Sensitivity of molecular dynamics simulations of lipids to the size of the ester carbon

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The dependence of molecular dynamics simulations of lamellar lipid systems on the potential energy parameters is examined. The radius of the ester carbon has a remarkable effect on the square area per lipid chain in contrast to the polarity of the glycerol ester, which does not. As a test system we make use of double-layers of the neutral triacylglycerol, trioctanoin, in the gel or α-phase.

MOLECULAR dynamics (MD) simulation is a powerful means of obtaining structural and dynamic information about complex bio-molecular systems at the atomic level. Simulations are generally sensitive to the functional form and parameter values of the force field. This is particularly evident for lamellar or micellar lipid systems, where interactions are repeated many times among similar molecules in the aggregates[1-4]. In a recent study, we examined the dependence of simulations of lipids on aliphatic alkane extended- or united-atom parameter sets[3], using the simulation program GROMOS[5,6]. The test system consisting of double-layers of the neutral triglyceride, trioctanoin, in the gel phase is particularly suited to study the force-field dependence of properties in simulation, as the lamellae are not hydrated and long-range electrostatic forces due to charged lipid head groups and counter ions dissolved in water are absent. In this report we focus on the surprising effect that the size of the ester carbon has on the geometric properties of trioctanoin systems in MD simulations.

The system we simulate consists of two 10×10 double-layers of trioctanoin, in the gel or α-phase. The molecule consists of three alkyl chains attached to a glycerol group by means of an ester linkage (Figure 1). In the double-layer arrangement characteristic of triglyceride gels[7], the molecules are stacked such that the layer repeat is equal to two chain lengths rather than three chain lengths seen in lipid bilayers (Figure 2). In all the simulations, the van der Waals parameters of the aliphatic alkane carbons as well as of the two oxygen atoms are identical and are taken from the latest GROMOS96 parameter set termed 45A3 (ref. 4). This is a united-atom parameter set where the hydrogen atoms are not treated explicitly, but included implicitly by

treating the carbons and their attached hydrogens as a single group centred at the carbon and has been specifically refined to fit the densities and heats of vapourization of a series of aliphatic alkanes. The geometry of the ester group is based on the consensus geometry arrived at by Chandrasekhar[8]. The corresponding parameters for the bonds and angles are identical in all the simulations and may be found listed in Chandrasekhar and van Gunsteren[3], where the simulation parameters and conditions are also detailed. The simulations are carried out under conditions of constant temperature and pressure, with an independent pressure coupling along the three Cartesian directions. Five simulations, differing in the van der Waals parameters of the ester carbon and the charges of the ester group are discussed. The simulation models referred to as M2 to M6 are detailed in Table 1. In each case the model number, the aliphatic alkane parameter set and the corresponding van der Waals parameters are given. Also listed are the partial charges assigned to the glycerol-ester group.

The mean square area per chain in the layer, A_c, is a characteristic of the phase of the lipid aggregate[9] and is often considered to be the order parameter of the phase transition of the system[10]. The value of A_c evaluated from 2.5 ns of the equilibrated trajectories for each of the simulations is listed in Table 1. The initial estimate of A_c was $0.192 \, nm^2$ (ref. 3). An inherent assumption in
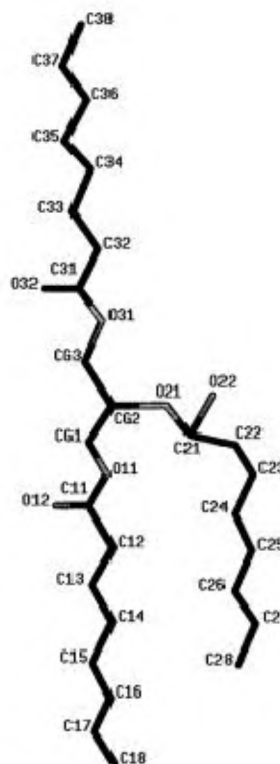

Figure 1. Trioctanoin molecule. The atomic nomenclature used is extended from a self-consistent nomenclature scheme developed in ref. 17.

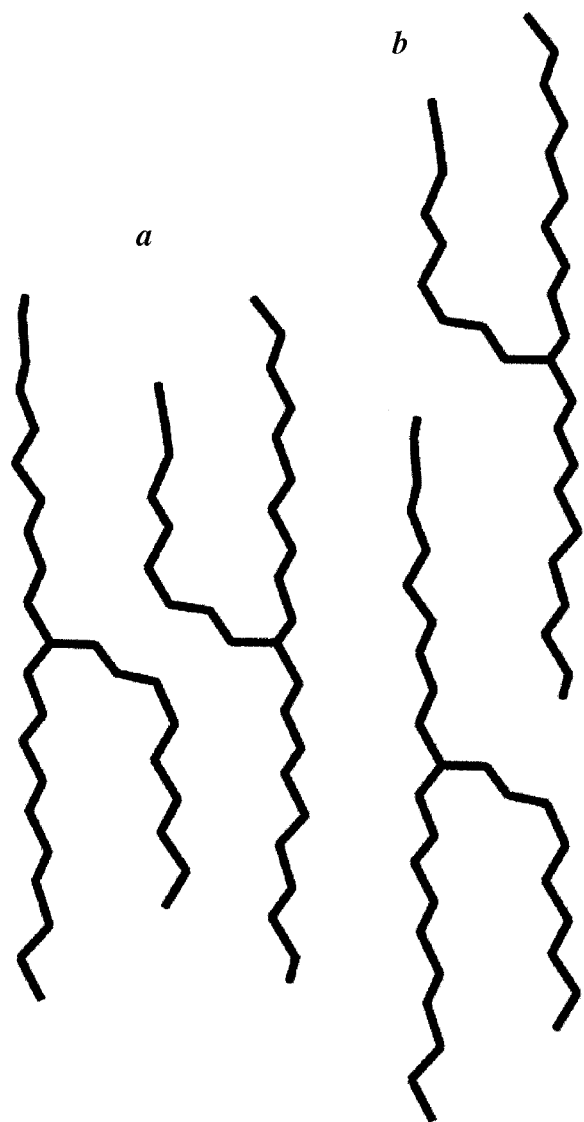*For correspondence. (e-mail: wfvgn@igc.phys.chem.ethz.ch)

Figure 2. Schematic of (*a*) the double-layer versus (*b*) the bilayer packing scheme of lipids.

Table 1. The models, the GROMOS96 parameter sets, the van der Waals parameters, ε and σ of the ester carbon and the resulting mean square area per chain, A_c, evaluated from 2.5 ns of equilibrated trajectory

Model	Parameter set (reference indicated in parentheses)	ε (kJ mol^{-1})	σ(nm)	A_c (nm^2) Initial estimate: 0.192 nm^2 (ref. 3)
M2	45A3-45 (19)	0.006995	0.6639	0.230
M3	[a]45A3 (4)	0.4059	0.3361	0.197
M4	[b]45A3 (4)	0.4059	0.3361	0.197
M5	[a]45A3 – 45 × 12 (19)	0.02577	0.5773	0.221
M6	[b]45A3 – 45 × 12 (19)	0.02577	0.5773	0.222

The van der Waals interaction is defined as $4\varepsilon[(\sigma/r)^{12} - (\sigma/r)^6]$.
[a]The partial charges (in *e*) for the glycerol ester group from GROMOS96 (ref. 5):
CGn = 0.2, On1 = –0.36, Cn1 = 0.54, On2 = –0.38, where *n* is the chain number (1, 2, 3).
[b]The partial charges (in *e*) for the glycerol ester group from ref. 18:
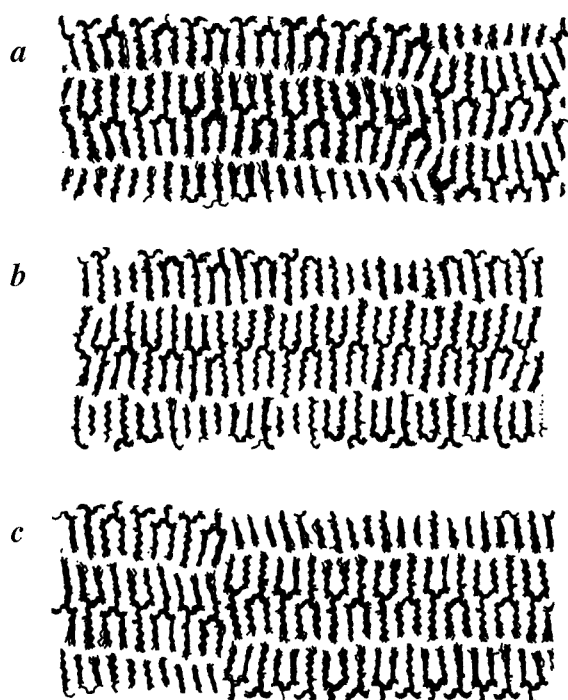CGn = 0.5, On1 = –0.7, Cn1 = 0.8, On2 = –0.6.

Figure 3. Equilibrated models at the end of 2.5 ns of simulation; *a*, M2; *b*, M3; *c*, M5. Models M4 and M6 are not shown, as they are similar to models M3 and M5, respectively.

arriving at this estimate is that the chains are in the fully-extended conformation. However this assumption is only acceptable as a first approximation, as triglyceride gels are believed to experience some form of chain relaxation: the reduced second moments of the ^{1}H-NMR spectra of triglyceride gels relative to the low temperature forms suggest that the molecules undergo reorientation about the long axis, attributed to either rotational diffusion and/or torsional relaxation[11]. Also, in triglyceride gels, the molecules are not interdigitated, but rather the chain termini undergo torsional reorientation and show chain melting at the layer interface[7]. Based on the above observations and the fact that the initial estimate of A_c is at the limit of the crystalline close-packed value[12–14], we anticipate an increase in A_c relative to the initial value.

The last column of Table 1 shows that there is indeed an increase in A_c in all the models. For model M2, where the ester carbon has the large radius of 0.66 nm, A_c is 0.23 nm^2. This is relatively high and falls in the range of the experimentally estimated square area per chain in a direction perpendicular to the lipid layer for hydrated dipalmitoylphosphatidylcholine (DPPC) in the gel phase, where the molecules are tilted relative to the

layer normal[13-15]. The square area per chain perpendicular to the layer normal for tilted systems is naturally larger than the square area per chain perpendicular to the chain direction, A_c. It is known, however, that triglyceride gels, unlike DPPC in the gel phase, are not tilted, as their thermodynamic properties do not show the chain length dependence characteristic of tilted systems[16]. Clearly, too large a radius for the ester carbon results in an uncharacteristically large A_c. Model M3 with an ester carbon radius of 0.34 nm, yields an A_c of 0.197 nm^2 that matches experimental A_c for fully hydrated DPPC in the gel phase, assuming a tilt per molecule of approximately 30° (refs 13 and 14). Model M4 differs from M3 in that the partial charges of the glycerol ester group are larger (Table 1). M4 has an A_c similar to M3, indicating that the charges do not play the same critical role in determining the system dimensions, as does the ester carbon size. The intermediate ester carbon radius of 0.58 nm in models M5 and M6 yields an A_c of 0.22 nm^2. In each of the models, the molecules in the layers show the overall conformational disorder characteristic of the gel phase of lipids[7,9]. However the form of the individual molecules is not significantly different between the models (Figure 3). As calculated properties are averaged over the entire array, the individual contributions of both the intra- as well as the inter-molecular interactions are reflected in the value.

In contrast to systems consisting of single molecules, the overall interactions of the triglyceride aggregates are dominated by the shear number of the methylene carbon interactions. As a consequence, we had not anticipated so significant an effect arising from the size of the carbonyl carbon. This is particularly so, as the van der Waals parameters for the aliphatic alkanes in parameter set 45A3 have been carefully refined against experimental data[4]. The fact that the polarity of the carbonyl group at the junction region of the molecule has little effect, is also surprising. In conclusion, MD simulations of simple neutral lipids reveal that the size of the ester carbon plays a critical role in determining the mean square area per chain even when the aliphatic al-kane parameters are identical, while the polarity of the glycerol ester group appears to have no effect on this quantity.

1. Chandrasekhar, I., in Biomembrane Structure and Function – State of the Art (eds Gaber B. P. and Easwaran, K. R. K.), Adenine Press, Albany, 1992, pp. 353–363.
2. Chiu, S. W., Clark, M., Jakobsson, E., Subramaniam, S. and Scott, H. L., J. Phys. Chem. B, 1999, 103, 6323–6327.
3. Chandrasekhar, I. and van Gunsteren, W. F., Eur. Biophys. J., 2001, in press.
4. Schuler, L. D., Daura, X. and van Gunsteren, W. F., J. Comput. Chem., 2001, 22, 1205–1218.
5. van Gunsteren, W. F. et al., Biomolecular Simulation: The GROMOS96 Manual and User Guide, vdf Hochschulverlag AG an der ETH Zürich and BIOMOS b.v., Zürich, Groningen, 1996.
6. Scott, W. R. P. et al., J. Phys. Chem. A, 1999, 103, 3596–3607.
7. Hernqvist, L., in Crystallisation and Polymorphism of Fats and Fatty Acids (eds Garti, N. and Sato, K.), Marcel Dekker, New York, 1988, pp. 97–137.
8. Chandrasekhar, I., Ph D thesis, Indian Institute of Science, Bangalore, India, 1986.
9. Small, D. M., The Physical Chemistry of Lipids from Alkanes to Phospholipids, Handbook of Lipid Research Series 4 (ed. Hanahan, D.), Plenum, New York, 1986, pp. 1–672.
10. Israelachvilli, J. N., Intermolecular and Surface Forces, Academic Press, New York, 1995.
11. Chapman, D., Richards, R. E. and Yorke, R. W., J. Chem. Soc., 1960, 436–444.
12. Tardieu, A., Luzzati, V. and Reman, F. C., J. Mol. Biol., 1973, 75, 711–733.
13. Wiener, M. C., Suter, R. M. and Nagle, J. F., Biophys. J., 1989, 55, 315–325.
14. Tristram-Nagle, S., Zhang, R., Suter, R. M., Worthington, C. R., Sun, W.-J. and Nagle, J. F., Biophys. J., 1993, 64, 1110–1112.
15. Nagle, J. F. and Tristram-Nagle, S., Biochim. Biophys. Acta, 2000, 1469, 159–195.
16. Hagemann, J., in Crystallisation and Polymorphism of Fats and Fatty Acids (eds Garti, N. and Sato, K.), Marcel Dekker, New York, 1988, pp. 9–95.
17. Chandrasekhar, I. and Sasisekharan, V., Mol. Cell. Biochem., 1989, 91, 173–182.
18. Chiu, S. W., Clark, M., Subramaniam, S., Scott, H. L. and Jakobsson, E., Biophys. J., 1995, 69, 1230–1245.
19. Schuler L. D., Ph D thesis, 14009 ETH-Zürich, Zürich, Switzerland, 2000.