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Peptide-induced bilayer thinning structure of unilamellar vesicles and the related binding behavior as revealed by X-ray scattering

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ABSTRACT

We have studied the bilayer thinning structure of unilamellar vesicles (ULV) of a phospholipid 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (di22:1PC) upon binding of melittin, a water-soluble amphipathic peptide. Successive thinning of the ULV bilayers with increasing peptide concentration was monitored via small-angle X-ray scattering (SAXS). Results suggest that the two leaflets of the ULV of closed bilayers are perturbed and thinned asymmetrically upon free peptide binding, in contrast to the centro-symmetric bilayer thinning of the substrate-oriented multilamellar membranes (MLM) with premixed melittin. Moreover, thinning of the melittin-ULV bilayer associates closely with peptide concentration in solution and saturates at ~4%, compared to the ~8% maximum thinning observed for the correspondingly premixed peptide-MLM bilayers. Linearly scaling the thinning of peptide-ULV bilayers to that of the corresponding peptide-MLM of a calibrated peptide-to-lipid ratio, we have deduced the number of bound peptides on the ULV bilayers as a function of free peptide concentration in solution. The hence derived X-ray-based binding isotherm allows extraction of a low binding constant of melittin to the ULV bilayers, on the basis of surface partition equilibrium and the Gouy–Chapman theory. Moreover, we show that the ULV and MLM bilayers of di22:1PC share a same thinning constant upon binding of a hydrophobic peptide alamethicin; this result supports the linear scaling approach used in the melittin-ULV bilayer thinning for thermodynamic binding parameters of water-soluble peptides.

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1. Introduction

Membrane-active peptides with genetic codes are known to interact directly with cell membranes, rather than via specific protein receptors, in carrying out their biological functions [1–3]. In the past decades, a great variety of peptides have been shown to form trans-membrane pores in cell membranes or mimic membranes of lipid vesicles [4–8]. As membrane pores are believed to be one of the primary causes for cell lysis and/or act as membrane crossing channels for ions/bio-molecules [9], understanding the pore formation process and mechanism is of broad interest. Compared to peptide activity measurements, structural studies of pores in thermodynamic equilibrium and/or formation dynamics can better address the mechanism of peptide–membrane interactions [8], as well as the regulation of ion/molecule transportation in and out of cells, thereby providing hints for drug design, disease therapy, nonviral gene transfer, and drug delivery [8,9].

Previously, membrane thinning structures upon peptide binding have been extensively studied with substrate-oriented multilamellar membranes (MLM) with premixed peptides using X-ray/neutron diffraction [5–8,10,11]. Accumulated evidences indicate that membrane thinning is, in general, a result of membrane deformation upon surface adsorption of peptides; at a critical membrane deformation (or thickness), peptides can insert into membranes and form membrane pores [6–8,12,13]. Applying contrast variation with deuterium substitution in small-angle neutron scattering [14] or Br-labeled lipid membranes in anomalous X-ray diffraction (XRD) [15], Huang et al. first illustrated peptide-induced pore structures and pore lattices in MLM and retrieved successfully the related electron density distribution [14,15]; which advanced significantly the understanding of peptide–membrane interactions.

Living cell membranes, however, comprise a single closed bilayer, and are subject to free peptides' binding in aqueous solution in certain cases. Moreover, pore formation in membranes caused by

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water-soluble amphipathic peptides typically occurs with a kinetic process [8]. Binding behavior of peptides with model membranes of single bilayer such as unilamellar vesicles (ULV) in solution would carry closer implication regarding thermodynamic or kinetic binding aspects of peptide–membrane interactions, compared to the correspondingly substrate-supported, planar peptide-MLM bilayers. Emerged recently are neutron/X-ray scattering methods that allow probing directly free-floating single bilayers of phospholipid unilamellar vesicles (ULV) in aqueous solution, in environments approaching that for real biological systems [10,11,16–19]. For instances, with neutron spin-echo spectroscopy ULV bilayers were shown to be drastically stiffened upon extensive formation of peptide-induced pores [18]. Revealed by small-angle X-ray/neutron scattering (SAXS/SANS), bilayer structures of pure phospholipid ULV bilayers were also shown to be similar (if not identical) to that of the corresponding planar MLM bilayers [10,11,17,19]. On the basis of the observations, bilayer structural changes of several phospholipids upon binding of hydrophobic alamethicin or embedment of cholesterol were revealed via integrated X-ray results obtained respectively from the ULV and MLM bilayers of the same lipids [10,11,16–19], and further complementary by molecular dynamics simulation [10,17].

In most of the ULV-based studies mentioned above, ULV bilayers were prepared with phospholipids premixed with peptides or cholesterol, of a hydrophobic nature. Nevertheless, for water-soluble amphipathic peptides, dynamic equilibrium between the peptides adsorbed to free-floating membranes and the free peptides in solution inevitably leads to a smaller peptide binding ratio onto membranes, χ_b (or P/L) than the ratio prescribed for mixing (termed as P_s/L). This should influence the bilayer thinning behavior of ULV of closed single bilayers upon water-soluble peptide binding in solution, compared to that of the corresponding substrate-supported MLM bilayers with premixed peptides. In the MLM case, kinetics of each individual bilayer may be significantly modulated by steric interactions between the stacked bilayers and further suppressed by the supporting solid substrate. Concerned here are (*i*) how a free-standing single bilayer of ULV responds to the solution binding of water-soluble amphipathic peptides and (*ii*) how binding affinity affects the dynamic equilibrium between bound peptides on the ULV bilayers and free peptides in the solution—hence the ultimate membrane thinning. Such information will not only shed lights on the underlying mechanism of water soluble peptide–membrane interactions, but also help to extract related thermodynamic parameters, such as binding constant and binding free energy [20].

In this study, using SAXS and XRD we address the two issues mentioned above by probing the bilayer thinning structures of phospholipid ULV upon solution binding of melittin, a water-soluble amphipathic peptide extracted from bee venom [8,13]. We compare the peptide-ULV bilayer thinning structures to that of the corresponding planar MLM with pre-mixed peptides, and show the similarity and dissimilarity of the ULV and MLM bilayer thinning behaviors upon binding of the same peptide. We further propose a linear scaling for the ULV bilayer thinning observed to that of MLM, to obtain the number of bound peptides. The hence established X-ray-based binding isotherm allows extraction of thermodynamic binding parameters of peptide–membrane interactions prior to membrane perforation. For an indirect support of the scaling approach used in the ULV and MLM bilayer thinning with the water-soluble melittin's binding, a hydrophobic peptide—alamethicin that can fully reside in either ULV bilayers in aqueous solution or MLM bilayers, is adopted to interact with the same model membrane [10,21]. From which result, we show that the ULV and MLM bilayers of a phospholipid 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (di22:1PC) share a same bilayer thinning constant when subject to binding of the same peptide. The phospholipid di22:1PC of a long chain length was chosen for a larger thinning range. Previous studies already showed that melittin-induced membrane pores are of the toroidal type [14], differing from the barrel-stave type induced by alamethicin [22]; both peptides are known to induce transmembrane pores in lipid bilayers [12,13].

2. Materials and methods

2.1. Materials and sample preparation

Phospholipids 1,2-dierucoyl-*sn*-glycero-3-phosphocholine (di22:1PC) were purchased from Avanti Polar Lipids (Alabaster, AL). The alamethicin of purity 98% HPLC (product no. A-4665) and melittin of purity 93% HPLC (product no. M-2272) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All materials were used as received without further purification. The lipids were dissolved in chloroform, dried under nitrogen flow for thin films, and then kept in vacuum for about 1 h. The lipid films were further hydrated in deionized water (pH ~ 6.8), followed by a vortex treatment for 15 min; the solutions were subjected to five rapid freeze–thaw cycles in the temperature range of −90 to 40 °C, followed by vortex-mixing for 40 min, then extruded through polycarbonate filters (with a pore size of 100 nm) at 40 °C for unilamellar vesicles. The mean diameter of the ULV determined by dynamic light scattering (Malvern Zetasizer Nano S90) was 60 nm. The aqueous solutions of di22:1PC ULV, 30–60 mM, were respectively mixed with a melittin solution for mixtures of various peptide-to-lipid ratios (P_s/L), ~10–30 min before SAXS measurements. These sample solutions were examined with preliminary SAXS measurements, ensuring no observable interference humps/peaks from residual multilamellar vesicles. For hydrophobic alamethicin, the peptide and di22:1PC were co-dissolved in solution of 1:1 v/v methanol and chloroform with several different P_s/L values (note that for hydrophobic peptides that can be fully embedded into the lipid bilayers, the mixture P_s/L is equivalent to the lipid membrane P/L). Aqueous solutions of the alamethicin-bound ULV of 30 mM were then prepared from the peptide–lipid mixtures, following the procedures used for pure di22:1PC ULV. Thin films of oriented peptide-MLM bilayers were cast onto silicon wafers from the organic solutions of different P/L; the sample films were vacuumed dried, then hydrated via water vapor.

2.2. SAXS and XRD

SAXS for the peptide-ULV solutions were conducted at the 23A SWAXS endstation of the National Synchrotron Radiation Research Center (NSRRC) [23]. With a beam of 15.0 keV (wavelength $\lambda = 0.8267$ Å) and a sample-to-detector distance of 1830 mm, SAXS data were collected using a pixel detector Pilatus-1MF of an active area of 169×179 mm^2 and a detector pixel resolution of 172 μm. This single instrument configuration could cover a reasonable q-range up to 0.5 Å^{-1} with excellent q-resolution; the scattering wavevector $q = 4\pi\lambda^{-1}\sin\theta$, defined by the scattering angle θ and λ, was calibrated with a standard sample of silver behenate. To minimize radiation damages, the 5-mm sample solution cell with thin (30 μm) quartz windows (5 mm in diameter) was gently rocked within an area of 1.5×1.5 mm^2 to avoid prolonged spot exposure (ca. 0.5 mm in beam diameter) of the sample solution at 30 °C. Each SAXS profile presented was averaged from ten SAXS data scans (each for 30 s); these ten successive scans could overlap well, suggesting negligible radiation damage effects and no structural transitions involved—hence a thermodynamically stable system. SAXS data were subtracted with water scattering measured under an identical environment as that used for the ULV sample solutions (with deionized water); the data were then corrected for incoming flux, sample thickness and electronic noise of the detector, as detailed in a previous report [23].

XRD for the peptide-bound MLM films on silicon wafers was conducted using either the 13A beamline of NSRRC with a 12 keV beam or an in-house X-ray source of 8.05 keV. The samples were respectively sealed in a humidity-controlled chamber for an environment of higher than 98% relative humidity during measurements at 30 °C [12].

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