SUPPORTING INFORMATION

for

Lipid Headgroups Modulate Membrane Insertion of pHLIP peptide

Alexander Kyrychenko,[†] Victor Vasquez-Montes,[†] Martin B. Ulmschneider,[‡]
and
Alexey S. Ladokhin[†]

[†]Department of Biochemistry and Molecular Biology, The University of Kansas Medical Center, Kansas City, KS 66160-7421, U.S.A.

[‡]Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, MD 21218-2608, U.S.A.

MATERIALS AND METHODS

Lipids.

All of the following lipids were purchased from Avanti Polar Lipids (Alabaster, AL): 1-palmitoyl-2-oleoyl-sn-glycero-3-phospholcholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol (POPG), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho ethanolamine (POPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS), 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (lyso-PC), phosphatidic acid (PA), cardiolipin (CL), cholesterol (Chol).

Sample preparation.

Large Unilamellar Vesicles (LUV) were prepared by extrusion as described in (1, 2). Briefly, appropriate volumes of lipid stocks in chloroform were properly mixed and dried under a stream of nitrogen before drying overnight under high vacuum. Dried lipid films were re-suspended and vortexed in 10 mM sodium phosphate buffer (pH 8) to a final concentration of 20 mM of lipid. LUV were prepared by repeated extrusion through a Mini-Extruder from Avanti Polar Lipids (Alabaster, AL) using Nuclepore polycarbonate membranes with 0.1 μm pore size. pHLIP peptide was solubilized in 10 mM phosphate buffer pH 8 to create a stock solution not exceeding 8 μM peptide concentration. For the measurements, the peptide was diluted to 1-2 μM and subsequently mixed with appropriate volume of LUV stock to achieve 1 mM lipid concentration. Membrane insertion was initiated by manual injection of the appropriate aliquots of the 2.5 M acetic buffer, pH 3.2. Samples were incubated for 20 minutes prior to taking measurements.

CD and fluorescence measurements.

CD measurements were performed using an upgraded Jasco-720 spectropolarimeter (Japan Spectroscopic Company, Tokyo). Normally, 20-40 scans were recorded using a 1-mm optical path cuvette. All CD spectra were corrected for background. Fluorescence was measured using an SPEX Fluorolog FL3-22 steady-state fluorescence spectrometer (Jobin Yvon, Edison, NJ) equipped with double-grating excitation and emission monochromators. The measurements were made in a 2×10mm cuvette oriented perpendicular to the excitation beam and maintained at 25°C using a Peltier device from Quantum Northwest (Spokane, WA). Excitation wavelength was 282 nm and the slits were 4 nm. The appropriate background spectra were subtracted in all cases. Spectral analysis was carried out using Origin 8.5 (OriginLab, MA).

Data analysis

pH-dependent changes in spectral position of fluorescence maximum, λ, fitted with the following equation:

$$\lambda = \frac{\lambda_N + \lambda_L \cdot 10^{n \cdot (pK_a - pH)}}{1 + 10^{n \cdot (pK_a - pH)}} \qquad \text{(Eq. S1)}$$

where λ_N and λ_L are the limiting values of the fluorescence maximum at neutral and low pH, respectively; pK_a is an apparent constant and n is a cooperativity coefficient.

Changes in free energy were calculated using a rearrangement of the classical Gibbs free energy formula:

$$\Delta G = 2.3RT(pK_a) \qquad \text{(Eq. S2)}$$

where R is the gas constant and T is the absolute temperature.

Membrane electric potential at bilayer interface, Ψ_o, was calculated using the Gouy-Chapman model(3):

$$\frac{\sigma(8N\varepsilon_o\varepsilon_r\kappa_B T)^{-1/2}}{\sqrt{C}} = \sinh\left(\frac{ze\Psi_o}{2\kappa_B T}\right) \qquad \text{(Eq. S3)}$$

$$\frac{\sigma}{\sqrt{8N\varepsilon_o\varepsilon_r\kappa_B TC}} = \sinh\left(-19.48\Psi_o\right)$$

$$\frac{\sigma}{\sqrt{8N\varepsilon_o\varepsilon_r\kappa_B TC}} = \frac{e^{-19.48\ V^{-1}(\Psi_o)} - e^{19.48\ V^{-1}(\Psi_o)}}{2}$$

where σ is the surface charge density of charges of the membrane created by anionic lipids, N is the Avogadro constant, ε_o is the permittivity in vacuum, ε_r is the dielectric constant of water (78.3), k_B is the Boltzman constant, C is the ion concentration of valence z, and e is the elementary charge. Solving this equation for the buffer system used (10 mM sodium phosphate) and assuming constant lipid charge throughout the titration, results in a surface electrostatic

potential of -73 mV, -96 mV and -124 mV for 10, 25 or 75% POPG in POPC matrix, respectively (Fig. 3).

The effect of the electric potential on protonation at the interface was described as suggested by Fernandez and Fromherz (4):

$$pK_a = pK^{\text{int}} - \frac{F_o \Psi_o}{2.3RT} \qquad \text{(Eq. S4)}$$

where pK_a is the apparent pK (experimental) and pK^{int} is the intrinsic pK, F_o is the Faraday constant, T is the absolute temperature, R is the gas constant, and Ψ_o is the membrane surface electrostatic potential calculated from Eq. S3.

Combining Eqs. S2 and S4 results in the following linear dependence of free energy vs surface potential, which was used to fit the data in Fig. 3:

$$\Delta G = 1.363 pK^{\text{int}} - 0.023 \tfrac{kcal}{mol \cdot mV} \Psi_o = \Delta G(0) - 0.023 \tfrac{kcal}{mol \cdot mV} \Psi_o \qquad \text{(Eq. S5)}$$

where $\Delta G(0)$ is the only free fitting parameter.

SUPPLEMENTAL RESULTS

Numerous studies demonstrate that pHLIP can interact with phosphatidylcholine bilayers in which it has been shown to be either associated interfacially at neutral pH (state II, according to designation of Engelman and co-workers) or inserted across the bilayer at low pH (state III) (5-7). These states are characterized by blue shifted tryptophan fluorescence and increased helical structure, which distinguishes them from unfolded state formed by pHLIP in solution at neutral pH (state I). Our measurements with POPC LUV accurately reproduce these features (Fig. 1). However, the presence of the state II was not observed in any of the LUV compositions tested, as indicated by the lack of changes in fluorescence and ellipticity between states I and II (Fig. 1, Table ST1). LUV containing LysoPC are the only other vesicles (in addition to pure POPC) that induce some spectral shift in pHLIP at neutral pH (Table ST1), however, it can't be excluded that in this case the peptide interacts with the lyso-lipid outside of the bilayer.

The obtained pK_a values were converted to free energy using Eq. S2. In the case of POPG vesicles the ΔG was plotted against the surface electrostatic potential (Ψ_o); which was obtained

using the Gouy-Chapman model (Eq. S3). These calculations were performed under the assumption that the protonation state of the lipids was stable. This assumption, however, does not hold for anionic lipids other than POPG (8-10), for which this analysis was not performed.

Table S1. Summary of pH-dependent interactions of pHLIP with LUV of various lipid compositions. Presence of pHLIP state II and III were determined by comparing shifts in tryptophan fluorescence maxima in the presence of vesicles at neutral (λ_N) and acidic pH (λ_L) with respect to the fluorescence maxima in state I (358nm), pHLIP in solution at neutral pH (Fig. 1). The four parameters characterizing pH-dependent insertion of pHLIP, namely λ_N, λ_N, pK_a and n were obtained by nonlinear least-square fit with Eq. S1 of pH titration of intrinsic fluorescence spectral data (e.g., Fig. 2). ΔG was calculated from the observed pK_a values using Eq. S2.

Lipids mol:mol	*State II*	λ_N *(nm)*	*State III*	λ_L *(nm)*	pK_a *± 0.1*	*n* *±0.3*	$\Delta G \pm 0.2$ *(kcal/mol)*
POPC	+	353	+	343	6.1	1.2	8.3
POPC:POPE 9:1	-	357	+	343	5.7	1.6	7.8
POPC:POPE 1:1	-	357	+	341	6.2	1.3	8.5
POPC:Chol 4:1	-	358	+	343	6.1	1.6	8.3
POPC:lysoPC 4:1	+/-	355	+	343	6.0	1.2	8.2
POPC:POPG 9:1	-	358	+	342	5.7	4.3	7.8
POPC:POPG 3:1	-	358	+	341	6.0	3.0	8.2
POPC:POPG 1:3	-	358	+	341	6.3	2.5	8.5
POPC:CL 6:1	-	358	+	341	6.1	2.6	8.3
POPC:CL 2:3	-	358	+	341	6.2	2.0	8.5
POPC:POPS 9:1	-	358	+	342	6.0	4.4	8.2
POPC:POPS 3:1	-	358	+	342	6.1	2.7	8.3
POPC:PA 9:1	-	358	+	341	5.8	2.5	7.9
POPC:PA 1:3	-	358	+	341	5.9	3.0	8.0

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