Mechanism of Solute Diffusion through Lipid Bilayer Membranes by Molecular Dynamics Simulation

Donna Bassolino-Klimas, Howard E. Alper,† and Terry R. Stouch*

Contribution from the Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, New Jersey 08543

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Abstract: This study extends previous studies of the mechanism of small molecule diffusion through lipid membranes. Atomic level molecular dynamics simulations of over 4 ns of benzene in fully hydrated dimyristoylphosphatidylcholine (DMPC) bilayers were performed at four different temperatures above the gel-to-Lα phase transition temperature. These studies confirm previous observations that small solutes diffuse at different rates in different locations in the bilayer. This difference in diffusion is likely to be due to "jumps" (single, large movements) between voids which are most common in the center of the bilayer. The benzene molecules appear to favor different regions of the bilayer at different temperatures. Although at 320 K the solutes show no regional preference, at 310 K they migrate to the center of the bilayer, while at 340 K they reside mostly near the head group region. This correlates with the distribution of free volume which concentrates at the bilayer center at low temperature but becomes more diffuse at higher temperatures. The mechanism of the diffusional process was found to be complex. Not only does the rate of diffusion depend on location within the bilayer, but the characteristics of this process appear to respond to temperature changes differently in the different regions of the bilayer. Only short time motions are dependent directly on the temperature. Longer time motions depend additionally on the size and availability of voids and the rate of torsional isomerization of the lipid molecules. It was found that an increase in kinetic energy was not always coincident with a jump; some jumps may be passive processes. This study provides further evidence that the interior of lipid bilayer membranes is not a homogeneous system analogous to pure alkane. Rather it is a structured system with different properties depending on the distance from the lipid/water interface.

Introduction

The transport of small molecules through lipid bilayer membranes is central to many biological processes. Insight into this process is vital for drug delivery and hence drug design. Unfortunately, little is known about the diffusion/permeation process at the atomic level. The earliest theory concerning this process dates back to Overton,[1] who found that the permeability of a molecule through a membrane can be correlated with its partition coefficient in water/octanol. However, it was found that the theory does not hold for small molecules.[2-4] Later, Walter and Gutknecht[5] found that small non-electrolyte molecules permeate inversely proportional to their molecular weight—i.e. small molecules permeate faster. This is consistent with the theories of Lieb and Stein,[6,7] who proposed that small molecules diffuse by "hopping" between empty voids in the bilayer. Smaller solutes can fit within smaller voids which are statistically more probable and so they diffuse faster. Unfortunately, it is difficult to confirm experimentally if this is the true mechanism at the atomic level.

Previously, using molecular dynamics (MD) simulations of atomic level lipid bilayers, we found that small solutes diffused at different rates depending on their location in the bilayers.[8] Initial results were based on crude correlations between the calculated diffusion coefficients for each of six benzene molecules and each molecule's general location within the membrane for several nanoseconds of simulation. This positional dependence was also found for the diffusion of water through a lipid membrane.[9] The results of these computer simulations are supported by experimental studies using ^{13}C relaxation studies of small nitroxide solutes in lecithin liposomes.[10]

Here, our previous MD studies[8] of small molecule diffusion within lipid bilayer membranes are extended and examined in more detail. This study includes 4 ns of molecular dynamics simulations at four temperatures above the gel-to-Lα transition temperature. The goal of this work was to elucidate the atomic level details of the mechanism of solute diffusion. This study shows conclusively that the rate of diffusion is dependent on location within the bilayer, demonstrates that diffusion through lipid membranes is a complex process, and shows that the lipid bilayer, even in the hydrocarbon region, is in many ways unlike bulk alkanes. The results support the theory of Lieb and Stein by showing that enhanced diffusion is correlated to an increased rate of jumps between regions of free volume.

Methods

A. Simulation Details. All atom models of explicitly hydrated dimyristoylphosphatidylcholine (DMPC) lipid bilayers at excess hydration containing four benzene molecules were employed. The details of the system were presented previously.[8,11] In summary, the system was composed of 36 DMPC molecules arranged in a bilayer with lateral

* Author to whom correspondence should be addressed.
† Current address: Moldyn Corp., Cambridge, MA 02138.
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dimensions of 34.5 Å × 34.5 Å in the $x-z$ plane, solvated by 962 water molecules. Two-dimensional periodic boundary conditions were employed in the $x-z$ plane to simulate an infinite bilayer. Repulsive walls were used to contain the waters in the y-direction (perpendicular to the bilayer plane) at the appropriate density, resulting in an effective "y" dimension of 61 Å. As noted previously,[12] in these studies the effect of the wall is negligible after 4 Å.

The number of benzene molecules included (4) was such that the ratio of solutes to lipids was comparable to that of experimental studies of clinical concentrations of anesthetics. The size of the simulation box was such that the closest approach of the benzene molecules was 10 Å; however, the benzene–benzene radial distribution functions showed almost all approaches to be greater than 15 Å.

All simulations were performed using a heavily modified version of DISCOVER V2.6 (Biosym Technologies Inc.). An all-atom representation of all molecules including all hydrogens was employed for a total of 7182 atoms. It has been shown that all-atom representation is necessary for the accurate estimation of diffusion coefficients.[13-16] The force fields were described previously.[8,17,18] These simulations took an average of 1.66 s/step on a single processor of a Cray Y-MP/2E-32.

The Verlet[19] algorithm was used to integrate the equations of motion with a time step of 1 fs. The simulations were run at four different temperatures: 310, 320, 330, and 340 K. Each temperature was maintained by coupling the system to an external bath.[20] Water was coupled to a separate temperature bath. As determined by the ratio of hydrocarbon chain torsions in the *gauche* vs *trans* rotamers (~0.3), all systems were above the gel → Lα transition temperature (determined experimentally as 297 K) for DMPC bilayers.[21]

The nonbonded neighbor list was updated every 20 time steps and evaluated to a distance 2 Å longer than the cutoff. A nominal 10 Å group-based cutoff was used for all interactions in all simulations. For interactions between waters, hydrocarbon chains, benzene molecules, and the glycerol moieties, nonbonded cutoffs were applied between small neutral groups of atoms (i.e. a "group"-based cutoff) as described previously.[8,12,17,22] A "residue"-based cutoff was employed for all interactions involving the zwitterionic phosphocholine head group, such that the whole group was treated as a single neutral moiety as described in Alper et al.[12] This residue-based cutoff results in a larger effective cutoff for the head groups (approximately 16 Å) which has been shown to be important for properly treating electrostatic interactions.[12]

A total of four simulations are discussed in this paper. The 1100 ps trajectory at 320 K was described previously.[8] (The first 100 ps were discarded as equilibration time and the remaining 1000 ps were used for analysis.) The final configuration of that simulation was used as the starting configuration for three other simulations at 310, 330, and 340 K. Each of these simulations was run for 100 ps of equilibration followed by 1000 ps of production run. Previously we found that the *trans/gauche* ratio of the lipid hydrocarbon chain torsions reach equilibrium values after 100 ps[11] and that convergence is seen for the hydrocarbon chain order parameters by approximately 800 ps.[23] Coordinate sets were saved every 100 fs for analysis. (Note that although these are constant volume simulations, experiment indicates that the change in the volume of the lipid over this temperature range is negligible.[24])

B. Calculation of Diffusion. The diffusion coefficient, D, was calculated from the Einstein relation:

$$D = {}^1/_6\{\lim\langle|R(t) - R(t=0)|^2\rangle\} \quad (1)$$

where $R(t)$ is the position at time t. D was calculated from a plot of the mean squared displacement (MSD) of the center of mass of each molecule averaged over all possible time origins vs time. For long t, the MSD becomes linear with time and the diffusion coefficient can be calculated from the slope. To determine if the diffusion has reached this limit and to determine if the Einsteinian model is appropriate, a plot of the log of the mean squared displacement vs log time was used. This plot gives a slope of 1.0 when in the region of Einsteinian diffusion, i.e. MSD $\propto t^n$ where $n = 1$. Non-Einsteinian diffusion results in different powers of n such as $n = 2$ for ballistic motion or $n = 0.5$ for anomalous diffusion.[13,25] This in turn is used to determine which portion of a plot of the MSD vs time to use in calculating the slope and hence the diffusion coefficient.

C. Computation of Distance Moved. The average distance moved in discrete periods of time was examined by calculating the displacement at various intervals corrected for periodic boundary conditions (eq 2):

$$d = \sqrt{\{|(R(t) - R(t-x)|^2\}} \quad (2)$$

where x is either 100 fs, 0.5 ps, 1 ps, 2.5 ps, or 5 ps. These data were plotted for each temperature in two ways. First, in order to probe the relationship between movement and position in the bilayer, the data were plotted as the mean distance moved (averaged over all solutes and over the entire trajectory) vs distance (in 1 Å slabs) from the bilayer center. Second, the data were plotted as a time series for each solute, in order to examine individual motions of solute atoms.

D. Computation of Free Volume. The free volume within the system was quantitated by a three-dimensional cubic grid of 0.5 Å spacing. Lattice points were first assigned as interior or exterior to lipid and water atomic volumes. The exterior points were labeled as concentric shells on the atoms as first, second, third, etc. (i.e. first neighbors were in shells immediately adjacent to interior points, second neighbors were adjacent to the first neighbors, third neighbors were adjacent to second neighbors, etc.). For this system, using a 0.5 Å grid, the highest valued points were fifth neighbors, meaning that they were 1.5–2.0 Å away from any atom. These highest valued points, being furthest from any atom, were used to locate the voids. The volume and centroid of the voids were then determined by expanding outward from those points to surrounding atoms. Volumes were computed from the sum of the surrounding points, and the centroids were determined from the mean position of all these points. First nearest neighbor points (the first shell surrounding the atoms) were not included in order to provide for an "excluded" volume around the atoms.[26]

Results and Discussion

A particularly interesting feature of these simulations was that, although the benzene molecules moved freely at all temperatures, at different temperatures the molecules preferred different regions of the bilayer. This is shown by Figure 1 which relates the positions of the benzene molecules relative to the center of the bilayer during the simulations to the temperature of the simulations. Figure 1a shows the mean position of each of the four solutes during the simulation and the overall mean position of all four solutes taken together. The simulations at 310, 330, and 340 K all started from the same configuration, the last configuration of the 320 K simulation, and so can be compared. During the simulation at 310 K, three

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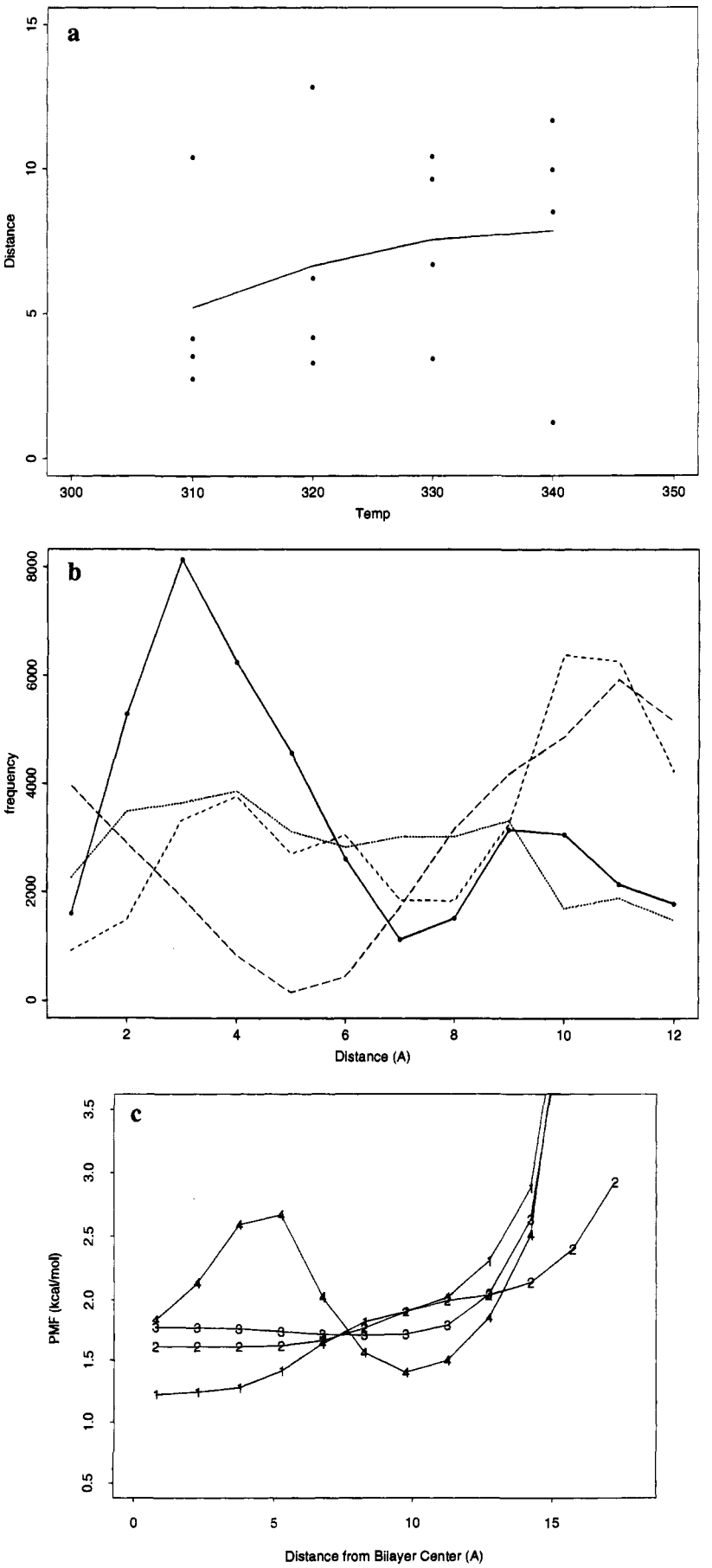

Figure 1. (a) Plot for all four benzene molecules in each simulation of the average distance from the bilayer center vs temperature of the simulation. The average positions of the individual benzene molecules are shown as points. (b) Plot of the frequency of occurrence of a benzene molecule as a function of distance from the center of the bilayer. The lines are as follows: 310 K, solid line; 320 K, dotted line; 330 K, small dashed line; and 340 K, large dashed line. (c) Plot of the potential of mean force calculated from the data in part (b) above: (1) 310, (2) 320, (3) 330, and (4) 3340 K.

of the solutes (#1, #2, and #4) moved quickly toward the bilayer center. The third (#3) initially moved away from the center by 8 Å, but moved back to the center about midway through the simulation. At 330 K, solute #1 gradually shifted farther from the center and #3 made several quick moves away from the center. Solute #2, although it made several moves toward the center, always reversed direction to remain about 10−12 Å away from the center. Solute #4 actually moved closer to the center after the first 300 ps and then oscillated about this position. At 340 K, solutes #2 and #4, although covering a range of 8−10 Å perpendicular to the bilayer plane and making several moves toward the center, always shifted back to positions 8−15 Å away from the center. Solute #1 moved rapidly 5 Å farther away from the center then gradually moved even farther away. Solute #3, although it made moves of about 5 Å in either direction away from the center, appeared trapped in a void and oscillated about its original position, near the bilayer center. In all simulations, benzene #2, on average, tended to stay farthest from the bilayer center, although this distance ranged from 7 to 15 Å. The overall results of these motions are summarized by the line in Figure 1a, which shows the mean position of all of the solutes. As the temperature of the simulation increased, the mean distance of the solutes from the bilayer center increased also.

This is further reflected by Figure 1b, which shows the frequency of occurrence of the solutes at different distances from the center. The probability of finding a solute near to the bilayer center is high at 310 K, but low at 340 K. The converse is true nearer the head group region (i.e. at a position about 10−12 Å from the bilayer center) where solutes were more prevalent at 340 K than at 310 K. Figure 1c further shows the potentials of mean force (PMF) calculated from the distributions. At 310 K, there was approximately a 1 kcal/mol difference between positions near to the center and those close to the interface. This preference was reversed at 340 K, although it is difficult to determine the magnitude of the difference (since the high peak for 340 K at 4−5 Å from the center is due to poor sampling in this region). The trends show the center to be increasingly more favorable for the solute as the temperature is lowered and the head group region to be increasingly favored as the temperature is raised.

These results agree with experimental observations of translational diffusion coefficients[27,28] which suggested that at low temperature solutes concentrate at the bilayer center and that an increasing range of positions become available to solutes with increasing temperature. The explanation of those authors was that, as the temperature is lowered, the bilayer center retains its fluidity longer than the regions near the head groups and consequently a lesser entropic penalty is paid if solutes move toward the center of the bilayer. Temperature-dependent structural changes in the bilayer and a consequential redistribution of free volume within the bilayer will be discussed later.

It is interesting to note that in Figure 1c the curves for 310, 320, and 340 K show inflection points at approximately the same place, near the center of the monolayers. This indicates a discontinuity in the rate of change of the properties of the hydrocarbon chains. This location roughly corresponds to that beyond which order parameters are known to decrease rapidly. A similar inflection in the rates of movement relative to position will be discussed presently.

Diffusion Coefficients. The diffusion coefficient of each benzene molecule was calculated according to the method described above and is shown in Table 1. (Figures 2a and 2b show plots from which the diffusion constant was calculated for one benzene molecule.) Table 2 shows the average distance from the center of the bilayer for each benzene molecule. These two tables show that, in general, as the benzene molecules move away from the center of the bilayer they diffuse more slowly. Previous studies have shown that simulations on the order of a nanosecond are required for diffusion to converge for these systems.[8,23] However, here there is some doubt whether, even at 1 ns, all of the benzene molecules exhibited Einsteinian diffusion particularly those near the head group region and those of lowest temperature. Near the head group region, the atoms are more tightly packed (as shown later) and more ordered. Hence, the benzene molecules' motions will be more restricted. It is possible that in this region it will require even longer times to reach the Einsteinian regime.

These qualitative trends in diffusion coefficients are clear. However, the complexity of the process of diffusion prevents the level of sampling that is needed to make direct quantitative comparison between the simulations and the different regions of the bilayer. These complexities are the following: First, as we noted, the molecules tend to reside in different regions of the bilayer at different temperatures. However, despite these tendencies, the movements of the molecules are not restricted and they were free to move between regions. Second, as we will show, the mechanism of diffusion changes with location in the bilayer. Third, the differences in structure of differen-tregions of the bilayer may prevent diffusion from becoming Einsteinian at some locations within the time scale of these simulations, as has been found for some polymer systems.[13]

Table 1. Diffusion Coefficients at Increased Temperature ($\times 10^6$ cm^2/s)

benzene molecule	310 K	320 K	330 K	340 K
BZ1	2.2	2.1	2.0[a]	1.5[a]
BZ2	0.96[a]	1.3[a]	0.97[a]	1.9[a]
BZ3	4.04	3.8	3.6	2.4
BZ4	1.1[a]	4.6	3.08	2.6[a]

[a] Non-Einsteinian diffusion.

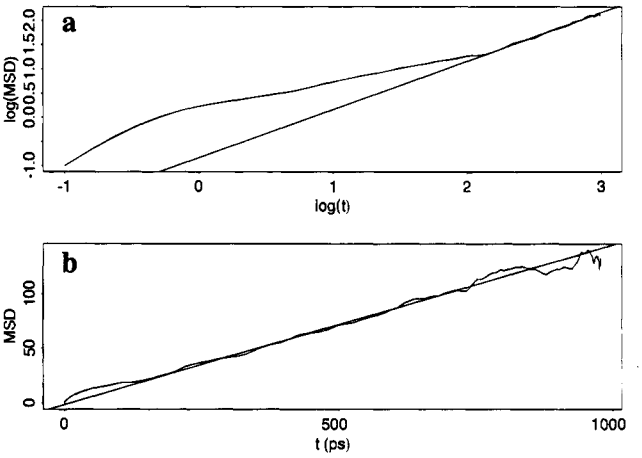

Figure 2. Example of the calculation of a diffusion constant: (a) log MSD vs log change in time (this shows the linear portion of the curve where diffusion is in the Einsteinian regime); and (b) MSD vs change in time (the slope of the portion in the Einsteinian regime identified in part (a) was used to calculate the diffusion constant).

Table 2. Average Distance (Å) from the Center of the Bilayer for Each Benzene at Different Temperatures

benzene molecule	310 K	320 K	330 K	340 K
BZ1	3.4	6.1	9.6	10.0
BZ2	10.4	12.8	10.4	11.7
BZ3	4.1	3.3	6.6	1.3
BZ4	2.8	4.2	3.5	8.5

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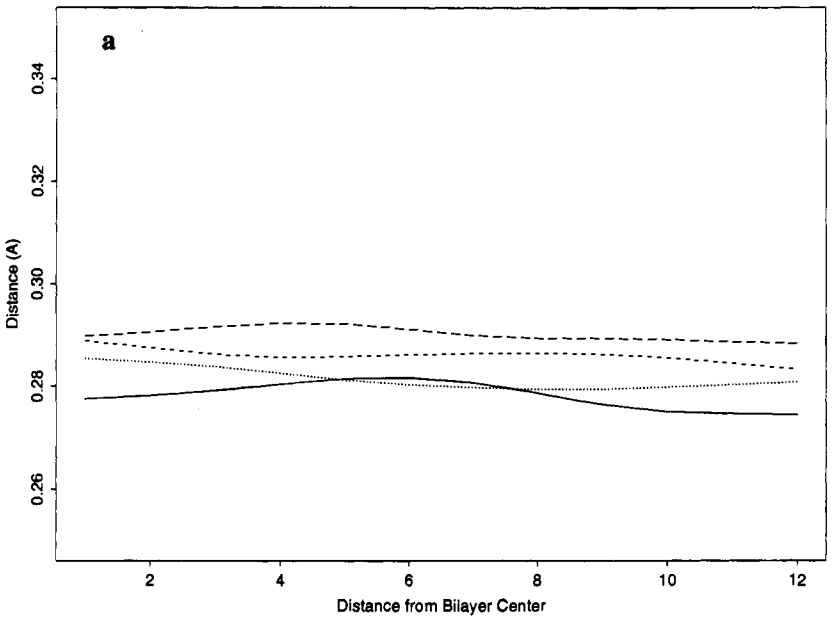
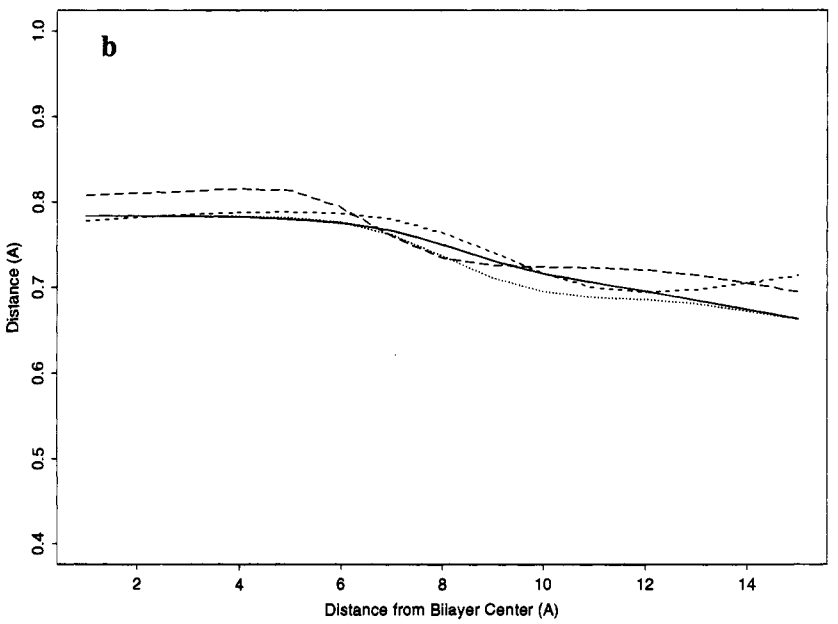
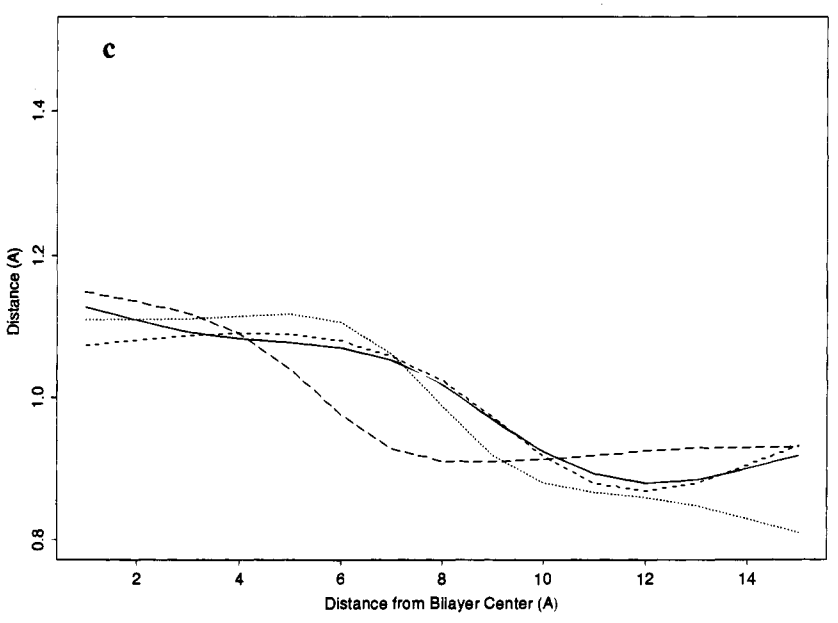

a
Distance (A)
0.26
0.28
0.30
0.32
0.34
2
4
6
8
10
12
Distance from Bilayer Center


b
Distance (A)
0.4
0.5
0.6
0.7
0.8
0.9
1.0
2
4
6
8
10
12
14
Distance from Bilayer Center (A)


c
Distance (A)
0.8
1.0
1.2
1.4
2
4
6
8
10
12
14
Distance from Bilayer Center (A)

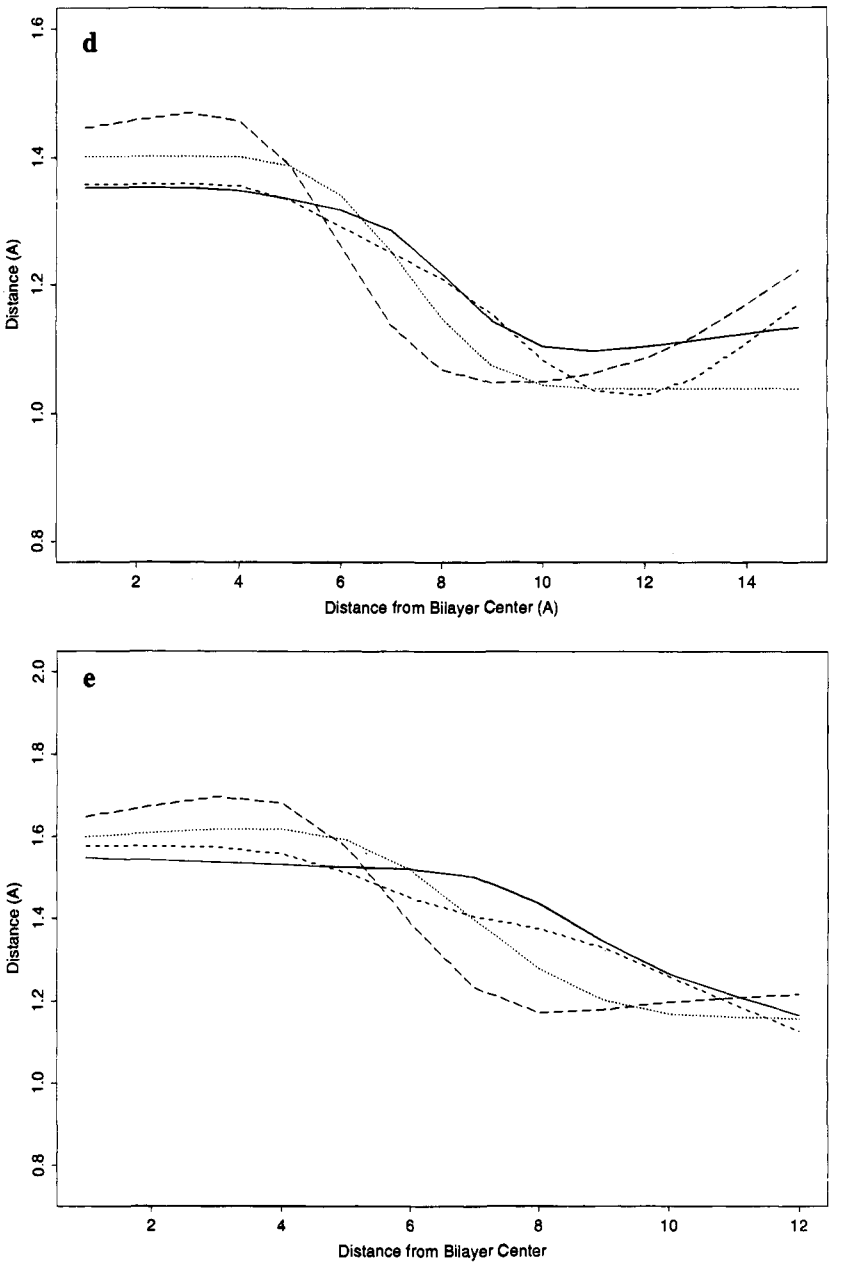


Figure 3. Average distance (all molecules over the entire 1000 ps) moved in a given time period as a function of the distance from the bilayer center: (a) 100 fs, (b) 0.5 ps, (c) 1.0 ps, (d) 2.5 ps, (e) 5 ps. The data presented have been smoothed for clarity. The line types are as in Figure 1.

Finally, as we will also show, the structure of the bilayer changes with temperature and this also affects the mechanism of diffusion. A similar change in diffusion mechanism with temperature has also been found for polymer systems.[15]

An additional possible factor for the non-Einsteinian diffusion seen here has already been addressed by others.[29-32] In studies of transfer across well-defined interfaces, it has been found that a substantial average force exists that can adequately be described only by the diffusion equations that incorporate the mean force. Although in this study the solute motion appears equivalent in all three Cartesian directions, it is possible that a net potential force (as hinted at by the PMF) could be manifested in differential rates of diffusion in longer simulations.

Diffusion, quantitated by the diffusion constant, measures average movement over long periods of time and is a composite measure of all aspects of a molecule's motion. As interpreted using theories of Lieb and Stein[6] and as we showed previously, the diffusion of benzene within the bilayer, especially in the low density bilayer center, is composed of at least two different phenomena: "rattling" within voids and "jumps" between voids in agreement with the "free volume" theory[33,2] that has been used to analyze the diffusion of the membrane lipids. The amount of motion in the rattling phase is determined by the size and fluctuations in the voids. The jumps are determined by the number, size, and proximity of the voids as well as the rate of torsional isomerization of the hydrocarbon chains which "gate" the passage of the solutes from one void to the next. This is similar to that found for penetrants in polymer systems.[13,25,26,34]

In order to more clearly understand the diffusional process and its dependence on temperature, we attempted to examine

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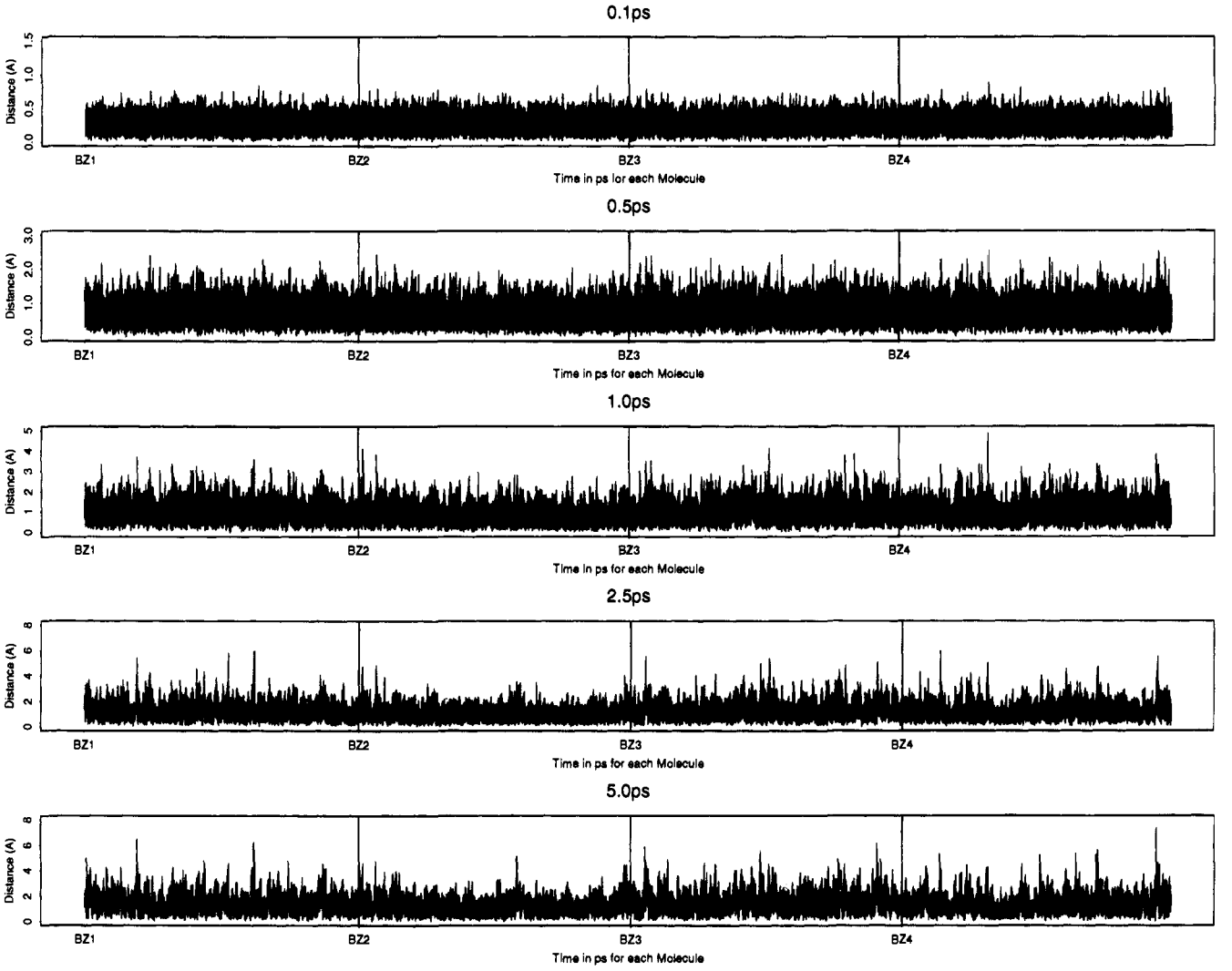

Figure 4. Distance moved in a given time period at 310 K, calculated for all starting points using eq 2 (see text) for time periods of (a) 100 fs, (b) 0.5 ps, (c) 1.0 ps, (d) 2.5 ps, and (e) 5 ps. The abscissa represents the full 1 ns time period for all four benzene molecules. The trajectories are deliminated by the molecule numbers (BZ1, BZ2, BZ3, BZ4).

these individual components of the process directly. We do this by examining the amount of movement that occurs over specific periods of time and how those movements relate to the position in the bilayer.

Position Dependence of the Average Distance Moved per Time Period. The average distance moved by each molecule during particular periods of time was examined as a function of distance from the center of the bilayer. Figure 3a shows this quantity for a 100 fs time period. (This is the shortest time frame possible for these simulations in which the coordinates were saved every 100 fs.) At this temperature, the magnitude of the distance traveled was dependent primarily on the temperature and, hence, the average velocities. Presumably, this time period approaches that of free flight. Figures 3b,c,d,e show this quantity at longer time intervals of 0.5, 1, 2.5, and 5 ps, respectively. At these longer times, the average distance traveled varies with position within the bilayer. Near the center of the bilayer the molecules move faster than toward the bilayer/water interface. Even at 0.5 ps, the distance traveled is 15% lower in the head group region than in the center of the bilayer. This increases to 20% at 1 ps and 35% at 5 ps.

The relationship between temperature and distance traveled at the longer times is not as clear cut as at 100 fs. The order of the curves is not always in line with expectations. This is probably due, in part, to the limited sampling within the 1 Å increments of these plots. As described above, the process is so complex that even these long simulations do not provide enough representation of the distribution of the molecules at different regions to provide adequate statistics for each 1 Å increment throughout the bilayer. Part of this complexity, as will be shown, is due to the redistribution of the free volume at different temperatures important to the process of diffusion. For this reason, we do not interpret these curves farther than to note the differential between the regions of the bilayer.

These plots confirm the qualitative trends seen for the diffusion constants and show, unequivocally, that the rate of movement is slower near the head group region than within the center of the bilayer, even at times as short as 1 ps. The change is not gradual or linear, but rather the rate of motion makes a steep increase toward the middle of each monolayer. Note that experimentally-determined order parameters for the methylene groups in bilayer hydrocarbon chains typically show a plateau of order which rapidly decreases at some point several methylene groups from the bilayer center.[35] This seems to parallel the behavior that we see for the rate of movement.

Mechanism of Diffusion—Nature of Jumps. The increased rate of diffusion for molecules in the bilayer center appears to be at least partly due to the fact that jumps occur more frequently and are larger at the bilayer center. Examination of the specific details of the molecular movements provides insight into how

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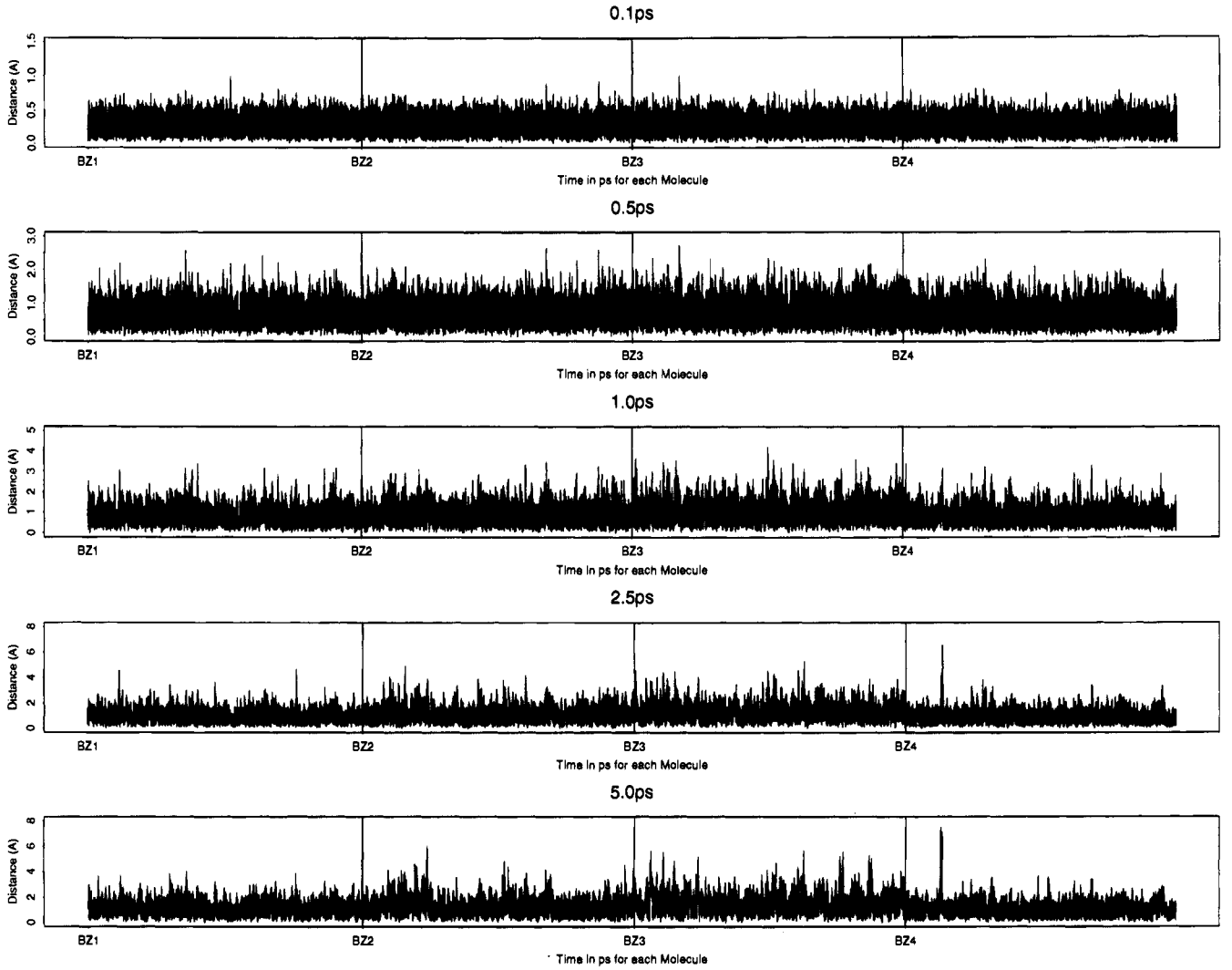

Figure 5. Distance moved in a given time period at 340 K, calculated for all starting points using eq 2 (see text) for time periods of (a) 100 fs, (b) 0.5 ps, (c) 1.0 ps, (d) 2.5 ps, and (e) 5 ps. The abscissa represents the full 1 ns time period for all four benzene molecules. The trajectories are deliminated by the molecule numbers (BZ1, BZ2, BZ3, BZ4).

the individual short time motions combine to give rise to longer time movements and the averages plotted in Figure 3. Here we define a jump as a rapid, significantly larger-than-average movement over a short period of time. In particular, the next few sections examine the nature of "jumps": their size, duration, relative rate of occurrence in different regions of the bilayer, and contribution to the overall rate of diffusion.

Figures 4 and 5 show the distance moved during each interval of 100 fs, 0.5 ps, 1.0 ps, 2.5 ps, and 5.0 ps for all four benzene molecules for the simulation at 310 and 340 K, respectively. The distance moved within any of the 100 fs intervals varies little. At 0.5 ps distinct spikes appear in the plot. These spikes become increasingly more pronounced and more frequent at 1 ps, 2.5 ps, and 5 ps. These significantly larger-than-average motions we previously referred to as "jumps" and were estimated to be motions of 5−8 Å in 5−10 ps. Figure 4 can be used to refine this estimate further. The magnitude of the jumps rapidly increases over time from ~2.5 Å at 0.5 ps, to 4 Å at 1 ps, until 2.5 ps when a maximum value of about 6 Å is achieved. At longer times, only relatively smaller increments are observed. In fact, distribution plots of the distances that the benzene molecules move during particular increments of time in most cases do not show many movements greater than 6−9 Å, even over 90 ps intervals. On this basis, we now refine our quantification of a jump as a 6 Å movement within 2.5 ps. This can be compared to jumps within homogeneous polyisobutylene of 7 Å/ps for H_2 and 5 Å/ps for O_2.[25] In a rigid zeolite structure jumps as large as 15 Å within 2−3 ps were observed for methane.[36] Of course, both matrices and solutes of these studies differ from benzene in a lipid bilayer, but they show physically meaningful trends.

As shown previously,[8] plots such as Figures 4 and 5 are diagnostic for location of a benzene molecule. For example, at 310 K one benzene molecule (BZ2) resided primarily near the head group region whereas the other benzene molecules (BZ1, BZ3, and BZ4) were located closer to the bilayer center. Although at time periods of 0.5 ps, or less, the profiles in Figure 4 are approximately the same, at longer times BZ2 experiences far fewer and far smaller jumps. At 340 K (Figure 5a−e), BZ3 is closer to the bilayer center than the others. As suggested by Figures 3a, 4, and 5, the short time motions appear equivalent regardless of position. It would seem that the increased rate of diffusion in the bilayer center is caused primarily by the presence of jumps.

Previously, we presented plots of the distribution of the magnitude of the movements of the benzene molecules at 320 K and related those distributions to the benzenes' positions within the bilayer (see Figure 8 of Bassolino-Klimas et al., ref 8). Figure 6, an expansion of part d of that figure, shows the distribution for a benzene molecule which spends most of its

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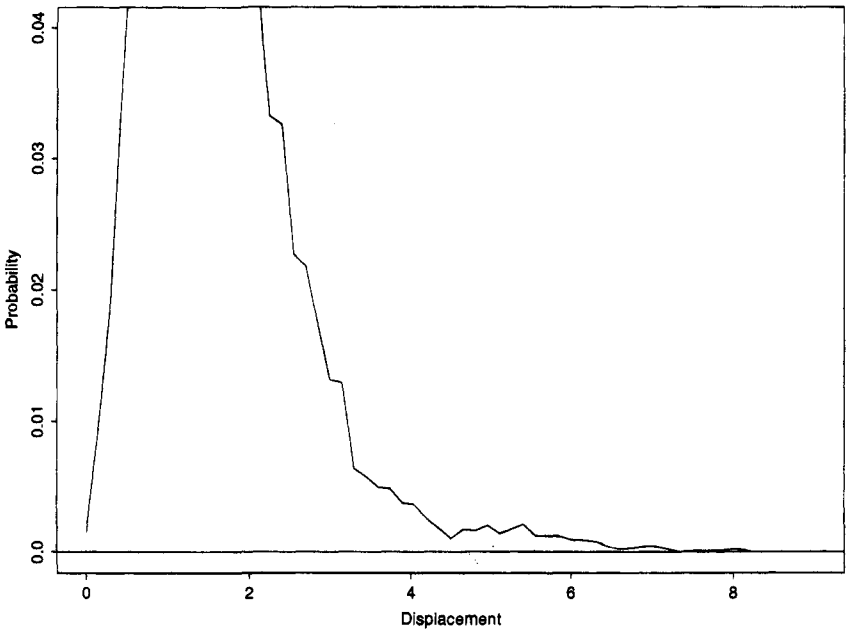

Figure 6. Probability distribution of the magnitude of movements over 10 ps for a benzene molecule near the bilayer center at 320 K. (Reprinted with permission from ref 8. Copyright 1993, American Chemical Society.)

time near the bilayer center from the 320 K simulation. Although the bulk of the movements for this time period (10 ps) are less than 4 Å, a second, small distribution of movements that range between 4.5 and 8 Å is also observed.

The notion of a jump implies a continuous motion. Our definition of a jump being 6 Å in 2.5 ps would require a velocity of 2.4 Å/ps (6 Å/2.5 ps), a value close to the average velocity of 2.7 Å/ps (at 310 K). At average velocity, if the motion were not continuous, it would require longer than 2.5 ps to travel 6 Å. Of course velocities leading to the 6 Å movements could be faster than the average velocity. However, as will be discussed, we do not always see an increase in kinetic energy of the benzene molecules prior to a jump. Considering the complexity and deformability of the bilayer, a jump as we define it could occur via any of a number of microscopic paths. Even within a rigid zeolite, jumps by propane between cavities were found to occur by a number of pathways.[36] On average, however, it appears that the jumps are single, continuous motions.

Types of Jumps. A molecular description of these jumps is complicated due to the complexity of the bilayer structure. The bilayer is composed of many lipid molecules, each capable of independent rotation, translation, and conformational fluctuation (which at the temperatures of these simulations is considerable). The hydrocarbon chains are quite intertwined and present a complicated matrix. As we mentioned, even within the relatively simple matrix of a rigid zeolite, the nature of jumps was found to be complex[36] and the details of particular short-time motions varied widely.

Previously, we noted that jumps can occur when a lipid hydrocarbon chain undergoes torsional isomerization and, by moving part of the chain aside, opens a path between two voids or enlarges a smaller void. This has been observed in many cases and may well be responsible for the majority of jumps seen in Figures 4 and 5. This is supported by the fact that torsional isomerizations occur rapidly (within 1 ps), albeit infrequently (residence time in a particular conformer is on the order of 10 to 100's of ps), similar to the infrequent occurrence but short time of a jump (2.5 ps). Also, the distance traveled during a jump corresponds with interchain distances of 6−8 Å seen at all levels within the bilayer as shown by carbon−carbon radial distribution functions (Figure 7). This is supported by experimental evidence which correlated water permeability through the skin with the number of gauche conformers, i.e. with hydrocarbon chain disorder.[37,38]

Yet not all jumps occur by that mechanism. Other cases are seen that are independent of torsional isomerization where a void becomes large enough such that a 6 Å or greater movement can occur within that single void. An example of this is the movement by benzene 4 at 340 K at about 100 ps (see Figure 5e). Anomolously, despite the fact that this benzene is near the head groups, it makes a large jump. This 8 Å movement occurs primarily in the direction perpendicular to the bilayer plane between two lipid molecules within an elongated void formed without the apparent benefit of torsional isomerization. It is likely that whole molecule movement by one of the lipid molecules enlarged the void. That the movement did not repeat itself suggests that the void closed fairly rapidly. Note that on-the-whole jumps are relatively infrequent; large jumps are usually hundreds of picoseconds apart. This would suggest that these single-void jumps are not responsible for the bulk of the jumps, since such movement would probably occur with a greater frequency than we observe.

We have not yet commented on the genesis of the jumps. For gases in polymers, some jumps by the gas molecules were found to be activated processed and were preceded by an increase in their kinetic energy.[13,25,26,39] However, the energy profiles of the jumps were not all the same. Indeed, in this system we noted a number of jumps that are preceded by a gain in kinetic energy of the individual benzene molecules. Presumably, this extra energy allows the molecule to transcend those physical barriers that separate two voids or prevent the expansion of a void. Interestingly, this energy gain was present only for a fraction of the jumps (~30%). This suggests that some of the jumps are passive and are due not to an activated process but simply to unimpeded motion. In other words, nothing gets in their way, either because no barriers are present to a particular direction of movement, as within a large void, or because a barrier has been removed, as in the gating action of a hydrocarbon chain torsional isomerization.

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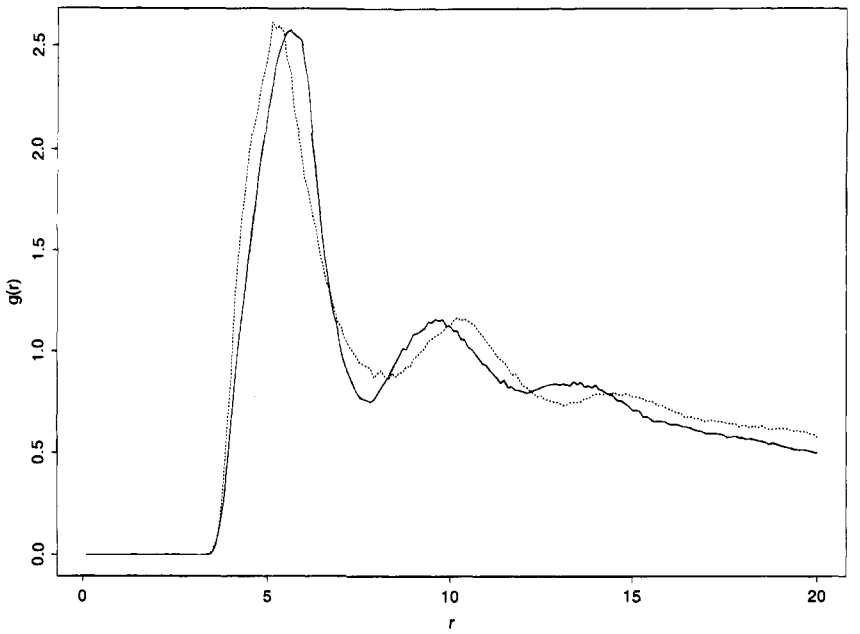

Figure 7. Radial distribution functions for the carbon atoms within the hydrocarbon chains of the lipid molecules showing the average chain–chain distances for the 4th (dotted line) and 13th (solid line) methylene carbon from the ester linkage.

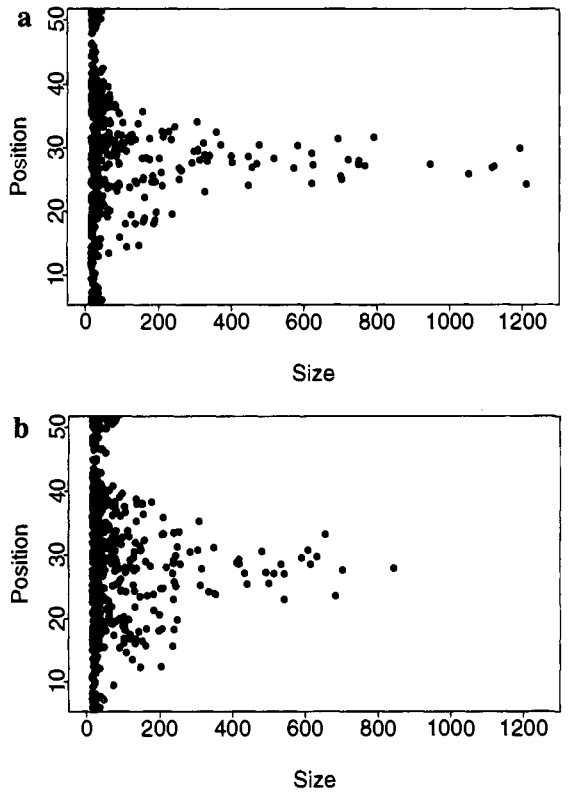

Figure 8. Distribution of free volume throughout the bilayer: (a) 310 K and (b) 340 K. The *x*-axis shows the size of the voids (Å^3). On the *y*-axis are the coordinates of the center of the void in the direction normal to the bilayer plane. The center of the bilayer is at 27.5 Å. These are composite plots showing data from 23 configurations each ~45 ps apart.

Free Volume/Voids. The size, distribution, and fluctuations of the voids are central to the "rattling"/"jumping" hypothesis for solute diffusion. Also, the redistribution of the location of the benzene molecules with temperature, and the sampling difficulties seen in the plots in Figures 1 and 3, prompted us to examine the distribution of the free volume in the bilayer at different temperatures. It is well-known that there is a large amount of free volume in lipid bilayers in the Lα phase. It was found previously that at 320 K the highest concentration of voids is in the center of the bilayer.[23]

A griding approach to quantitating and locating the voids was used as described in the Methodology. Figure 8 shows the void location in the direction perpendicular to the bilayer plane vs void size for the temperature extremes of 310 and 340 K. The figures are a composite of the voids found in 23 configurations, each separated by 45 ps. Two things are clear. First, as in the 320 K simulation, at these two temperatures most of the free volume is concentrated in the center of the bilayer in a number of voids. These voids can become large and many are well above the 82 Å^3 volume of a benzene molecule. However, it should be noted that these voids are not spherical in shape and may be irregular shapes connected by channels. This helps to explain why sometimes high concentrations of small molecules[40] can exist within lipid bilayers without causing much perturbation to bilayer properties; the small molecules can fit into pre-existing and spontaneously arising voids.

Second, these plots show that the distribution of the voids changes with temperature. At 310 K a number of very large voids are concentrated in the center and there is virtually no free volume in the head group region. At 340 K the free volume has become more disperse and voids are distributed more evenly throughout the hydrocarbon region. In the center, although still large and numerous, the voids do not become as large as they do at 310 K. Additionally, a number of small voids occur in the head group region.

The redistribution is physically reasonable. 310 K is only 12 K above the gel to Lα phase transition. At this temperature, the hydrocarbon chains could experience some freezing and increased packing. This would be most prevalent nearer the head groups where the chains are most ordered. Even at the center, however, van der Waals interactions could start to overcome the kinetic energy of the chains and initiate increased packing and consequent consolidation of the free volume. At 340 K, well above the transition temperature, the chains have more kinetic energy and move about more freely, decreasing the packing, sweeping through the bilayer more frequently, and causing a wider distribution of the free volume. The increase

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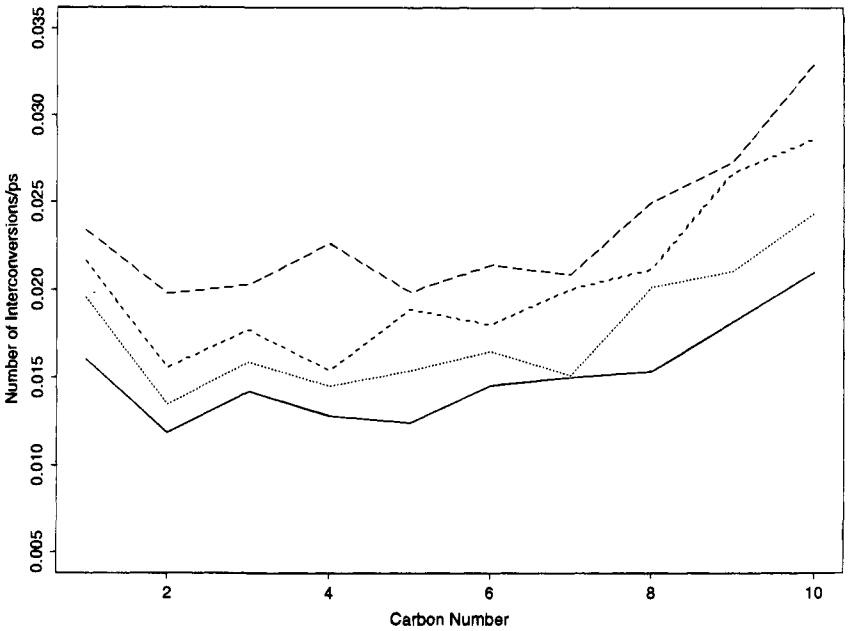

Figure 9. Frequency of torsional interconversion (number of guache → trans/trans → gauche interconversions divided by the length of the run) for each torsion along the hydrocarbon chain as a function of temperature. Line types as in Figure 1.

in torsional isomerization both with temperature and over the length of the hydrocarbon chains is shown in Figure 9.

This differing distribution of free volume helps to explain several phenomena which at first seemed inconsistent. First, it shows why the benzene molecules reside in different regions at 310 K vs 340 K. At 310 K virtually all of the free volume is at the center. At 340 K, the more disperse free volume, the smaller voids at the center, and the presence of more and larger voids near the head groups allow the solutes to penetrate into that region. Also as noted above, at higher temperatures the hydrocarbon chains experience increased motion. Since this is particularly true in the bilayer center, the presence of the solutes could exact an entropic penalty. Second, it helps to explain the fact that diffusion does not increase directly with temperature. Everything else being equal, an increase in temperature should result in an increase in the rate of diffusion. However, the larger voids at 310 K lead to longer times of unimpeded motion and more jumps in the bilayer center and enhanced movement relative to that which would be expected if the voids were of the same size as they are at 340 K.

Conclusion

These studies show that diffusion of solutes through biomembranes is a complex process. The inhomogeneous structure of the lipid bilayer causes the mechanism of diffusion to change with position relative to the water interface. Despite the complexity, the range of movements that contribute to the overall diffusion constant roughly correspond to either rattling within voids or jumps between the voids, as suggested by Leib and Stein.

We confirm previous estimation that diffusion of small solutes is faster within the hydrocarbon center than in the head group region. This appears to be partly due to the increased quantity and size of voids at the center which allow larger and more frequent "jumping" events (which we quantitate as an uninterupted 6 Å movement within ~2.5 ps). In addition, the rate of torsional isomerization is more rapid in the hydrocarbon center as we show here and as was shown by experiment.[35] This increases the frequency of "gated" jumps and is likely related to the "end-effects" found in polymer systems.[41] Since short time motions (~0.1−0.5 ps) appear similar in all regions of the bilayer, the increased rate of diffusion in the bilayer center appears to be due primarily to the increased frequency of jumps.

Although we previously hypothesized a gradient for the rate of diffusion relative to distance from the water interface, the change in rate, at least over short times, appears to take a steep inflection midway toward the center. The position of the inflection roughly corresponds to the position of the well-known decrease in the methylene order parameters.

The inhomogeneity of the bilayer structure and the changes observed with changing temperature make the relationship between the rate of diffusion and temperature difficult to quantify. Although the activation energy of "jump" events of gases in polymers[42] and in zeolites[36] has been quantitated, the complexity of the diffusional process studied here makes similar values difficult to determine for benzene molecules within the bilayer. Pant and Boyd[15] previously observed similar non-Arrhenius behavior in polyethylene where the mechanism of diffusion of methane was also observed to change with temperature.

The "jumps" themselves are of different types. Rare instances of large movements within single very large (and apparently short-lived) voids can be of a size and rate to classify as jumps. Some jumps appear to be facilitated by an increase in kinetic energy prior to the jump event. However, many jumps appear to result from the removal of barriers to solutes moving at a constant velocity. Well-defined cases are seen where a jump is preceded by an interconversion of a hydrocarbon chain torsion between conformational states. The interconversion removes a physical barrier between two voids between which the solute can then move. Evidence suggests that these are the most numerous type of jump.

Although the simulations were started at the same configuration, the benzene molecules migrated to different regions at different temperatures. Although this seems related to the distribution of free volume, energetic considerations could play a role and these are being studied.

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Explicit in both our current and previous experimental observations (as well as those of White[43,44] and Dill[45,46]) is the fact that the internal structure of lipid bilayers makes their properties distinct from those of a bulk-hydrocarbon/bulk-water interface. Previously, we showed that only at a position intermediate between the head group region and the bilayer center did the process of diffusion for small solutes resemble that within bulk alkane. The high concentration of terminal methyl groups at the bilayer center decreases the density of the region and makes it more fluid than bulk alkane. Toward the head group regions, the restricted motion of the hydrocarbon chain also makes this region unlike bulk alkane. In the center of the chain, however, the hydrocarbon mix takes on the properties similar to bulk alkane.[8,9,47] These differences should be considered during extrapolations between solubility parameters determined in bulk and permeability estimates for biomembranes.

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