Modeling Order-Disorder Transition in Low-Density Lipoprotein

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***Abstract*—Low Density Lipoproteins (LDL) undergo a reversible order-disorder thermal transition close to biological temperature due to cooperative melting of the cholesteryl esters (CE) in the core of the LDL particle. We have noticed that chain-chain interactions between CE molecules are responsible for the stability of the ordered smectic phase; thus, we formulated a simple "coarse-grained" two-state model to describe the melting process. In this model only nearest neighbor interactions are allowed. On the basis of these assumptions we performed Metropolis Monte Carlo (MC) simulation in order to obtain the heat capacity curve. The resulting profile reveals well-known features of the systems with a finite size.**

I. Introduction

The main purpose of Low Density Lipoproteins (LDL) is to transport cholesterol through blood vessels and deliver it to the cells. To accomplish this goal, LDL is structured as a spherical hydrophilic outer shell with hydrophobic cargo in the core. While Apolipoprotein B-100 (ApoB-100), phospholipids and cholesterol constitute hydrophilic outer shell, LDL's hydrophobic core contains cholesteryl ester (CE), triglycerides (TG) and some of the cholesterol. In general, LDL particles are very heterogeneous in their size and lipid composition [1]. The size of the LDL particles varies from 18nm to 25nm and a normal core-lipid concentration involves a CE-to-TG ration larger than four [1], [2]. A sample of human plasma LDL particles with a CE-rich core will reveal a reversible order-disorder transition close to biological temperature [3]. X-ray scattering, differential scanning calorimetry (DSC) and ^{13}C NMR experiments show that this transition is related to cooperative melting of CE molecules from smectic liquid crystal phase to liquid phase [3], [4]. Furthermore, the CE cooperative unit size was estimated to be between 50 and 100 molecules based on the van't Hoff equation [3]. These experimental results require a region in the core of the LDL that is rich in CE molecules and capable of supporting smectic ordering at low temperatures [3]. This conclusion is in agreement with more recent image evidence provided by cryo-electron microscopy (cryo-EM) that verifies that CE form lamellae-like layers in the core of the LDL below the transition temperature [5], [6]. The absence of the cholesteric phase, normally observed in isolated pure systems of cholesteryl oleate (CO) and cholesteryl linoleate (CL), is attributed to the small amount of TG present in the core and to the phospholipid chains situated in the outer shell. In particular, these factors will destabilize ring-ring interactions, responsible for the cholesteric ordering of CO and CL, in favor of chain-chain interactions responsible for the stability of the smectic phase [4].

The same fundamental interactions govern ordering process for the pure isolated systems of CEs. Based on these interactions and experimental evidence Ginsburg was able to propose a model for the smectic phase of CE. This model is derived from the monolayer type II crystal structure of CE and assumes that CE molecules are in antiparallel arrangement, contributing their chains to adjacent layers (Fig.1) [7].

A large number of small interacting systems will exhibit behavior which can be analyzed through methods of statistical mechanics [8]. In this paper we will formulate a minimal model to obtain heat capacity curve of order-disorder transition in LDL using Metropolis Monte Carlo (MC) and known experimental thermodynamic information.

II. Methods

As previously mentioned, the presence of small amount of TG, cholesterol molecules, and penetrating phospholipid chains from the particles surface will prevent preordering of the sterol ring in the isotropic state necessary for the formation of the cholesteric phase. These conclusions are based on ^{13}C NMR investigation of temperature dependent motion of CE in LDL [4]. This means that the physical state of the system is controlled by the hydrocarbon chains. It is known that the portion of the chain between the ring and double bond is responsible for the formation and stability of the smectic phase. Furthermore, the reminder of the chain is excluded from the phase ordering and may disorder it [7]. Recent findings suggest that although the chain is in an extended conformation in the liquid state, it is highly disordered. Chain motions are present in the region that supports stability of the smectic phase. These motions are accompanied with some degree of bond isomerism. Motions are suppressed in double bond region of the chain, but after that, they again become strong and increase toward the end of the chain [9]. Thus we conclude that the final order-disorder melting process is driven by liberated motion of acyl chains in a liquid state, and increased disorder possibly due to some degree of the bond isomerism.

Although the motion of the chain is complex we will assume that, on sufficiently long time scales, the system will explore all the relevant chain states so that two major states will emerge: the "ordered" state and "disordered" state. Molecules in the "ordered" state will be responsible for the formation and stability of the smectic phase and molecules in the "disordered" state will disrupt the smectic layers. Furthermore, a molecule in a particular state will have distinct conformational energy, and it will interact with

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neighboring molecules. This will allow us to formulate a model with effective interactions between molecules capable of reproducing experimental findings. Similar approaches were used by Doniach [10], Caillé [11] and Pink [12] to simplify a large molecular chain to a "coarse-grained" two-state system in order to explain the phase transition in lipid membranes as well as the phase transition from nematic to liquid state in triglycerides.

In pure, isolated systems, CO and CL, major CE of the LDL particle, form a smectic-A phase [13], [14]. In the smectic-A phase, the long axis of the molecule is perpendicular to the layer's plane. Layers are flexible and glide freely over one another with low viscosity. If we add to this picture that, as in LDL, the ring portion of the molecule is excluded from the ordering process in the LDL, one may conclude that layers are uncoupled and that they could be treated independently of each other (Fig.1). We assume that in each layer, each molecule is surrounded by six nearest neighbors. Now we can express the actual conformation of the system in terms of matrix $\boldsymbol{S} = [s_{ij}]$. Furthermore, we assume an ensemble with constant number of particles, pressure and temperature (NPT) that is in thermal equilibrium with surroundings. For computational simplicity, we will work with periodic boundary conditions, though this choice has no significant effect on the result. The Gibbs free energy of a particular configuration can be expressed as:

$$G(S) = N_s G_s + N_i G_i + N_{ss} G_{ss} + N_{si} G_{si} + N_{ii} G_{ii} \quad (1)$$

The first two terms represent the internal energy of the system. G_s and G_i are the internal energies of the molecules in the ordered smectic state and in the disordered isotropic state, respectively. N_s and N_i are the number of molecules in the ordered and in disordered state. The last three terms represent the dispersive van der Waals interaction. G_{ss} is the interaction energy between two nearest-neighbor molecules in the smectic phase, and N_{ss} is the number of nearest-neighbor smectic-smectic contacts. G_{si} is interaction energy between two molecules in different states, and G_{ii} is the interaction energy between two molecules in isotropic state. N_{si} is the number of nearest-neighbor contacts between molecules in smectic and isotropic state, and N_{ii} is the number contacts between two isotropic nearest-neighbor molecules. Taking into account the relationships between number of molecules in smectic and isotropic state and their mutual contacts [15]:

$$N = N_i + N_s; N_{ss} = (zN_s - N_{si})/2; N_{ii} = (zN_i - N_{si})/2 \quad (2)$$

It is possible to rewrite the equation for the free energy of the system as [16]:

$$G(S) = N\left(G_s + \frac{zG_{ss}}{2}\right) + N_i\left(\Delta H - T\Delta S\right) + N_{si} w \quad (3)$$

Here, ΔH is the change in enthalpy associated with the transition and ΔS is the change in entropy. These two parameters are measured during the phase transition. The only unknown parameter is the so-called cooperativity parameter, which consists of an enthalpic term and an entropic term: $w = w_H - Tw_S$. This parameter needs to be fitted in order to describe the curve of the phase transition. If the cooperativity parameter is greater than zero, molecules tend to form clusters; when the cooperativity parameter is equal to zero, cooperativity between molecules vanishes. In the case where the cooperativity parameter is below zero, the system will tend to bring molecules of opposite states in contact [16].

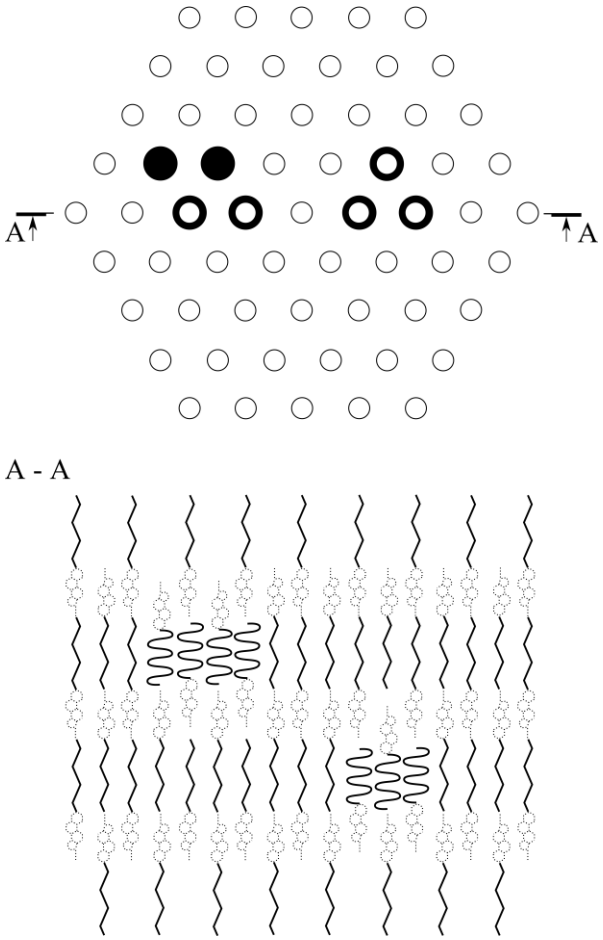

Figure 1. Schematic representation of a portion of LDL core modeled by two-state coarse-grained system. Although the motions of the chains are complex on sufficiently long time scales, the system will explore all the relevant chain states so that two major states will emerge: the "ordered" state and "disordered" state. Upper image shows top view of the portion of the LDL internal core. Smaller white circles indicate molecules in the ordered state and larger black circles represent molecules that disrupt order. Bottom image shows cross section A-A. Ring moiety of the CE molecule is represented by a dotted line indicating that this part of the molecule is excluded from the ordering and melting process in LDL. Hydrocarbon chains are represented by a continuous dark line indicating that they contribute to the order-disorder melting process. Zig-zag line represents molecules that support ordering inside LDL and wavy line depicts rapid chain motions of molecules that support disorder.

MC sampling was employed to obtain ensemble averages at equilibrium. For this purpose a triangular lattice with 25x25 sites was explored. Each simulation began with generation of an arbitrary initial configuration. Equilibration of the initial configuration is obtained through a change of the state of the single chain at each MC step. Therefore, a

trial configuration was formed by changing the state of the randomly selected chain from 0 to 1 if the initial chain state was 0, or from 1 to 0 in the opposite case. We accepted the trial configuration when the Boltzmann factor $exp(-\Delta G/k_BT)$ was greater than or equal to the randomly generated number in the interval from 0 to 1, otherwise the trial configuration was rejected and the initial configuration was retained. This process was repeated for a few million steps in order to ensure proper equilibration. Once equilibrium has been reached we sample conformational fluctuations of the system. This data collection entails recording the number of molecules in disordered state N_i and the number of unlike nearest-neighbor contacts N_{si}. Averaging N_i and N_{si} values and obtaining enthalpy from (3) allows us to calculate the heat capacity using fluctuation-dissipation theorem [16]:

$$C_p = \frac{\langle H^2 \rangle - \langle H \rangle^2}{k_B T^2} \quad (4)$$

III. Results and Discussion

Implementation of the previously described model requires an experimental value of enthalpy and melting temperature as input data. The enthalpy of the reversed transition of LDL is known. For LDL CE, this enthalpy is $\Delta H=0.69\pm0.06$ cal/g [17]. We take the peak of the phase transition as a melting temperature, $T_M=303.45\pm2.3$K [17]. Finally, we estimated the effective cooperative parameter, $w=313$ cal/mol, by fitting the mean onset temperature of the phase transition, 290.45 ± 3.3K, and the end temperature, 314.15 ± 1.7K [17]. The resulting normalized heat capacity curve shows a finite width over a range of temperatures (Fig.2). This feature is a well-known characteristic of the large sample of systems with finite number of particles.

Additionally, we are interested in the number of molecules that had broken away from the ordered phase at a particular temperature (Fig.3). We obtained this value by dividing the average number of molecules in disordered state $\langle N_i \rangle$ by the total number of molecules in the system at a given temperature. Again, instead of abrupt, stepwise change between smectic and isotropic state, we see a smooth phase transition typical for the large number of small interacting systems.

The outcomes of the proposed computational model agree well with experimental findings. Experimental data obtained by independent techniques, DSC and X-ray scattering, show that temperature dependent relative intensity of scattering, corresponding to the Bragg spacing of 36 Å, closely correlates with structural transition detected by calorimetry. In more detail, constructive scattering is present below the structural transition and its relative intensity is temperature dependent. Upon heating this signal progressively decreases until the melting temperature where it completely disappears. Since the structural transition is completely reversible, the same signal will reappear again during cooling process [17]. Bragg spacing of 36 Å is also present in smectic phase of pure CO and CL and it is related to the molecular length [13], [14]. In our study we assume that layer thickness is comparable with the length of the CE molecules. Further, the shape of the curve that represents the number of molecules in the disordered state may imply that phase transition is continuous. This leads to the disappearance of the order above the melting temperature which would correspond to the experimentally observed X-ray intensity.

In biological sense, the obtained results support the idea that the heat capacity curve is produced by a sample of LDL particles similar in composition. This implies that melting of the core in each individual particle occurs over the span of temperatures.

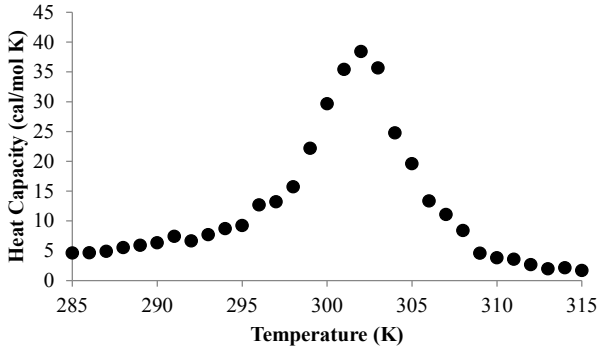

Figure 2. Specific heat capacity obtained with MC simulations of 625 latice sites. Each site is ocupated by a CE chain and surrounded by six nearest neighbors. Phase transition temperature is $T_M=303.45$K, and phase transition entalpy $\Delta H=0.69$cal/g.

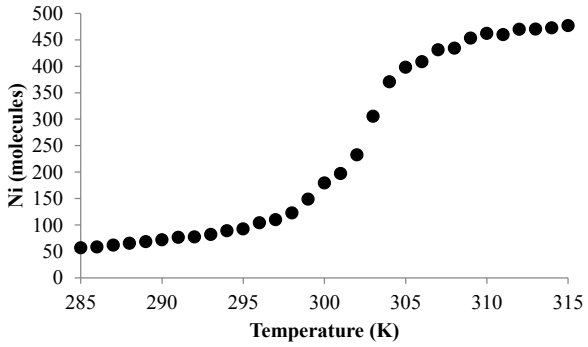

Figure 3. Number of molecules in the disorder state as a function of temperature.

IV. Conclusion

In this study, we present a model for order-disorder melting in the core of LDL particles close to biological temperature. Based on experimental reports that CE chains are responsible for the smectic formation in the core of LDL particle at low temperatures [3], [4], [5], [6], we made an assumption that each CE chain may be present either in an "ordered" state or in an "disordered" state within the LDL core. While the ordered state is responsible for stability of the smectic phase, the disordered state represents a highly mobile CE chain that loses its stiffness in the region between ring and double bond necessary for the smectic ordering.

Although this "coarse-grained" two-level approximation appears crude it is quite robust since it combines a statistical model, actual experimental results and single fitting parameter. Thus, it leads to surprisingly good agreement between experimental heat capacity measurement and numerical results. The predicted phase transition does not have a sharp prominent top that leads to a discontinuity of heat capacity as is characteristic for a bulk sample. Instead, it is rounded and spreads over a finite temperature interval, which is a characteristic of a sample consisting of small systems with finite numbers of molecules [8]. The same trend is observed on the curve that shows a fraction of molecule in isotropic phase.

Finally, we note that this model has the potential for further investigation of the thermal transition in the core of LDL. For example, the model can be useful in the study of the clustering of core lipids especially because detailed calculations of these dynamics are particularly complex and intense when using other simulation methods, such as Molecular Dynamics. Elucidation of connection of these fundamental processes with phase transition will lead toward further clarification of atherogenicity [2] and oxidation resistance [18] of LDL particles.

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