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COMPUTER SIMULATION STUDY OF PROPERTIES OF UNSATURATED LIPID MEMBRANES

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Biomembranes surround cells: a membrane separates the interior of a cell from the outside environment. Being selectively permeable, membranes participate in control of the movement of various compounds (substances) into and out of cells. Biomembranes are very complex heterogeneous systems consisting of many different types of lipids, sterols, proteins, carbohydrates and various membrane associated molecules which are involved in a variety of cellular processes. Consequently, membranes play an active part in the life of the cell, they exist as dynamic structures. Lipid molecules differ with respect to the type of hydrophilic head-group and occur with a wide variety of hydrophobic hydrocarbon chains of fatty acids (FAs). Usually the most abundant phospholipid in animal and plants is phosphatidylcholine (PC), it is the key building block of membrane bilayers. Knowledge of physical-chemical properties of lipid bilayers is a key element of our general understanding of biomembrane functioning, which is one of the greatest challenging problems in biochemical, biophysical and biomedical sciences. This information is also relevant and essential in new biotechnological and biomedical applications.

Experimental measurements of structural and dynamical properties are obtained as averages over a large number of lipids and over a certain time interval, which not always can give an unambiguous picture of individual lipids and their interactions. During the last decades computer simulations have become a well established tool of modern investigations of molecular structure. Monte Carlo (MC) or molecular dynamics (MD) can provide three-dimensional real-time imaging of the system with atomistic-level resolution, and hence can give essential structural and dynamical information which otherwise is hardly accessible by any experimental method. The rapid development of the accessible computer power has made simulations of more and more complicated systems feasible, and allowed also increase the size of the simulated systems. The amount of works on simulations of lipid membrane systems has increased tremendously, and a number of reviews appeared accounting for this in the past decade (Damodaran, Merz, 1994; Mouritsen, Jørgensen, 1994; Pastor, 1994; Mouritsen et al., 1996; Jacobsson, 1997; Merz, 1997; Tieleman et al., 1997; Tobias et al., 1997; Berendsen, Tieleman, 1998; Feller, MacKerell, 2000; Feller, 2000; Forrest, Sansom, 2000) and more recently (Feller, 2001; Tobias, 2001; Scott, 2002; Hansson, 2002; Saiz, Klein, 2002; Saiz et.al., 2002; Vigh et. al., 2005; Chan, Boxer, 2007; Vermeer et al., 2007; Feller, 2008; Marrink et al., 2009; Pandit, Scott, 2009).

It was mentioned that a typical biological membrane contains many species of lipid molecules, with different head groups and hydrocarbon tails. The most commonly occurring FA chains may contain 1 – 6 carbon – carbon double bonds of the cis configuration in different positions. In most cases, at least half of the FA chains are unsaturated. The double bonds of polyunsaturated (PU) chains are, as a rule, methylene-interrupted. The PUFA tails of lipids are of great importance for the structure and functioning of biomembranes (Dratz, Deese, 1986; Rabinovich, Ripatti, 1994; Gawrisch et al., 2003; Stillwell, Wassall, 2003; Rabinovich et al., 2003; Valentine, Valentine, 2004; Feller, Gawrisch, 2005; Gawrisch et al., 2008; Stillwell, 2008; Wassall, Stillwell, 2009). Docosahexaenoic acid, 22:6(n-3)cis, is the longest and most unsaturated FA commonly found in nature. It is known that membranes that are active metabolically have high levels of PU chains. PU chains have been linked to the great number of biochemical processes, to an enormous variety of human afflictions. Evidently the basis of these phenomena is the specific chemical structure of PU chains, which results in their specific physical properties, which are in its turn cause their specific functioning in living organisms. Full understanding of the effects of lipid unsaturation on various physical properties of membranes at the molecular level, affecting their functioning, is not yet achieved. The mechanisms of many biological functions of PUFAs remain a subject of much debate.

We have carried out series of MD simulations of 16 hydrated liquid crystalline phase PC bilayers consequently changing the number of double bonds in the *sn*-2 chain of phospholipids with *sn*-1 saturated and *sn*-2 unsaturated chains: 18:0/18:1(n-9)cis PC, 18:0/18:2(n-6)cis PC, 18:0/18:3(n-3)cis PC, 18:0/18:4(n-3)cis PC, 18:0/18:5(n-3)cis PC, 18:0/20:4(n-6)cis PC, 18:0/20:5(n-3)cis PC, 18:0/22:6(n-3)cis PC, 16:0/18:1(n-9)cis PC, 16:0/18:2(n-6)cis PC, 16:0/18:3(n-3)cis PC, 16:0/18:4(n-3)cis PC, 16:0/18:5(n-3)cis PC, 16:0/20:4(n-6)cis PC, 16:0/20:5(n-3)cis PC, 16:0/22:6(n-3)cis PC. The main goal was to study their physical properties and the features of PU bilayers.

The main idea of MD simulations of a many-particle system is the solution of the Newton's equations of motion for a set of particles (atoms or molecules) that comprises the system. This procedure includes a model description of the atomic system, atom-atom interaction potentials, boundary conditions of the system, and an approximate step-by-step technique for solving the classical equations of motion. Bilayer system setup and simulation details were as follows: each bilayer was simulated in a rectangular periodic box within NPT ensemble, i.e., with constant number of lipid molecules N, pressure P (1 atm) and temperature T (303 K); 128 PC lipids per bilayer (64 lipids in each leaflet) with explicit hydrogens were used; the two hydrocarbon tails, the glycerol section and the head group of the lipid molecules were treated in accordance with their known chemical structure; the lipids were hydrated by 3840 water molecules (30 waters per lipid) which were approximated by anharmonic flexible SPC water model; the electrostatic interactions were treated by the Ewald summation method. To calculate the energy of the bilayer systems CHARMM27 force field with a scaling factor for electrostatic interactions between 1...4 neighbours and corrections of partial atom charges were used (Hőgberg et al., 2008). The double time step algorithm (Tuckerman et al., 1992) was used to treat separately fast (covalent bonds, angles, torsions, collision Lennard-Jones forces within 5 A distance) and slow forces: 0.25 fs time step for the fast, 2 fs – for the slow forces.

The average area per lipid defined in constant pressure – zero tension simulations, is a parameter which is most often used to define the quality of the force field used in the simulations. Area per lipid molecule A_p_m is one of the most fundamental properties of a lipid bilayer and one of the most common ways to determine whether the bilayer system has reached equilibrium. When the area per lipid reaches a stable value, other structural properties (density distributions, NMR order parameters) do not change either. Simulated area per lipid can be also compared with experimental values available from X-ray or neutron diffraction and volumetric data. A collection of average lipid areas for several bilayers composed from different lipids and computed from different force fields, as well as experimental areas, is available in Table 1 of paper (Poger, Mark, 2010). More reliable validation of a force field can be done by comparison of simulated and experimental structure factors as it was shown in paper (Benz et al., 2005). Additional important source of data for validation of a force field used in lipid bilayer simulations is NMR bond order parameters.

The temporal behavior of the A_p_m ($Å^2$) of the bilayers is shown in Fig.1(*a* and *b*). It is clear from this figure that MD simulations of the bilayers should be at least of the order of tens of nanoseconds to reach equilibrium and surpass the longest characteristic timescales for A_p_m fluctuations. For this reason, the first 20 ns of the total 60 ns were considered as equilibration, and the last 40 ns were used for analysis and calculations. Consecutive configurations of the bilayers were sampled every 1 ps.

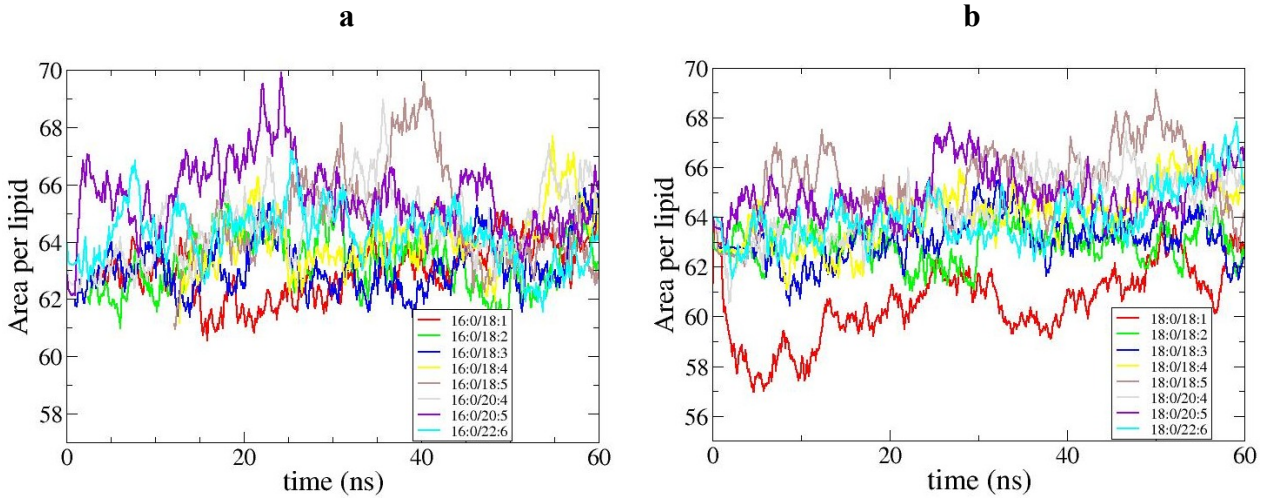

Fig.1. The temporal behavior of the average area per lipid A_p_m ($Å^2$) of the PC bilayers with 16:0 (*a*) and 18:0 (*b*) *sn*-1 chains

Different equilibrium structural and dynamic parameters of the bilayers were defined, such as the average area per lipid head group and its fluctuations, bond order parameters of lipid molecules with respect to the bilayer normal, the orientational fluctuations of the bond vectors, the root mean square values of the positional fluctuations of all lipid atoms relative to the average atomic coordinates, mass density distributions of various atoms of lipids relative to the bilayer middle plane, interpenetration of lipid tails of the opposite monolayers into each other, etc. In this paper the main goals are the C-C bond order parameters to characterize the order in lipid bilayers. These quantities are sensitive to the degree of unsaturation in the chains. The order parameter for each C-C bond of the chains may be defined as:

$$S_{CC} = (3\cdot\langle\cos^2\beta_{CC}\rangle - 1)/2,$$

where β_{CC} is the angle between a C-C-bond and the bilayer normal. Fig. 2 (*a, b, c, d*) shows the C-C bond order profiles for the sixteen bilayers and marked differences in the S_{CC} order parameter between odd and even running numbers for hydrocarbon chains *sn*-1 and *sn*-2 of the bilayers.

The 'ordinary' odd-even effect for saturated *sn*-1 chains 16:0 and 18:0 (Fig.2) is well known: this is because the rotations of the CH_2 groups about their local axes are anisotropic. The main difference in behavior and properties of unsaturated lipid molecules is that the S_{CC} order parameters of single C-C-bonds next to the cis double bond C=C in all unsaturated *sn*-2 chains are lower than that for the double bond C=C and for the corresponding single bond C-C (i.e. C-C bond **with the same number**) in the saturated chains *sn*-1.

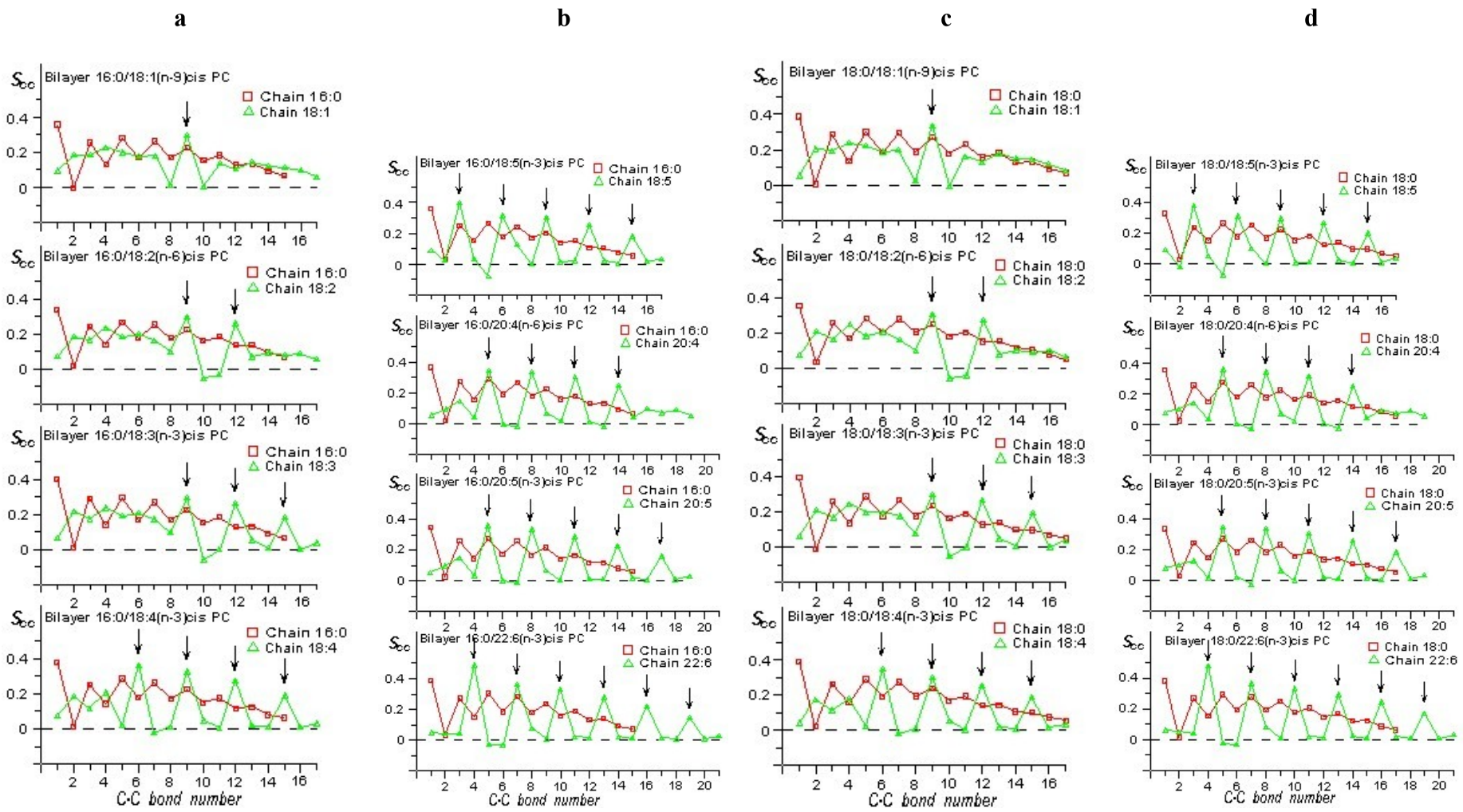

Fig. 2. Order parameter S_{CC} as a function of the C-C bond number of the lipid hydrocarbon chains of the eight PC bilayers with *sn-1* chain 16:0 (*a,b*) and the eight PC bilayers with *sn-1* chain 18:0 (*c,d*) as determined from the MD simulations. The arrows indicate the double bonds

It should be noted that the main qualitatively characteristic features of the profiles of S_{CC} for a given acyl chain (*sn*-2) of the bilayers are highly characteristic for the level of unsaturation. It means that a close relationship between the investigated order parameters and the structure of the chains is elucidated. The number of the chain carbons, the number of *cis* double bonds and their position in the chain determine principally the calculated order properties: they are similar both for the bilayers with 16:0 and 18:0 chains *sn*-1. The understanding of the molecular basis of the physical properties of the lipids allows one to narrow down the list of hypotheses under consideration about the possible function of various acyls in lipid membranes.

The temperatures of the lamellar gel – liquid crystalline phase transition in fully hydrated PCs of different *sn-2* chain unsaturation (Koynova, Caffrey, 1998) show that increased chain unsaturation above a certain number of double bonds does not necessarily translate into increased membrane fluidity: a fluid lipid bilayer could be attained by lipids having only mono- and diunsaturated FAs (e.g., 18:1 and 18:2) and hence the influence of PU chains of lipids is much more than the simple 'fluidization' of the matrix of lipid membrane. The effects of PU chains are more profound than would be observed by solely controlling the temperature. The results obtained in this work supplement the information about PU lipids, these data can be used for the analysis of the relations between properties and functions of PU lipids that play a key role in functioning of biomembranes (Rabinovich, Ripatti, 1994; Rabinovich, 2008).

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