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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ABSTRACT

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
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 caused 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 an antigen [23]. For 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 (IgE550) 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 vacuum for 1 h to remove traces of oxygen. The lipid bilayer used here is composed of zwitterionic lipids (POPC and PE), delamination is likely not the main mechanism. It is also required further investigation. For example, a relatively high amphipathic helix concentration could delaminate the supported 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 [8,10] 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 IgE550 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 IgE550 (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

2.4. Confocal microscopy

A Leica TCS SP5 confocal laser scanning microscope with a 63 × oil immersion objective was used to simultaneously image IgE550 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 IgE550 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 IgE550 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.
possible that the formation of amphipathic helix-induced tubules will force FcεRI receptor complexes to redistribute because the signaling complexes cannot pack inside thin tubules. An experimentally determined FcεRI receptor signaling complex diameter is 7 nm [29], which is smaller than ~50 nm diameter tubules formed from charged liposomes by amphipathic helices [9]. Further studies are needed to investigate this possibility. Finally, the observed receptor reorganization may be due to changes in surface energy of the cell membrane after the addition of curvature-generating aN. In this case, FcεRI receptor complexes preferentially accumulate at membrane locations with curvature favorable to their shape [4,30]. In the last decade, there have been many studies investigating the mechanism of curvature-based membrane protein sorting. For a recent review of this topic, refer to Refs. [5,31,32].

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References

[1] K. Takahashi, U.I. Heine, J.L. Junker, N.H. Colburn, J.M. Rice, Role of cytoskeleton changes and expression of the H-ras oncogene during promotion of neoplastic transformation in mouse epidermal JB6 cells, Cancer Res. 46 (1986) 5923–5932.

[2] P. Behnam-Motlagh, K. Granqvist, R. Henriksen, K.G. Engström, Response in shape and size of individual p31 cancer cells to cisplatin and ouabain: a computerized image analysis of cell halo characteristics during continuous perfusion, Cytometry 40 (2000) 198–208, http://dx.doi.org/10.1002/1097-0223(20000701)40:3<198::AID-CYTO4>3.0.CO;2-1.

[3] H.T. McMahon, J.L. Gallop, Membrane curvature and mechanisms of dynamic cell membrane remodelling, Nature 438 (2005) 590–596, http://dx.doi.org/10.1038/nature04396.

[4] T. Baumgart, B.R. Capraro, C. Zhu, S.L. Das, Thermodynamics and mechanics of membrane curvature generation and sensing by proteins and lipids, Annu. Rev. Phys. Chem. 62 (2011) 483–506, http://dx.doi.org/10.1146/annurev.physchem.012809.103450.

[5] A. Callan-Jones, P. Bassereau, Curvature-driven membrane lipid and protein distribution, Curr. Opin. Solid State Mater. Sci. 17 (2013) 143–150, http://dx.doi.org/10.1016/j.cossms.2013.08.004.

[6] P. Wigge, H.T. McMahon, The amphiphysin family of proteins and their role in endocytosis at the synapse, Trends Neurosci. 21 (1998) 339–344, http://dx.doi.org/10.1016/S0166-2236(98)01264-8.

[7] B.J. Peter, H.M. Kent, I.G. Mills, et al., BAR domains as sensors of membrane curvature: the amphiphysin BAR structure, Science 303 (2004) 495–499, http://dx.doi.org/10.1126/science.1092586.

[8] A. Arkhipov, Y. Yin, K. Schulten, Membrane-bending mechanism of amphipathic N-BAR domains, Biophys. J. 97 (2009) 2727–2735, http://dx.doi.org/10.1016/j.bpj.2009.08.051.

[9] C. Low, U. Weininger, H. Lee, et al., Structure and dynamics of helix-0 of the N-BAR domain in lipid micelles and bilayers, Biophys. J. 95 (2008) 4315–4323, http://dx.doi.org/10.1529/biophysj.108.134155.

[10] F. Fernandes, L.M. Loura, F.J. Chichon, J.L. Carrascosa, A. Fedorov, M. Prieto, Role of helix 0 of the N-BAR domain in membrane curvature generation, Biophys. J. 94 (2008) 3065–3073, http://dx.doi.org/10.1529/biophysj.107.113118.

Fig. 1. Confocal microscope images of mast cells in contact with a fluid lipid POPC bilayer with 25 mol% DNP-Cap PE lipid. Shown confocal slices are taken from the cell-substrate contact zone. A and B: cells loaded with fluorescent IgE-receptor complexes (IgE550, red) after settling under gravity for approximately 30 min. Big central receptor patch is the mast cell synapse. C to F: cells loaded with fluorescent IgE-receptor complexes (IgE550, red) and NBD labeled N-terminal amphipathic helix of human Amphiphysin (aN, green). E and F show that aN peptides form tubules that appear to emanate from the cell membrane. Insert in E, shows an optical slice through the cell. Scale bars represents 5 μm.
K. Spendier, K.A. Lidke, D.S. Lidke, J.L. Thomas, Single-particle tracking of
K. Spendier, A. Carroll-Portillo, K.A. Lidke, B.S. Wilson, J.A. Timlin, J.L. Thomas,
F. Quemeneur, J.K. Sigurdsson, M. Renner, P.J. Atzberger, P. Bassereau,
R.M. Weis, K. Balakrishnan, B.A. Smith, H.M. McConnell, Stimulation of
S. Aimon, A. Callan-Jones, A. Berthaud, M. Pinot, G.E. Toombes, P. Bassereau,
A. Carroll-Portillo, K. Spendier, J. Pfeiffer, et al., Formation of a mast cell
P.D. Blood, R.D. Swenson, G.A. Voth, Factors in
M. Simunovic, A. Srivastava, G.A. Voth, Linear aggregation of proteins on the
B. Sorre, A. Callan-Jones, J. Manzi, et al., Nature of curvature coupling of
V.K. Bhatia, K.L. Madsen, P.Y. Bolinger, et al., Amphipathic motifs in BAR
domains are essential for membrane curvature sensing, EMBO J. 28 (2009)
3303–3314, http://dx.doi.org/10.1038/emboj.2009.261.
B. Soare, A. Callan-Jones, J. Manzi, et al., Nature of curvature coupling of
amphipathy with membranes depends on its bound density, Proc. Natl. Acad.
Sci. USA 109 (2012) 173–178, http://dx.doi.org/10.1073/pnas.1103594108.
M. Simunovic, A. Srivastava, G.A. Voth, Linear aggregation of proteins on the
membrane as a prelude to membrane remodelling, Proc. Natl. Acad. Sci. USA
110 (2013) 20396–20401, http://dx.doi.org/10.1073/pnas.1308191110.
P.D. Blood, R.D. Swenson, G.A. Voth, Factors influencing local membrane cur-
vature induction by N-BAR domains as revealed by molecular dynamics
simulations, Biophys. J. 95 (2008) 1866–1876, http://dx.doi.org/10.1529/
biophysj.107.121160.
S. Aimon, A. Callan-Jones, A. Berthaud, M. Pinot, G.E. Toombes, P. Bassereau,
Membrane shape modulates transmembrane protein distribution, Dev. Cell 28
(2014) 212–218, http://dx.doi.org/10.1016/j.devcel.2013.12.012.
F. Quemeneur, J.K. Sigurdsson, M. Renner, P.J. Atzberger, P. Bassereau,
D. Lacoste, Shape matters in protein mobility within membranes, Proc. Natl.
Acad. Sci. USA 111 (2014) 5083–5087, http://dx.doi.org/10.1073/pnas.
1321054111.
R.M. Weiss, K. Balakrishnan, B.A. Smith, H.M. McConnell, Stimulation of fluor-
escence in a small contact region between rat basophil leukemia cells and
planar lipid membrane targets by coherent evanescent radiation, J. Biol. Chem.
257 (1982) 6440–6445.
A. Carroll-Portillo, K. Spendier, J. Pfeiffer, et al., Formation of a mast cell
synapse: Fc epsilon RI membrane dynamics upon binding mobile or immo-
obilized ligands on surfaces, J. Immunol. 184 (2010) 1328–1338, http://dx.doi.
org/10.4049/jimmunol.0903071.
K. Spendier, A. Carroll-Portillo, K.A. Lidke, B.S. Wilson, J.A. Timlin, J.L. Thomas,
Distribution and dynamics of rat basophilic leukemia immunoglobulin E
receptors (FcepsilonRI) on planar ligand-presenting surfaces, Biophys. J. 99
(2010) 388–397, http://dx.doi.org/10.1016/j.bpj.2010.04.029.
K. Spendier, K.A. Lidke, D.S. Lidke, J.L. Thomas, Single-particle tracking of
immunoglobulin E receptors (FcepsilonRI) in micron-sized clusters and
receptor patches, FEBS Lett. 586 (2012) 416–421, http://dx.doi.org/10.1016/j.
febslet.2012.01.013.
C.R. Monks, B.A. Freiberg, H. Kupfer, N. Siaky, A. Kupfer, Three-dimensional
segregation of supramolecular activation clusters in T cells, Nature 395 (1998)
82–86, http://dx.doi.org/10.1038/25764.
H.W. Sohn, F. Tolar, S.K. Pierce, Membrane heterogeneities in the formation of
B cell receptor-lyn kinase microclusters and the immune synapse, J. Cell. Biol.
182 (2008) 367–379, http://dx.doi.org/10.1083/jcb.200802007.
A. Carroll-Portillo, J.L. Cannon, J. Te Riet, et al., Mast cells and dendritic cells
form synapses that facilitate antigen transfer for T cell activation, J. Cell. Biol.
210 (2015) 851–864, http://dx.doi.org/10.1083/jcb.201412074.
J.H. Werner, G.A. Montano, A.L. Garcia, et al., Formation and dynamics of
supported phospholipid membranes on a periodic nanotextured substrate,
Langmuir 25 (2009) 2986–2993, http://dx.doi.org/10.1021/la802249f.
C. Drin, B. Antonny, Amphipathic helices and membrane curvature, FEBS Lett.
584 (2010) 1840–1847, http://dx.doi.org/10.1016/j.febslet.2009.10.022.
P.A. Janney, P.K.J. Kinnunen, Biophysical properties of lipids and dynamic
membranes, Trends Cell. Biol. 16 (2006) 538–546, http://dx.doi.org/10.1016/j.
tcb.2006.08.009.
S. Martin, I. Pombo, P. Poncet, B. David, M. Arock, U. Blank, Immunologic sti-
mulation of mast cells leads to the reversible exposure of phosphatidylserine
in the absence of apoptosis, Int. Arch. Allergy Immunol. 123 (2000) 249–258,
doi:iaa23249.
N.M. Rysavy, L.M.N. Shimoda, A.M. Dixon, et al., Beyond apoptosis: the
mechanism and function of phosphatidylserine asymmetry in the membrane
of activating mast cells, BioArchitecture 4 (2014) 127–137, http://dx.doi.org/
10.1016/j.iaa23249.
B. Baird, Y. Zheng, D. Holowka, Structural mapping of IgE-Fc epsilon.RI, an
immunoreceptor complex, Acc. Chem. Res. 26 (1993) 428–434, http://dx.doi.
org/10.1021/ar00032a006.
V.S. Markin, Lateral organization of membranes and cell shapes, Biophys. J. 36
(1981) 1–19, http://dx.doi.org/10.1016/S0006-3495(81)84713-3.
B. Bozic, S.L. Das, S. Svetina, Sorting of integral membrane proteins mediated
by curvature-dependent protein-lipid bilayer interaction, Soft Matter 11
(2015) 2479–2487, http://dx.doi.org/10.1039/c4sm02289k.
S. Svetina, Curvature-dependent protein-lipid bilayer interaction and cell
mechanosensitivity, Eur. Biophys. J. 44 (2015) 513–519, http://dx.doi.org/
10.1007/s00249-015-1046-5.