Membrane binding of intrinsically disordered proteins

Critical importance of an appropriate membrane model

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Intrinsically disordered proteins (IDPs) are proteins that lack a well-defined three-dimensional structure under physiological conditions. 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), and γ signaling subunit of Fc ϵ RI receptor (Fc ϵ RI γ_{cyt}) represent a novel class of IDPs.¹⁻³ These regions all have one or more copies of an immunoreceptor tyrosine-based activation motif (ITAM), tyrosine residues of which are phosphorylated upon receptor engagement in an early and obligatory event in the signaling cascade. Considering a crucial role of ζ_{cyt} , CD3 ϵ_{cyt} and Fc ϵ RI γ_{cyt} in immune signaling and their close proximity to the cell membrane, the question whether or not membrane binding of these IDPs can promote folding of ζ_{cyt} , CD3 ϵ_{cyt} and Fc ϵ RI γ_{cyt} ITAMs and thus lead to inaccessibility of the ITAM tyrosines for phosphorylation is of fundamental importance in our understanding of receptor triggering. However, little is known about lipid-binding activity of the ITAM-containing cytoplasmic domains and the existing data are strikingly contradictory (Fig. 1).^{1,2,4}

Our recent study⁵ provides, for the first time, molecular explanation of discrepancies in the literature and demonstrates that the use of an inappropriate membrane model can result in misleading conclusions regarding membrane-binding activity of proteins and its physiological relevance.

DMPG Vesicles as a Membrane Model

In 2000,² using fluorescence, circular dichroic (CD) spectroscopy, and an in vitro kinase assay, it has been shown that α -helical folding transition of ζ_{cyt} upon binding to acidic detergents and phospholipids [lysomyristoyl-phosphatidylglycerol (LMPG) micelles and dimyristoyl-phosphatidylglycerol (DMPG) vesicles, respectively] prevents ITAM phosphorylation. The authors concluded that this folding transition can represent a conformational switch to regulate TCR triggering.² Later, formation of partial helices within the ζ_{cyt} ITAM motifs upon binding to LMPG micelles has been analyzed using CD and multidimensional nuclear magnetic resonance (NMR) spectroscopy.⁶

The possible role of membrane binding in TCR-mediated cell activation has been further addressed in the recent studies of Xu et al.⁴ that have extended the previously reported findings and mechanisms suggested for ζ ^{2,6} to CD3 ϵ . Using purified CD3 ϵ_{cyt} and membrane models such as DMPG vesicles and bicelles composed of 1-palmitoyl-2-oleoyl-phosphatidylglycerol (POPG) and dihexanoyl-phosphatidylcholine (DHPC), the authors showed that CD3 ϵ_{cyt} binds to acidic phospholipid-formed bilayers and that this binding is mediated by electrostatic interactions between the N-terminal basic residues of CD3 ϵ_{cyt} and polar lipid groups.⁴ As shown by CD and NMR spectroscopy, this binding is accompanied by folding of the protein and formation of partial helices within the ITAM region, leading to burying of the two ITAM tyrosines into the

Key words: intrinsically disordered protein, T-cell receptor, membrane binding, conformational change, membrane model, cell activation model

Submitted: 01/30/10

Revised: 02/17/10

Accepted: 02/17/10

Previously published online:

www.landesbioscience.com/journals/selfnonself/article/11547

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Figure 1. Mode of binding of intrinsically disordered ζ_{cyt} , CD3 ϵ_{cyt} and Fc ϵ RI γ_{cyt} to lipid bilayers depends on the membrane model. On the left, DMPG vesicles are used as a membrane model. With this model, binding mediated by the N-terminal basic residues is coupled to folding of the cytoplasmic domain (A) and formation partial helices within the ITAM motif (the signature sequence of the ITAM region is shown in green) with the tyrosines buried in the bilayer (B). In this state, the ITAM tyrosines are inaccessible for Src kinase phosphorylation. On the right, POPG vesicles are used as a membrane model. With this model, binding is not accompanied by folding (A) and therefore the inaccessibility of the ITAM tyrosines to kinases is questionable. (C) Dynamic light scattering (DLS) and electron microscopy (EM) of DMPG (left) and POPG (right) vesicles in the absence or presence of protein. The DLS and EM data clearly demonstrate that binding to the proteins of interest induces membrane fusion and rupture in DMPG but not POPG vesicles.

hydrophobic area of the lipid bilayer (Fig. 1A and B, left) such that these residues are inaccessible for phosphorylation by LCK in an in vitro kinase assay.⁴ Based on these findings and results of the fluorescence energy resonance transfer (FRET)

experiments performed in Jurkat cells, the authors concluded that TCR triggering in vivo is regulated by dynamic membrane binding of the CD3 ϵ_{cyt} ITAM motif and that “sequestration of key tyrosines into the lipid bilayers represents a previously

unrecognized mechanism for control of receptor activation”.⁴

Thus, for both cytoplasmic domains, CD3 ϵ_{cyt} ⁴ and ζ_{cyt} ,² lipid-dependent helical folding transition was suggested to represent the main regulator of TCR in vivo,

resulting in the development of a conformational model of T cell activation.^{2,4,7}

There are several serious concerns regarding these studies. The major concern is that in the studies by Aivazian and Stern² and Xu et al.⁴ binding of CD3 ϵ_{cyt} and ζ_{cyt} to acidic phospholipid bilayers was assumed always to be coupled with protein folding and formation of helices within the ITAMs making their tyrosines inaccessible for kinase phosphorylation (Fig. 1A and B, left). However, this assumption is not consistent with our current understanding of lipid-binding activity of intrinsically disordered cytoplasmic domains of TCR signaling subunits. First, binding mediated by electrostatic interactions of positively charged CD3 ϵ_{cyt} and ζ_{cyt} residues with negatively charged polar groups of acidic phospholipids and folding mediated by hydrophobic interactions of ITAMs with lipid tails are not necessarily coupled.^{1,5} Further, helical folding of the ζ_{cyt} ITAM motifs can be induced by principally different kinds of agents such as LMPG micelles and DMPG vesicles,² and trifluoroethanol,⁸ indicating that electrostatic interactions are not necessary to induce helical structure formation. Similarly, for CD3 ϵ_{cyt} , folding can be induced by LMPG micelles, DMPG vesicles and POPG/DHPC bicelles.⁴ Interestingly, based on the NMR data reported by Xu et al.⁴ CD3 ϵ_{cyt} helical folding induced by LMPG micelles structurally differs from that induced by POPG/DHPC bicelles, suggesting that the structural features of folded CD3 ϵ_{cyt} depend on the model lipid system. In addition, as reported,⁴ the CD3 ϵ_{cyt} ITAM residues are not important for binding to acidic phospholipid bilayers. Thus, these observations collectively indicate that for CD3 ϵ_{cyt} and ζ_{cyt} , binding and folding are mediated by different interactions (electrostatic and hydrophobic, respectively) and are not necessarily coupled.

Our recent study⁵ confirms the existence of two different membrane binding modes of intrinsically disordered ζ_{cyt} , CD3 ϵ_{cyt} and Fc ϵ R γ_{cyt} , depending on the cell membrane model: binding followed by helical folding of the ITAMs (DMPG vesicles) and binding without a disorder-to-order transition (POPG vesicles).

POPG Vesicles as a Membrane Model

Recently, we have shown that binding of CD3 ϵ_{cyt} , ζ_{cyt} and Fc ϵ R γ_{cyt} to POPG vesicles, which would be expected to be a better model to mimic the cell membrane than micelles, DMPG vesicles, and likely bicelles, is not accompanied by a disorder-to-order transition.¹ The proteins remain similarly disordered in free and lipid-bound forms, thus suggesting a principally different mode of their lipid-binding activity: binding without folding (shown for CD3 ϵ_{cyt} in Fig. 1B, right). Considering also the importance of the clusters of N-terminal basic residues and the non-importance of the ITAM residues for binding of CD3 ϵ_{cyt} and ζ_{cyt} to acidic phospholipid bilayers,^{4,5} these findings suggest that in POPG-bound proteins, the ITAM tyrosines can remain accessible to kinase phosphorylation (Fig. 1B, right), thus questioning the physiological relevance of the ITAM helical folding suggested for CD3 ϵ_{cyt} ⁴ and ζ_{cyt} .^{2,6}

Interestingly, phosphorylation of ζ_{cyt} resulting in fully phosphorylated protein with a net charge of -5.5 does not alter its random coil conformation and only slightly weakens but does not block its binding to acidic phospholipid bilayers,¹ showing that partitioning of this protein onto POPG vesicles is driven mostly by the clusters of basic residues rather than the overall net charge. Thus, in our study,¹ we investigated lipid binding of fully phosphorylated ζ_{cyt} and not the phosphorylation of the lipid-bound protein. Therefore, the interpretation of that work by Xu et al.⁴ that "...lipid binding only partially inhibited ITAM phosphorylation (Sigalov et al. 2006)" is incorrect.

Further, the results of FRET studies performed in T cells show that CD3 ϵ_{cyt} can be membrane-bound in vivo.⁴ However, this does not necessarily mean that membrane binding of CD3 ϵ_{cyt} is accompanied by a folding of the CD3 ϵ_{cyt} ITAM. Importantly, our previous¹ and more recent⁵ observations suggest that should membrane binding of the ITAM-containing cytoplasmic domains of TCR signaling subunits occur in vivo, it would not necessarily be coupled to helical folding of ITAMs and therefore block phosphorylation of ITAM Tyr

residues. In addition, our findings also suggest that phosphorylated CD3 ϵ_{cyt} and ζ_{cyt} can still remain bound to the membrane.¹ Thus, the data of FRET studies⁴ can not be considered as experimental evidence indicating that the CD3 ϵ_{cyt} ITAM motif is folded in vivo (Fig. 1B, left), thus questioning a role of α -helical folding transition of the CD3 ϵ_{cyt} ITAM in TCR triggering and challenging the suggested model of TCR activation.^{2,4,7}

Integrity of DMPG and POPG Lipid Bilayers Upon Protein Binding

Our recent study⁵ resolves the long-standing structural puzzle and demonstrates that binding of highly positively charged ζ_{cyt} , CD3 ϵ_{cyt} and FcR γ_{cyt} (net charges are +5, +11 and +3, respectively) to acidic phospholipids can destabilize and disrupt lipid bilayers. Using electron microscopy (EM) and dynamic light scattering (DLS), we have shown that upon protein binding, unstable DMPG vesicles fuse and rupture (Fig. 1C, left). In contrast, stable POPG vesicles remain intact under these conditions (Fig. 1C, right). Thus, protein binding-induced membrane perturbation and disruption can represent a molecular basis for the observed formation of the ITAM helices in the presence of DMPG vesicles.^{2,4,5}

Importantly, our findings⁵ demonstrate, for the first time, that depending on lipid type, the use of vesicles of the same size and surface charge can result in opposite conclusions regarding membrane-binding activity of proteins and its physiological relevance. The study also underlines the importance of ensuring the integrity of model membranes upon protein binding, especially in studies of IDPs.

Conclusion

In summary, I would like to highlight that the studies discussed clearly show the critical importance of the choice of an appropriate membrane model in studies of protein-lipid interactions and how substantially our improved understanding of basic biochemical mechanisms underlying both fundamentally and clinically important processes such as receptor triggering depends upon critical evaluation of the data and observations accumulated to date.

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