Biochimica et Biophysica Acta, 510 (1978) 209—215

BBA 78054

MONTE CARLO STUDIES OF PHOSPHOLIPID LAMELLAE

EFFECTS OF PROTEINS, CHOLESTEROL, BILAYER CURVATURE, AND LATERAL MOBILITY ON ORDER PARAMETERS

H.L. SCOTT and SHEAU-LIN CHERNG

Department of Physics, Oklahoma State University, Stillwater, Okla. 74074 (U.S.A.)

(Received October 31st, 1977)

Summary

In this paper we present the results of a Monte Carlo study of the effects of protein, cholesterol, bilayer curvature, and mobility on the chain order parameters of a lipid layer. The Monte Carlo method used is identical to the version developed earlier (Scott, Jr., H.L. (1977) Biochim. Biophys. Acta 469, 264—271). Simulations of protein and cholesterol effects are accomplished by insertion of a rigid stationary cylinder into the lipid matrix. The protein studies show the presence of boundary lipid (Jost, P., Griffith, O.H., Capaldi, R.H. and Vanderkooi, G. (1973) Biochim. Biophys. Acta 311, 141—152). The effect of cholesterol is dependent upon the length of the lipid hydrocarbon chains relative to the cholesterol depth of penetration. Our computer studies of bilayer curvature show the manner in which this curvature disrupts chain packing and are consistent with experimental results (Chrzeszczyk, A., Wishnia, A. and Springer, C.S. (1977) Biochim. Biophys. Acta, 470, 161—171). We also find that restricting lateral motion in chains, the simplest manner in which head group interactions can affect hydrocarbon chain order, does not measurably alter the order parameters. We argue that this provides some support for an earlier hypothesis by Scott (Scott, Jr., H.L. (1975) Biochim. Biophys. Acta 406, 329—346) regarding head group-chain interaction in monolayer experiments.

Introduction

In an earlier paper [1] the Monte Carlo method was applied to the study of linked hydrcarbon chains interacting through hard-core repulsive forces with one end attached to an interface. In this work, the objective was the study of the effect chain packing has upon the order parameters at each chain

position. The results were in agreement with NMR measurements of the order parameters, and provided a measure of the perturbing effects of spin labels in the terminal regions of chains. In the present paper we now apply our Monte Carlo method to the study of the effects other membrane components have upon the lipid order parameters.

First, we present the results of our study of lipid-protein hard-core interactions and compare them with experimental studies of boundary lipid [2]. We then consider the hard-core interactions between lipid and cholesterol molecule, at different penetration depths for the latter. Next, we consider the affect of both positive and negative bilayer curvature upon the order parameters, and compare our results with recent NMR data [3]. We finally analyze the effects of restricted lateral motion on the order in the hydrocarbon region. Our motivation for this latter study is to test an hypothesis put forward by one of us [4,5] which states that, in a continuous-compression type monolayer experiment, the head group region and the hydrocarbon region are effectively thermodynamically isolated.

Method

The details of the Monte Carlo method used here are described in detail in ref. 1. The method consists of picking a chain at random and first translating the chain a random, but small, distance in a random direction in the plane of the layer. Then a bond is picked at random on the chain and *gauche* rotations are performed with proabilities derived from standard Monte Carlo importance sampling theory [1]. Next, the new state is checked for hard-core overlaps between chains and other objects in the layer. If none are present, the new state is accepted. If an overlap does occur, the new state is rejected, and the system is returned to the original state. In either case the order parameters are determined, and a running Monte Carlo average is kept. In the limit of a very large number of steps this average approaches the thermodynamic average. In all simulations periodic boundary conditions were imposed. In this paper we now discuss in turn the modifications used in each of the simulations we performed.

Lipid-protein interactions. We simulate the presence of a protein molecule by an impenetrable cylinder placed in the lipid array. We the arrange the lipid molecules around the cylinder so that certain lipids are located in a nearest neighbor annulus around the protein, and others are located in a next-nearest neighbor annulus. In order to economically simulate the closely packed protein-lipid system, no translational motion of lipid occurred in these runs, and the protein radius is only a few times larger than the radius of a hydrocarbon chain.

Lipid-cholesterol interactions. These simulations are similar to the lipid-protein simulations described above, except the cylinders which now simulate cholesterol are smaller (approx. 2 × chain size) than those which simulate protein and do not completely penetrate the hydrocarbon region. Two values of the penetration depth were used, corresponding to 70 and 30% of the all *trans* chain length.

Bilayer curvature effects. In these simulations we considered lipids confined

to arrays which are not rectangular, but trapezoidal in cross-sectional shape, with the top (head-group region) either wider or narrower that the bottom, corresponding to the two types of curvature. In these runs, as well as all runs described above, periodic boundary conditions are imposed. In the curvature simulations, however, the application of the boundary conditions is of prime importance. In this case we require that a segment of a chain leaving the paralelepiped re-enter at a point $\vec{r}$ determined by the location the segment would have in an adjacent volume in a curved layer made by packing the trapezoidal boxes with their larger faces on the same side of the layer.

Restricted lateral motion. This analysis is identical to that of ref. 1, except we restrict the translocation of hydrocarbon chains by either performing translations on only a fraction of the Monte Carlo steps, or by performing no translations at all. In all cases, the initial chain positions and configuration were the same.

Results and Discussion

Lipid-protein interactions. In Fig. 1 we plot the order parameters for systems containing eight hydrocarbon chains and one cylinder at the center of the array. The radius of the cylinder is 2.25 times the carbon-carbon bond length. This is very small for a protein, but we must use this size in order to observe boundary lipid in our chain system, since the number of molecules we can

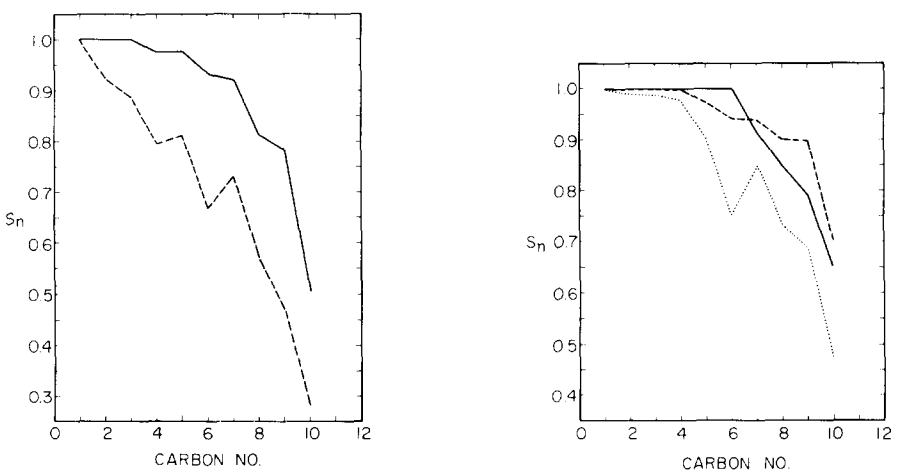

Fig. 1. Plot of order parameter, S_n, vs. carbon number, n, for a system of 8 chains, each 10 links long, confined to a 7 X 7 array. At the center of this array is a cylinder of radius 4.5 times larger than the chain hard-core radius, which completely penetrates the available volume. 4 of the 8 chains are confined to a nearest neighbor annulus (———) and the other to a next-nearest neighbor annulus (- - - - -). Runs were 100 000 steps.

Fig. 2. Plot of S_n vs. n for a system of 8, 10-link chains confined to a 6 X 6 array. In the center of this array is a cylinder of radius 2 times the carbon-carbon bond length, but which does not completely penetrate the available volume. Chains are completely mobile in these runs, which were 100 000 steps. - - - - -, penetration to 70% of all *trans* chain length; · · · · ·, penetration to 30% of all *trans* chain length. For comparison a plot of S_n vs. n for a 5 X 5 array of 10 chains only is also shown (———).

simulate is necessarily less than 10. The results should, however, be qualitatively the same for larger systems at the same molecular area. The differences in the order parameters in the nearest and next-nearest neighbor annuli are clear. In a more closely packed arrangement, which we would expect in a bilayer, the lipids in the nearest-neighbor annulus should have $S_n \approx 1$ for all n, while the lipids in successive annuli would have successively smaller S_n until the free lipid order parameters are reached [2,6]. For reasons discussed ref. 1, systems for which more than about 35% of the surface area is filled cannot be easily simulated by our method. However, our results show clearly that the presence of a large object in the lipid layer produces boundary lipid layers with lipid flexibility and mobility increasing with distance from the protein, simply because it encludes the lipid from the volume it coupies. It appears that protein-protein interactions are then mediated by this boundary lipid [6].

Lipid-cholesterol interactions. In Fig. 2 we present the results of our simulation of the steric portion of lipid-cholesterol interactions. Here we depict the cholesterol molecule as a cylinder of radius 2 times the carbon-carbon bond length which penetrates the lipid layer only partially [7]. Of course the cholesterol-lipid interaction is more complex than this, involving possible hydrogen bonding as well [8]. However, the extent to which cholesterol disrupts the order and packing in the hydrocarbon region should depend mainly upon the penetration depth, relative to the chain length. Thus, in Fig. 2 we present two curves, for 70% and 30% penetration relative to the length of an all *trans* chain. As can be seen, the former case yields order parameters which are, within our error limits, nearly equal to those for simple chain systems. The latter case, however, shows that for shorter relative penetration (i.e., for longer chain lipids) the free volume below the cholesterol leads to reduced parameters in the lower parts of the chains (from $n = 6$ to 10 for 30% penetration). This is consistent with the results of studies of the effect of cholesterol on lipid phase transitions in which the enthalpy change deacreases with increasing cholesterol content [9]. The rigid portion of the cholesterol molecule disrupts the cooperative rotations (not simulated here) which lead to disorder in the upper chain regions. Also, increased free volume in the lower regions of the chains allows for some disorder to exist here even below the phase transition temperature. The simulation depicted in Fig. 2 corresponds to a 1 : 8 cholesterol : chain concentration (or 1 : 4 cholesterol : phospholipid) and does not consider any preferred 1 : 1 associations which may exist in bilayers [10]. The effect of such an increased cholesterol concentration would be to make the chains more rigid above the penetration depth, and still allow for fluidity in the regions below this depth. Thus, the S_n vs. n plot would resemble a horizontal line with a precipitous drop at some value of n.

The system simulated here does not appear to resemble an actual lipid/cholesterol mixture for several reasons. The lipid chains are only 10 carbons long and the penetration of cholesterol therefore cannot reach the depth of 10—12 carbons, as some data suggest [7]. However, this is a problem of scale only so that there is no need to simulate the longer chain system. A system with chains 10 carbon atoms long, and cholesterol penetrating to the depth of 7 carbons should be very similar to a system with chains 16 carbons long, and the cholesterol cylinders reaching a depth of 11—12 carbons. We also do

not consider the effect of the cholesterol side chain in this study.

Effects of bilayer curve. In Fig. 3 we plot the results of our simulations of bilayer curvature and, for comparison, a plot for an uncurved system. Chrezsczyk et al. [3] report that the molecular area in the inner monolayer in curved vesicles varies from 68 $Å^2$ at the head groups to 94 $Å^2$ at the terminal region and from 76 $Å^2$ at the head groups to 51 $Å^2$ at the terminal region for the outer monolayer. Therefore, we have used arrays of size 5.5 X 5.5 (head group region) → 4.5 X 4.5 (tail region) and 6.5 X 6.5 (head group region) → 7.5 X 7.5 (tail region) to simulate the outer and inner monolayers, respectively. We find that while the relative area differences between inner and outer monolayers are greater than those used by Chrezsczyk et al. (although the percentage changes in area from head to tail are about the same), the average chain length only increases by about 20% in our studies, compared to 25% reported in ref. 3 for the hydrocarbon chains from inner to outer monolayer. This differences may be due to the less efficient packing available for the small (10 chains) system we simulated, or to the shape of the volumes we used, which do not exactly conform to the truncated pyramids of ref. 3. More realistic volumes are difficult to economically simulate. However, our results are at least qualitatively consistent with the conclusions drawn from NMR studies by Chrezsczyk et al. [3].

Restricted lateral mobility. In Fig. 4 we present the results of our simulations of the effect of restricted translocational motion, presumably caused by interactions between head groups, upon the segmental order parameters. Within the errors inherent in the Monte Carlo method, for fixed array size, the order parameters are the same for runs in which translocational motion is (i) allowed in all steps, (ii) not allowed in any step and (iii) allowed in any given

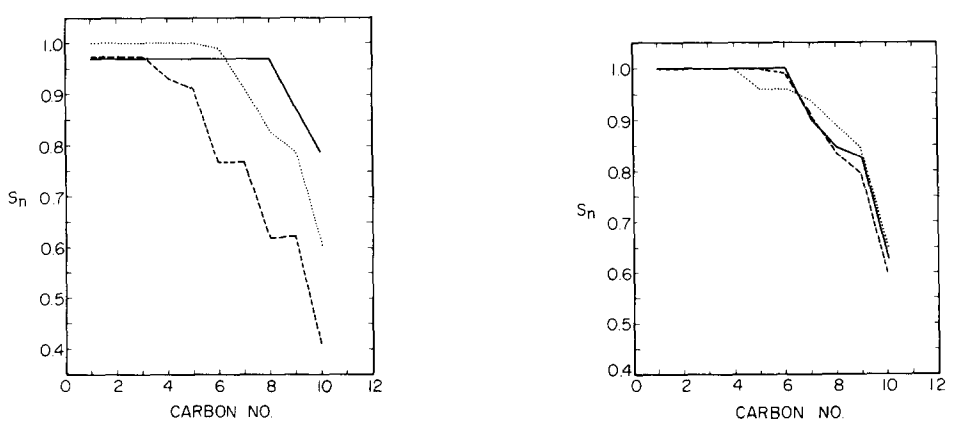

Fig. 3. Plot of S_n vs. n for systems of 10, 10-link chains confined to arrays of tapered cross-section as described in the text. All runs are 100 000 steps. ———, 5.5 X 5.5 (top) to 4.5 X 4.5 (bottom); - - - - - -, 6.5 X 6.5 (top) to 7.5 X 7.5 (bottom). The dotted line corresponds to the 5 X 5 untapered array simulation also shown in Fig. 2.

Fig. 4. Plot of S_n vs. n showing the effect of translational freedom on order parameters. ———, no restrictions; · · · · · ·, translations allowed with probability $\frac{1}{2}$; - - - - - -, no translations allowed. All simulations were performed using the same initial configuration for 10 chains, each 10 links long on a 5 X 5 array, with 100 000 Monte Carlo steps.

step with probability $\frac{1}{2}$. By not allowing translation to occur we limit the states sampled by the computer to those accessible via rotational motions only with the uppermost sphere in each chain held fixed. The results shown in Fig. 4 suggest that sampling these states only is sufficient to produce the same thermodynamic properties (i.e., order parameters) that are obtained when translocations are allowed. That is, the two samples are nearly identical. The results then imply that the order parameters in lipid mono- or bilayer are determined primarily by the area per molecule. In our simulations, Van der Waals forces were not included, but the long-range, nonpolar nature of these forces suggests that they make little direct contribution to the local molecular order.

In a lipid bilayer, the area per molecule is determined by head group-water interactions, in conjunction with chain packing and Van der Waals forces, to produce the molecular area which minimizes the total free energy. In a monolayer experiment, however, the molecular area is continuously changed by the experimenter so that the one way in which the head groups can interact with the hydrocarbon chains (by forcing the system into some fixed molecular area) is not available. Since the most direct form of head group-chain interaction is now removed, it is feasible that head group/water region is not able to influence the hydrocarbon region as easily as in bilayers. Then in continuous compression experiment equilibrium between these regions may never be attained. We feel, therefore, that the results in Fig. 4 support the hypothesis that, in a continuous monolayer experiment, the hydrocarbon region is effectively dependent of the head group/water region. Theoretical calculations based upon this hypothesis lead [4,5] to monolayer isotherms with the characteristic kinks seen experimentally.

A consequence of the above reasoning is that the phase changes observed in monolayer films are mediated by the hydrocarbon chains alone. This implies that at a given temperature the molecular area at which the isotherm kink appears depends upon chain length but not upon head-group type. The pressures in films of different lipids such as the phosphatidylcholines and the ethanolamines may, however, be quite different due to different contributions from the head-group region. Published data relevant to the above predictions do not appear to support this hypothesis, but the results are not conclusive, since the surface pressure between ethanolamine and phosphatidylcholine monolayers are considerable. For example, using the data of Phillips and Chapman, [11] dimyristoyl phosphatidylathenolamine shows a kink at 22°C at about 10 dynes/cm, 65 $Å^2$/mol, whereas dimyristoyl phosphatidylcholine shows no kink and at 65 $Å^2$/mol has a pressure of about 25 dynes/cm with a steeply rising isotherm at 22°C. Since 22°C is close to but still below the dimyristoyl dispersion melting temperature [12] (23.9°C) it is possible that a very slight undetected kink may exist in this isotherm at an area close to 65 $Å^2$/mol.

Acknowledgements

We thank Professor C.S. Springer for suggesting that we include bilayer curvature effects in our studies, and for a reprint of ref. 3 prior to publication.

We also thank Dr. J.F. Nagle and K.L. Carraway for comments on the manuscript. H.L.S. was supported in part by the U.S. National Science Foundation Grant No. PCM 76-20679.

References

1 Scott, H.L., Jr. (1977) Biochim. Biophys. Acta 469, 264—271
2 Jost, P., Griffith, O.H., Capaldi, R.A. and Vanderkooi, G. (1973) Biochim. Biophys. Acta 311, 141—152
3 Chrezeszczyk, A., Wishnia, A. and Springer, C.S. (1977) Biochim. Biophys. Acta 470, 161—171
4 Scott, H.L., jr. (1975) Biochim. Biophys. Acta 406, 329—346
5 Scott, H.L., Jr. and Cheng, Wood-Hi (1977) J. Col. Int. Sci. 62, 125—130
6 Marćelja, S. (1976) Biochim. Biophys. Acta 455, 1—7
7 Rothman, J.E. and Englemen, D.M. (1972) Nat. New Biol. 237, 42—44
8 Huang, C.H. (1976) Nature 259, 242—244
9 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 28, 42
10 Phillips, M.C. and Finer, E.G. (1974) Biochim. Biophys. Acta 356, 199—206
11 Phillips, M.C. and Chapman, D. (1968) Biochim. Biophys. Acta 163, 301—313
12 Mabrey, S. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. U.S. 73, 3862—3866