Vol. 165, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1989 Pages 1-6

MONTE CARLO STUDIES OF LIPID CHAINS AND GRAMICIDIN A IN A MODEL MEMBRANE

J. Xing and H. L. Scott*

Department of Physics, Oklahoma State University
Stillwater, Oklahoma 74078

Received July 28, 1989

Summary The Monte Carlo method has been used to simulate the equilibrium properties of a planar array of 94 saturated lipid chains and one monomer of Gramicidin A. Chains are free to move laterally in the layer plane and to change conformation via *gauche* rotations and long axis rotations in a continuum. All non-hydrogen atoms on chains and on the Gramicidin A monomer interact via 6-12 potentials, and periodic boundary conditions are imposed. Calculated results consist of order parameter profiles for C-14 and C-16 chains. Profiles are calculated for chains which are neighbors to the Gramicidin A molecule and for chains which are not neighbors to the peptide. The main conclusion is that the average conformations of the chains neighboring the Gramicidin A monomer are very similar to those of the bulk chains.

While there have been a large number of experimental and theoretical studies of the structure and properties of the gramicidin A model transmembrane channel (for reviews, see references [1,2]) less is known about the interactions between gramicidin and its surrounding lipid molecules in a model membrane. Deuterium NMR experiments indicate that the quadrupole splittings of the C–D bonds in the lipid hydrocarbon region are only slightly affected by the presence of gramicidin in concentrations up to lipid/gramicidin (4:1) [3,4]. There is, however, evidence for significant lipid-gramicidin interactions which affect the conductance of the gramicidin channel [1], and the lipid phase transition [5]. Changes in both head group and acyl chain composition can alter the channel conductance by as much as a factor of two [1]. At low concentrations of peptide ^{2}H NMR and differential scanning calorimetry indicate the presence of two coexisting phases in phosphatidylcholine bilayers. At higher concentrations the two phase region seems to disappear [5].

Due to the complex nature of the intermolecular interactions between even a simple polypeptide and lipid molecules the construction of theoretical models for lipid-protein interactions is a formidable project (for a review, see reference [6]). Simple models based on hard-core interactions which work fairly well for the description of lipid phase transitions are not so successful in predicting the observed properties of lipid-peptide or protein mixtures [7,8]. Thus a microscopic picture of these mixtures is not yet available, even though the structure and conformation of the gramicidin A dimer in a lipid bilayer is fairly well understood. For this reason we have performed Monte Carlo computer calculations of the equilibrium properties of lipid hydrocarbon chains interacting with a monomer of gramicidin A in a planar array. In this paper we present the results of our calculations.

* To whom correspondence should be addressed.

0006-291X/89 $1.50

Methods

The Monte Carlo algorithm used in the simulations is identical to that used earlier by Scott and Kalaskar to study lipid- cholesterol interactions [9], and is described in full detail in reference [10]. To briefly summarize, the cooordinates of all the carbon atoms in a 10 × 10 array of chains with the top carbons constrained to lie in a plane (but which are free to move laterally in this plane) are generated using rotation operations repeatedly. Initially the chains are positioned with hexagonal symmetry, and with an area per chain of $31Å^2$. To accomodate the gramicidin A monomer six chains in the center of the array are removed, and the atomic coordinates for the peptide are entered. The model used for the polypeptide is that of a left-handed β-helix with the amino terminus nearest the top surface of the monolayer and the carbonyl terminus nearest the terminii of the lipid chains. Co-ordinates were generously supplied by Professor Eric Jakobsson.

There are no clear experimental guidelines for the placement of the peptide in the lipid layer relative to the layer plane. Since both C-14 and C-16 saturated chains have greater length than a gramicidin monomer, and since all the side chains on the peptide are hydrophobic it is unlikely that the topmost chain carbon lies *below* the upper Trp-15 residue. In fact it seems likely that, in membranes, the gramicidin dimers are positioned in the center of the hydrophobic core and this requires either considerable chain folding or "dimpling" of the membrane surface. In this study the top chain carbons and the Trp-15 residue lie in the same plane. This position places headgroups and lipid carbonyls above the peptide and allows for optimal hydrophobic interactions. Interactions between all non-hydrogen atoms on all molecules were calculated as in reference [9] using optimized 6-12 potentials, and a cutoff of 15 Åwas employed. Periodic boundary conditions were imposed on the array to reduce finite size and boundary effects. A typical step in the Monte Carlo procedure consists of a lateral translation of the entire chain by a small distance in a random direction in the bilayer plane, a long-axis rotation of the entire chain, and the insertion of one or two *gauche* rotations at randomly chosen bonds.

The Monte Carlo algorithm was used to generate 6 million configurations at a temperature of 300 K (sufficient for all chains to disorder in the absence of external constraints). The first 3 million configurations were then discarded and averages over the final 3 million configurations were calculated. The physical quantities of interest for the averaging were the segmental order parameter profiles defined by:

$$< S_n >= \frac{1}{2} < 3cos^2\theta_n - 1 > \qquad (1)$$

where θ_n is the angular deviation of bond n from its orientation when the chain is in the non-tilted all-*trans* conformation. At regular intervals in the calculations, the configurations were written to a file and later displayed using the program pdViewer (A. Crofts and H. Robinson, Biotechnology Center, University of Illinois). All calculations were carried out on the Cray X/MP-48 machine at the National Center for Supercomputing Applications, Urbana, Ill.

Results

Figure 1 shows a plot of $< S_n >$ vs n as calculated for C-16 chains. In earlier work [9] it was demonstrated that profiles calculated by the present algorithm agree well with experimental data after the experimental order parameters are scaled by a factor of ~ 0.5 to allow for whole-chain tilting not included in the simulations. The figure shows that for bonds 1-9 there is no systematic difference between the order parameters for the chains which neighbor the gramicidin and the remaining (bulk) chains. At bond 10 there is a significant dip in the profile for the gramicidin neighbor chains, compared to the bulk chains. Then, for the remaining bonds 11-15 there is again no difference in order between bulk and gramicidin neighboring chains.

Figure 2 shows a plot of $< S_n >$ vs n as calculated for C-14 chains. For bonds 1-8 there is again little difference in order parameter between bulk and peptide neighboring chains. At bond 9 there is a sharp drop in the order parameter for the gramicidin neighbor chains, and from bonds 9-12 the $< S_n >$ remain somewhat lower than those for the bulk chains. At the terminal bond the order parameters are again equal.

Errors in the calculated profiles were estimated by calculating standard deviations in averages of the $< S_n >$ over subsets of the 3 million configurations used in the averaging

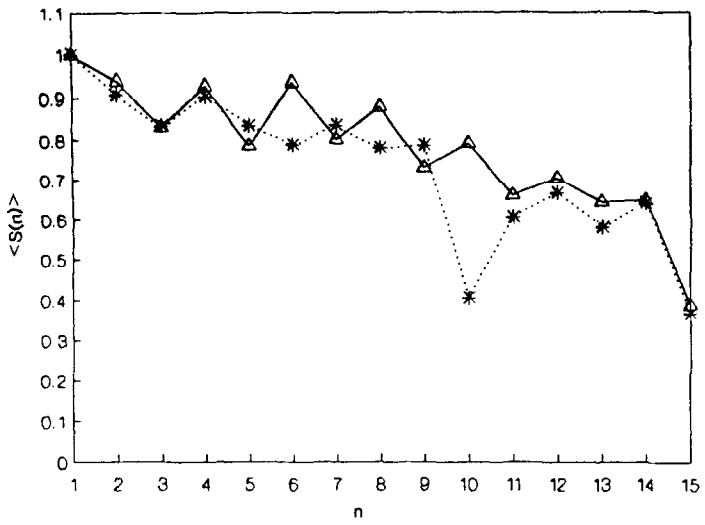

Figure 1. A plot of order parameter vs bond number for averages calculated in C-16 simulations: (△) averages for bulk chains; (*) averages for chains which are nearest neighbors of the gramicidin monomer.

for both the C-14 and C-16 simulations, and by carrying out a full independent second simulation for the C-16 system. Table I lists the standard deviations for the calculations based on a single run, and estimated errors determined from comparison of results from the two independent C-16 simulations. As the right-hand column in Table I shows, in simulations of systems with restricted geometries and strong excluded volume effects the variation in order parameters between two completely different runs is greater than the variations within a single run. This is a consequence of the complex excluded volume problem which severely limits the conformational changes allowed to the chains. In a system of 100 or more chains it is possible to generate numerically a large variety of chain conformations, and this is why the independent simulations calculate similar profiles for the bulk chains. However,

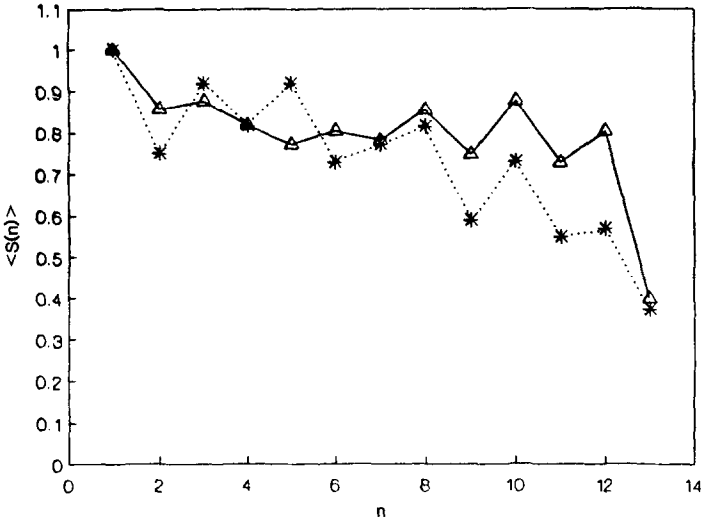

Figure 2. Same as Figure 1, with results from C-14 calculations. (△) averages for bulk chains; (*) averages for chains which are nearest.

TABLE I

STANDARD DEVIATIONS AND ESTMATED ERRORS IN $< S_n >$

bond no.	C-14: bulk chains	Single Run GA neighbor chains	C-16: bulk chains	Single Run GA neighbor chains	C-16: bulk chains	Two Runs GA neighbor chains
2	0.013	0.003	0.004	0.002	0.02	0.02
3	0.001	0.014	0.014	0.000	0.02	0.05
4	0.011	0.005	0.004	0.040	0.05	0.05
5	0.012	0.023	0.019	0.000	0.05	0.05
6	0.022	0.024	0.008	0.018	0.05	0.18
7	0.005	0.031	0.014	0.000	0.05	0.10
8	0.021	0.049	0.005	0.008	0.05	0.10
9	0.024	0.029	0.014	0.065	0.05	0.10
10	0.014	0.050	0.007	0.081	0.05	0.15
11	0.006	0.053	0.010	0.029	0.05	0.08
12	0.035	0.056	0.027	0.026	0.05	0.02
13	0.031	0.034	0.020	0.039	0.02	0.02
14			0.030	0.022	0.02	0.02
15			0.073	0.053	0.02	0.02

the gramicidin neighbor chains are much smaller in number, so the fluctuations are much greater and this is reflected in the profiles for these chains.

Discussion

Figure 3 shows schematically the relative lengths of the gramicidin A monomer and the C-14 and C-16 chains in the juxtaposition used in these calculations. It is apparent that bonds 8 and/or 9 are located at a level such that *gauche* rotations about these bonds can fold a chain into the open volume under the peptide. This is the reason for the reduced order parameters for the gramicidin neighboring bonds (10 and 9 in Figures 1 and 2 respectively). In independent calculations the large dip in $< S_n >$ did not always occur at the same bond but it did occur between bonds 5 and 10 in all cases considered. This is the reason for the uncertainties in the $< S_n >$ for the gramicidin neighbors in the right-hand column in Table I. The significance of bond 5 is that this bond is, as shown in Figure 3, just at the level of

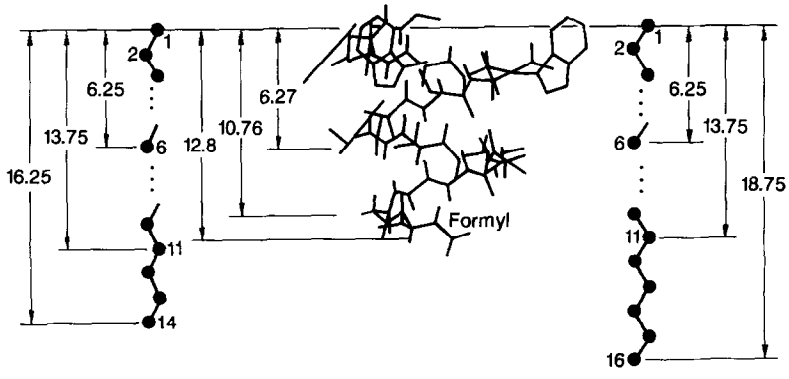

Figure 3. Schematic drawing of a gramacidin A monomer and two lipid chains of length 14 and 16 carbons, respectively. Distances are in angstroms.

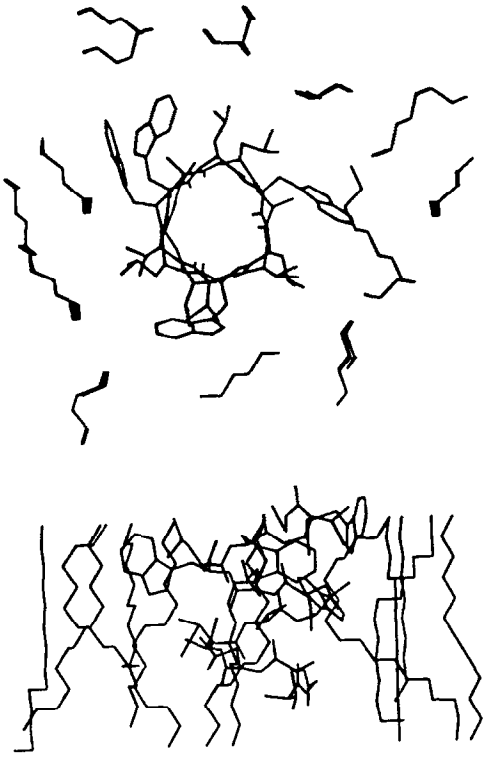

Figure 4. Two-dimensional projection of a typical configuration of the gramicidin monomer and neighboring chains. Top: Top view; Bottom: side view.

Trp-9, the lowest of the bulky tryptophan residues. It is possible for chains to occasionally fold under the Trp-9 residue via a *gauche* rotations about bond number 5 or 6 into free volume underneath this residue.

Figure 4 shows a plot of a typical configuration of gramicidin and its neighboring C-16 chains. The snapshot shows several chains folded under the peptide. In a full bilayer simulation with gramicidin *dimers* there would be no such free volume. Therefore we may conclude that in a model membrane lipid chains which neighbor the gramicidin dimer have, on average, the same conformational degrees of freedom as the bulk lipid in the bilayer. The existence of standard deviations which are equal to or very close to zero for gramicidin neighbor bonds 2 and 4 in the C-14 system and 2,3, and 5 in the C-16 system suggests some hindrance of rotational isomerism by the peptide. The magnitude of this effect is very small compared to that of cholesterol [9].

Our main conclusions from the simulations are (*i*): the average chain configuration as reflected in the order parameter profiles is identical for the bulk chains and the gramicidin neighboring chains except for the effect of chain folding underneath the Trp-9 residue and underneath the monomer itself; (*ii*) the monomer only slightly hinders rotational isomerism in its neighboring chains. In a bilayer containing gramicidin A dimers we expect almost no difference in order parameter profiles between gramicidin neighboring chains and bulk chains. The calculations and the conclusions are in accord with available experimental information on lipid-gramicidin bilayers [3,4]. Since the gramicidin does not appear to impose any specific conformational states on the lipid chains the likely mechanism for disruption of the lipid phase transition is the hindrance of *cooperative* rotational isomerism and of other cooperative lipid-lipid interactions such as chain tilting. All the simulations reported here were carried out at a 94:1 chain: gramicidin ratio, or a 47:1 lipid:gramicidin ratio (about 5.4 % gramicidin by weight). At smaller ratios effects due to hindered rotation may be more

marked as lipid cooperativity is reduced. We plan to examine smaller lipid:gramicidin ratios in the future. Even at higher peptide concentrations it is likely that the rough peptide surface will allow for significant conformational freedom among the lipid chains. This conclusion is also likely to be valid in the more general case of interactions between lipid chains and helical hydrophobic membrane proteins.

Acknowledgment

This work was supported in part by NSF Grant no. DMB 8703644.

References

[1] Andersen, O. S. (1984) Ann. Rev. Physiol. **46**, 531-548.
[2] Jordan, P. C. (1987) J. Phys. Chem. **91**, 6582-6591.
[3] Seelig, J., and Macdonald, P. M., (1987) Accounts Chem. Res. **20**, 221-228.
[4] Rice, D., and Oldfield, E., (1979) Biochemistry **18**, 3272-3279.
[5] Morrow, M. R., and Davis, J. H. (1988) Biochemistry **27**, 2024-2032.
[6] Abney, J. R., and Owicki, J. C. (1985) in *Progress in Protein-Lipid Interactions* (A. Watts and J. J. H. H. M. DePont,Eds.) pp 1-60, Elsevier Science, Amsterdam.
[7] Scott, H. L., and Cheng, W. H. (1979) Biophys. J. **28**, 117-132.
[8] Scott, H. L., and Coe, T. J. (1983) Biophys. J. **42**, 219-224.
[9] Scott, H. L., and Kalaskar, S. (1989) Biochemistry **28**, 3687-3691.
[10] Scott, H. L. (to appear) in *Molecular Description of Biological Membrane Components by Computer Aided Conformational Analysis* (R. Brasseur, Ed.), CRC Press, Orlando, Fl.