Cholesterol Shows Preference for the Interior of Polyunsaturated Lipid Membranes

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · FEBRUARY 2008

Impact Factor: 12.11 · DOI: 10.1021/ja076641c · Source: PubMed

SUPPORTING INFORMATION

Cholesterol shows preference for the interior of polyunsaturated lipid membranes

Siewert J. Marrink[*,1], *Alex H. de Vries*[1], *Thad. A. Harroun*[2], *John Katsaras*[2,3], *Stephen R. Wassall*[4]

[1]Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands, [2] Dept. of Physics, Brock University, St. Catharines, ON L2S 3A1, Canada, [3]Canadian Neutron Beam Centre, National Research Council, Chalk River, ON K0J 1J0, Canada, [4]Dept. of Physics, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202-3273, USA.

The supporting information contains details of the simulation model, the analysis methods, and preliminary results obtained using all-atom models.

Simulation model

Mapping: All systems were simulated using the MARTINI coarse grained force field[1], version 2.0. The MARTINI model is based on a four-to-one mapping, i.e. on average four heavy atoms are represented by a single interaction center, with an exception for ring-like molecules. To map the geometric specificity of small ring-like fragments or molecules such as cholesterol, the general four-to-one mapping rule is insufficient. Ring-like molecules are therefore mapped with higher resolution (up to

two-to-one). The model considers four main types of interaction sites: polar (P), non-polar (N), apolar (C), and charged (Q). Within a main type, subtypes are distinguished either by a letter denoting the hydrogen bonding capabilities (d=donor, a=acceptor, da=both, 0=none), or by a number indicating the degree of polarity (from 1=low polarity, to 5=high polarity). In the coarse grained representation, the PC head group consists of two hydrophilic groups: the choline (type Q_0) and the phosphate group (Q_a). The former bears a positive charge, the latter a negative one. Two sites of intermediate hydrophilicity (N_a) are used to represent the glycerol ester moiety. Palmitoyl tails are modeled by four, oleoyl, stearoyl, and arachidonyl tails by five, and the C_{24} tail by six hydrophobic CG particles. For saturated chains, the particle type is C_1. The single double bond of the oleoyl tail is modeled by a central more hydrophilic C_3 particle to account for the polarizable nature of the double bond. For the arachidonyl chains, the yet slightly more hydrophilic C_4 type is used for all tail particles except for the terminal tail bead which is of type C_1. In case of the C_{24} chain another C_1 particle is added. Cholesterol is mapped onto eight CG particles (see Figure 1C in the main manuscript). The hydrophilic head group is modeled as SP_1 (where the S denotes the special ring type), the sterol body by four SC_1 and one SC_3 particle accounting for the presence of a double bond, and two tail particles (SC_1 attached to the sterol body, and C_1 for the terminal group). The solvent is modeled as a single type P_4 site, representing four water molecules.

Non-bonded interactions: Non-bonded interactions are described by a Lennard-Jones (LJ) potential. The strength of the pair interaction, determined by the value of the well depth of the LJ potential, depends on the interacting particle types. The value of the LJ well depth ranges from $\varepsilon = 5.6$ kJ/mol for interactions between strongly polar groups to $\varepsilon = 2.0$ kJ/mol for interactions between polar and apolar groups mimicking the hydrophobic effect. The effective size of the particles is governed by the LJ parameter $\sigma = 0.47$ nm for all normal particle types. For the special class of particles used for ring-like molecules such as cholesterol, slightly reduced parameters are defined to model ring-ring interactions; $\sigma = 0.43$ nm, and ε is scaled to 75% of the standard value. The full interaction matrix can be found in

the original publication[1]. In addition to the LJ interaction, charged groups (type Q) bearing a charge q interact via a Coulombic energy function with a relative dielectric constant ε_{rel} = 15 for explicit screening. Note that the non-bonded potential energy functions are used in their shifted form. The non-bonded interactions are cutoff at a distance r_{cut} = 1.2 nm. The LJ potential is shifted from r_{shift} = 0.9 nm to r_{cut}. The electrostatic potential is shifted from r_{shift}= 0.0 nm to r_{cut}. Shifting of the electrostatic potential in this manner mimics the effect of a distance-dependent screening.

Bonded interactions: Bonded interactions are described by a standard set of harmonic functions. The force constants are generally weak, inducing flexibility of the molecule at the coarse grained level resulting from the collective motions at the fine grained level. A two body bonded potential is used for chemically bonded sites, and a three body angle potential to represent chain stiffness. A four body improper dihedral angle potential is used to prevent out of plane distortions of the sterol moiety. Standard bonded parameters were used for the lipids as well as for cholesterol; details can be found elsewhere[1]. Parameters for poly-unsaturated chains were not available, and have been optimized using all-atom simulations of Feller *et al.*[2]. From quantum mechanical calculations these authors concluded that poly-unsaturated chains show an unusually high degree of conformational flexibility compared to mono-unsaturated or saturated chains. To mimic this behavior in the CG model, both the force constant K and the equilibrium angle θ of the angle potential for the CG particles constituting the poly-unsaturated lipid tails were reduced from the standard values of K=45 kJ mol^{-1} to 10 kJ mol^{-1} and θ = 120^0 to 100^0.

Simulation parameters: The simulations described in this paper were performed with the GROMACS simulation package[3], version 3.3. The parameters and example input files of the applications described in this paper are available at http://md.chem.rug.nl/~marrink/coarsegrain.html. Four different systems were simulated: POPC (16:0-18:1 PC), SAPC (18:0-20:4 PC), DAPC (20:4-20:4 PC), and DTPC (24:4-

24:4 PC) bilayers, each at 10 mol% cholesterol, and at a hydration level of 14 waters per lipid. This composition matches that of the experimental systems[4]. Each system consists of 38 lipids, 4 cholesterols, and 142 CG water particles. The system size is rather small to allow extended simulation times. Test runs performed for two and four times larger systems did not show any noticeable effect on the properties analyzed, however. The starting structures of the simulations were obtained by modification of previous lipid/cholesterol systems[1]. The initial placement of the cholesterol molecules did not matter; different starting structures led to the same equilibrium configurations on a time scale much shorter than the total simulation time. In all simulations the solvent molecules, the lipids, and cholesterol were independently coupled to 300 K with a relaxation time of τ_T=0.1 ps. The pressure was weakly coupled to 1 bar with a relaxation time of τ_p=0.5 ps following a semi-isotropic scheme in which the (*x,y*) plane and the *z* direction are coupled to separate baths. This approach results in a tensionless bilayer. All systems were simulated for $5*10^7$ steps, using an integration time step of 30 fs and an update of the neighbor list every 10 steps. Due to the smoothness of the CG potentials, the effective time scale sampled is larger than the actual simulation time. Based on a comparison of diffusion constants in CG systems and systems modeled at atomic detail, the effective time sampled by the CG model was found to be two- to tenfold larger. When interpreting the simulation results with the CG model, one can to a first approximation simply scale the time axis. Somewhat surprisingly, a similar scaling factor appears to describe the general dynamics present in a variety of systems quite well[1]. The standard conversion factor we use is a factor of four, which is the speed up factor in the diffusional dynamics of CG water compared to real water[5]. The time scale quoted in the main manuscript of the paper is therefore an effective time scale, which should be interpreted with care. The total effective time sampled is 6 μs for the DAPC, SAPC, and DTPC systems. The POPC simulation was extended to 60 μs in order to get more accurate data on the cholesterol flip flop rate.

Data analysis

Neutron scattering profile: The neutron scattering profiles were calculated from the simulation trajectory by time averaging the lateral density profiles, weighing the contribution from each atom by the neutron scattering length density (NSLD) per atom. The average scattering factor for the system was subtracted from the profile. Subsequently, arbitrary scaling of the density was performed to match the peak heights of the experimental profiles. The scattering from the cholesterol head group only was obtained by subtracting the profiles arising from specifically deuterated cholesterol and undeuterated cholesterol. Note that we did not perform additional simulations (the CG model is not detailed enough to discriminate between normal and deuterated cholesterol), but simply recalculated the neutron scattering profiles using a different assignment of the atom NSLDs. In the CG model, the contribution of the six deuterium atoms (the experimental measurements[4] use [2,2,3,4,4,6-$^2H_6$]-cholesterol) are distributed equally among the cholesterol head group bead and the first bead of the sterol body.

Structural parameters: The bilayer repeat distance was simply taken from the box dimension in the *z*-direction, and the area/lipid as the product of the box dimensions in the *x* and *y* directions (divided by 21, the total number of lipids and cholesterol per monolayer). The bilayer thickness is defined as the distance between the two maxima of the neutron scattering profile. The accuracy of the structural parameters reported in Table 1 of the main manuscript is within 0.1 nm. In order to test the hypothesis that the increased flip-flop rate of cholesterol in polyunsaturated bilayers results from an increase in free volume, i.e. an intrinsic membrane property rather than an effect arising from lipid-cholesterol interactions, we also calculated the total lipid volume of the DAPC and SAPC bilayers. The lipid volume was obtained by subtracting the volume taken by the solvent, assuming a volume per water molecule equal to the bulk value, from the total volume of the simulation box. We found the volume of

DAPC to be somewhat larger than the volume of SAPC, in agreement with the expected increase in free volume upon unsaturation. The difference is very small (0.4%), however, and therefore not likely to be the cause of the increased flip-flop rates.

Free energy calculation: The estimate of the free energy difference between cholesterol in its standard position aligned with the lipid tails, and in the unusual position in between the leaflets, is obtained from their relative populations, using $\Delta G = kT \ln \rho^{standard}/\rho^{unusual}$. The populations were obtained by fitting the neutron scattering profiles to a sum of three Gaussians (see Figure 1A of the main manuscript). Integration of the individual terms gives their relative populations. The average for both usual populations in each of the two monolayers was used, implying that the free energy difference measures the free energy change going from parallel to the bilayer normal *in a particular monolayer* to a position in between the monolayers. Since each bilayer consists of two monolayers, the free energy difference between a position in between the monolayers and a position *anywhere else* in the bilayer would be larger by a term ln 2. The standard error estimate of the free energy values reported in Table 1 of the main manuscript is 1 kJ mol^{-1} for DAPC, DTPC, and SAPC, and 2 kJ mol^{-1} for POPC. The error estimate was obtained based on the differences between individual cholesterol molecules and by dividing the trajectory into independent blocks of 1 μs.

Tilt angle: The orientation of cholesterol was defined as the angle between the bilayer normal (z-axis), and the vector connecting the head group site to the tail site that is directly attached to the sterol body. Histograms of this angle were obtained by binning the angles using a bin width of 1^0. These histograms were also fitted to a sum of multiple Gaussians, from which the most probable "tilt" angle and the standard deviation were determined. The tilt angles presented in Table 1 of the main manuscript reflect data obtained for the cholesterol population in their usual orientation only, i.e. aligned with the lipid tails.

Flip-flop rates: The average monolayer residence time of cholesterol, or flip-flop time, was estimated by counting the number of crossings observed during the entire 6 μs simulation trajectory. A crossing was defined as a tilt angle changing from a value $\theta > 120^0$ to a value $\theta < 60^0$ or vice versa. To remove fast reorientations not necessarily indicating a flip-flop, the time series were filtered by applying a running average over 10 ns before counting the number of flip-flop events. Visual inspection of the trajectories resulted in similar residence times. The data were averaged over the four cholesterol molecules present in the system, and over the two monolayers. The standard errors in the times reported in Table 1 of the main manuscript is less than 10 ns for the DAPC and DTPC bilayers, 100 ns for SAPC, and 500 ns for POPC. These error estimates (and standard deviations) were obtained from the distribution of flip-flop times of individual cholesterol molecules. For example, for POPC a total number of 55 flip-flop events were observed during the 60 μs simulation, with individual flip-flop times ranging from 100 ns to 15 μs.

Visual images: Visual images were prepared using the VMD (Visual Molecular Dynamics) software[6], version 1.8.6.

Comparison to all-atom simulations

In order to see whether more detailed models could also provide evidence for the depletion of cholesterol from its usual position aligned with the lipid tails and the hydroxyl group located near the aqueous interface, we performed additional simulations for the DAPC system using an atomistic force field. The force field used for the atomistic simulations was based on the Gromos 53A6 force field[7] with reparameterized ester and head group moieties[8]. Parameters for the bond lengths, angles, and dihedrals around the polyunsaturated tail atoms were based on the study[2] by Feller *et al*. The set-up and

force field files are available on request. Simulations using the Berger force field[9] for lipids did not show significant differences regarding the position and orientation of cholesterol. The system set-up and simulation conditions were chosen similar to those of the CG simulations. Due to the increased computational effort to simulate at an atomic level of detail, only a 150 ns trajectory has been generated up till now. During this time, no cholesterol flip-flops were observed. The cholesterols retained their starting orientation, which was the usual cholesterol position. Alternative starting conditions with the cholesterols embedded in the unusual position in between the monolayer leaflets resulted in a reorientation of the cholesterol molecules toward their usual position on a time scale of 10 to 40 ns. Although the time scale of these simulations is too small to draw significant conclusions, the preliminary data point to three aspects that are in line with the results obtained with the CG model: *i)* the preferred orientation appears to be the standard (parallel to the bilayer normal), rather than the unusual position, *ii)* cholesterol can be present in the unusual position for periods of 10s of nanoseconds, and *iii)* cholesterol flip-flops require at least 100 ns. The average angle of orientation, $\langle\theta\rangle = 25 \pm 15^0$ obtained from the atomistic data is somewhat smaller than the value of $\langle\theta\rangle = 30 \pm 22^0$ obtained for the CG model. Both values are indicative of a strongly tilted and very broadly distributed cholesterol orientation, however. The area/lipid for the all-atom simulation ($A=0.77$ nm^2) is close to the CG estimate ($A=0.79$ nm^2).

---

[1] Marrink, S.J., Risselada, H.J., Yefimov, S., Tieleman, D.P., de Vries, A.H. *J. Phys. Chem. B* **2007**, *111,* 7812.

[2] Feller, S.E., Gawrisch, K., MacKerell, A.D., Jr., *J. Amer. Chem. Soc*. **2002**, *124,* 318.

[3] van der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A.E., Berendsen, H.J.C. *J. Comput. Chem*. **2005**, *26,* 1701.

[4] Harroun, T.A., Katsaras, J., Wassall, S.R. *Biochemistry* **2006**, *45,* 1227.

[5] Marrink, S.J., de Vries, A.H., Mark, A.E. *J. Phys. Chem. B* **2004**, *108,* 750.

[6] Humphrey, W., Dalke, A., Schulten, K. *J. Mol. Graphics* **1996**, *14,* 33.

[7] Oostenbrink, C., Villa, A., Mark, A.E., van Gunsteren, W.F. *J. Comp. Chem.* **2004**, *25,* 1656.

[8] de Vries, A.H., Marrink, S.J., *to be published.*

[9] Berger, O., Edholm, O., Jahnig, F. *Biophys. J.* **1997**, *72,* 2002.