of the membranes consisted of 32 lipids and

had an area per lipid equal to 0.64 nm^2. Each lipid molecule in the plain bilayer was rotated to a random angle to avoid structuredness of the system. In the case of mixed bilayers two types of lipids were placed randomly. The systems were solvated by SPC water aqueous solution. Na^+ ions were added to neutralize the systems charge. Both membranes were equilibrated during 200 ps.

After equilibration zervamicin molecule was faced by its hydrophilic side to the membrane surface on the distance of 0.7 nm. Starting conformation of zervamicin molecule was obtained from Protein Data Bank (1IH9.pdb). In the system with two zervamicin molecules they were parallel to each other on the distance of 2.5 nm between mass centers.

In simulation the periodic boundary conditions were applied in the NPT ensemble with Berendsen barostat. The following parameters were used: 1 bar towards membrane normal and −30 bar towards membrane surface, temperature (300 K) was maintained via Langevin dynamics with thermostat coefficient 0.2 ps. Van der Waals and Coulomb cut-offs were 2.5 nm. Simulations were performed with time step of 1 fs without constraints. The trajectory length was 10 ns.

During the simulation we investigated the orientation of zervamicin relatively to the membrane surface. We calculated the position of Cα-atoms at the beginning and after 10 ns of the simulations. The center of mass of lipids phosphorus atoms was accepted for water-membrane boundary.

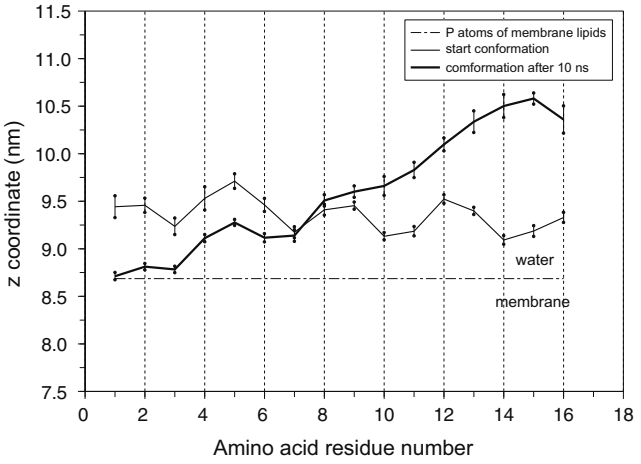

Fig. 3. Position of Ca-atoms relatively to the membrane surface in the starting conformation and after 10 ns of dynamics (POPE/POPG lipid bilayer).

3. Results and discussion

According to the BS-model peptaibols do not embed in the hydrophobic area of the membrane and interact only with lipid headgroups (Tieleman et al., 1999). At the starting conformation zervamicin was faced by its hydrophilic side to the lipids at the distance of 0.7 nm. In all three systems zervamicin changed its orientation relatively to the membrane surface, but did not penetrate deep in the bilayer and stayed in the water surrounding. Zervamicin maintained helical structure during the whole simulations.

3.1. Interaction with POPC lipid bilayer

In the case of POPC lipid bilayer zervamicin turned on its axis through 180°, so that the hydrophobic side of the peptide was faced to the lipids. The peptide stayed parallel to the membrane surface and did not move closer to the membrane (Fig. 1). The minimal distance between peptide and membrane during the simulation was 0.5 nm.

Hydrogen bonds between Gln3, Gln10 and lipid headgroups stabilized this position of zervamicin molecules. Other polar amino acid residues were oriented towards water phase.

Carboxyl oxygen of Gln11 side chain interacted with positively charged $(CH_3)_3N^+$ group of POPC lipid. Hydrogen atoms of Gln11 amine group formed hydrogen bonds with oxygens of POPC phosphate. Because all four oxygen atoms of POPC phosphate group are able to form hydrogen bonds, we calculated the distance between N (of Gln11 amine group) and P (of POPC phosphate group). Probability distribution of the distance between N and P had wide peak because of the various combinations of hydrogen bonds (Fig. 2). Carboxyl oxygen of Gln3 formed intramolecular hydrogen bond and hydrogen atoms of its amine group interacted with phosphate of lipid. In this case, probability distribution of the distance between N and P had two peaks, which were more pronounced, because the intermolecular hydrogen bond restricted the rotation of the amine group.

3.2. Interaction of one ZrvIIB molecule with POPE/POPG membrane

Zervamicin IIB position after 10 ns of dynamics is represented in Fig. 3. After simulation peptide was placed angularly to the membrane surface so that its N-termini was located in the area of the lipid heads. During the dynamics ZrvIIB turned on its axis through 180° just as well as in the case of POPC lipid bilayer. But it did not stay parallel to the membrane surface and rotated by 40° relatively to the surface.

Zervamicin molecule has dipole moment that is pointed from C-terminus (minus) towards N-terminus (plus) due to the direction of the intermolecular hydrogen bonds. This dipole moment is equal to 50D, which corresponds to +0.4e and −0.4e on the N- and C-termini, respectively.

During the dynamics negatively charged PG lipids attracted positively charged N-terminus and it turned relatively to the membrane surface. As in the case of POPC membrane, zervamicin interacted only with the polar part of the lipid bilayer and did not penetrate into the hydrophobic area. This peptide position was stabilized by 4 hydrogen bonds.

Carbonyl oxygen of Ace0 interacted with positively charged NH_3^+ group of POPE lipid. Hydrogen atoms of Gln3 amino groups and Thr6 hydroxyl group formed hydrogen bonds with oxygen atoms of lipid phosphates. Probability distribution of the distance between atoms (Fig. 4) had one strongly pronounced maximum, in contrast to analogous graphics for POPC membrane, where were several wide maximums. This can be considered as an evidence of the fact that interactions of ZrvIIB with prokaryotic membrane are stronger than those with eukaryotic one.

3.3. Dimerization of the ZrvIIB on the membrane surface

In the experimental studies it was shown that covalent binding of two alamethicin molecules by N-termini increases life time of alamethicin channel to 100 ms (Duclohier et al., 1999). In this

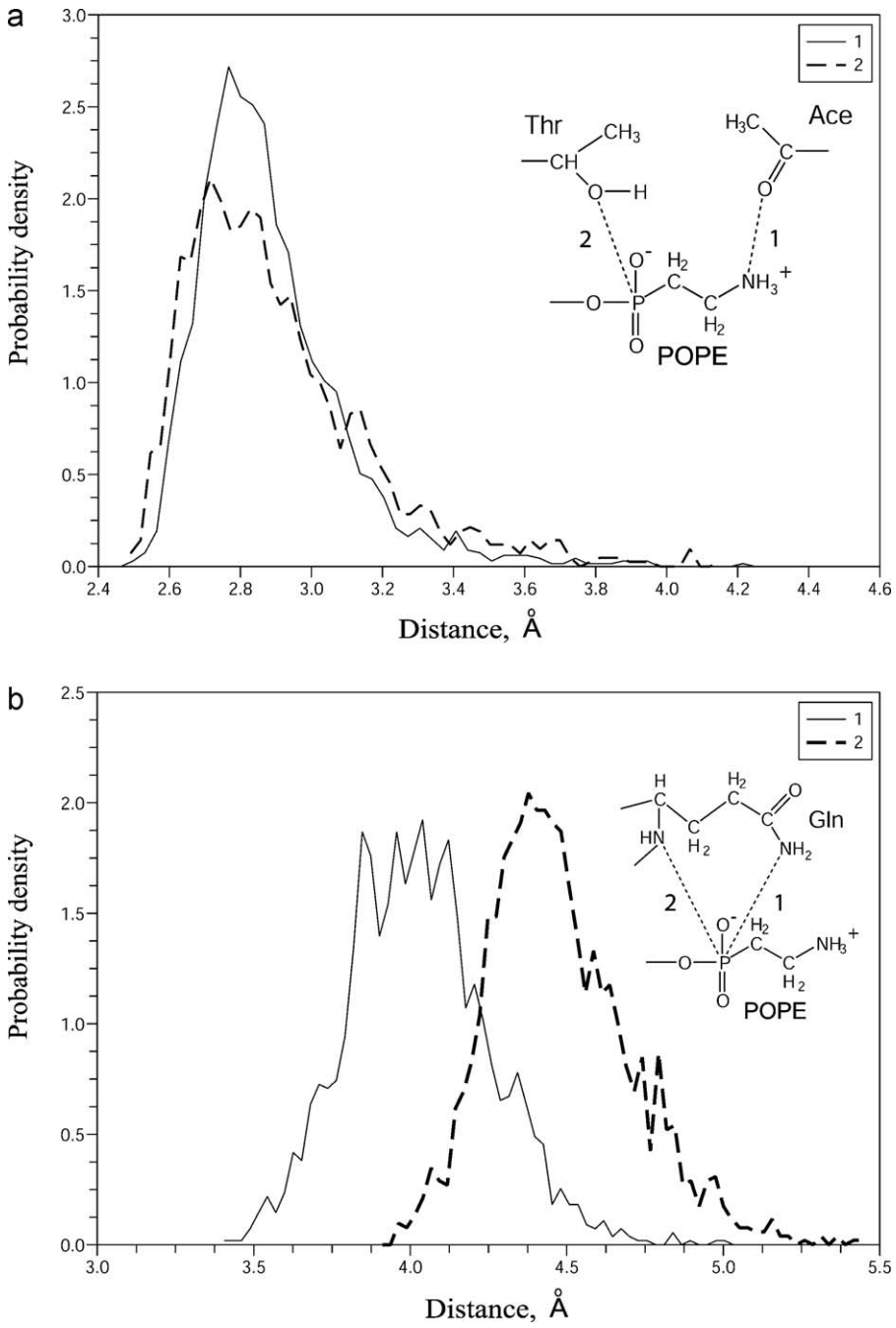

Fig. 4. (A) Distance between carbonyl oxygen of Ace0 and nitrogen atom of POPE (1), between hydroxyl oxygen of Thr6 and phosphate oxygen of POPE (2). (B) Distance between nitrogen atom of Gln3 side chain and phosphorus atom of POPE (1) and between nitrogen of Gln3 and phosphorus of POPE (2).

work we have studied the influence of intermolecular interaction of two ZrvIIB on binding with prokaryotic membrane. In the starting conformation ZrvIIB molecules were parallel to each other at distance of 2.5 nm. Each molecule was faced by its polar side to the lipids.

After 6 ns of the dynamics the distance between ZrvIIB molecules decreased from 2.5 nm to 1 nm and the peptides formed dimer (Fig. 5). Hydrophobic surfaces of both molecules were oriented to each other, so nonpolar amino acids were isolated from water surrounding. Presumably, formation of this dimer can promote ZrvIIB embedding.

After 10 ns the interaction between ZrvIIB and lipid bilayer was stronger than in the case of the single molecule. As it is seen in Fig. 5, both molecules rotated by 20° relatively to the membrane surface. N-terminus of one peptide (Fig. 5B) penetrated into the lipid bilayer deeper than to the level of the phosphorus atoms. The first 7 amino acids of the other peptide placed in the area of the lipid headgroups. 8 hydrogen bonds stabilized the interaction of the 2 ZrvIIB molecules with the lipids. Ace0, Gln3, Thr6 and Gln11 participated in the formation of hydrogen bonds. Probably, during the subsequent embedding ZrvIIB molecules rotate relatively to each other so that hydrophobic amino acids can interact with the lipid tails.

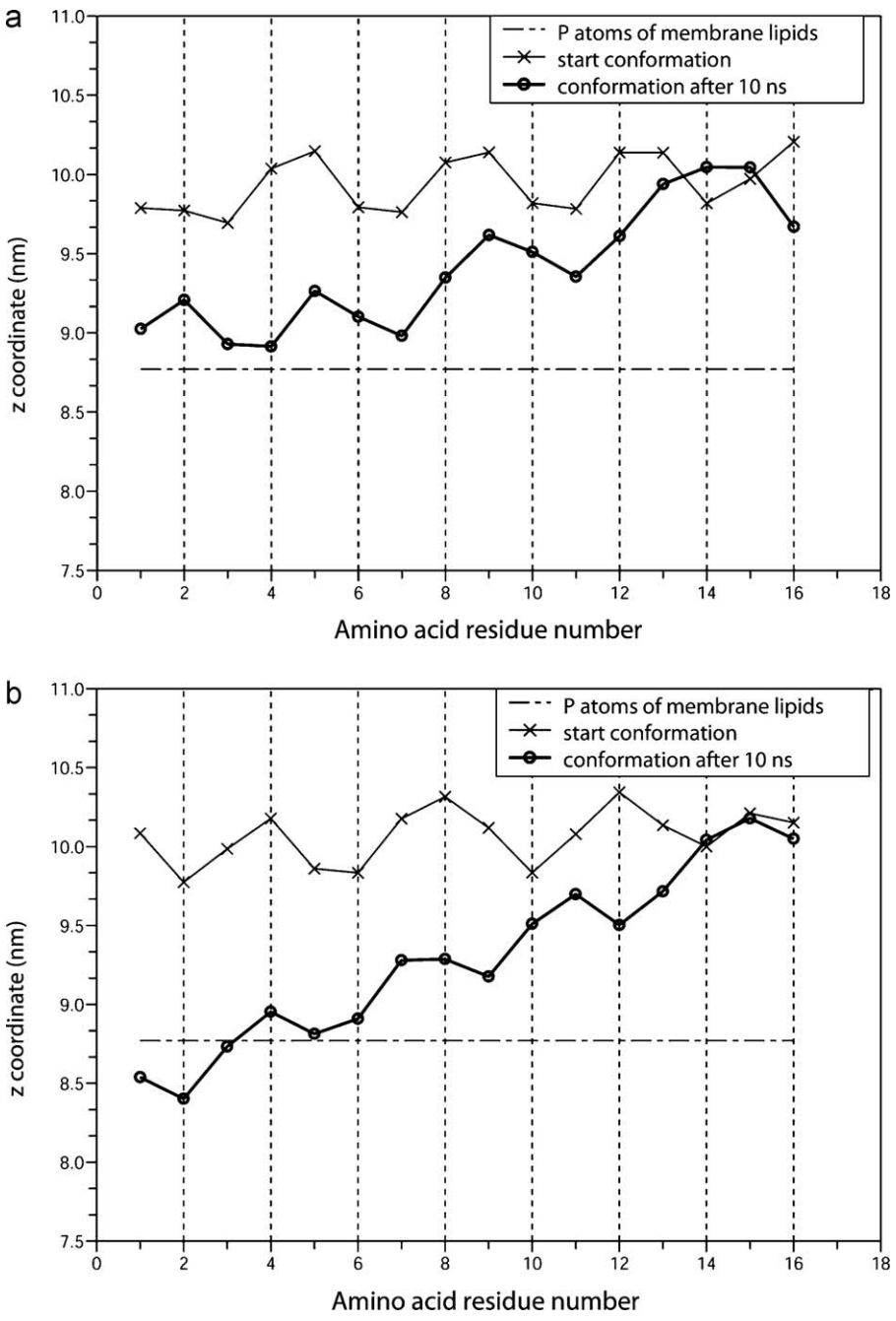

Fig. 5. Position of Ca-atoms relatively to the membrane surface in the starting conformation and after 10 ns of dynamics for two peptides (A and B).

3.4. Conformation stability of the zervamicin

In this work we compared the conformational changes of zervamicin molecules in the four surroundings: water, methanol, the surface of POPC and POPE/POPG bilayers. Cα-atoms root mean square deviation (RMSD) from the starting structure was accepted for a measure of structural stability for zervamicin molecules in different systems. As opposed to long peptaibols (Tieleman et al., 1999), helical structure of ZrvIIB was stable and in all four environments and we observed only slight conformational changes (Fig. 6).

The lowest RMSD for zervamicin IIB was observed in water and methanol (~0.15 nm). RMSD for ZrvIIB near the membrane surface changed from ~0.18 nm at the beginning of the simulation to ~0.35 nm at the end for POPC bilayer. These high RMSD values for ZrvIIB on the surface of POPC membrane can be explained by bend of Gln3 to the lipid headgroups, which causes formation of new hydrogen bonds. In the case of POPE/POPG bilayer RMSD rose up to ~0.4 nm during the first 6 ns of the simulation and then decreased to ~0.2 nm. The increase of RMSD was provoked by the rotation of the peptide relatively to the membrane surface. This process was attended by formation and breaking of new hydrogen bonds. After

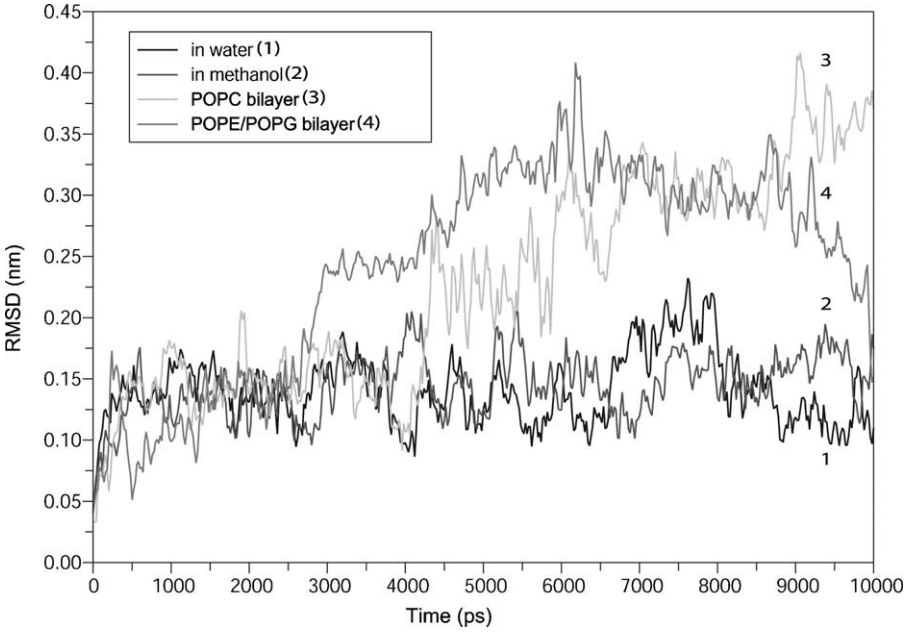

Fig. 6. RMSD of ZrvIIB in different systems.

position of the peptide stabilized, its conformation returned to the starting one, which led to RMSD decreasing.

4. Conclusion

In all our simulations zervamicin molecule interacted only with lipid headgroups but did not penetrate the hydrophobic core of the bilayers.

During the interaction with the prokaryotic membrane zervamicin placed by its N-termini towards the lipids and rotated at an angle of 60° relatively to the bilayer surface. The first 3 amino acid residues were situated at the level of the lipid phosphate groups. This conformation was stabilized by 4 hydrogen bonds between amino acids and lipids. Two zervamicin molecules on the membrane surface formed dimer and penetrated deeper in the area of lipid headgroups. In the case of the eukaryotic membrane, all amino acid residues of zervamicin stayed in the water surrounding and peptide located parallel to the membrane surface.

In our study zervamicin demonstrated higher conformation stability in water and methanol surrounding than that of long peptaibols like alamethicin. Helical structure of peptide creates dipole moment that plays a crucial role in interaction with membrane surface. Peptaibols presumably affect prokaryotic cells due to presence of negatively charged lipids in prokaryotic membranes.

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References

Agarwalla, S., Mellor, I.R., Sansom, M.S., Karle, I.L., Flippen-Anderson, J.L., Uma, K., Krishna, K., Sukumar, M., Balaram, P., 1992. Zervamicins, a structurally characterised peptide model for membrane ion channels. Biochem. Biophys. Res. Commun. 186, 8–15.

Argoudelis, A.D., Dietz, A., Johnson, L.E., 1974. Zervamicins I and II, polypeptide antibiotics produced by emericellopsis salmosynnemata. J. Antibiot. (Tokyo) 27, 321–328.

Balaram, P., Krishna, K., Sukumar, M., Mellor, I.R., Sansom, M.S., 1992. The properties of ion channels formed by zervamicins. Eur. Biophys. J. 21, 117–128.

Balashova, T.A., Shenkarev, Z.O., Tagaev, A.A., Ovchinnikova, T.V., Raap, J., Arseniev, A.S., 2000. NMR strucrure of the channel-former zervamicin IIB in isotropic solvents. FEBS Lett. 466, 333–336.

Duclohier, H., Kociolek, K., Stasiak, M., Leplawy, M.T., Marshall, G.R., 1999. C-terminally shortened alamethicin on templates: influence of the linkers on conductances. Biochim. Biophys. Acta 1420, 14–22.

Laver, D.R., 1994. The barrel-stave model as applied to alamethicin and its analogs reevaluated. Biophys. J. 66, 355–359.

Oh, S.U., Yun, B.S., Lee, S.J., Kim, J.H., Yoo, I.D., 2002. Atroviridins A–C and neoatroviridins A–D, novel peptaibol antibiotics produced by Trichoderma atroviride F80317 I. Taxonomy, fermentation, isolation and biological activities. J. Antibiot. (Tokyo) 55, 557–564.

Shenkarev, Z.O., Paramonov, A.S., Balashova, T.A., Yakimenko, Z.A., Baru, M.B., Mustaeva, L.G., Raap, J., Ovchinnikova, T.V., Arseniev, A.S., 2004. High stability of the hinge region in the membrane-active peptide helix of zervamicin: paramagnetic relaxation enhancement studies. Biochem. Biophys. Res. Commun. 325, 1099–1105.

Tieleman, D.P., Berendsen, H.J., Sansom, M.S., 1999. Surface binding of alamethicin stabilizes its helical structure: molecular dynamics simulations. Biophys. J. 76, 3186–3191.