Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Melittin-induced cholesterol reorganization in lipid bilayer membranes☆,☆☆

Shuo Qian [a,b], William T. Heller [a,*]

[a] Biology and Soft Matter Division, Oak Ridge National Laboratory, P.O. Box 2008, MS-6473, Oak Ridge, TN 37831, USA
[b] Center for Structural Molecular Biology, Oak Ridge National Laboratory, P.O. Box 2008, MS-6473, Oak Ridge, TN 37831, USA

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 5 June 2015
Accepted 10 June 2015
Available online xxxx

Keywords:
Membrane active peptides
Lipid bilayers
Small-angle neutron scattering
Melittin
Cholesterol

ABSTRACT

The peptide melittin, a 26 amino acid, cationic peptide from honey bee (*Apis mellifera*) venom, disrupts lipid bilayer membranes in a concentration-dependent manner. Rather than interacting with a specific receptor, the peptide interacts directly with the lipid matrix of the membrane in a manner dependent on the lipid composition. Here, a small-angle neutron scattering study of the interaction of melittin with lipid bilayers made of mixtures of dimyristoylphosphatidylcholine (DMPC) and cholesterol (Chol) is presented. Through the use of deuterium-labeled DMPC, changes in the distribution of the lipid and cholesterol in unilamellar vesicles were observed for peptide concentrations below those that cause pores to form. In addition to disrupting the in-plane organization of cholesterol, melittin produces vesicles having inner and outer leaflet compositions that depend on the lipid–Chol molar ratio and on the peptide concentration. The changes seen at high cholesterol and low peptide concentration are similar to those produced by alamethicin (Qian, S. et al., *J. Phys. Chem. B* 2014, *118*, 11200–11208), which points to an underlying physical mechanism driving the redistribution of Chol, but melittin displays an additional effect not seen with alamethicin. A model for how the peptide drives the redistribution of Chol is proposed. The results suggest that redistribution of the lipids in a target cell membrane by membrane active peptides takes places as a prelude to the lysis of the cell.

1. Introduction

Membrane active peptides (MAPs) are relatively short peptides, generally less than 40 amino acids in length, which interact directly with the lipid matrix of cellular membranes, rather than with a specific protein target [1–4]. MAPs display diverse functions, with most of the commonly-studied examples being toxins and components of the immune system [2]. Biophysical studies to determine their mechanism of action have found that while a direct interaction with the lipid bilayer of the cell membrane is a common feature, its specific nature depends on the sequence of the MAP and on the composition of the membrane [1–4].

Melittin is a hemolytic peptide from the venom of the European honey bee (*Apis mellifera*) and is one of the most extensively studied MAPs [5,6]. It is 26 amino acids in length and has the sequence GIGAVLKVLTTGLPALISWIKRKRQQ. While predominantly hydrophobic, it carries a net charge of +6 that is concentrated in the KRKR sequence near the C-terminus (residues 21–24). When associated with lipid bilayers, melittin folds into an α-helix that is broken at residue 12 (glycine), creating a helix–hinge–helix structure [7–9]. The folded peptide is amphipathic along its length, a motif that is shared by other MAPs, such as magainin, alamethicin and the cecropins [1,3,4].

The interaction of melittin with lipid bilayer membranes strongly depends on the membrane composition and the peptide concentration. In fluid phase bilayers, melittin is monomeric when the peptide-to-lipid ratio (P/L) is less than 1/200 [10]. At higher concentrations in phosphatidylcholine bilayers, the resulting state of the system depends on the structural phase of the lipid bilayer. Below P/L = 1/20, fluid phase bilayers remain largely intact, but break into disk-shape micelles if the temperature decreases below the gel phase transition temperature of the lipid [11–13]. At higher P/L, the bilayer breaks into small structures regardless of the state of the lipid [14–16]. This dependence of the behavior of melittin on the structural phase of the lipid raises interesting questions about the role of the order of the hydrocarbon core of the bilayer on the mechanism of action of melittin.

Cholesterol (Chol) exerts a strong effect on the structure and dynamics of lipid bilayers. Chol resides within phospholipid bilayers in a manner that depends on the lipid composition [17–19]. It creates a

☆ *Notes* The authors declare no competing financial interests.

☆☆ Notice: This manuscript has been authored by UT-Battelle, LLC, under Contract No. DE-AC0500OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for the United States Government purposes.

* Corresponding author at: Oak Ridge National Laboratory, PO Box 2008, MS-6473, Oak Ridge, TN 37831, USA.
E-mail address: hellerwt@ornl.gov (W.T. Heller).

http://dx.doi.org/10.1016/j.bbamem.2015.06.012
0005-2736/

more rigid bilayer [20–22], while simultaneously increasing the fluidity of the membrane [23]. Chol has a condensing effect on the structure of the hydrocarbon core of the bilayer [24], driving increased order in the lipid chains to ultimately produce the liquid-ordered (L_O) phase of the lipid [25]. Molecular dynamics simulations indicate that lipid bilayers made of mixtures of dimyristoylphosphatidylcholine (DMPC) and Chol are laterally inhomogeneous [26,27], which was supported by small-angle neutron scattering measurements [28].

The presence of Chol in lipid bilayer membranes alters the behavior of melittin. For example, 30 mol% Chol increases the resistance of dipalmitoyl phosphatidylcholine (DPPC) membranes to disruption into discoidal micelles or large vesicles by melittin [16,29]. NMR studies indicated that the introduction of melittin to DPPC:Chol mixtures having 35 mol% Chol increases disruption of the lipid bilayers into micelles when P/L > 1/100, even though the affinity of melittin for the membrane decreases [30]. Studies of the interaction of melittin with 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and dioleoyl phosphatidylcholine (DOPC) also found that Chol confers a greater resistance to lysis by melittin [30–32]. Later work indicated that the decreased penetration of melittin into the bilayer caused the reduced potency of melittin [31,32].

To better understand how the structure of a Chol-containing lipid bilayer is altered by melittin, small-angle neutron scattering (SANS) was applied to vesicles composed of DMPC:Chol mixtures in the presence of melittin at peptide concentrations below those known to form transmembrane pores. Through the use of deuterium labeling and solvent contrast variation [33], melittin-driven changes in the distribution of Chol were observed. The structure of the lipid bilayers of the DMPC:Chol mixtures studied responds strongly to low concentrations of the peptide. Melittin disrupts the lateral organization of DMPC and Chol and redistributes Chol between the leaflets of the bilayer, much like alamethicin [28], but an additional effect is indicated by the SANS data. Interestingly, the change in the localization of Chol in the vesicles depends on the DMPC:Chol molar ratio in the vesicle, leading to the proposal of an underlying physical mechanism that drives the Chol distribution in response to peptide. Taken together with the results of previous work [28,34], the results demonstrate that low concentrations of MAPs remodel the distribution of lipids in a membrane, suggesting a key step leading up to the eventual lysis of a cell by a MAP.

2. Materials and methods

2.1. Materials

Chain-perdeuterated dimyristoylphosphatidylcholine (d54-DMPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, U.S.A.), D_2O (99.9% atomic D) was purchased from Cambridge Isotope Laboratories (Andover, MA, U.S.A.), while melittin (product M2272; >85% purity) and cholesterol (product C8667; >99% purity) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Materials were not further purified prior to use.

2.2. Vesicle sample preparation

Vesicle solutions for SANS and CD experiments were prepared using previously described procedures [28,34]. Briefly, d54-DMPC, Chol and melittin were co-dissolved in D_2O at a 2% w/w concentration. After vigorous mixing, the suspension was subjected to at least 3 freeze–thaw cycles using a water bath (40 °C) and a freezer (−20 °C). Immediately prior to vesicle extrusion, melittin was added to the lipid suspension and subjected to vortex mixing. A mini-extruder from Avanti Polar Lipids (Alabaster, AL, U.S.A.) fit with a porous polycarbonate membrane having an average pore diameter of 100 nm was used for the vesicle extrusion. Samples were passed through the extruder at least 10 times. The extruder was maintained at 40 °C to ensure that the lipids were in the fluid phase. The process produced vesicles with radii uniformly distributed around 60 nm, as measured by dynamic light scattering (Wyatt Technology Corp., Santa Barbara, CA, U.S.A.). Samples not used immediately were stored at 4 °C for up to 24 h, which did not alter the samples.

d54-DMPC:Chol molar ratios of 8:2 and 6:4 were studied. The P/L employed was the molar ratio of melittin to the d54-DMPC:Chol mixture rather than d54-DMPC. P/Ls of 1/200 and 1/500 were used for the SANS experiments. P/L = 1/200 was used for the vesicle CD measurements. At P/L = 1/500, the CD signal was too weak to be reliable. At 2% w/w, the 8:2 d54-DMPC:Chol solution corresponds to 34 mM lipid. The resulting peptide concentrations were 169 μM (P/L = 1/200) and 68 μM (P/L = 1/500). Similarly, the 6:4 d54-DMPC:Chol concentration corresponds to 38 mM lipid, yielding melittin concentrations of 189 μM (P/L = 1/200) and 76 μM (P/L = 1/500). The SANS samples were measured as-prepared, while the samples for CD measurements were diluted 10-fold prior to measurement to reduce absorption by the sample to improve the signal-to-noise ratio.

2.3. Planar lipid bilayer sample preparation

Samples for oriented circular dichroism (OCD) experiments were prepared as described previously [28,35]. Briefly, appropriate amounts of DMPC and Chol were co-dissolved at the desired molar ratio in a 1:1 (v/v) mixture of chloroform and trifluoroethanol. Melittin dissolved in the same solvent was added to the DMPC:Chol solution at peptide-to-lipid ratios of 1/20 and 1/200. The combined solution was deposited on thin quartz substrates and the solvent was allowed to evaporate. The dry samples were placed in a vacuum for at least 1 h to ensure total removal of the solvent. After being removed from the vacuum, the samples were incubated with H_2O or D_2O-saturated water vapor at ambient temperature for several hours prior to measurement.

2.4. Circular dichroism (CD) and oriented circular dichroism (OCD)

All CD and OCD data were measured using a Jasco J-810 CD spectropolarimeter (Tokyo, Japan). Dilutions from the samples used in SANS experiments were loaded into 1 mm path length cells and were scanned from 190 nm to 260 nm using a wavelength step of 0.5 nm. The sample temperature was maintained at 37 °C. OCD is a powerful tool for

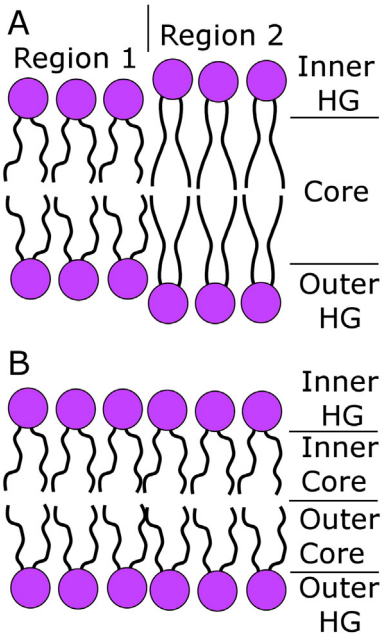

Fig. 1. (A) Schematic of the d54-DMPC:Chol bilayer defining the regions for the two profile model. (B) Schematic of the d54-DMPC:Chol bilayer as a guideline defining the regions of the 4-shell model used for fitting the SANS data when melittin is present.

Please cite this article as: S. Qian, W.T. Heller, Melittin-induced cholesterol reorganization in lipid bilayer membranes, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbamem.2015.06.012