

Inequivalence of single CH_a and CH_b methylene bonds in the interior of a diunsaturated lipid bilayer from a molecular dynamics simulation

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Abstract

Orientational order parameters for individual CH_a and CH_b bonds are local measures for the alignment of the bonds in a membrane interior. Experimental values exist for some lipid systems but no results are available from molecular dynamics (MD) simulations, although they are increasingly used to study biomembranes. We present such detailed analysis of a one nanosecond MD simulation for a PLPC (16:0/18:2 ^{$\Delta 9,12$}) bilayer. The results show marked inequivalence for the CH_a and CH_b bonds of the methylene segments in the beginning and in the double bond region of the diunsaturated sn-2 chain. They also suggest slight inequivalences in the saturated chain.

1. Introduction

Molecular dynamics (MD) simulations are increasingly used as an independent tool for biomembrane studies [1]. Several successful lipid membrane simulations have recently been carried out, e.g. for saturated DPPC [2–5] and monounsaturated POPC systems [6]. Methylene segment order parameters are good measures to characterise the structure of the hydrophobic fatty acid region of the membrane and they are available for a variety of lipid systems from both MD simulations [3–6] (and references therein) and ²H NMR experiments [7] (and references therein). The order parameter of a segment describes

local order with respect to an external reference direction. The most detailed description is obtained with a separate order parameter for each individual CH bond. Some experiments indicate a difference in the order parameters of the two CH bonds of the same carbon segment at some positions of the hydrocarbon interior [8–14]. However, we are unaware of any reported MD results for this kind of CH bond inequivalence.

The inequivalence provides additional information on the local structural details of a membrane interior and it can also be used as complementary data in the comparison of MD results and experiments. Additionally, evaluation of these parameters may be used to control the adequacy of the length of an MD simulation. In this Letter we report a detailed analy-

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sis of the orientational order of the CH_a and CH_b bonds in a bilayer interior based on a one nanosecond MD simulation of a diunsaturated phospholipid membrane.

2. Calculations

A PLPC molecule, 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphatidylcholine, consists of a glycerol backbone into which the saturated palmitic acid chain (16:0), the diunsaturated linoleic acid chain (18:2 ^{$\Delta^{9,12}$}) and the phosphatidylcholine (PC) head group are attached to the positions sn-1, sn-2 and sn-3, respectively. A phospholipid bilayer was modelled by constructing a monolayer system of 36 PLPC molecules plus 1368 water molecules, and by applying rotation–reflection in the bilayer normal direction. Details of the layer construction have been reported elsewhere [15]. Lipid force field parameters were used [16], except for the partial charges of the linoleic acid double bonds, which were estimated from an ab initio local density functional calculation [17] (charges were partitioned using the method of Hirshfeld [18]) and adapted to the charge distribution of the rest of the molecule. For water molecules the TIP3P parameters [19] were used. All atoms were independently taken into account. The system was simulated using molecular dynamics in constant volume and energy (an *NVE* ensemble). It was first heated and allowed to equilibrate for 200 ps, after which a 1 ns simulation was performed using the CHARMM software [20]. The average simulation temperature was 321 K. The simulation time step was 1 fs (using the SHAKE algorithm [21] for lengths of the bonds involving hydrogen atoms) and the nonbonded list was updated every 10 fs. During the simulation the coordinates and velocities were saved every 25 fs.

The orientational order is described by the order parameter S_j , which is defined as

$$S_j = \frac{1}{2} \langle 3 \cos^2 \theta_j - 1 \rangle,$$

where θ_j is the angle between the orientation vector and the reference direction. The brackets denote an ensemble and time average. A ^2H NMR observable order parameter, S_j^{CD} , is obtained by defining the orientation vector along the CH_j bond, and the bi-

layer normal as the reference direction. According to this definition we calculated the order parameters from the PLPC simulation for the CH_{aj} and CH_{bj} bonds. It is usual in MD simulations to define the orientation vector along the normal of the HC_jH plane or from C_{j-1} to C_{j+1} , when the molecular order parameter S_j^{mol} is obtained. When isotropic rotations around the HC_jH plane normal are assumed, the CH bonds and corresponding S_j^{CD} values become equivalent, and S_j^{CD} can be compared with the S_j^{mol} values according to the relation $-2S_j^{\text{CD}} = S_j^{\text{mol}}$ [22].

The S_j^{mol} may vary between $-1/2$ and 1 corresponding to the variation between perpendicular and parallel orientation with respect to the bilayer normal. A single orientation angle of 54.7° in the sample results in a zero value of S_j , but an isotropic orientation distribution also has a zero value.

The standard error of the mean (SEM, 68%) of the time averages was evaluated to estimate the accuracy of the S_j^{CD} values. In addition, the distributions of the angles between the individual CH_{aj} and CH_{bj} bonds and the bilayer normal were calculated to assess the origin of the order parameters.

3. Results and discussion

The data presented here are part of the results of the analysis of a 1 ns MD simulation for a PLPC (16:0/18:2 ^{$\Delta^{9,12}$}) bilayer. In order to ensure a sound basis for the present investigation, various simulation results [15,23] have been compared with the available experiments as well as with MD simulations for other lipid systems. In general, good agreement has been found. For instance, the average S^{CD} order parameter profile of the saturated sn-1 chain shows higher order for the PLPC system than is observed for the corresponding profile of DPPC and POPC systems [24,25] by ^2H NMR at approximately the same reduced temperature. This agrees with experimental observations of the stiffening effects of the sn-2 double bonds on the saturated chains [7,11,24,25]. Moreover, the average S^{CD} order parameter profile of the sn-2 linoleoyl chains in the simulated PLPC membrane and the corresponding ^2H NMR profile of the sn-2 isolinoleoyl chains in a

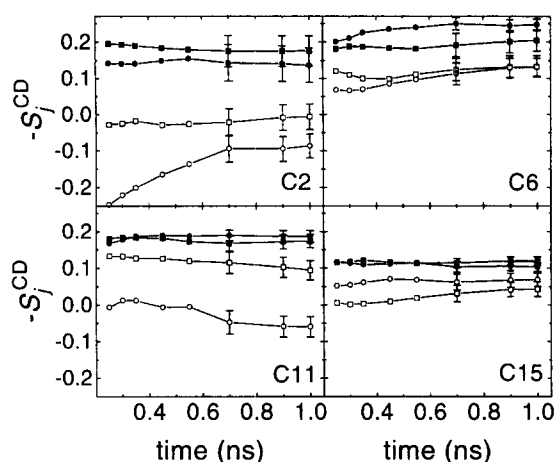


Fig. 1. The separate order parameter values $-S_j^{\text{CD}}$ and the SEMs of the time averages for the single hydrogens $\text{H}_{\text{a}j}$ and $\text{H}_{\text{b}j}$ of the sn-1 chains (■ and ●, respectively) and $\text{H}_{\text{a}j}$ and $\text{H}_{\text{b}j}$ of the sn-2 chains (□ and ○, respectively) averaged from the beginning to several time points of the simulation for the C2, C6, C11 and C15 carbon segments.

PiLPC (16:0/18:2 $^{\Delta 6,9}$) membrane [13] are remarkably similar at the region of the double bonds.

The separate S_j^{CD} order parameter values associated with the single hydrogens $\text{H}_{\text{a}j}$ and $\text{H}_{\text{b}j}$ of the methylene groups were averaged from the beginning to several time points of the simulation; the behaviour of the C2, C6, C11 and C15 methylenes is shown, as an example, in Fig. 1. In the beginning (< 0.5 ns) of the simulation some fluctuations occur but at the end (≥ 0.7 ns) all the order parameter values stay almost constant. This is an additional indication of the simulation integrity and suggests that the following orientational interpretations have a sound basis. Bearing in mind the inevitable time limitation and statistical nature of this kind of simulation, the slight differences at the end cannot be interpreted with statistical significance.

In Fig. 2 the average of $-S_j^{\text{CD}}$ for $\text{CH}_{\text{a}j}$ and $\text{CH}_{\text{b}j}$ bonds at different carbon segments are shown together with the S_j^{mol} based $-S_j^{\text{CD}}$ order parameter values. For the sn-1 chains these order parameter profiles are almost equal except for slight differences for the hydrogens related to the carbons C4–C8. In the case of the sn-2 chains differences are apparent for almost all of the segments, especially for the methylenes attached to the carbons C2–C4 and C9–C14. As the relation $-2S_j^{\text{CD}} = S_j^{\text{mol}}$ [22] holds for

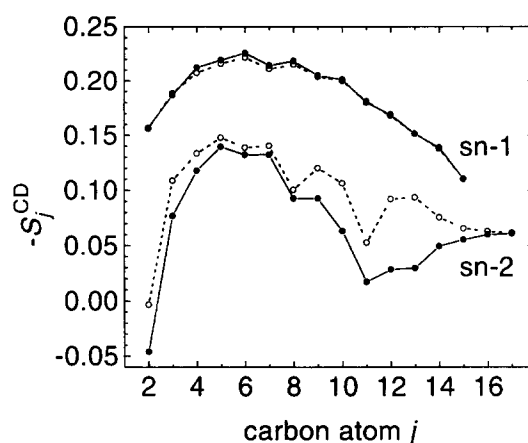


Fig. 2. The average of the $-S_j^{\text{CD}}$ order parameters for the $\text{CH}_{\text{a}j}$ and $\text{CH}_{\text{b}j}$ bonds at different carbon segments (●) together with the S_j^{mol} based $-S_j^{\text{CD}}$ order parameter values calculated as $-S_j^{\text{CD}} = S_j^{\text{mol}}/2$ (○).

isotropic rotations around the HC_jH plane normal, the results indicate that the rotation of the carbon segments in the diunsaturated chains is remarkably restricted but for the saturated chains only slight restrictions take place.

Absolute values of the differences between the S_j^{CD} values for the $\text{CH}_{\text{a}j}$ and $\text{CH}_{\text{b}j}$ are shown in Fig. 3. The SEMs of the time averages for the differences

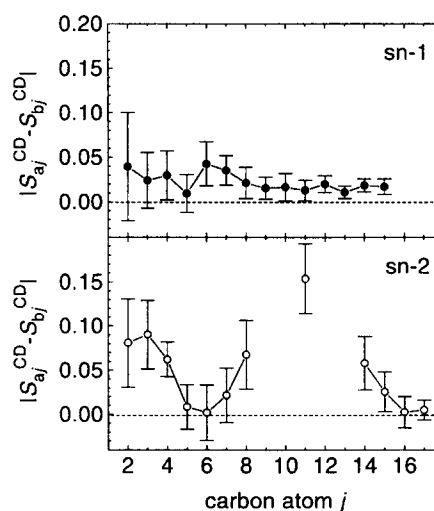


Fig. 3. The absolute values of the differences between the S_j^{CD} values for the $\text{CH}_{\text{a}j}$ and $\text{CH}_{\text{b}j}$ bonds of the sn-1 (●) and sn-2 chains (○). The SEMs (68%) of the time averages are shown to indicate the significance of the inequivalence.

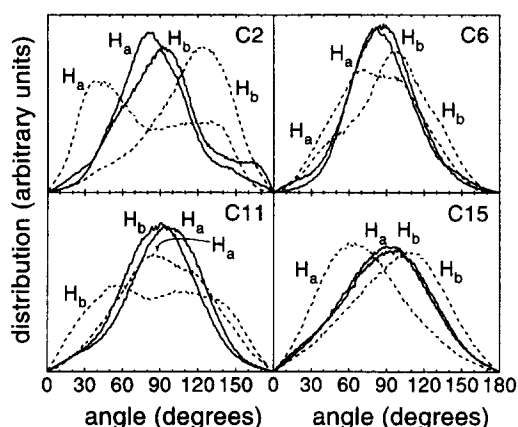


Fig. 4. The distributions of the orientation angles for the CH_{a_j} and CH_{b_j} bonds of the carbon segments C2, C6, C11 and C15 for the saturated sn-1 (—) and for the diunsaturated sn-2 chains (---).

are also shown to enable estimation of their significance. The inequivalence is clearly statistically significant for the carbon segments C2–C4, C8, C11 and C14 of the sn-2 chain. Also in the sn-1 chain some smaller inequivalences are observed. This indicates that the inequivalence of the CH_{a_j} and CH_{b_j} bonds is more evident for the diunsaturated chains. Supporting evidence comes from the distributions of the orientation angle for the CH_{a_j} and CH_{b_j} bonds, which are illustrated for the carbon segments C2, C6, C11 and C15 of both chains in Fig. 4. It is seen that the CH_{a_j} and CH_{b_j} distributions differ more for the sn-2 chains than for the sn-1 chains. Also comparison of the average orientation angle of the hydrogens with respect to the bilayer normal is in accordance with this: in the sn-1 chains the maximum difference of the average angles between H_{a_j} and H_{b_j} is 7.7° and the maximum deviation of the average angle from the perpendicular orientation (90°) is $\pm 6^\circ$, while for the sn-2 chains the corresponding values are 43.4° and $\pm 23^\circ$.

The difference of the order parameters S_j^{CD} in the sn-1 chain is largest for the carbon segments C2 and C6–C7. For C6–C7 the values are also larger than the SEMs. Interestingly, several ^2H NMR experiments have been performed determining the S^{CD} order parameters for saturated sn-1 chains (16:0) [7–9,11,24–28], but to our knowledge only Haberkorn et al. [9] and Paddy et al. [11] have observed the splitting of the deuterium labelled C2

resonance into two lines when studying a (16:0/16:0)PC system. Paddy et al. also observed the splitting for the (16:0/16:1)PC and found it to be larger for the monounsaturated than for the saturated system. They suggested this showed greater motional inequivalence resulting from the ordering effect of the cis double bond [11]. We have proposed a similar explanation for the ordering effect of the sn-2 chain double bonds on the sn-1 chains also in the PLPC system [15]. Most likely the inequivalence at the C2 position originates from the connection to the rigid glycerol backbone and the unsaturation of the sn-2 chains strengthens the effect by causing rigidity also at the lower parts of the chains. This explanation may also hold for the inequivalence observed in our simulation for C6–C7 of the sn-1 chain, which lie at the same depth in the bilayer as the sn-2 double bonds [15]. Also the slight difference between the above-mentioned order profiles of the sn-1 chains (Fig. 2) supports this idea of restricted motions due to the unsaturation.

In the diunsaturated sn-2 chain the inequivalence is clear for C2–C4 in the beginning of the chain and for C8, C11 and C14 in the vicinity of the double bonds. Plenty of observations exist of the hydrogen inequivalence at the C2 segment of the sn-2 chain [8–10,14,25–28], and the S^{CD} values have also been measured separately for C2, providing strong evidence for the orientational inequivalence of the hydrogens [10,14]. The inequivalence has been suggested to be caused by the tilted conformation in the beginning of the chain. This was also noticed in the simulation: the most prevailing angles of the vector C1–C3 with respect to the bilayer normal were 24° for the sn-1 and 60° for the sn-2 chains. Moreover, the orientation distributions of the CH_{a_2} and CH_{b_2} bonds were different (see Fig. 4), revealing their orientational inequivalence in the simulation. As in the case of C2 in the sn-1 chain, the connection to the rigid glycerol backbone probably also contributes to the observed inequivalence in C2 of the sn-2 chains. It is interesting to note that there are no experimental observations of the inequivalence for the C3 and C4 segments although in the simulation their inequivalence is as clear as in the case of C2.

The difference of the order parameters in the simulation for the C8 and C14 segments of the sn-2 chain is clearly caused by the orientational inequiva-

lence, since the average orientations of the CH_a and CH_b bonds are 69.9° and 100.1° for C8, and 114.0° and 70.6° for C14. Interestingly, for C11, which has the strongest inequivalence in the simulated lipid system (even above 3SEM, i.e. the 99% probability) both hydrogens have average orientations almost perpendicular to the bilayer normal. However, the distribution of the H_a is clearly narrower than that of H_b (see Fig. 4), emphasising the effect of the width of the orientation distribution on the observed S^{CD} value. In fact, Baenziger et al. [12,13] observed a splitting of the ^2H NMR resonance for the deuterium labelled C8 of an isolinoleoyl chain ($18:2^{\Delta 6,9}$), which is located between the double bonds similarly as C11 in the linoleoyl chain ($18:2^{\Delta 9,12}$). They suggest the inequivalence to be due to the restricted motional freedom caused by the rigid cis double bonds. Our simulated orientation distributions for C11 of the sn-2 chain substantiate this conclusion.

The inequivalences observed in the simulation are not quantitatively comparable with the experimental ones. For instance, for C11 of the sn-2 chain the difference of the S^{CD} values from ^2H NMR measurements for $(16:0/18:2^{\Delta 6,9})\text{PC}$ is about 0.003 [12], while the corresponding simulated value for the $(16:0/18:2^{\Delta 9,12})\text{PC}$ is 0.154. This discrepancy could be partly explained by the lack of experimental data for the simulated PLPC bilayer, since slight differences in the fatty acid composition may lead to notable changes in the structure of the membrane interior. Moreover, the different time scales, $< 10^{-6}$ s for the NMR versus 10^{-9} s in the simulation, and the different sizes of the systems may partly explain these quantitative differences. It is also possible that some of the inequivalences seen in the simulation, but not experimentally reported, may vanish if a longer simulation for a larger amount of PLPCs could be performed. On the other hand, e.g. for C6–C7 of the sn-1 chain, the reason can also be the lack of suitable experiments. Earlier measurements with specific deuteration have resulted in quite poor signal-to-noise ratios and resolutions causing considerable difficulties in resolving adjacent resonances. Recent measurements have usually been done using perdeuteration, i.e. all the methylenes of a hydrocarbon chain have been deuterated at the same time. This results in spectra with many overlapping peaks, thus making reliable assignment and analysis of pos-

sible inequivalences problematic, especially in the beginning of the sn-1 chain. These aspects may also explain why only in some of the reported experiments the inequivalence for the C2 hydrogens of the sn-1 chain have been observed.

4. Conclusion

In the present MD simulation of the PLPC ($16:0/18:2^{\Delta 9,12}$) system, a clear inequivalence of the order parameters for the CH_{aj} and CH_{bj} bonds is found at the carbon segments C2–C4, C8, C11 and C14 of the diunsaturated sn-2 chain. Also slight inequivalences are observed in the saturated sn-1 chain, especially for C2 and C6–C7. The observed inequivalences most likely originate from the stiffening effects of the glycerol backbone, the special tilted conformation in the sn-2 chain beginnings and the two cis double bonds. Based on ^2H NMR experiments the inequivalence has been reported for C2 of the sn-2 chain in various PC systems, for C8 of the sn-2 chain in PiLPC ($16:0/18:2^{\Delta 6,9}$) (corresponding C11 in PLPC) and additionally in $(16:0/16:0)\text{PC}$ and $(16:0/16:1)\text{PC}$ for C2 of the sn-1 chain, for which slight inequivalence was also observed in our simulation. This suggests that some of the inequivalences seen in our simulation, even though not reaching statistical significance, may be experimentally observable.

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References

- [1] K.M. Merz Jr. and B. Roux, eds., *Biological membranes: a molecular perspective from computation and experiment* (Birkhäuser, Boston, MD, 1996).
- [2] R.M. Venable, Y. Zhang, B.J. Hardy, R.W. Pastor, *Science* 262 (1993) 223.
- [3] E. Egberts, S.-J. Marrink, H.J.C. Berendsen, *Eur. Biophys. J.* 22 (1994) 423.
- [4] W. Shinoda, T. Fukada, S. Okazaki, I. Okada, *Chem. Phys. Lett.* 232 (1995) 308.
- [5] K. Tu, D.J. Tobias, M. Klein, *Biophys. J.* 69 (1995) 2558.
- [6] H. Heller, M. Schaefer, K. Schulten, *J. Phys. Chem.* 97 (1993) 8343.
- [7] M.A. McCabe, G.L. Griffith, W.D. Ehringer, W. Stillwell, S.R. Wassal, *Biochemistry* 33 (1994) 7203.
- [8] A. Seelig, J. Seelig, *Biochim. Biophys. Acta* 406 (1975) 1.
- [9] R.A. Haberkorn, R.G. Griffin, M.D. Meadows, E. Oldfield, *J. Am. Chem. Soc.* 99 (1977) 7354.
- [10] A.K. Engel, D. Cowburn, *FEBS Lett.* 126 (1981) 169.
- [11] M.R. Paddy, F.W. Dahlquist, E.A. Dratz, A.J. Deese, *Biochemistry* 24 (1985) 5988.
- [12] J.E. Baenziger, I.C.P. Smith, R.J. Hill, H.C. Jarrel, *J. Am. Chem. Soc.* 110 (1988) 8229.
- [13] J.E. Baenziger, H.C. Jarrel, R.J. Hill, I.C.P. Smith, *Biochemistry* 30 (1991) 894.
- [14] J.-P. Douliez, A. Léonard, E.J. Dufourc, *Biophys. J.* 68 (1995) 1727.
- [15] M. Hyvönen, M. Ala-Korpela, J. Vaara, T.T. Rantala, J. Jokisaari, *Chem. Phys. Lett.* 246 (1995) 300.
- [16] M. Schlenkrich, J. Brickmann, A.D. MacKerell Jr. and M. Karplus, in: *Biological membranes: a molecular perspective from computation and experiment*, eds. K.M. Merz Jr. and B. Roux (Birkhäuser, Boston, MD, 1996).
- [17] DMol Version 2.3 (Biosym Technologies, San Diego, CA, 1993).
- [18] F.L. Hirshfeld, *Theoret. Chim. Acta* 44 (1977) 129.
- [19] W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, *J. Chem. Phys.* 79 (1983) 926.
- [20] CHARMM Version 23.1 (Molecular Simulations, Waltham, MA, 1992).
- [21] W.F. van Gunsteren, H.J.C. Berendsen, *Mol. Phys.* 34 (1977) 1311.
- [22] J. Seelig, W. Niederberger, *J. Am. Chem. Soc.* 96 (1974) 2069.
- [23] M. Hyvönen, T.T. Rantala and M. Ala-Korpela, submitted.
- [24] A. Seelig, J. Seelig, *Biochemistry* 16 (1977) 47.
- [25] J. Seelig, J.L. Browning, *FEBS Lett.* 92 (1978) 41.
- [26] J. Seelig, N. Waespe-Sarcevic, *Biochemistry* 17 (1978) 3310.
- [27] H.U. Gally, G. Pluschke, P. Overath, J. Seelig, *Biochemistry* 18 (1979) 5605.
- [28] A. Salmon, S.W. Dodd, G.D. Williams, J.M. Beach, M.F. Brown, *J. Am. Chem. Soc.* 109 (1987) 2600.