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The role of caveolin-1 in lipid droplets and their biogenesis

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ABSTRACT

We address unresolved questions of the energetics and mechanism of lipid droplet (LD) biogenesis, and of the role of caveolins in the endoplasmic reticulum (ER) and in mature LDs. LDs are eukaryotic repositories of neutral lipids, which are believed to be synthesised in the ER. We investigate the effects of a curvature-inducing protein, caveolin-1, on the formation and structure of a spontaneously aggregated triolein (TO) lipid lens in a flat lipid bilayer using molecular dynamics (MD) simulations. A truncated form of caveolin-1 (Cav1) localises on the interface between the spontaneously formed TO aggregate and the bulk bilayer, and thins the bilayer at the edge of the aggregate, which may contribute to lowering the energy barrier for pinching off the aggregate from the host bilayer. Simulations of fully mature LDs do not conclusively establish the optimal localisation of Cav1 in LDs, but when Cav1 is in the LD core, the distribution of both neutral lipids in the LD core, and of phospholipids on the engulfing monolayer are altered significantly. Our simulations provide an unprecedented molecular description of the distribution and dynamics of various lipid species in both mature LDs and in the nascent LD inside the bilayer.

1. Introduction

Lipid droplets (LDs) regulate intracellular lipid storage and lipid metabolism of neutral lipids such as cholesteryl esters and triglycerides (TGLs) such as triolein (TO). A mature LD is composed of a phospholipid monolayer surrounding a neutral lipid core (Martin and Parton, 2006). The monolayer is composed of different phospholipids. The main constituents are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol, and ether-linked phosphatidylcholine (ePC) phospholipids (Bartz et al., 2007). The monolayer can also contain free cholesterol (Ohsaki et al., 2014) and from 50 to 200 different proteins (Wilfling et al., 2014) while the core of the LD is mainly composed of TGLs, cholesteryl esters such as cholesterol oleate (CO) and diacylglycerols such as dioleoylglycerol (DOG). Cholesteryl esters such as cholesterol oleate and TGLs such as triolein (TO) in LDs can be hydrolysed to free cholesterol and fatty acids by enzymatic machinery when required as, for example, a source of energy. The size of LD is variable, and ranges from a few nanometers to micrometers in diameter (Wilfling et al., 2014).

Although the clear mechanism of their formation is still unknown, LDs are believed to form at the surface of the endoplasmic reticulum (ER). According to the current working model, TGLs synthesised in the ER self assemble into ellipsoidal discs, (also called lenses) inside the ER membrane. Once the lenses reach a threshold size, the aggregate can bud off from the ER membrane along-with a monolayer of lipids acquired from the ER membrane. The small length scales of the budding process make it impossible for light microscopy to observe the budding process in live cells. Theoretical calculations and simulations have been used to study the mechanism and energetics of the budding of nascent LDs from the ER membrane (Khandelia et al., 2010; Thiam and Foret, 2016). Although budding may not require external agents (Thiam and Foret, 2016), several proteins localise in both the ER and in LDs, and can affect the efficient formation of LDs (Guo et al., 2008). Examples are COP1 (Thiam et al., 2013; Wilfling et al., 2014), oleosins (Murphy and Vance, 1999) and caveolins (Le Lay et al., 2006). COP1 was shown to capable of budding off droplets from a reconstituted phospholipid-triglyceride interface (Thiam et al., 2013). Similarly, caveolins (CAVs) are of particular interest because they can curve plasma membranes to form caveolae, and thus could serve a similar purpose on the surface of LDs, where they were discovered to be present in several studies published in 2001 (van Meer, 2001; Fujimoto et al., 2001; Ostermeyer et al., 2001; Pol et al., 2001). Like in caveolae, caveolins can support the curvature of, and can eventually promote the pinching off of the TGL lens in the ER membrane. Caveolin consists of about 178 amino-acids and can be found in three isoforms: caveolin-1, -2, -3, and caveolin-1 is known to be the most ubiquitous form. Caveolin-deficient

Abbreviations: POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine; TO, triolein; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; CHOL, cholesterol; CO, cholesteryl oleate; DOG, dioleoyl glycerol; MD, molecular dynamics; LD, lipid droplet; Cav1, truncated version of caveolin-1; CG, coarse-grained; TGL, triglyceride; ER, endoplasmic reticulum

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mouse adipocytes can still form LDs, but with a different protein and lipid composition as well as a different size distribution (Blouin et al., 2010). During liver regeneration, caveolin-1 deficient mice produced very low concentrations of LDs. Thus, although LDs can form without CAVs, CAVs still seem to play an important role in LD maturation and biogenesis. In mature LDs, there is evidence that caveolins can be found both on the outer monolayer and in the core (Robenek et al., 2005).

Previously, we showed that triolein (TO) spontaneously aggregates into an aggregate lens inside a POPC lipid bilayer, without completely being expelled from the bilayer (Khandelia et al., 2010). In the current study, we investigate the effects of a truncated form of caveolin-1, (Cav1) on the properties of the TO lens. Furthermore, we investigate the properties of a fully matured LD consisting of POPC, POPE, TO, DOG, CO and Cav1. Some of the specific questions we have attempted to address are: Does Cav1 have an impact on the curvature of the TO lens? Where is Cav1 localised in mature LDs? What effect does Cav1 have on the properties of mature spherical LDs?

We find that (1) Cav1 tends to aggregate and localise at the edges of the spontaneously formed TO lens inside a lipid bilayer (2) the curvature of the lipid lens is not significantly affected by Cav1 (3) Cav1 thins the membrane patch at the interface between the self-assembled triolein lens and the bulk lipid bilayer and (4) Cav1 modifies the surface distribution of both neutral lipids and phospholipids on the LD surface.

2. Methods

2.1. Modeling caveolin-1

Caveolin-1 contains a highly variable N-terminal domain (residues 1–81) and a membrane attaching C-terminal domain (residues 135–178) which is possibly acylated. The two termini domains are bridged by the caveolin scaffolding domain (residues 82–101) and the transmembrane domain, TMD (residues 102–134). The TMD has been shown to adopt a U-shaped conformation inside the lipid bilayer (Liu et al., 2016) and the segment 82–136 is sufficient to curve model lipid bilayers (Rui et al., 2014). To obtain the correct conformation of the Cav1 (residues 82–136) protein, we performed an all-atom molecular dynamics (MD) simulation of Cav1 in a POPC bilayer using NMR and MD data from Rui et al. (2014). The all-atom simulation is described in the next section. We selected the three most representative conformations of Cav1 from the all-atom simulation using a clustering analysis. These three conformers were then used to make three models of coarse-grained Cav1. We used the pairwise root mean squared deviations between all the conformations available in the all-atom simulation, to perform a hierarchical cluster analysis using the Ward's minimum variance method Hierarchical Grouping to optimise an Objective Function (Ward, 1963). Then we selected three clusters from which we determined three centroids corresponding to the three Cav1 conformers used in the coarse-grained (CG) simulations. The all-atom and CG models of Cav1 contain 927 and 138 particles respectively. We used the MARTINI force field (Marrink et al., 2007) for the CG simulations.

2.2. Molecular dynamics

2.2.1. All-atom simulations

A 800 ns all atom molecular dynamics simulation of single caveolin protein in a POPC lipid bilayer was performed using Gromacs version 5.0.4 (Abraham et al., 2015; Spoel et al., 2005) and the Charmm36 force field for lipids (Klauda et al., 2010) and proteins (Best et al., 2012). The details of the simulations are provided in the SI. Using the trajectories of this simulation, three conformations of Cav1 were selected for use in the subsequent CG simulations.

2.2.2. Coarse-grained simulations

We used the MARTINI force field (Marrink et al., 2004, 2007) for all CG simulations. We performed two kinds of CG simulations: simulations of flat bilayers, and simulations of mature LDs. The flat bilayers always contained POPC and 5% TO. In addition, systems were simulated where Cav1 was added in two different concentrations. The list of simulations, along-with the composition of the bilayer/droplets are described in Table 1. In all systems the TO and Cav1 were initially mixed randomly in a lipid bilayer.

Table 1
Description of the simulations. The number of water molecules is not mentioned. POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, TO: triolein, POPE: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine, CHOL: cholesterol, CO: cholesteryl oleate, DOG: dioleoyl glycerol. For the CG simulations, the MARTINI simulation times are shown. Real simulation times are 4 times the value in the table.

Denomination	Composition	Time
All-atom simulation		
aa_cav	1 Cav1 – 288 POPC	800 ns
Coarse-grained simulations – lipid bilayer		
POPC	2450 POPC	10 μs
POPC TO	2268 POPC – 126 TO	10 μs
POPC TO 9Cav1(3 replicas)	9 Cav1 – 2290 POPC – 126 TO	10 μs
POPC TO 18Cav1	18 Cav1 – 2401 POPC – 132 TO	20 μs
Coarse-grained simulations – lipid droplet		
CO30 DOG20	1607 POPC – 536 POPE – 1429 TO – 857 CO – 571 DOG	10 μs
CO30 DOG20 9Cav1	9 Cav1 – 1539 POPC – 521 POPE – 969 TO – 610 CO – 355 DOG	20 μs
CO30 DOG20 6Cav1-surf	6 Cav1 – 1607 POPC – 536 POPE – 1429 TO – 857 CO – 571 DOG	16 μs
CO30 DOG20 18Cav1-surf	18 Cav1 – 1244 POPC – 464 POPE – 1373 TO – 768 CO – 552 DOG	20 μs

Mature LDs were simulated with TO, CO and DOG in the core of the droplet, which was surrounded by a monolayer containing POPC and POPE. In addition, 6 or 18 Cav1 molecules were placed on the surface of the droplet initially. When Cav1 was initially placed in the droplet core, it could never reach the surface (see later).

2.3. Simulation conditions

Details about the simulation conditions are provided in the SI. Most simulations were carried out for 20 μs or more MARTINI time, corresponding to a total simulation time of nearly 0.6 ms.

2.4. Analysis

To prepare the simulations, we wrote Python scripts using popular scientific Python packages (MDTraj (McGibbon et al., 2015), NumPy (van der Walt et al., 2011), SciPy (Jones et al., 2001), matplotlib (Hunter, 2007).

The average properties such as the mean curvature and thickness were calculated over the last 2 μs of the CG simulations. For calculating the local curvature of the different monolayers of the bilayer, we used an in-house C++ code based on both Legendre polynomial fitting and Fourier analysis. First we obtained the position of each monolayer surface numerically from the trajectory of the simulations. From this surface, the local mean curvature of each monolayer was obtained as $C_0(x, y) = -\frac{1}{2}\nabla \cdot \hat{n}$, where $\hat{n}$ is a unit vector that is normal to the obtained surface. In this formulation, an upward bending with respect to the z-axis (bilayer normal) will be referred to as negative curvature, and inward bending will be referred to as positive curvature. The monolayer surfaces were found by fitting the phosphate particle (CG simulations) or the phosphorus atoms (all-atom simulation) coordinate of the POPC lipids in each monolayer to a Fourier series in two dimensions as

$$z_n(x, y) = \sum_{i=-N}^{N} \sum_{j=-M}^{M} A_{i,j}(\cos[p_i x + q_j y] + \sin[p_i x + q_j y]) \quad (1)$$

where n is an index to distinguish the different monolayers, $p_i = 2\pi i/L_x$,