Biochimica et Biophysica Acta, 1106 (1992) 227–232

BBAMEM 75620

Monte Carlo studies of a model for lipid-gramicidin A bilayers

J. Xing and H.L. Scott

Department of Physics, Oklahoma State University, Stillwater, OK (USA)

(Received 5 December 1991)

Key words: Gramicidin A; NMR; Monte Carlo simulation; Bilayer

This paper presents results of Monte Carlo simulations of a full bilayer of 200 lipid chains and one gramicidin A dimer. Simulations are described for systems with lipid chains of 14, 16, and 18 carbons, respectively. Using accepted potential functions to calculate interactions between all non-hydrogen atoms a Monte Carlo configuration sampling is generated from which order parameter profiles are calculated and specific configurations are displayed. Results are compared with experimental data for lipid-gramicidin bilayers.

Introduction

Gramicidin A is a linear hydrophobic pentadecapeptide which exists in lipid bilayers in a well characterized right-handed β-helical conformation. When two molecules of gramicidin A, one in each monolayer, form a linear dimer, an ion-specific channel through the bilayer is created [1,2]. Because the structure of gramicidin is simple, and because its conformation in a lipid bilayer is fairly well established, it serves as a natural model system for more complex membrane proteins. The properties of the gramicidin dimer and the ion channel have been studied by many investigators (for reviews, see Refs. 1 and 2). It is known that lipid–gramicidin interactions affect the conductance properties of the gramicidin channel [2], altering the conductivity by up to a factor of two. Additional evidence that the lipid matrix strongly affects the properties of the gramicidin channel has been given by Sawyer et al. [3], who show that nonionic and zwitterionic detergents may induce changes in the properties of the channel.

In contrast to the number of structural and channel studies, at present there is considerably less direct information available on the nature of the interactions between *lipid chains* and the gramicidin A dimer. Early deuterium NMR experiments indicated that the quadrupole splittings of the C-D bonds are only slightly affected by gramicidin A up to fairly high lipid-to-gramicidin ratios in DMPC [4,5]. A more recent comparative study of the effects of gramicidin A on dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), and distearoylphosphatidylcholine (DSPC), was carried out by Watnick et al. [6] using ^{31}P- and ^{2}H-NMR. The conclusions from this work were that, for gramicidin A concentrations below about 6 mol% (where gramicidin induces formation of significant amounts of H_{II} phase in DSPC) the effect of the peptide on relaxation rates and on quadrupole splittings are greatest for the shorter chain DMPC lipids and least for the longest chain lipids, DSPC. The data are interpreted by Watnick et al. [6] in terms of a hydrophobic mismatch in length between chains and gramicidin dimers. The greatest mismatch occurs, in this interpretation, for DMPC. In the fluid lipid phase the 14-carbon DMPC chains are somewhat shorter in length than a gramicidin monomer, so these chains must incorporate more trans bonds to match the length of the gramicidin. However, fully extended DMPC chains are still longer than a monomer of gramicidin A, so some *gauche* segments must still be present.

The hydrophobic length of a gramicidin A dimer is between 26 Å and 30 Å, depending on the conformations of the tryptophan residues and the flexibility of the helical backbone of the peptide. This range in length encompasses the fluid state hydrophobic lengths of DMPC (about 27 Å) and, marginally, DPPC (about 29 Å), but probably not DSPC (about 30 Å). It is considerably shorter than a bilayer of ordered chains of even DMPC (about 32 Å). Since the lipids in the experiments were all in their respective fluid phases it may be argued that the shorter DMPC chains are in fact better able to match the hydrophobic length of the

Correspondence: H.L. Scott, Department of Physics, Oklahoma State University, Stillwater, OK 74078-0444, USA.

peptide and to pack tightly against the gramicidin than are longer chain lipids, even though a modest increase in chain ordering may be required. For longer chains to match the hydrophobic length of the gramicidin an increase in the number of *gauche* rotations over the average number per chain in the fluid lipid phase appears to be necessary. At the relatively high hydrocarbon densities present in fluid lipid bilayers steric interactions will make additional chain folding (beyond the average number of rotamers per chain in the fluid lipid phase) difficult. On the other hand chains which can match the hydrophobic length of the gramicidin without an increase, or with a small decrease, in the number of *gauche* rotamers per chain should be best able to accomodate higher concentrations of the peptide in the bilayer.

In the above picture tightly packed lipids which are neighbors to peptide molecules will be less rotationally and translationally mobile and this will increase relaxation times and disrupt interactions between the gramicidin neighbor chains and the 'bulk' chains in the pure lipid regions of the bilayer. Other dynamical studies of gramicidin–lipid interactions indicate that gramicidin disrupts the slow, cooperative lipid motions to the greatest extent, especially near the lipid headgroups [7,8].

There have been many numerical studies of the dynamical properties of the gramicidin peptide and the ion channel [1,10–13]. However the only numerical investigations of the microscopic (i.e. atom-by-atom) interactions between gramicidin monomers and lipid chains are those of Xing and Scott [14] and Wang and Pullman [15]. In the simulation of Xing and Scott a monolayer array of lipid chains and a single gramicidin monomer were analyzed using the Monte Carlo (MC) method. The main conclusion from these calculations was that the lipid chain conformations are, on average, very similar to those of chains in pure lipid bilayers. Hindrance of lipid chain rotational mobility by the gramicidin was found, although this effect was not as pronounced as was found in lipid-cholesterol MC simulations [17]. Wang and Pullman [15] carried out an energy minimization of a gramicidin A monomer surrounded by an annulus of glycerol momooleate molecules. They found that interaction energies between lipid and gramicidin are not as strong as lipid–lipid interactions, and that the tryptophan residues interact most strongly with the lipids. They also observed that chain conformations are not substantially altered by the gramicidin. The MC of Xing and Scott and the energy minimization work of Wang and Pullman yield a picture in which lipid chains attempt to fill free volume around a gramicidin monomer, but in which steric interactions with the residues prevent ideal molecular packing while leaving chains conformationally disordered. Both of the above studies involved only *monomers* of gramicidin and *monolayers* of lipid. In a bilayer environment there is an additional degree of freedom, namely motion perpendicular to the bilayer plane, which may be significant in the packing of chains next to the peptide.

The purpose of the present paper is to report results of new Monte Carlo simulations of a model for the interactions between hydrocarbon chains and gramicidin dimers in *full bilayer* geometries. By using full bilayer simulation cells and running simulations for hydrocarbon chains of length 14, 16, and 18 carbons, the hydrophobic mismatch proposal of Watnick et al. [6] can be examined numerically.

Method

The model systems to be studied by Monte Carlo simulation consists of two layers of 100 chains each with a gramicidin dimer in the center of the array. All simulations involved interactions between all non-hydrogen atoms on all chains and on the gramicidin dimer. The Monte Carlo (MC) algorithm is basically the same as was used by the authors earlier [14,16]. The MC procedure was to either pick a chain at random or (on a vector machine) pass through the entire simulation array systematically and attempt a move at each chain (including the gramicidin). Hydrocarbon chains are allowed to translate laterally in the bilayer plane and *gauche* rotations are attempted on one or two randomly chosen bonds. The rotational-isomeric model is used, so that there are only three allowed rotational states per bond, 0°, ±120°. MC moves of the gramicidin dimer were limited to small random rotations of randomly chosen residues about a randomly chosen bond (excluding tryptophan ring bonds). The helical structure of the gramicidin peptide was kept rigid during the simulations. The inter-atomic interaction functions were optimised 6–12 potentials [18]. As is true in most simulations of dense fluids, no real lateral diffusion of molecules occurs during a simulation run, so that the chains which are initially neighbors to the gramicidin remain neighbors throughout the MC run. Due to conformational changes in the chains, however, the neighbor list for a given chain can change during a simulation. A 15 Å cutoff was used in defining the neighbor list to allow for larger scale lateral motions of chain tails during conformational changes.

The above MC procedure was used earlier to study a model system consisting of a layer of pure lipid chains. In this case direct comparison of the generated results with experiment is possible [16]. After scaling the data to allow for cooperative chain tilting (which is not included in the simulations) the order parameter profiles (see below for a definition) agreed very well with experimental data [19].

In the present set of calculations whole-chain motion perpendicular to the bilayer plane was also allowed. A restraint was added as an effective hydrophobic constraint to keep chains from leaving the bilayers. Simulations were run for models with chains of length 14, 16, and 18 carbons at temperatures 10–15 C° above the chain melting temperature for lipid bilayers composed of phosphatidylcholines of the respective chain lengths. Generally 10–12K MC steps per chain were run for equilibration and another 10K for the final average calculations. A very large number of chain conformations were generated as part of the MC procedure which were utilized to calculate equilibrium average bond order parameters and to generate specific chain and peptide conformations for graphic visualization.

The gramicidin dimers were generated from coordinates generously supplied by Dr. Eric Jakobsson. Models for the gramicidin dimer have undergone some modification [13] since these coordinates were first sent to the authors, but these changes are sufficiently small that no effects on the calculations reported here are likely. While flexibility of the helical backbone may be very important-to the ion conduction process it is probably far less important for the interactions between gramicidin and neighboring lipids. This is because the amplitudes of the structural motions are small compared to the steric size of the lipid chains and the peptide side chains. In the interest of model simplicity as well as computational economy these motions were not considered. A possibly significant degree of freedom which was not included in the calculations reported here is axial rotational motion of the full gramicidin dimer.

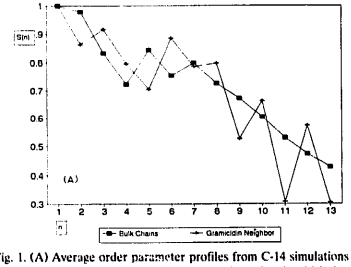
The most useful results of the simulations are average C-C bond order parameter profiles. The definition utilized for the order parameter for bond n is

$$\langle S_n \rangle = \tfrac{1}{2}\langle 3\cos^2\theta_n - 1 \rangle \qquad (1)$$

where θ_n is the angular deviation of bond n from its orientation in the all-*trans* chain conformation. Profiles of $\langle S_n \rangle$ vs. n were generated separately for chains which were nearest neighbors to the gramicidin dimer and for the remaining ('bulk') chains. The majority of the calculations were carried out using the Cray Y-MP machine at the National Center for Supercomputing Applications at the University of Illinois, with some runs also performed on the Oklahoma State University IBM 3090-200S mainframe.

Results and Discussion

Figs. 1–3 show order parameter profiles and typical snapshots from each of the three sets of simulations. The calculated profiles reveal that the most significant differences between gramicidin neighbor chains and 'bulk' chains occur for the C-14 chains. The large zig-zags in Fig. 1A for chains which are gramicidin neighbors are indicative of chains which are not rotationally mobile (although not in all-*trans* conforma-



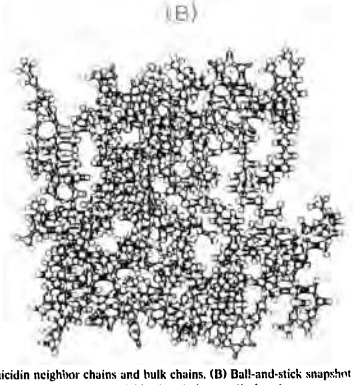


Fig. 1. (A) Average order parameter profiles from C-14 simulations for gramicidin neighbor chains and bulk chains. (B) Ball-and-stick snapshot of a typical C-14-gramicidin configuration, in which the gramicidin dimer and selected neighboring chains are displayed.

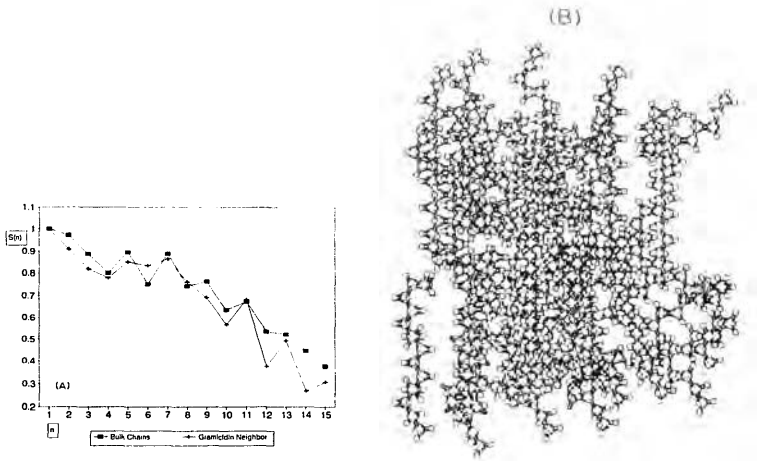


Fig. 2. (A) Average order parameter profiles from C-16 simulations for gramicidin neighbor chains and bulk chains. (B) Ball-and-stick snapshot of a typical C-16-gramicidin configuration, in which the gramicidin dimer and selected neighboring chains are displayed.

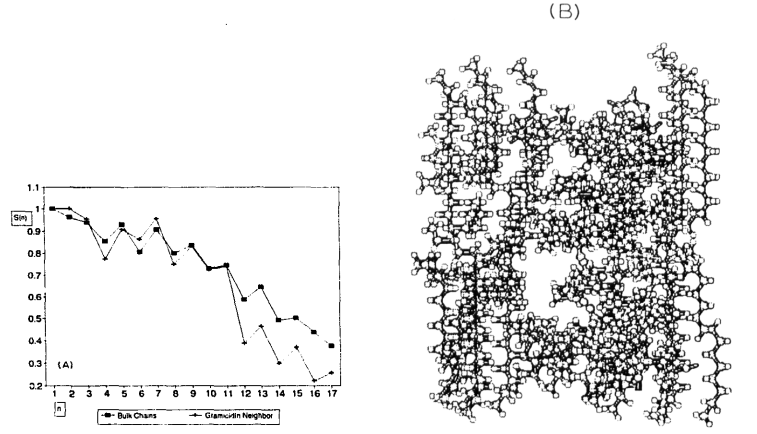


Fig. 3. (A) Average order parameter profiles from C-18 simulations for gramicidin neighbor chains and bulk chains. (B) Ball-and-stick snapshot of a typical C-18-gramicidin configuration, in which the gramicidin dimer and selected neighboring chains are displayed.

tions). That is, for the chains near the gramicidin, bonds 9 and 11 are in lower states of order than bonds 10 and 12. This is indicative of stable kink-like structures which have formed in these chains during the equilibration process, and cannot easily be altered. The snapshot in Fig. 1B shows that the C-14 chains are, after some isomeric disordering, able to pack around the gramicidin dimer in a manner which allows close contact between all of the segments of the chains and the gramicidin.

By contrast, the profiles of bulk and gramicidin neighbor C-16 chains are nearly the same. A slight decrease in the C-16 order parameter profile for gramicidin neighbors at bonds 12–15 is due to increased chain folding under the tryptophan side chains (the gross steric profile of a gramicidin monomer is a truncated inverted cone). This same decrease is seen more clearly in the C-18 profiles in Fig. 3 Å. Snapshots for C-16 and C-18 chains in Figs. 2B and 3B also show that these chains are generally too long to make contact with the peptide along their entire length (they extend above and below the gramicidin in Figs. 2B and 3B), while C-14 chains seem to be able to fold and pack closely to the gramicidin dimer along their entire length (Fig. 1B).

The quadrupolar splittings measured by Watnick et al. [6] at the C-9 and C-10 carbon positions for DMPC increase from 26 kHz at zero gramicidin concentration to about 32 kHz at 5 mol% gramicidin. This corresponds to a change in order parameter of less than 0.03. (It is of course possible that deuteration at a lower carbon on the chain might show a greater change in the splitting due to chain folding under the tryptophan residues. This effect is likely to be small for DMPC, but could be significant for DSPC, as our data in Fig. 3A suggest.) The data presented in Fig. 1A for C-14 chains at 1 mol% gramicidin show, for gramicidin neighbor chains, a small increase in order for bonds 3, 4, and 6, and the zig-zag behavior described above which begins at bond 9. The increased order in the upper parts of the gramicidin neighbor chains was persistent throughout the computer runs. The increase in the transverse relaxation rate observed by Watnick et al. [6] for C-14 lipid bilayers with increasing gramicidin concentration is consistent with the hindered rotational mobility of lipid chains which neighbor the gramicidin dimers seen in the simulations, although this hindrance is small for the C-14 model system at the concentration used in the calculations.

The effects of using a full bilayer model system rather than a monolayer are quite apparent. By allowing chains to move in the direction perpendicular to the bilayer plane the simulations have allowed chains of all lengths in a bilayer to fill voids in the bilayer center caused by *gauche* rotations of chains in the opposite monolayers. Equally significantly, chains which are near neighbors to the gramicidin dimer can utilize perpendicular motional freedom to locate optimal packing positions relative to the side chains of the peptide. The degree to which the chains are able to accomplish this seems to depend critically on their length relative to the hydrophobic polypeptide. The simulations all begin with all the chains in all-*trans* states. After MC equilibration a C-14 chain is comparable in length to a gramicidin monomer, while C-16 and C-18 chains are unable to undergo enough *gauche* rotations at lipid bilayer chain densities to match the peptide length. The simulation data therefore favor a picture in which a DMPC bilayer can most easily incorporate the peptide, but with increasing loss of chain mobility due to tight chain-peptide packing. DPPC and DSPC bilayers are unable to effectively incorporate the peptide because the chains are too long for efficient packing to take place (possibly this leads to instabilities which favor formation of an H_{II} phase). This is a slightly different picture of the microscopic interactions between lipid and gramicidin than the hydrophobic mismatch proposal of Watnick et al. [6]. In our view the C-14 chains undergo a small increase in the number of *trans* bonds per chain to match the hydrophobic length of the gramicidin. The C-14 chains are better able to perform this match than are the C-16 and C-18 chains, which must under go more *gauche* rotations than are sterically possible in a bilayer geometry. In this picture the greater mismatch occurs between the gramicidin and the longer chain lipids. It must be cautioned, however, that no lipid headgroups are included in the simulations. Before any truly definitive conclusions can be drawn simulations must be run using full lipid molecules, and probably also water.

The inclusion of perpendicular chain motions has also produced a certain amount of interdigitation of the chains between the two monolayers of the bilayer, usually involving 1–4 carbons on about 10% of the chains. It is not likely that this is an artifact of the single-chain nature of the model. Steric interactions prevent deeper interdigitation, and it is reasonable to suppose that this is also the case in real bilayers. But it is also possible that, in bilayers with chains in *sn*-1 and *sn*-2 positions, somewhat more interdigitation between the two monolayers occurs.

The calculations described in this paper may be considered as an initial step in building a microscopic theoretical understanding of lipid–protein interactions. The models studied in the simulations represent gradual, stepwise increases in the complexity over earlier models and, thereby, progress towards realistic models for lipid–protein interactions. This gradualist approach is essential for complete understanding of the real lipid-protein-water systems. It is hoped that the present calculations on a relatively simple model can pro-

vide a benchmark for future MC or Molecular Dynamics studies of more detailed models for lipids and proteins in bilayers.

Acknowledgement

We thank the referee of this paper for several useful suggestions. This work utilized the Cray YMP system at the National Center for Supercomputing Applications at the University of Illinois at Urbana-Champaign.

References

1 Jordan, P.C. (1987) J. Phys. Chem. 91, 6582–6591.
2 Andersen, O.S. (1984) Annu. Rev. Physiol. 46, 531–548.
3 Sawyer, D.B., Koeppe, R.E. and Andersen, O.S. (1989) Biochemistry 28, 6571–6583.
4 Seelig, J. and Macdonald, P.M. (1987) Acc. Chem. Res. 20, 221–228.
5 Rice, D. and Oldfield, E. (1979) Biochemistry 18, 3272–3279.
6 Watnick, P.I., Chan, S.I. and P. Dea (1990) Biochemistry 29, 6215–6221.
7 Peng, Z.Y., Tjandra, N., Simplaceanu, V. and Ho, C. (1989) Biophys. J. 56, 877–885.
8 Cornell, B.A. and Keniry, M. (1983) Biochim. Biophys. Acta 732, 705–710.
9 Morrow, M.R. and Davis, J.H. (1988) Biochemistry 27, 2024–2032.
10 Jakobsson, E. and Chiu, S. (1987) Biophys. J. 52, 33–45.
11 Chiu, S., Subramaniam, S., Jakobsson, E. and McCammon, J.A. (1989) Biophys. J. 56, 253–261.
12 Roux, B. and Karplus, M. (1991) Biophys. J. 59, 961–981.
13 Chiu, S.W., Nicholson, L.K., Brenneman, M.T., Subramaniam, S., Teng, Q., McCammon, J.A., Cross, T.A. and Jakobsson, E. (1991) Biophys. J. 60, 974–978.
14 Xing, J. and Scott, H.L. (1989) Biochem. Biophys. Res. Commun. 165, 1–6.
15 Wang, J. and Pullman, A. (1991) Chem. Phys. Lipids 57, 1–16.
16 Scott, H.L. (1990) in Molecular Description of Biological Membrane Components by Computer Aided Conformational Analysis (Brasseur, R., ed.), 49 pp, CRC Press, Boca Raton.
17 Scott, H.L. (1991) Biophys. J. 59, 445–455.
18 Jorgensen, W. (1982) Chem. Phys. 77, 4156–4163.
19 Seelig, J. (1977) Q. Rev. Biophys. 10, 353–418.