Biochemical and Biophysical Research Communications 452 (2014) 422–427

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Surface features of the lipid droplet mediate perilipin 2 localization

Arthur Sletten, Alison Seline, Andrew Rudd, Michelle Logsdon, Laura L. Listenberger *

Department of Biology, St. Olaf College, 1520 St. Olaf Avenue, Northfield, MN 55057, United States
Department of Chemistry, St. Olaf College, 1520 St. Olaf Avenue, Northfield, MN 55057, United States

ARTICLE INFO

Article history:
Received 15 August 2014
Available online 27 August 2014

Keywords:
Lipid droplet
Perilipin 2
Phospholipid monolayer

ABSTRACT

All eukaryotic organisms store excess lipid in intracellular lipid droplets. These dynamic structures are associated with and regulated by numerous proteins. Perilipin 2, an abundant protein on most lipid droplets, promotes neutral lipid accumulation in lipid droplets. However, the mechanism by which perilipin 2 binds to and remains anchored on the lipid droplet surface is unknown. Here we identify features of the lipid droplet surface that influence perilipin 2 localization. We show that perilipin 2 binding to the lipid droplet surface requires both hydrophobic and electrostatic interactions. Reagents that disrupt these interactions also decrease binding. Moreover, perilipin 2 binding does not depend on other lipid droplet-associated proteins but is influenced by the lipid composition of the surface. Perilipin 2 binds to synthetic vesicles composed of dioleoylphosphatidylcholine, a phospholipid with unsaturated acyl chains. Decreasing the temperature of the binding reaction, or introducing phospholipids with saturated acyl chains, decreases binding. We therefore demonstrate a role for surface lipids and acyl chain packing in perilipin 2 binding to lipid droplets. The ability of the lipid droplet phospholipid composition to impact protein binding may link changes in nutrient availability to lipid droplet homeostasis.

1. Introduction

The ability to esterify cholesterol or fatty acids and store these neutral lipids in cytosolic lipid droplets is characteristic of all eukaryotic organisms [1]. In mammals, lipid droplets are most abundant in adipose tissue. The triacylglycerol-rich lipid droplets of adipocytes provide large energy stores in the form of esterified fatty acids that are exported to other tissues for metabolism through β-oxidation. Lipid droplets in steroidogenic cells of the adrenal cortex, testes, and ovaries supply cholesteryl esters for steroid hormone synthesis. The role of lipid droplets in other tissues is equally important. Lipid droplets serve as local energy depots or lipid reservoirs for membrane homeostasis. Furthermore, lipid droplets protect cells from lipotoxicity by sequestering toxic lipid species from apoptotic pathways [2,3].

Each lipid droplet consists of a neutral lipid core (primarily triacylglycerol and/or cholesteryl ester) separated from the cytosol by a monolayer of amphipathic lipids [1]. Proteins that associate with the surface of lipid droplets are in close proximity to the underlying hydrophobic lipid and may interact with this lipid directly. Perilipin 2 (previously known as adipocyte differentiation-related protein, or ADRP) is a prominent lipid droplet protein that coats the surface of lipid droplets in all mammalian cells, except mature white adipocytes where a similar protein, perilipin 1, is found [4,5]. Perilipin 2 prevents lipase association with the surface of lipid droplets and slows triacylglycerol turnover [6]. Consequently, the level of perilipin 2 expression is intimately tied to lipid droplet accumulation [7–9].

It is currently unknown how perilipin 2 interacts with the lipid droplet surface. Efforts to identify targeting domains within the protein have found multiple sequences that may facilitate its localization [10–12]. In our study, we take a different approach. We identify features of the lipid droplet surface that mediate perilipin 2 binding. We show that perilipin 2 does not require other lipid droplet proteins to bind with lipid droplets. Instead, perilipin 2 interacts directly with the amphipathic lipid at the lipid droplet surface. We show that physicochemical features of the lipid droplet surface, including hydrophobicity, electrostatic forces, lipid composition, and lipid packing play a substantial role in perilipin 2 binding. Our findings demonstrate the importance of the lipid droplet surface composition in mediating the binding of specific proteins and suggest a model for protein–lipid droplet interactions.

Abbreviations: CCT1, CTP: phosphocholine cytidylyltransferase 1; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; Laurdan, 6-dodecanoyl-2-dimethylamino-naphthalene; LUV, large unilamellar vesicle; MC540, merocyanine 540; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

* Corresponding author. Fax: +1 507 786 3968.
E-mail address: listenbe@stolaf.edu (L.L. Listenberger).

http://dx.doi.org/10.1016/j.bbrc.2014.08.097
0006-291X/

2. Materials and methods

2.1. Cell culture

Creation of the perilipin 2-overexpressing HEK293 cell line has been described [6]. Perilipin 2-expressing and untransfected HEK293 cells were cultured as reported [6]. Where indicated, culture medium was supplemented with 400 μM oleate (Nu-Check Prep) complexed to BSA as described [13].

2.2. Immunoblotting

Perilipin 2 was resolved by SDS–PAGE and probed with anti-perilipin 2 rabbit polyclonal antibodies (Novus Biologicals) and peroxidase-conjugated secondary antibodies (Sigma). Blots were subsequently incubated with Supersignal West Femto chemiluminescence reagent (Fisher Scientific) or Opti-4CN colorimetric reagent (Bio-Rad) and imaged with film (Kodak X OMAT) or a FotoDyne FOTO/Analyst Luminary/FX workstation.

2.3. Lipid droplet isolation

Eight confluent 10 cm plates of perilipin 2-expressing or untransfected HEK293 cells were supplemented with 400 μM oleate overnight. Cells were scraped into cold PBS, pelleted, and resuspended in HEPES buffer (10 mM HEPES, 5 mM EDTA, pH 7.4, 1 μg/mL pepstatin, 1 μg/mL leupeptin, 100 μg/ml PMSF). Cell suspensions were incubated 10 min on ice and homogenized with a 25 g needle. The homogenate was adjusted to 20% sucrose, overlaid with HEPES buffer, and centrifuged at 280,000*g*, 4 °C, for 60 min. Lipid droplets were harvested in the floating fraction.

2.4. Disruption of electrostatic and hydrophobic interactions

Isolated lipid droplets were centrifuged with 100 mM sodium carbonate (as in [14]) or 60 mM $MgSO_4$ following the same protocol. Alternatively, isolated lipid droplets were resuspended in NP-40 buffer (150 mM NaCl, 1% NP-40, 50 mM TrisHCl [pH 8], as in [15]), adjusted to 20% sucrose, and incubated on ice for 30 min. Lipid droplets were collected after centrifugation at 26,000*g*, 4 °C, for 30 min. Perilipin 2 was detected in lipid droplet fractions by immunoblotting. Following treatment, lipid droplets were stained with 1 mg/mL BODIPY 493/503 (Invitrogen) and visualized with an Olympus BX40 fluorescence microscope.

2.5. Proteolysis of lipid droplet proteins

Isolated lipid droplets were incubated with 1 mg/ml proteinase K for 1 h at 37 °C. Five mM PMSF was added to halt each reaction. Samples were adjusted to 25% sucrose, overlaid with 10% sucrose and centrifuged at 26,000*g*, 4 °C, for 30 min. Equivalent volumes of untreated and proteinase K-treated lipid droplets were resolved on 11% SDS–polyacrylamide gels and proteins were visualized by silver staining.

2.6. Isolation of perilipin 2-enriched cytosol

Eight 10 cm plates of perilipin 2-expressing HEK293 cells were cultured and lysed as described for lipid droplet isolations. The homogenate was adjusted to 10% sucrose, overlaid with HEPES buffer, and centrifuged at 280,000*g*, 4 °C, for 3 h. The void volume, corresponding to the cytosol, was collected with a 22 g needle.

2.7. Preparation of lipid vesicles

For temperature dependent experiments, 10–13 μmol of dioleoylphosphatidylcholine dissolved in chloroform (DOPC, Avanti Polar Lipids) were dried under nitrogen, dissolved in chloroform, and dried again under nitrogen for a minimum of 60 min. Following 10 min at 70 °C, prewarmed HEPES buffer was added, and samples were incubated at 70 °C for 10 min. Lipid films were hydrated on a shaking platform at 50 °C for 1 h. Large unilamellar vesicles (LUVs) were formed by 5–10 freeze/thaw cycles in an ethanol/dry ice bath.

For measurements of phospholipid packing and binding to DOPC and palmitoyloleoylphosphatidylcholine (POPC) vesicles, 30 μmol aliquots of DOPC or POPC (Avanti Polar Lipids) were transferred to a round bottom flask and dried under vacuum at 60 °C, resuspended in chloroform:methanol (2:1), and dried again under vacuum at 60 °C for at least 45 min. Prewarmed HEPES buffer was added, and lipid films were hydrated on a rocking platform at 50 °C overnight. LUVs were formed by ten freeze/thaw cycles in an ethanol/dry ice bath. Vesicle suspensions were extruded through a 0.1 μm filter (Avanti Polar Lipids) using a thermobarrel extruder. The phospholipid content of the extruded vesicles was determined as described by Ames and Dubin [16].

2.8. In vitro binding assay

LUVs or isolated lipid droplets were combined with perilipin 2-enriched cytosol and placed in an orbital mixer for 1 h at 37 °C (unless otherwise noted). Samples were adjusted to 10% sucrose, loaded into a centrifuge tube, overlaid with HEPES buffer and centrifuged at 280,000*g*, 4 °C, for a minimum of 3 h. Lipid droplets (top fraction) and void volumes (unbound protein) were collected with a 22 g needle and perilipin 2 detected by immunoblotting.

2.9. Measurements of phospholipid packing

LUVs were labeled with 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan, Molecular Probes) at a probe:lipid ratio of 1:333 and a final Laurdan concentration of 0.3 μM. Immediately after Laurdan addition, labeled vesicles were rigorously vortexed for 3 min and placed on a shaking platform for 1 h in the dark. Fluorescence spectra were acquired upon excitation at 360 nm with an emission and excitation slit width of 3.5 nm and 7 nm, respectively.

In other experiments, LUVs were titrated with 10 μg/mL merocyanine 540 (MC540) in HEPES buffer. MC540 fluorescence spectra were acquired with an emission slit width of 2.5 nm and excitation slit width of 15 nm.

All fluorescence measurements were acquired in a Perkin-Elmer Luminescence LS50B Spectrophotometer at a speed of 60 nm min^{-1}.

2.10. Statistical analyses

Values are means ± SEM. Statistical analyses included the Student *t*-test or the Wilcoxon signed-rank test where appropriate (GraphPad Prism). Values with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Perilipin 2 binds to lipid droplets in vitro

We developed an *in vitro* binding assay to explore how the lipids and proteins on the lipid droplet surface allow perilipin 2 to

recognize and bind to the lipid droplet. Many lipid droplet proteins, including perilipin 2, are synthesized on free ribosomes and associate with lipid droplets post-translationally. We hypothesized that perilipin 2 would be capable of binding to a suitable surface outside of the cell as long as necessary co-factors were present.

Our binding assay required a surface of amphipathic lipid for the protein to bind and a source of perilipin 2. Our initial experiments used lipid droplets from untransfected HEK293 cells as the recipient lipid surface. Endogenous perilipin 2 was not detected on lipid droplets from HEK293 cells (Fig. 1A, lane 2), although the presence of a floating white layer was visual confirmation of lipid droplet isolation. This result is consistent with reports that HEK293 cells express extremely low levels of endogenous perilipin 2 [6].We utilized cytosol from perilipin 2-overexpressing HEK293 cells as a source of perilipin 2 protein. While perilipin 2 is efficiently targeted to lipid droplets in this engineered cell line, the cytosol retains significant levels of soluble perilipin 2 [6].

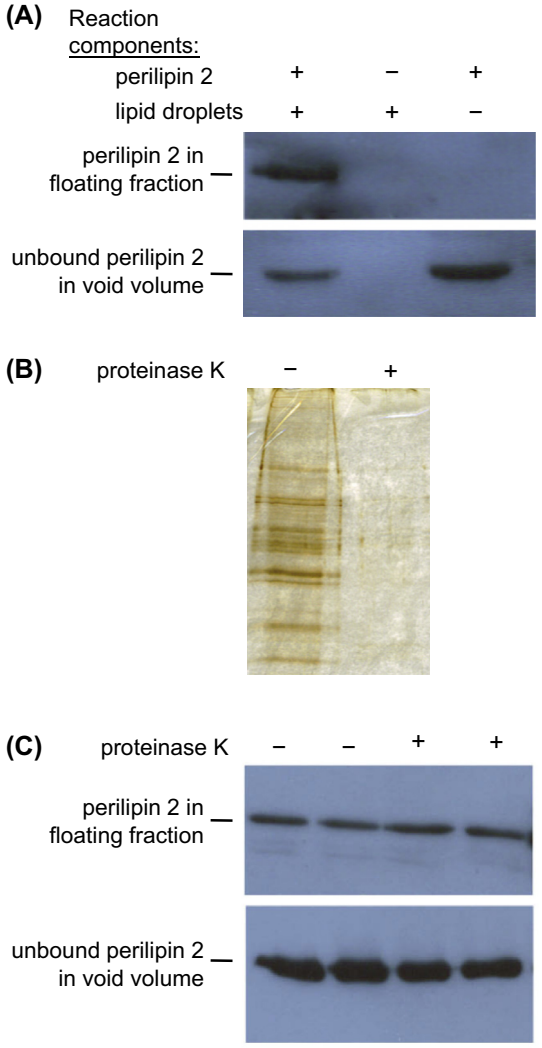

Fig. 1. *In vitro* binding of perilipin 2 to lipid droplets does not require other lipid droplet proteins. (A) Cytosolic perilipin 2 was incubated with isolated (perilipin-free) lipid droplets. Following ultracentrifugation, lipid droplets with bound protein were harvested from the top of each centrifuge tube. Unbound protein remained in the void volume. Perilipin 2 in equivalent volumes of floating fractions (top blot) or void volumes (bottom blot) was detected by immunoblotting. (B) SDS–PAGE and silver staining of lipid droplet-associated proteins, from untreated (–) or proteinase K-treated (+) lipid droplets. (C) Immunoblotting for perilipin 2 in floating fractions (top blot) or void volumes (bottom blot) following binding to untreated (–) or proteinase K-treated (+) lipid droplets.

To determine if perilipin 2 would bind to lipid droplets *in vitro*, perilipin 2-enriched cytosol was incubated with lipid droplets isolated from untransfected HEK293 cells. Lipid droplets were separated from unbound perilipin 2 by ultracentrifugation and perilipin 2 in both the floating (lipid droplet-bound) and void (unbound) fractions was visualized by immunoblotting. Perilipin 2 was found in the floating fraction only when both perilipin 2-enriched cytosol and lipid droplets were included in the binding reaction (Fig. 1A, lane 1). Thus, the features of the lipid droplet that allow perilipin 2 to recognize and bind to the lipid droplet surface are retained in our *in vitro* assay. Subsequent experiments aimed to identify the specific features of the lipid droplet surface that mediate the interaction with perilipin 2.

3.2. Perilipin 2 binding does not require other lipid droplet proteins

Many proteins associate with lipid droplets [17]. Some interact directly with perilipin 2 [18,19]. To investigate whether such proteins are necessary for perilipin 2 binding, lipid droplets were isolated from untransfected HEK293 cells and treated with proteinase K to degrade native lipid droplet-associated proteins (Fig. 1B). Protein-depleted lipid droplets were mixed with perilipin 2-enriched cytosol and binding was examined with our *in vitro* assay. Protein-depleted lipid droplets retained the ability to bind perilipin 2 (Fig. 1C).

3.3. Perilipin 2 binds to DOPC vesicles in a temperature dependent manner

Because perilipin 2 binding did not require other lipid droplet proteins, we hypothesized that this protein interacts directly with the phospholipids at the lipid droplet surface. To demonstrate that amphipathic lipids are sufficient for binding perilipin 2, we

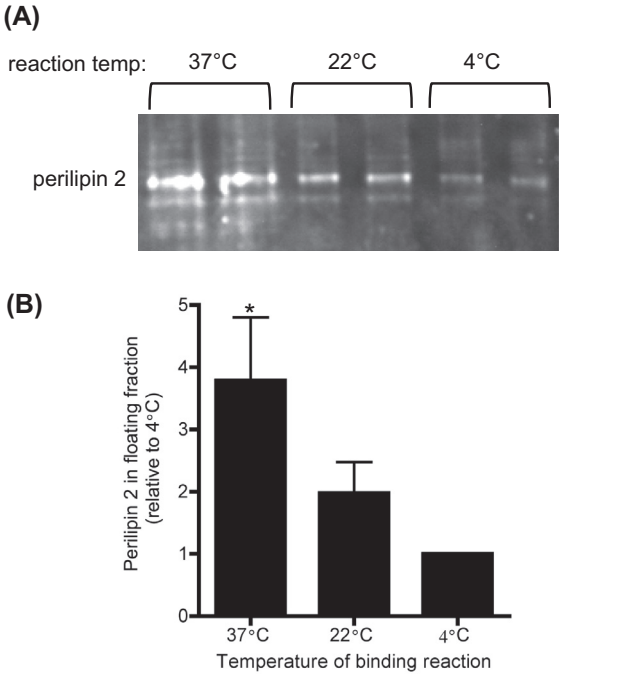

Fig. 2. Perilipin 2 binds to lipid membranes. Cytosolic perilipin 2 was incubated with DOPC LUVs for 1 h at 37 °C, 22 °C, or 4 °C. LUVs were separated from unbound protein by sucrose gradient centrifugation. (A) A representative Western blot of perilipin 2 in the LUV (floating) fraction, with duplicate samples at each temperature. (B) Ave. ± SEM from 3 (22 °C) or 7 (37 °C, 4 °C) experiments. Asterisk indicates a statistically significant difference relative to 4 °C ($p < 0.008$, Wilcoxon signed-rank test).

adapted our *in vitro* assay to examine perilipin 2 binding to LUVs. LUVs were synthesized from DOPC, an unsaturated phospholipid, and incubated with perilipin 2-enriched cytosol. LUVs were separated from unbound perilipin 2 by flotation through a sucrose gradient and perilipin 2 in the floating fraction was detected by immunoblotting. Perilipin 2 was found in LUV fractions when the binding reaction was performed at 4 °C. However, vesicle-bound perilipin 2 was nearly 4-fold more abundant when binding was performed at 37 °C (Fig. 2).

3.4. Phospholipid packing modulates perilipin 2 association with lipid droplets

Our observation that perilipin 2 binding to LUVs was influenced by temperature, suggests that phospholipid fluidity or packing may be involved in moderating the binding of perilipin 2 to the lipid droplet. To test this hypothesis, we first prepared 100 nm diameter LUVs with DOPC or POPC and assayed molecular packing in these membranes using two fluorescent probes, Laurdan and MC540.

Laurdan is a lipophilic naphthalene derivative that integrates into the nonpolar environment of a phospholipid membrane. The emission spectrum of Laurdan is dependent on the polarity of its physical environment [20]. In loosely-packed bilayers where lipids are hydrated, Laurdan emissions at 490 nm increase. Conversely, few water molecules penetrate into densely-packed bilayers and the emissions at 440 nm increase. We labeled DOPC and POPC vesicles with Laurdan and observed the emission spectra (Fig. 3A). Spectra were acquired at 22 °C, a temperature well above the T_m for both lipids. Consistent with the membrane condensing effects of saturated acyl chains, we observed an increase in I_{440}/I_{490} in POPC vesicles compared to DOPC vesicles (Fig. 3B).

We also measured molecular packing with MC540. MC540 readily partitions into the hydrophobic environment at the surface of a phospholipid vesicle. When MC540 binds membranes, its fluorescence emission spectrum shifts from an aqueous maximum of 570–590 nm. This red shift is also accompanied by an increase in quantum yield [21]. We observed a dramatic red shift in emission maxima and increase in fluorescence yield when MC540 was

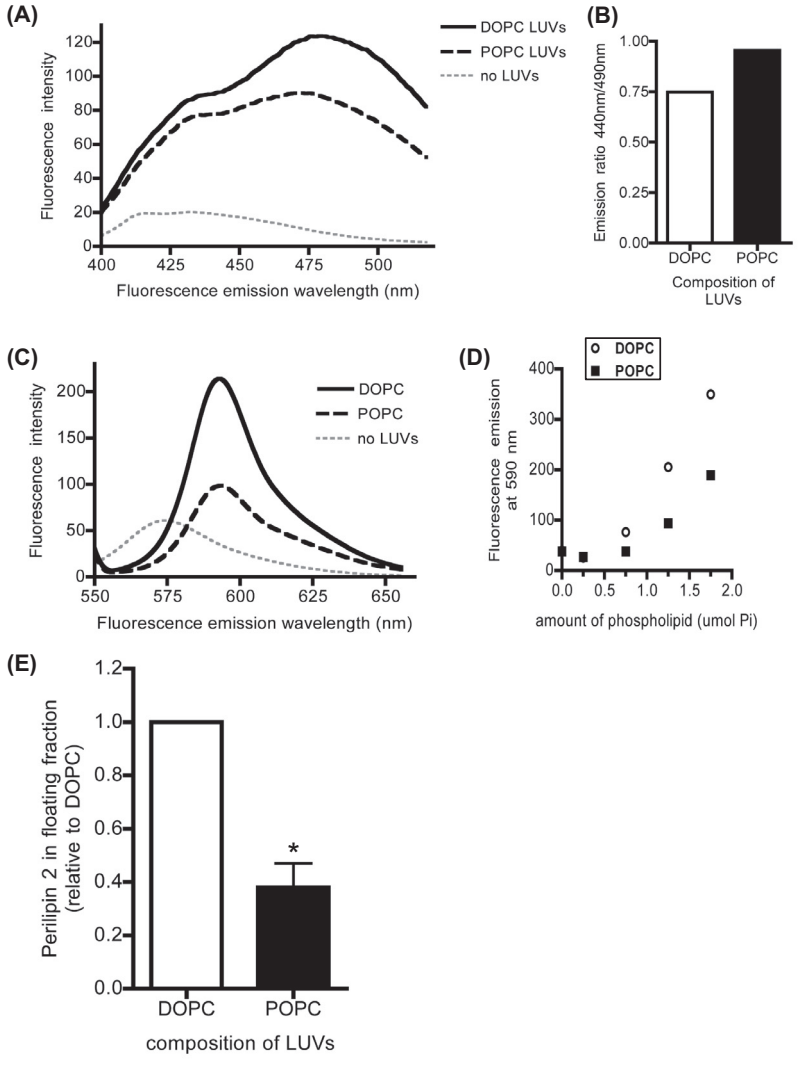

Fig. 3. Phospholipid composition influences perilipin 2 binding to LUVs. Laurdan or MC540 was incorporated into DOPC and POPC LUVs for measurements of acyl chain packing. A representative scan of emitted Laurdan fluorescence (A) and the ratio of emitted Laurdan fluorescence at 440–490 nm (B) is shown. A representative scan of emitted MC540 fluorescence (C) and the intensity of MC540 fluorescence emitted at 590 nm following incubation with different amounts of DOPC or POPC vesicles is shown. (E) Cytosolic perilipin 2 was incubated with DOPC or POPC LUVs. SDS–PAGE and Western blotting detected perilipin 2 in the LUV (floating) fraction after binding. Ave. ± SEM from 4 experiments is shown (*$p = 0.002$, Wilcoxon signed-rank test).

titrated with DOPC vesicles. These spectral changes were less pronounced with POPC vesicles (Fig. 3C and D). While a red shift did occur, the quantum yield of MC540 in POPC bilayers was reduced compared to equivalent amounts of DOPC vesicles. Taken together, data from these fluorescence studies indicate that we have created LUVs with differences in surface lipid packing.

To isolate the effects of changes in phospholipid packing on perilipin 2 binding, we repeated our *in vitro* binding assay with 100 nm LUVs synthesized from DOPC or POPC. Following incubation with soluble perilipin 2, the LUVs were separated from unbound perilipin 2 by flotation through a sucrose gradient. Perilipin 2 bound readily to LUVs composed of unsaturated DOPC. POPC vesicles bound 60% less perilipin 2 (Fig. 3E). Based on measurements of lipid packing in these LUVs (Fig. 3A–D), our findings suggest that increasing the molecular packing of the lipids at the phospholipid surface negatively impacts perilipin 2 binding.

3.5. Hydrophobic and electrostatic interactions mediate perilipin 2 binding to lipid droplets

Because of the relationship between perilipin 2 binding and phospholipid packing, we hypothesize that nonpolar domains of perilipin 2 interact directly with hydrophobic regions of surface lipids. Notably, when we disrupt hydrophobic interactions with NP-40 detergent, isolated lipid droplets lose their coating of perilipin 2 (Fig. 4A). While it is possible that treatment with a mild detergent could solubilize the lipid droplet phospholipid monolayer, NP-40 did not alter lipid droplet size and structure in our experiments (Fig. 4B). Moreover, similar experiments with NP-40 failed to dissociate histone H2B from lipid droplets, suggesting that lipid droplets retain some lipid droplet-associated proteins following incubation with NP-40 [15]. Surprisingly, our experiments also show that disrupting electrostatic interactions with either alkaline carbonate or magnesium sulfate reduced the amount of perilipin 2 on isolated lipid droplets without disrupting lipid droplet ultrastructure (Fig. 4A and B). Together, these results suggest that perilipin 2 localizes to the lipid droplet surface by a mechanism involving both hydrophobic and electrostatic interactions.

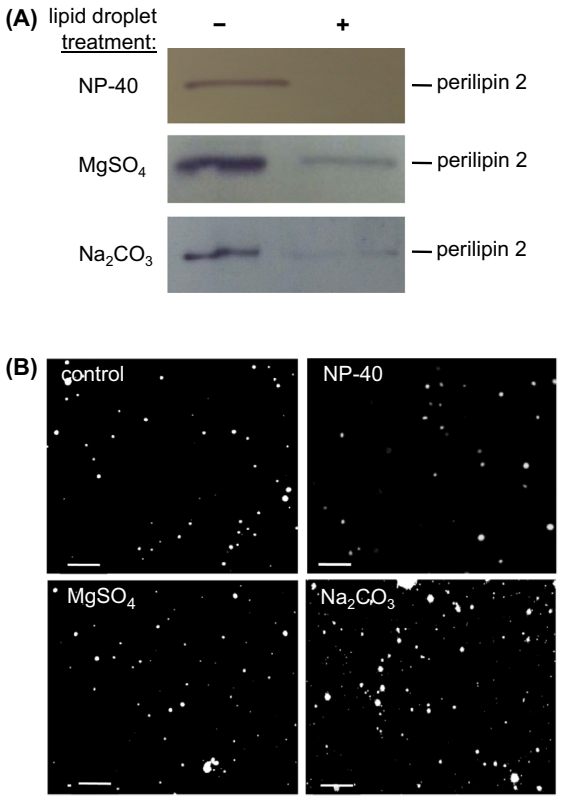

Fig. 4. Hydrophobic and electrostatic interactions anchor perilipin 2 to lipid droplets. (A) Lipid droplets were isolated from HEK293 cells modified to stably express perilipin 2. Lipid droplets were mock-treated or treated with reagents that disrupt hydrophobic interactions (1% NP-40) or electrostatic interactions (100 mM Na_2CO_3 or 60 mM $MgSO_4$). Perilipin 2 remaining on lipid droplets was detected by immunoblotting. (B) Treatment of lipid droplets did not disrupt their morphology as assessed by BODIPY 493/503 staining. Bars: 10 μm.

4. Discussion

Lipid droplets are decorated with numerous proteins, which impact lipid droplet structure and function [1,17]. Lipid droplet proteins control lipid synthesis, initiate lipid droplet fusion and promote lipid hydrolysis. Despite this intimate relationship between protein targeting and lipid droplet metabolism, very little is known about how specific proteins associate with lipid droplets.

We utilize perilipin 2 as a model lipid droplet protein to examine protein-lipid droplet interactions. While the three-dimensional structure of perilipin 2 has not been solved, modeling suggests that it belongs to a family of lipid droplet proteins with amphipathic alpha helices [22]. Other proteins in this family include HCV core protein, viperin, and CTP:phosphocholine cytidylyltransferase 1 (CCT1) (reviewed in [1]).

Our work aims to complement ongoing efforts to identify targeting domains in lipid droplet-associated proteins. We report features of the lipid droplet that modulate perilipin 2 binding. Importantly, targeting is not reliant on other lipid droplet proteins. We show perilipin 2 binds protein-depleted lipid droplets as well as LUVs that lack proteins and the neutral lipid core. Though our data show that perilipin 2 can bind lipid membranes directly, we do not disregard a possible contribution from the neutral lipid core. Indeed, we hypothesize more generally that perilipin 2 requires access to a hydrophobic environment. Abrogation of perilipin 2 binding by detergent treatment of lipid droplets supports the model where interactions between nonpolar domains of the protein and lipid droplet surface are necessary for binding. Additionally, preference for hydrophobic environments offers a potential explanation for the selective localization of perilipin 2 to lipid droplets.

If perilipin 2 must access hydrophobic lipid domains to bind to lipid droplets, then it is not surprising that surface lipid packing influences perilipin 2 binding. We show that perilipin 2 binds best to LUVs composed of unsaturated phospholipids. Decreasing temperature or introducing saturated acyl chains, manipulations that condense membranes, impairs perilipin 2 binding. The role of phospholipid packing on protein binding to lipid droplet surfaces *in vivo* is currently obscure. However, Storey et al. find perilipin 2 on a population of lipid droplets enriched in unsaturated fatty acids that exhibit a highly fluid phospholipid monolayer [23]. Perilipin 2 was largely excluded from a second population of lipid droplets with increased surface lipid rigidity. Also, descriptions of the phospholipid content of lipid droplets note more unsaturated phospholipid relative to other cell membranes [24,25].

Accessibility of hydrophobic lipid may be a broader mechanism for recruiting lipid droplet-associated proteins. CCT1, an enzyme in the phosphatidylcholine synthesis pathway, is recruited to lipid droplets during lipid droplet biogenesis at times when incorporation of neutral lipid is outpacing phospholipid synthesis [26]. Putative "gaps" in the lipid droplet surface may provide hydrophobic surface to bind CCT1. This model of protein binding to exposed hydrophobic lipid is reminiscent of models of exchangeable lipoprotein binding to lipoprotein particles. The role of amphipathic

alpha helices in mediating these interactions has been discussed [27].

Multiple features of the lipid droplet likely influence protein binding. The preference for specific surface phospholipids may extend beyond those that impact surface lipid packing. Our data suggest a role for electrostatic interactions, which may involve interactions with specific phospholipids. While there is no current data on the role that specific surface lipids may play in protein binding to lipid droplets, there is evidence that diacylglycerol may help recruit a similar protein, perilipin 3, to the ER membrane [28].

We propose a model where the lipid content of the lipid droplet surface influences protein binding. The significance of this model is far-reaching. There is ample evidence that the lipid content of lipid droplets responds to changes in the metabolic state of the cell [29,30]. We suggest the lipid content of the lipid droplet recruits specific proteins that promote lipid storage or hydrolysis, thus linking nutrient abundance to lipid droplet metabolism.

Acknowledgments

This work was supported by a National Institutes of Health Ruth L. Kirschstein National Research Service Award (Grant 1 F32 GM-074453-01) to L.L.L. and an award from Research Corporation for Science Advancement.

References

[1] T.C. Walther, R.V. Farese, Lipid droplets and cellular lipid metabolism, Annu. Rev. Biochem. 81 (2012) 687–714.
[2] L.L. Listenberger, X.L. Han, S.E. Lewis, S. Cases, R.V. Farese, D.S. Ory, J.E. Schaffer, Triglyceride accumulation protects against fatty acid-induced lipotoxicity, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 3077–3082.
[3] I. Tabas, Consequences of cellular cholesterol accumulation: basic concepts and physiological implications, J. Clin. Invest. 110 (2002) 905–911.
[4] P.E. Bickel, J.T. Tansey, M.A. Welte, PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores, Biochim. Biophys. Acta 1791 (2009) 419–440.
[5] C. Londos, D.L. Brasaemle, C.J. Schultz, J.P. Segrest, A.R. Kimmel, Perilipins, ADRP, and other proteins that associate with intracellular neutral lipid droplets in animal cells, Semin. Cell Dev. Biol. 10 (1999) 51–58.
[6] L.L. Listenberger, A.G. Ostermeyer-Fay, E.B. Goldberg, W.J. Brown, D.A. Brown, Adipocyte differentiation-related protein reduces the lipid droplet association of adipose triglyceride lipase and slows triacylglycerol turnover, J. Lipid Res. 48 (2007) 2751–2761.
[7] M. Imamura, T. Inoguchi, S. Ikuyama, S. Taniguchi, K. Kobayashi, N. Nakashima, H. Nawata, ADRP stimulates lipid accumulation and lipid droplet formation in murine fibroblasts, Am. J. Physiol. Endocrinol. Metabol. 283 (2002) E775–E783.
[8] G. Larigauderie, C. Furman, M. Jaye, C. Lasselin, C. Copin, J.C. Fruchart, G. Castro, M. Rouis, Adipophilin enhances lipid accumulation and prevents lipid efflux from THP-1 macrophages: Potential role in atherogenesis, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 504–510.
[9] B. Magnusson, L. Asp, P. Bostrom, M. Ruiz, P. Stillemark-Billton, D. Linden, J. Boren, S.O. Olofsson, Adipocyte differentiation-related protein promotes fatty acid storage in cytosolic triglycerides and inhibits secretion of very low-density lipoproteins, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 1566–1571.
[10] J.L. McManaman, W. Zabaronick, J. Schaack, D.J. Orlicky, Lipid droplet targeting domains of adipophilin, J. Lipid Res. 44 (2003) 668–673.
[11] N. Nakamura, T. Fujimoto, Adipose differentiation-related protein has two independent domains for targeting to lipid droplets, Biochem. Biophys. Res. Commun. 306 (2003) 333–338.
[12] P. Targett-Adams, D. Chambers, S. Gledhill, R.G. Hope, J.F. Coy, A. Girod, J. McLauchlan, Live cell analysis and targeting of the lipid droplet-binding adipocyte differentiation-related protein, J. Biol. Chem. 278 (2003) 15998–16007.
[13] L.L. Listenberger, D.S. Ory, J.E. Schaffer, Palmitate-induced apoptosis can occur through a ceramide-independent pathway, J. Biol. Chem. 276 (2001) 14890–14895.
[14] V. Subramanian, A. Garcia, A. Sekowski, D.L. Brasaemle, Hydrophobic sequences target and anchor perilipin A to lipid droplets, J. Lipid Res. 45 (2004) 1983–1991.
[15] S. Cermelli, Y. Guo, S.P. Gross, M.A. Welte, The lipid-droplet proteome reveals that droplets are a protein-storage depot, Curr. Biol. 16 (2006) 1783–1795.
[16] B.N. Ames, D.T. Dubin, The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid, J. Biol. Chem. 235 (1960) 769–775.
[17] L. Yang, Y.F. Ding, Y. Chen, S.Y. Zhang, C.X. Huo, Y. Wang, J.H. Yu, P. Zhang, H.M. Na, H.N. Zhang, Y.B. Ma, P.S. Liu, The proteomics of lipid droplets: structure, dynamics, and functions of the organelle conserved from bacteria to humans, J. Lipid Res. 53 (2012) 1245–1253.
[18] N. Nakamura, T. Akashi, T. Taneda, H. Kogo, A. Kikuchi, T. Fujimoto, ADRP is dissociated from lipid droplets by ARF1-dependent mechanism, Biochem. Biophys. Res. Commun. 322 (2004) 957–965.
[19] T. Yamaguchi, N. Omatsu, S. Matsushita, T. Osumi, CGI-58 interacts with perilipin and is localized to lipid droplets. Possible involvement of CGI-58 mislocalization in Chanarin-Dorfman syndrome, J. Biol. Chem. 279 (2004) 30490–30497.
[20] L.A. Bagatolli, T. Parasassi, G.D. Fidelio, E. Gratton, A model for the interaction of 6-lauroyl-2-(N,N-dimethylamino) naphthalene with lipid environments: implications for spectral properties, Photochem. Photobiol. 70 (1999) 557–564.
[21] P. Williamson, K. Mattocks, R.A. Schlegel, Merocyanine 540, a fluorescent probe sensitive to lipid packing, Biochim. Biophys. Acta 732 (1983) 387–393.
[22] B.M. Chong, P. Reigan, K.D. Mayle-Combs, D.J. Orlicky, J.L. McManaman, Determinants of adipophilin function in milk lipid formation and secretion, Trends Endocrinol. Metab. 22 (2011) 211–217.
[23] S.M. Storey, A.L. McIntosh, S. Senthivinayagam, K.C. Moon, B.P. Atshaves, The phospholipid monolayer associated with perilipin-enriched lipid droplets is a highly organized rigid membrane structure, Am. J. Physiol. Endocrinol. Metabol. 301 (2011) E991–E1003.
[24] R. Bartz, W.H. Li, B. Venables, J.K. Zehmer, M.R. Roth, R. Welti, R.G.W. Anderson, P.S. Liu, K.D. Chapman, Lipidomics reveals that adiposomes store ether lipids and mediate phospholipid traffic, J. Lipid Res. 48 (2007) 837–847.
[25] K. Tauchi-Sato, S. Ozeki, T. Houjou, R. Taguchi, T. Fujimoto, The surface of lipid droplets is a phospholipid monolayer with a unique fatty acid composition, J. Biol. Chem. 277 (2002) 44507–44512.
[26] N. Krahmer, Y. Guo, F. Wilfling, M. Hilger, S. Lingrell, K. Heger, H.W. Newman, M. Schmidt-Supprian, D.E. Vance, M. Mann, R.V. Farese, T.C. Walther, Phosphatidylcholine synthesis for lipid droplet expansion is mediated by localized activation of CTP: phosphocholine cytidylyltransferase, Cell Metab. 14 (2011) 504–515.
[27] H. Saito, S. Lund-Katz, M.C. Phillips, Contributions of domain structure and lipid interaction to the functionality of exchangeable human apolipoproteins, Prog. Lipid Res. 43 (2004) 350–380.
[28] J.R. Skinner, T.M. Shew, D.M. Schwartz, A. Tzekov, C.M. Lepus, N.A. Abumrad, N.E. Wolins, Diacylglycerol enrichment of endoplasmic reticulum or lipid droplets recruits perilipin 3/TIP47 during lipid storage and mobilization, J. Biol. Chem. 284 (2009) 30941–30948.
[29] D.L. Brasaemle, G. Dolios, L. Shapiro, R. Wang, Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes, J. Biol. Chem. 279 (2004) 46835–46842.
[30] A.E. Crunk, J. Monks, A. Murakami, M. Jackman, P.S. Maclean, M. Ladinsky, E.S. Bales, S. Cain, D.J. Orlicky, J.L. McManaman, Dynamic regulation of hepatic lipid droplet properties by diet, PLoS ONE 8 (2013) e67631.