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Micro article

N-terminal amphipathic helix of Amphiphysin can change the spatial distribution of immunoglobulin E receptors (FcεRI) in the RBL-2H3 mast cell synapse

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Biomembranes undergo extensive shape changes as they perform vital cellular functions or become diseased. To understand the mechanisms by which lipids and proteins control membrane curvature during various processes, researchers have identified and engineered many curvature-inducing and curvature-sensing proteins and peptides. In this paper, a simple experiment was performed to show qualitatively how membrane remodeling by N-terminal amphipathic helix of Amphiphysin affects the spatial distribution of the transmembrane Fc receptor protein (FcεRI) in mast cells. Results indicate that an elevated concentration of amphipathic helices can interfere with the formation of a typical mast cell synapse.

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1. Introduction

Cellular membranes exhibit different curvatures depending on the function they perform. For example, normal cells are known to change their membrane shape when they become cancerous or are exposed to toxic drugs [1,2]. Cell membrane topography also changes to form various vesicles (closed membrane shells) in order to synthesize and traffic materials such as proteins [3]. Dynamic remodeling (restructuring) of the membrane is a consequence of several different mechanisms that include, but are not limited to, changes in lipid composition, specific protein-membrane interactions that drive the expansion of one lipid membrane layer, changes in cytoskeletal polymerization and pulling of tubules by motor proteins, direct and indirect scaffolding of the lipid bilayer, and active amphipathic helix insertion into one leaflet of the bilayer [3–5]. Numerous curvature-sensing and curvature-generating proteins in the cell have been identified, each having a specific application [3]. For example, the Amphiphysin protein of the BAR (Bin/amphiphysin/RVs) domain is a brain-enriched protein with an N-terminal lipid interaction, dimerization and membrane bending BAR domain, a middle clathrin and adaptor binding domain and a C-terminal SH3 domain [6]. In the brain, its primary function is thought to be the recruitment of dynamin to sites of clathrin-mediated endocytosis [6,7]. BAR domains generate and sense membrane curvature by binding the negatively charged membrane to their positively charged and concave surfaces [7–9]. To understand the basic mechanism of curvature generation and sensing by the Amphiphysin protein, researchers have experimentally studied full-length Amphiphysin, N-BAR domains, as well as N-terminal amphipathic helices [9–14]. For example, N-BAR domains are found to shape low-curvature microvesicles into high-curvature tubules in vitro [7,12] and can tubulate the cell plasma membrane when overexpressed in COS cells [7]. Moreover, computer simulations have shown that sufficient high concentrations of bound N-BAR domains [12] as well as embedded amphipathic helices [12,14] will cause increases in membrane remodeling. Besides the growing recognition that proteins can generate membrane curvature, there have been experimental efforts in understanding how membrane shape modulates transmembrane protein distribution and mobility. In recent studies, the integral membrane protein potassium channel KvAP was shown to prefer highly curved membrane tubes to the practically flat membrane of cell-sized giant unilamellar vesicles [15] and showed a significant increase in mobility under tension [16].

In this study, the effect of curvature-remodeling N-terminal amphipathic helix of Amphiphysin on transmembrane Fc receptor protein (FcεRI) distribution in mast cells was investigated when

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cells were in contact with a ligand-modified fluid supported-lipid bilayer using confocal microscopy. For mast cells, the rat basophilic leukemia 2H3 cell line (RBL) is typically used as a model system [17–20]. Mast cells store granules with chemical mediators of inflammation. These mediators are released when high affinity FcεRI specific for immunoglobulin E (IgE) are brought into close proximity, i.e. aggregated. In model systems, receptor aggregation is usually accomplished by cross-linking IgE-loaded receptors with multivalent ligands or by monovalent ligands bound to a fluid lipid bilayer [17–20]. In the latter model system, the receptors are first aggregated in microclusters by diffusion-limited trapping of IgE-receptor complexes at close cell-substrate contact points [19]. After aggregation, micron-sized clusters move by apparently performing diffusion or a random walk motion to eventually coalesce to form a big central receptor patch, called the mast cell synapse [18,19]. This large receptor patch resembles the immunological synapse formed by T cells and B cells, when in contact with a ligand presenting bilayer [21,22]. Similar to the immunological synapse, there is evidence that the mast cell synapse can play a critical role in immune cell signaling between contacting cells. For example, a recent experiment showed that a synapse formed by mast cells and dendritic cells can facilitate antigen transfer in T cell activation [23].

Experiments presented here suggest that FcεRI receptor distribution in the RBL-2H3 synapse can be significantly changed in the presence of curvature-remodeling N-terminal amphipathic helix of Amphiphysin.

2. Materials and methods

2.1. Cells

The RBL-2H3 cell line was purchased from ATCC. RBL-2H3 cells were maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% Penicillin Streptomycin (Pen-Strep), and 1% L-glutamine (L-glut). Anti-DNP IgE was purchased from Sigma-Aldrich and labeled with DyLight 550 Amine-Reactive Dye (Thermo Scientific). Before microscopy, cells were fluorescent IgE (IgE_{550}) primed by incubation with 0.5 mg/mL of fluorescent IgE overnight.

2.2. Supported lipid bilayer

Prior to use, microscope glass cover slips were cleaned of organic residues with a mixture of sulfuric acid and hydrogen peroxide (piranha solution). Supported lipid bilayers were made by spontaneous liposome fusion [18,19,24]. Lipids, obtained from Avanti, were dissolved in chloroform, dried under air flow, then placed under a vacuum for 1 h to remove traces of oxygen. The lipid film was then suspended in PBS to 1.3 mM and sonicated for 5 min using a probe sonicator in an ice bath. Laterally mobile bilayers were formed from zwitterionic 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 25 mol% N-dinitrophenyl-aminocaproyl phosphatidylethanolamine (DNP-Cap PE) on piranha cleaned cover glass for 15 min on a slide warmer at 37 °C. Each bilayer coated coverslip was water immersed during transfer to the imaging chamber. Prior to adding cells to the bilayer, the chamber was flushed with Hanks' buffered saline solution.

2.3. Amphiphysin peptide

Fluorescent N-terminal amphipathic helix of human Amphiphysin, residues 1-38 (aN) was provided by Dr. Hubert Yin, University of Colorado, Boulder. The fluorescent tag, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD), was attached to the N-terminus of this peptide (NBD-aN).

2.4. Confocal microscopy

A Leica TCS SP5 confocal laser scanning microscope with a 63 × oil immersion objective was used to simultaneously image IgE_{550} and NBD-aN in the cell-substrate contact zone. The confocal pinhole size was set to 95.6 μm (1 airy unit) and images were taken with a scanning speed of 400 Hz (660 ms). Cell samples were maintained at 37 °C using an objective heater.

3. Results and discussion

After the addition of RBL-2H3 mast cells loaded with IgE_{550} to the microscope imaging chamber, cells were allowed to settle under gravity onto a 25 mol% DNP-Cap PE bilayer for 30 min. Fig. 1A and B show typical spatial distribution of IgE_{550} receptor complexes (red) forming a large central receptor patch. This spatial pattern is referred to as mast cell synapse [18]. Fig. 1A shows a receptor patch that is in process of spreading. Fig. 1B shows a typical mast cell synapse with most receptors near the center forming a large patch with an irregular boundary that is surrounded by a receptor cluster depletion zone [18,19]. It is typical that holes in the receptor pattern persist.

Previous experiments showed that N-terminal amphipathic helices of Amphiphysin bind avidly to anionic liposomes [9,10] and can constrict them into tubules about 50 nm in diameter at a concentration of 10 μM (peptide:lipid or P:L ratio in the range of ~1:10) [9]. N-BAR domains are known to induce membrane deformation at a lower P:L ratio than their cognate amphipathic helices [25]. Although nearly all anionic lipids in eukaryotic cells face the cytoplasm [26], some cell functions require anionic lipids to flip. For example in mast cells, anionic phosphatidylserine has been shown to flip from the plasma membrane inner to outer monolayer after antigenic stimulation [27,28]. Therefore, to test how a curvature-remodeling peptide affects FcεRI receptor distribution in the mast cell synapse, NBD-aN was added after the mast cell synapse was formed. Cells loaded with IgE_{550} settled under gravity on a 25 mol% DNP-Cap PE bilayer for 30 min. After 30 min of cell settling, 20 μM of NBD-aN was added to the adherent cells and allowed to incubate for 5 min before the unbound peptide was removed by sample washes with Hanks' buffered saline solution. Fig. 1C and D show that the addition of NBD-aN (green) appears to interfere with the formation of the mast cell synapse. Specifically, NBD-aN seems to prevent spreading of IgE_{550} (red) into a large central receptor patch as shown in Fig. 1B. When NBD-aN is allowed to incubate for more than 10 min, NBD-aN tubules appear to emanate from the cell membrane as shown in Fig. 1E and F. Due to the limited spatial resolution of confocal microscopy, further investigations are needed to confirm tubule formation. The small inset in Fig. 1E depicts an optical slice through the same cell showing clear binding of NBD-aN to the cell membrane. Finally, it is noted that the effect of aN on FcεRI receptor distribution in the absence of an antigen and for lower peptide concentrations remain to be investigated.

Possible mechanisms for the observed receptor reorganization require further investigation. For example, a relatively high amphipathic helix concentration could delaminate the supported lipid bilayer due to helix insertion causing redistribution of FcεRI receptor complexes. However, it has been shown that amphipathic helices partition weakly to zwitterionic bilayers at peptide concentrations ranging from 5 to 50 μm [10]. Since the supported lipid bilayer used here is composed of zwitterionic lipids (POPC and PE), delamination is likely not the main mechanism. It is also

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