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Membrane binding mode of intrinsically disordered cytoplasmic domains of T cell receptor signaling subunits depends on lipid composition

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Abstract

Intrinsically disordered cytoplasmic domains of T cell receptor (TCR) signaling subunits including ζ_{cyt} and CD3 ϵ_{cyt} all contain one or more copies of an immunoreceptor tyrosine-based activation motif (ITAM), tyrosine residues of which are phosphorylated upon receptor triggering. Membrane binding-induced helical folding of ζ_{cyt} and CD3 ϵ_{cyt} ITAMs is thought to control TCR activation. However, the question whether or not lipid binding of ζ_{cyt} and CD3 ϵ_{cyt} is necessarily accompanied by a folding transition of ITAMs remains open. In this study, we investigate whether the membrane binding mechanisms of ζ_{cyt} and CD3 ϵ_{cyt} depend on the membrane model used. Circular dichroic and fluorescence data indicate that binding of ζ_{cyt} and CD3 ϵ_{cyt} to detergent micelles and unstable vesicles is accompanied by a disorder-to-order transition, whereas upon binding to stable vesicles these proteins remain unfolded. Using electron microscopy and dynamic light scattering, we show that upon protein binding, unstable vesicles fuse and rupture. In contrast, stable vesicles remain intact under these conditions. This suggests different membrane binding modes for ζ_{cyt} and CD3 ϵ_{cyt} depending on the bilayer stability: 1) coupled binding and folding, and 2) binding without folding. These findings explain the long-standing puzzle in the literature and highlight the importance of the choice of an appropriate membrane model for protein-lipid interactions studies.

Keywords

Intrinsically disordered protein; T cell receptor; membrane binding; conformational change; membrane model

Intrinsically disordered proteins (IDPs) are proteins that lack a well-defined three-dimensional structure under physiological conditions [1]. In this context, cytoplasmic regions of signaling subunits of immune receptors, including those of ζ and CD3 ϵ signaling subunits (ζ_{cyt} and CD3 ϵ_{cyt} , respectively) of T cell receptor (TCR), represent a novel class of IDPs [2–5]. These regions all have one or more copies of an immunoreceptor tyrosine-based activation motif (ITAM) [6], tyrosine residues of which are phosphorylated upon receptor engagement in an early and obligatory event in the signaling cascade. IDPs are thought to undergo coupled binding and folding upon interaction with their partners [7]. In contrast, random coil ζ_{cyt} remains unfolded upon homodimerization [3,4,8] or interaction with the well-structured core domain of simian immunodeficiency virus Nef [9], as shown by circular dichroic (CD) and nuclear magnetic resonance (NMR) spectroscopy. Perhaps even more intriguing is the fact that no chemical shift changes and significant changes in peak intensities are observed in the ^1H - ^{15}N heteronuclear single quantum coherence spectra of ^{15}N -labeled ζ_{cyt} in ζ_{cyt} dimer

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[8] or ζ_{cyt} -Nef complex [9], thus highlighting unusual biophysical features of this and, possibly, other ITAM-containing proteins. Considering a crucial role of ζ_{cyt} and CD3 ϵ_{cyt} in TCR signaling and their close proximity to the cell membrane, the question whether or not lipid binding of these IDPs promotes folding of ζ_{cyt} and CD3 ϵ_{cyt} ITAMs and thus leads to inaccessibility of the ITAM tyrosines for phosphorylation is of fundamental importance. However, little is known about lipid-binding activity of the ITAM-containing cytoplasmic domains and the existing data are contradictory [2, 4, 10].

In 2000 [2], it has been shown that α -helical folding transition of ζ_{cyt} upon binding to acidic phospholipids prevents ITAM phosphorylation. The authors concluded that this folding transition can represent a conformational switch to regulate TCR triggering [2]. Later, the other group of investigators extended these findings to CD3 ϵ_{cyt} and showed that binding of this protein to acidic phospholipids is accompanied by folding of ITAM, leading to inaccessibility of the ITAM tyrosines for phosphorylation *in vitro* [10]. This led the authors [10, 11] to the conclusion that the conformational model of TCR activation previously suggested for ζ_{cyt} [2] can be extended to CD3 ϵ_{cyt} . In contrast, we have previously shown that binding of ζ_{cyt} and CD3 ϵ_{cyt} as well as ITAM-containing cytoplasmic domain of Fc receptor, FcR γ_{cyt} , to acidic phospholipids is not accompanied by a disorder-to-order structural transition [4]. This questions the possibility of ITAM folding upon membrane binding *in vivo* and challenges the suggested model of TCR activation [2, 10, 11].

Lipid bilayers are self-assembled structures, the mechanical properties of which are derived from noncovalent forces such as the hydrophobic effect, steric forces, and electrostatic interactions. In this context, the electrostatic force is of special interest because biological membranes are rich in anionic lipids [12] and are therefore charged in aqueous solution. Importantly, electrostatic interactions play the critical role in membrane stability [13]. Thus, considering that net charges of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} are + 5, + 11, and + 3, respectively, binding of these proteins to acidic phospholipids can potentially destabilize and disrupt lipid bilayers.

In this study, using CD and fluorescence spectroscopy, we demonstrated that α -helical folding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} upon binding to acidic lipids depends on the membrane model structures used (lipid-mimetic detergent micelles vs. lipid vesicles) and the lipid composition of vesicles. For ζ_{cyt} , we used synthetic peptides and a lipid-binding assay employing sucrose-loaded large unilamellar vesicles (LUVs) of palmitoyl-oleoylphosphatidylglycerol (POPG) [14] to map the lipid-binding region(s) of ζ_{cyt} . The assay revealed that not ITAMs but clusters of basic residues outside ITAMs are involved in protein binding to stable acidic phospholipid vesicles. As shown by electron microscopy (EM) and dynamic light scattering (DLS), binding of ζ_{cyt} to small unilamellar vesicles (SUVs) and LUVs of dimyristoylphosphatidylglycerol (DMPG) but not POPG promotes vesicle fusion and rupture, highlighting the importance of ensuring the integrity of model membranes upon protein binding.

Materials and methods

Reagents and proteins

Peptides A-G (Table 1) were from Sigma. Lysomyristoylphosphatidylglycerol (LMPG), DMPG, and POPG were from Avanti Polar Lipids. Cytoplasmic domains of CD3 ϵ , ζ , and FcR γ were expressed and purified as described previously [4].

Vesicle Binding Assay

Sucrose-loaded LUVs of POPG were prepared and the membrane binding assay was performed as described [4, 14].

Vesicle preparation

LUVs of DMPG and POPG were made after five freeze-thaw cycles of the hydrated lipids by extruding multilamellar vesicles 10 times through a stack of two polycarbonate filters (100 nm pore diameter) in an Avanti Mini-Extruder (Avanti Polar Lipids). Filters with 30 nm pore diameter were used to prepare extruded SUVs of DMPG and POPG. Alternatively, SUVs were prepared by sonication of the hydrated lipids to clarity in a high intensity bath sonicator (Laboratory Supplies).

CD and fluorescence spectroscopy

Far-UV CD spectra were recorded on an Aviv 202 spectropolarimeter (AVIV Instruments) as described previously [4]. The intrinsic tyrosine fluorescence spectra were taken at 25 °C using a Spex Fluoromax-2 spectrofluorimeter as reported [4].

Dynamic light scattering

Scattering data were collected at 20°C with a DynaPro-MS800 instrument (Protein Solutions) and hydrodynamic radius values were calculated as described previously [4].

Electron microscopy

Samples of 3 mM extruded LUVs of POPG and DMPG as well as sonicated SUVs of DMPG alone or in the presence of 10 μ M ζ_{cyt} were prepared in 20 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), 0.1 M NaCl, pH 7.0. Negatively stained grids were prepared by applying 8- μ l drops to carbon-coated Formvar 300-mesh gold grids, blotting away excess sample after 1 min, then soaking the grid on 6 successive drops of 1% (w/v) uranyl acetate, blotting, and allowing to dry. Micrographs were taken at an initial magnification of 28,500 with a Philips CM10 transmission electron microscope (Philips) operating at 80 kV.

Results

Mapping of ζ_{cyt} domains involved in lipid binding

To map the lipid-binding ζ_{cyt} sites, we used a sucrose-loaded vesicle-binding assay [14] and synthetic ζ_{cyt} peptides of varying length that cover the entire protein sequence (Fig. 1). The binding data clearly show that the peptides A, C, and E that correspond to the regions outside ITAMs and contain clusters of basic amino acids bind to POPG LUVs while the ITAM peptides (B, D, and F) – do not (Table 1).

These findings experimentally prove our early hypothesis that binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} to acidic phospholipid LUVs is driven by the clusters of basic amino acid residues rather than the overall net charge [4]. In addition, we specifically show that ITAMs do not contribute to membrane binding by ζ_{cyt} . As recently reported [10], CD3 ϵ_{cyt} ITAM is not involved in binding to DMPG LUVs. Thus, taken together, these findings suggest that binding of ITAM-containing ζ_{cyt} , CD3 ϵ_{cyt} , and highly possibly, FcR γ_{cyt} , to acidic phospholipid bilayers involves the protein regions outside ITAMs. This raises an important question: if ITAMs do not contribute substantially in binding, then what determines lipid binding-mediated folding of these functionally important domains and therefore accessibility (or inaccessibility) of the ITAM tyrosines for phosphorylation? To answer this question, we further investigated the induction of secondary structure upon binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} to acidic micelles and vesicles of different size and composition.

Structure of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} in the presence of micelles and vesicles

Considering current discrepancies in the literature on whether or not lipid binding of intrinsically disordered ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} is accompanied by a disorder-to-order

transition [2, 4, 10], we hypothesized that membrane binding mode of these proteins can depend on the membrane model used. To test this hypothesis, we examined binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} to micelles and different vesicles: LMPG micelles, extruded or sonicated DMPG and POPG SUVs as well as LUVs of DMPG and POPG. Among vesicles, POPG LUVs likely represent the best model to mimic the cell membrane because of higher vesicle stability (POPG vs. DMPG) and lower degree of membrane curvature (LUVs vs SUVs).

The CD data clearly show that ζ_{cyt} remains unfolded upon binding to POPG SUVs obtained by sonication (Fig. 2a) or extruding (data not shown) and POPG LUVs (Fig. S1a). Similarly, CD studies of CD3 ϵ_{cyt} and FcR γ_{cyt} do not reveal any detectable secondary structure induction upon binding to POPG SUVs (Figs. S2a and S3a) and LUVs (data not shown). In contrast, the CD spectra of ζ_{cyt} taken in the presence of LMPG micelles (Fig. 2a), DMPG SUVs obtained by sonication (Fig. 2a) and extruding (data not shown) as well as DMPG LUVs (Fig. S1a) are typical of proteins with a high helical content. Similar induction of helical conformation is observed for CD3 ϵ_{cyt} and FcR γ_{cyt} in the presence of DMPG SUVs (Figs. S2a and S3a) and LUVs (data not shown). Thus, in the lipid systems studied, induction of helical conformation of intrinsically disordered ζ_{cyt} , CD3 ϵ_{cyt} and FcR γ_{cyt} upon lipid binding depends on lipid structure (micelles vs. vesicles) and composition (DMPG vs. POPG) but not vesicle size and preparation technique. In addition, for each protein studied, the percentage of helicity induced by LMPG micelles is similar to that by DMPG vesicles, providing indirect evidence for similar mode of protein-lipid interactions in these systems.

Interestingly, in 1998 [15], using CD and infrared spectroscopy, the ζ_{cyt} ITAM peptides have been reported to adopt an α -helical conformation in the presence of trifluoroethanol (TFE). In this study, the ITAM1 peptide contained the smallest percentage of α -helix in TFE, while the ITAM2 and ITAM3 peptides were predominantly helical. Later, similar helical conformational changes of ζ_{cyt} ITAMs were observed in NMR studies of full-length ζ_{cyt} in the presence of LMPG micelles [16], showing that micelles and TFE induce similar helical folding of ITAMs but not other domains. Results of our titration studies (not shown) are similar to previously reported data [16] and indicate that the percentage of helicity reaches a maximal value of ~35–40% at 1:50 ζ_{cyt} /LMPG molar ratio. Considering an LMPG micelle aggregation number of approximately 55 detergent molecules, this protein/detergent ratio corresponds to one molecule per micelle. In summary, considering also the results of our mapping studies (Table 1 and Fig. 1), these findings suggest that in contrast to lipid binding that is mediated by clusters of basic amino acids outside ζ_{cyt} ITAMs, helical folding occurs within ITAMs and can be induced by physiologically irrelevant agents such as TFE. Similarly, it has been reported recently for CD3 ϵ_{cyt} that basic residues outside ITAM contribute to binding of this protein to DMPG LUVs whereas helical folding is induced within ITAM [10].

Our fluorescence studies demonstrate that changes in intrinsic tyrosine fluorescence accompany binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} to all membrane models studied: LMPG micelles, DMPG and POPG SUVs and LUVs (Fig. 2b, and Figs. S1b, S2b, and S3b). However, quantitative analysis reveals that fluorescence changes are substantially larger in the presence of LMPG micelles and DMPG vesicles as compare to POPG vesicles (independent on vesicle size and method of preparation). A reasonable molecular explanation for this phenomenon is that ITAMs of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} all contain two tyrosines [4] that upon helical folding of ITAMs, can associate with LMPG and DMPG molecules, resulting in a substantial increase in tyrosine fluorescence. In contrast, binding to POPG that is mediated by the regions outside ITAMs and is not accompanied by a disorder-to-order transition can affect the fluorescence intensity of only non-ITAM tyrosines (1 in ζ_{cyt} , 1 in FcR γ_{cyt} , and none in CD3 ϵ_{cyt}), thus changing an overall tyrosine fluorescence in much less extent. This is well supported by the data obtained (Fig. 2b, and Figs. S1b, S2b, and S3b). In addition, as with the CD data, for each protein studied, the amplitude of the fluorescence changes induced by LMPG micelles is similar

to that promoted by DMPG vesicles, suggesting further evidence of similar mode of protein-lipid interactions in these systems.

Thus, the CD and fluorescence results suggest the existence of two different membrane binding modes of intrinsically disordered ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} , depending on the cell membrane model: binding followed by helical folding of the ITAMs (LMPG micelles and DMPG vesicles) and binding without a disorder-to-order transition (POPG vesicles). Importantly, in both modes, initial binding of these proteins to acidic phospholipids is driven by electrostatic interactions between polar heads and clusters of positively charged amino acids outside ITAMs. In contrast, formation of helices within ITAMs in the presence of LMPG micelles and DMPG vesicles is likely mediated by hydrophobic interactions of these domains with detergent and lipid tails. The observed difference between DMPG and POPG vesicles (independent on vesicle size and preparation procedure) in their ability to induce helical structure formation led us to suggest that binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} to phospholipid vesicles may or may not affect the bilayer integrity. To test this hypothesis, we used DLS and EM to investigate lipid-binding activity of ζ_{cyt} as related to vesicle stability.

Lipid bilayer stability in the presence of ζ_{cyt}

Cationic peptides are well known to induce aggregation of negatively charged lipid vesicles [17]. Thus, it is important to assess acidic phospholipid vesicle stability upon binding to positively charged ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} . DLS is a rapid and nondestructive method to measure vesicle hydrodynamic radius (R_H) and size distribution. Our previous DLS studies demonstrated that binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} does not disturb the lipid bilayer structure and integrity of the sucrose-loaded POPG LUVs used [4]. Here, we describe the results of DLS measurements of phospholipid vesicles of different size and composition in the presence or absence of ζ_{cyt} . Importantly, in DLS studies, particle size distributions can be displayed as the percent mass distributions that show how much mass of that particular peak would explain the correlation function or as the percent scattering intensity distributions that reflect the actual contribution of signal to the measurement. Graphically, mass- and intensity-weighted size distributions can be significantly different due to the exponentially stronger scattering from large particles. Thus, conversion of the percent scattering distributions into percent mass distributions allows to correct for the dominance of larger aggregates.

The DLS data do not reveal any substantial changes in the mass- and intensity-weighted size distributions of POPG LUVs and SUVs upon adding ζ_{cyt} , suggesting that protein binding does not disturb the lipid bilayer structure in these particles and does not cause vesicle fusion (Figs. 2c and 2d). For POPG SUVs, similar results were obtained by using the vesicles prepared by sonication (Fig. 2d) or extruding (Fig. S1c), demonstrating the lack of any significant dependence on the method of vesicle preparation. In contrast, binding of ζ_{cyt} to DMPG LUVs and SUVs leads to vesicle fusion and formation of large vesicle aggregates (Figs. 2c and 2d). The results for DMPG SUVs do not depend on the method of preparation, and the vesicles prepared by sonication or extruding fuse and aggregate similarly in the presence of ζ_{cyt} (Fig. 2d and Fig. S1c). Interestingly, adding of ζ_{cyt} to LMPG micelles results in narrowing the initial R_H distributions (Fig. S1d). This can be explained by formation of relatively uniform micelles due to structuring and stabilizing effect of ζ_{cyt} . According to the results of our CD titration studies, these micelles contain one protein molecule per particle.

To further investigate structure and morphology changes of vesicles of different size and composition upon adding ζ_{cyt} , we used negative staining transmission EM. Our EM studies clearly show that protein binding-dependent membrane disruption is observed for DMPG LUVs and SUVs (Fig. 3a). In contrast, POPG LUVs (Fig. 3a) and SUVs (not shown) remain intact upon binding to ζ_{cyt} . These results provide direct evidence that interactions of a positively

charged ζ_{cyt} with acidic phospholipids can disturb membrane integrity and that this effect depends on vesicle composition rather than size.

In summary, the DLS and EM data clearly demonstrate that binding to ζ_{cyt} induces membrane fusion and rupture in DMPG vesicles. Importantly, the destructive effect of protein binding on bilayer lipid membrane is not dependent on vesicle size (SUV vs. LUV) or technique of SUV preparation (sonication vs. extruding). The membrane rupture is known to result in monolayer fusion of the membranes, i.e., in the formation of a bridge connecting the monolayers, which is usually named the stalk or hemifusion intermediate [18]. Interestingly, tight coupling between the loop-to-helix structural transition and stalk formation as a result of deformation of the target and viral membranes has been reported for influenza hemagglutinin [19,²⁰]. Thus, protein binding-induced membrane perturbation and disruption can represent a molecular basis for formation of the ITAM helices in the presence of DMPG vesicles.

Discussion

Here we analyzed membrane binding of intrinsically disordered ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} , and found that there are two different modes of their lipid-binding activity toward acidic phospholipids (Figs. 3b and S2c): 1) coupled binding and folding that is characteristic for micelles and those vesicles that are unstable upon protein binding (DMPG), and 2) binding without folding that is observed in the presence of stable vesicles (POPG). As we suggest, in both modes, initially, clusters of basic amino acids in the regions outside ITAMs bind to polar heads of acidic phospholipids while the ITAM residues do not contribute to binding at this stage. Then, in micelles (mode I), hydrophobic interactions between ITAMs and detergent tails promote folding of ITAMs, thus making ITAM tyrosines inaccessible for kinases as it has been shown for ζ_{cyt} /LMPG micelles system [2,¹⁶]. In vesicles, depending on the bilayer stability, initial protein binding to the membrane may (mode I) or may not (mode II) induce vesicle fusion and rupture and promote formation of ITAM helices stabilized by hydrophobic interactions with lipid tails in ruptured bilayers.

Instability of the bilayers of DMPG LUVs or POPG/dihexanoylphosphatidylcholine bicelles upon binding to CD3 ϵ_{cyt} (net charge of +11) can explain α -helical folding of CD3 ϵ_{cyt} ITAM (mode I) and inaccessibility of the ITAM tyrosines for kinases observed in the presence of these membrane models [10]. It should be also noted that in mode II, ITAMs do not participate in binding to lipid bilayers and the ITAM tyrosines are therefore likely to be easily accessible for phosphorylation (Fig. 3b and Fig. S2c).

The existence of two different modes of membrane binding raises an important question: which mode of action is of physiological relevance? Considering that folding of ITAMs is not observed in the presence of those vesicles that are stable upon protein binding, this folding transition is unlikely to play a significant role in transmembrane signaling and cell activation. However, it does not necessarily mean that mode II (binding without folding) is also physiologically irrelevant. For example, in a recently proposed novel model of immune signaling, the signaling chain homooligomerization (SCHOOL) model [21,²²], homointeractions between cytoplasmic domains of the ITAM-containing receptor signaling subunits are suggested to be critical in receptor-mediated cell activation. Thus, membrane binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} might prevent homooligomerization of these cytoplasmic domains [3] in receptor clusters on the surface of resting cells and during random encounters of receptors diffusing in the cell membrane.

In summary, our data first provide molecular explanation for current discrepancies in the literature on whether or not lipid binding of ζ_{cyt} , CD3 ϵ_{cyt} , and FcR γ_{cyt} is accompanied by helical folding of ITAMs [2,^{4,10}]. Second, we show that the use of lipid vesicles of the same

size and surface charge can result in opposite conclusions regarding membrane-binding activity of proteins and its physiological relevance. The questions raised here highlight the importance of the choice of an appropriate membrane model and how substantially our improved understanding of important biological processes such as receptor triggering and cell activation depends upon critical evaluation of the data and observations accumulated to date.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Model depicting ζ_{cyt} and synthetic ζ_{cyt} peptides A–G used to map the lipid-binding sites. ITAM domains are shown as gray boxes. The peptides A, C, and E shown to bind to sucrose-loaded POPG LUVs are indicated in bold.

Fig. 2.

Different interaction of ζ_{cyt} with lipid micelles and vesicles. Far-UV CD (A) and the intrinsic tyrosine fluorescence (B) spectra of $10 \mu\text{M} \zeta_{\text{cyt}}$ in 20 mM MOPS, 0.1 M NaCl, pH 7.0 alone (thin continuous line) or in the presence of 2 mM LMPG micelles (dotted line) and sonicated SUVs of 3 mM DMPG or POPG (broken and thick continuous lines, respectively). (C) Distribution of hydrodynamic radii of LUVs in terms of percent mass. The peak positions are 44.5 ± 9.1 and 33.4 ± 7.6 nm (mean $\pm$ SD), respectively, for 3 mM POPG (thin continuous line) and DMPG (dotted line) extruded LUVs alone and 40.8 ± 7.6 and 38.9 ± 12.0 nm, respectively, in the presence of $10 \mu\text{M} \zeta_{\text{cyt}}$ (thick continuous and broken lines, respectively). The inset shows the corresponding distributions in terms of percent scattering intensity. The peak positions, obtained from percent scattering intensity distributions, are 50.8 ± 11.2 and 39.4 ± 9.8 nm, respectively, for 3 mM POPG (thin continuous line) and DMPG (dotted line) LUVs alone and 45.6 ± 9.5 and 53.7 ± 19.0 nm, respectively, in the presence of $10 \mu\text{M} \zeta_{\text{cyt}}$ (thick continuous and broken lines, respectively). (D) Distribution of hydrodynamic radii of SUVs in terms of percent

mass. The peak positions are 16.0 ± 6.0 and 17.8 ± 7.3 nm, respectively, for 3 mM POPG (thin continuous line) and DMPG (dotted line) sonicated SUVs alone and 17.2 ± 6.1 and 15.3 ± 7.6 nm, respectively, in the presence of $10 \mu\text{M } \zeta_{\text{cyt}}$ (thick continuous and broken lines, respectively). The inset shows the corresponding distributions in terms of percent scattering intensity. The peak positions, obtained from percent scattering intensity distributions, are 25.3 ± 10.4 and 33.7 ± 18.9 nm, respectively, for 3 mM POPG (thin continuous line) and DMPG (dotted line) sonicated SUVs alone and 25.8 ± 10.0 and 41.3 ± 29.2 nm, respectively, in the presence of $10 \mu\text{M } \zeta_{\text{cyt}}$ (thick continuous and broken lines, respectively).

Fig. 3.

Lipid bilayer stability and the membrane binding mode of ζ_{cyt} . (A) EM of POPG LUVs and DMPG LUVs or SUVs in the absence or presence of ζ_{cyt} . (B) Schematic representation of the proposed dual mode of ζ_{cyt} lipid-binding activity. Mode I: Binding of intrinsically disordered ζ_{cyt} to negatively charged micelles or unstable bilayer vesicles. Initially mediated by electrostatic interactions of head groups with clusters of basic residues outside ITAMs, this binding is accompanied by a helical folding of ITAMs promoted by hydrophobic interactions with lipid tails in the micelle core or the membrane stalk intermediates of fusion. Mode II: Binding of ζ_{cyt} to stable negatively charged bilayer vesicles. This binding is mediated by electrostatic interactions and is not accompanied by a helical folding of ITAMs. Polar head

groups and hydrophobic tails of detergent and lipid molecules are denoted by gray filled circles and lines, respectively.

Table 1Binding of T cell receptor ζ cytoplasmic domain peptides to POPG LUVs

	Peptide/protein	net charge	% of bound peptide/protein
A	LRV <u>K</u> FSRSADAPAYQQGQNQL	+2	50
B	LYNELNLGR <u>R</u> EEYDVL	-2	3
C	D <u>KRR</u> GRDPEMGG <u>KPRRK</u> NPQEL	+4	42
D	LYNELQKDKMAEAYSEI	-2	0
E	GM <u>K</u> GE <u>RRR</u> G <u>K</u> GHDGL	+3	54
F	LYQGLSTAT <u>K</u> DTYDAL	-1	2
G	LYQGLSTAT <u>K</u> DTYDALHMQALPP <u>R</u>	0	8
	ζ_{cyt}	+5	90