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Phase behavior and the partitioning of caveolin-1 scaffolding domain peptides in model lipid bilayers

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Abstract

The membrane binding and model lipid raft interaction of synthetic peptides derived from the caveolin scaffolding domain (CSD) of the protein caveolin-1 have been investigated. CSD peptides bind preferentially to liquid-disordered domains in model lipid bilayers composed of cholesterol and an equimolar ratio of dioleoylphosphatidylcholine (DOPC) and brain sphingomyelin. Three caveolin-1 peptides were studied: the scaffolding domain (residues 83–101), a water-insoluble construct containing residues 89–101, and a water-soluble construct containing residues 89–101. Confocal and fluorescence microscopy investigation shows that the caveolin-1 peptides bind to the more fluid cholesterol-poor phase. The binding of the water-soluble peptide to lipid bilayers was measured using fluorescence correlation spectroscopy (FCS). We measured molar partition coefficients of 10^4 M^{-1} between the soluble peptide and phase-separated lipid bilayers and 10^3 M^{-1} between the soluble peptide and bilayers with a single liquid phase. Partial phase diagrams for our phase-separating lipid mixture with added caveolin-1 peptides were measured using fluorescence microscopy. The water-soluble peptide did not change the phase morphology or the miscibility transition in giant unilamellar vesicles (GUVs); however, the water-insoluble and full-length CSD peptides lowered the liquid–liquid melting temperature.

Keywords: Lipid rafts; Fluorescence microscopy; Fluorescence correlation spectroscopy; Cholesterol; Bilayers; Lipid domains; Membranes

1. Introduction

Lipid rafts, or detergent-insoluble domains of the plasma membrane enriched in cholesterol and sphingolipids, are thought to play a role in sequestering various molecules to facilitate cell signaling. Caveolae are a specialized type of lipid raft with flask-like invaginated morphology enriched in the protein caveolin-1 that participate in cell signaling and lipid metabolism [1–5]. Caveolin-1 has been shown to bind cholesterol [6] and associate with sphingolipids [7] and may have a structural role in the formation of caveolae [8,9].

Model lipid rafts in synthetic lipid bilayers have provided a basis for understanding cholesterol-enriched phases of the plasma membrane. Lipids extracted from cell membranes and reconstituted in giant unilamellar vesicles (GUVs) exhibit microscopic phase coexistence with lipid domains resembling ternary lipid mixtures of cholesterol, phosphatidylcholine, and sphingomyelin [10]. In this widely-studied model system, liquid-ordered (L_o) domains are formed from the packing among saturated lipid acyl chains and cholesterol and are immiscible with liquid-disordered (L_d) domains enriched in phosphatidylcholine [11]. These L_o lipid domains are considered models of lipid rafts in the plasma membrane. Various physical properties of model lipid rafts, including composition, morphology, and molecular mobility, have been studied [11,12]. There has also been an increasing effort to understand how proteins partition into either the L_o phase or the L_d phase [13]. What remains to be well studied, however, is how peptides and proteins influence the lipid phase behavior of these model lipid rafts.

Proteins are a significant part of the composition of cell membranes and it is therefore important to consider proteins in model studies of lipid rafts and caveolae. Studying the in-

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terplay between proteins and lipid phase separation may give insight into the formation of lipid rafts, as proteins have been suggested to promote domain formation by associating with certain lipids [14]. It has also been suggested that lipid molecules can organize around proteins and modulate phase separation [15,16]. Using model lipid membranes, researchers have studied how proteins and peptides can cause lateral redistribution of lipids in bilayer membranes using differential scanning calorimetry (DSC) [14] and fluorescence microscopy [17]. An advantage of fluorescence microscopy is that it allows one to directly observe the lipid-phase partitioning of labeled molecules as well as microscopic phase separation.

In this study we investigated the partitioning and phase behavior of lipid bilayer membranes containing caveolin-1. We selected caveolin-1 because caveolae are enriched in the lipid raft components cholesterol and sphingomyelin and the membrane interaction of caveolin-1 is not well understood. Mutagenesis experiments have identified the caveolin scaffolding domain (CSD) as the region of caveolin-1 responsible for membrane binding and targeting the full-length protein to caveolae [18]. The CSD comprises amino acids 82–101 of the N-terminal domain of caveolin-1 and has been shown to associate with detergent-insoluble membrane fractions assayed in vivo [19]. We have selected model peptides derived from the CSD of caveolin-1 to study phase separation and the influence of cholesterol concentration on peptide–lipid interactions in lipid bilayers.

In previous model membrane experiments, the full-length CSD formed cholesterol-enriched domains in model membranes composed of DOPC, the acidic lipids phosphatidylserine and phosphatidylinositol-4,5-biphosphate (PIP_2), and cholesterol [20]. Subregions of the CSD and their membrane interactions have also been previously investigated. In live-cell mutagenesis experiments, KYWFYR was shown to be the membrane-attachment sequence [19] of caveolin-1, and in recent model membrane experiments, authors have demonstrated, using DSC, that KYWFYR does not promote local high cholesterol concentrations, nor does it bind cholesterol in phosphatidylcholine membranes [21]. DSC analysis has been used to study the peptide *N*-acetyl-VTKYWFYR amide, which was shown to promote local cholesterol crystal formation and depletion from other domains, though this effect was more pronounced with the full-length CSD [22].

While the effect of acidic lipids [20,23] and cholesterol [20,22] on the spatial organization and binding of CSD peptides has been investigated, to our knowledge the interaction of CSD peptides with putative model lipid rafts containing sphingomyelin has not yet been investigated. Therefore, our primary goal was to study caveolin-1 in model membranes with defined L_o and L_d domains and to investigate how the CSD can impact the phase behavior of L_o and L_d phases. Sphingomyelin was recently shown to be a component of caveolae in vivo [7] and thus we focused on a membrane containing sphingomyelin in order to understand caveolin and lipid interactions.

2. Materials and methods

2.1. Commercial reagents

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), cholesterol, and brain sphingomyelin (BSM) were purchased from Avanti Polar Lipids (Alabaster, AL). Texas Red 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (TR-DPPE) and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine 4-chlorobenzenesulfonate salt (DiD) were purchased from Invitrogen (Eugene, OR). Rhodamine 6G and Cy5 dyes in sugar solutions (Merck, Darmstadt, Germany) were used for FCS system calibration. Lipids were dissolved in high-performance liquid chromatography-grade chloroform and methanol from either Fluka (Switzerland) or Mallinckrodt (Phillipsburg, NJ). All other chemicals used were reagent grade. Fluorescently labeled synthetic peptides containing sequences derived from the caveolin scaffolding domain (CSD) were purchased from SynPep (Dublin, CA) and the MIT Biopolymers Laboratory. The CSD peptide labeled at the N terminus with fluorescein isothiocyanate is FITC-CGIWKASFTTFTVTKYWFYR-acetyl (CAV-CSD). A shorter fluorescently labeled peptide containing the membrane-attachment segment amino acid sequence residues 89–101 is FITC-FTTFTVTKYWFYR-acetyl (CAV-INSOL). The soluble peptide containing these residues was synthesized with a FITC label at the N-terminus and the sequence SGS between the FITC and CSD residues to improve peptide water solubility without adding net charge, resulting in a final peptide structure of FITC-SGSFTTFTVTKYWFYR-acetyl (CAV-SOL). All peptides were purified using HPLC. The p*I*'s of the three peptides were estimated to be in the range 9.5–10.5 [24]. The structures of the three peptides are shown schematically in Fig. 1.

2.2. Preparation of vesicles

We prepared giant unilamellar vesicles (GUVs) with the electroformation technique [25]. Approximately 40 μL of lipids

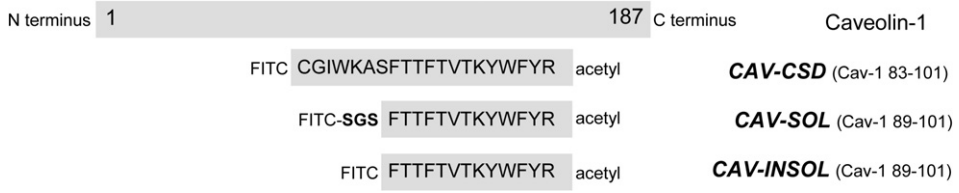

Fig. 1. Schematic of fluorescently labeled synthetic peptides used in experiments. CAV-CSD contains the caveolin scaffolding domain (residues 83–101); CAV-SOL contains residues 89–101 and is solubilized at the N terminus by a serine–glycine–serine (SGS) sequence. CAV-INSOL contains the same residues as CAV-SOL but lacks the SGS sequence.