COMMUNICATIVE & INTEGRATIVE BIOLOGY
2017, VOL. 10, NO. 4, e1343772 (3 pages)
https://doi.org/10.1080/19420889.2017.1343772

Taylor & Francis
Taylor & Francis Group

ARTICLE ADDENDUM

OPEN ACCESS Check for updates

Enhanced synaptotagmin plasticity derived from pairing intrinsic disorder with synaptic vesicle lipids

Michael E. Fealey, David D. Thomas, and Anne Hinderliter

Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, Minneapolis, MN, USA; Department of Chemistry and Biochemistry, University of Minnesota Duluth, Duluth, MN, USA

ABSTRACT

Synaptotagmin 1 (Syt 1) is an integral membrane protein responsible for sensing the calcium ion (Ca^{2+}) influx in neurons that triggers synaptic vesicle exocytosis. How Syt 1's intrinsically disordered region (IDR), a ~60 residue sequence located between the protein's transmembrane helix and two Ca^{2+}-sensing C2 domains, contributes to protein function is not well understood. The same is true of analogous IDRs located in the other synaptotagmin isoforms. Recently, we found that the Syt 1 IDR is structurally responsive to vesicles whose lipid composition mimics that of a synaptic vesicle organelle and that this sensitivity allosterically influences binding and folding behavior of the adjacent C2 domain. We believe these observations may be applicable to the study of other synaptotagmin isoforms and discuss generally how an IDR-membrane interaction could contribute to modulation of C2 domain function.

ARTICLE HISTORY
Received 2 June 2017
Accepted 14 June 2017

KEYWORDS
allostery; lipids; intrinsically disordered region; synaptic vesicle synaptotagmin

A recent coalescence of fields in biophysics has led to a way of viewing a synaptic protein region often regarded as a hinge as something much more: a source of structural and functional plasticity that contributes on the molecular level to neuronal plasticity. When studies on vesicle-localized integral membrane protein p65 (now known as synaptotagmin 1, Syt-1) first began, the method used to discern structural organization of its domains was proteolysis. In this method, any unfolded flexible regions of the protein are readily hydrolyzed thus releasing the more modular folded domains for subsequent mass analysis. With this method, investigators discovered that Syt-1 had 3 major segments: a lumenal domain, a transmembrane domain, and a cytosolic domain of roughly 39 kDa.[1] What these initial studies also reported, in addition to location of domain segments, was the first experimental evidence for an intrinsically disordered region (IDR) in Syt-1. Specifically, they identified the residue region 90–110 to be highly susceptible to proteolytic cleavage when treated with pronase and trypsin.[1] At the time, intrinsic disorder had not yet been developed as a field of biophysics and structural biology. As such, a significant portion of synaptotagmin research has since been focused on the protein's downstream C2 domains, β-sandwich folds that chelate calcium ions (Ca^{2+}) as they enter axon terminals through voltage-gated Ca^{2+}-channels to trigger exocytosis. C2 domains are largely considered the main functional unit of the protein and when the IDR (often referred to as the "juxta-membrane linker") has been considered, it has typically been viewed as just a flexible tether, a site of oligomerization or a site for palmitoylation.

While short linker regions in single-pass membrane proteins can certainly provide adjacent folded domains more flexibility when exploring their local restricted volumes, with the current establishment of intrinsic disorder as a field, it may now be worth considering the juxta-membrane linker through a different lens in an attempt to gain functional insights that might otherwise be overlooked.[2] IDRs are gaining wide spread recognition for their role in modulating the function of adjacent folded domains, including allosteric mechanisms. If the functional plasticity of IDRs is applied to Syt-1, it opens up new potential avenues for understanding the protein's complex role in neurotransmission. Additionally, such investigation would have a direct impact on the understanding of other synaptotagmins within the protein

CONTACT Michael E. Fealey feale005@d.umn.edu 321 Church Street SE, Minneapolis MN 55455, USA; Anne Hinderliter ahinderl@d.umn.edu 1039 University Drive, Duluth MN 55812, USA.

Addendum to: Fealey ME, Mahling R, Rice AM, Dunleavy K, Kobany SE, Lohese KJ, Horn B, Hinderliter A. Synaptotagmin I's intrinsically disordered region interacts with synaptic vesicle lipids and exerts allosteric control over C2A. Biochemistry 2016;55(21):2914–26. doi: 10.1021/acs.biochem.6b00085

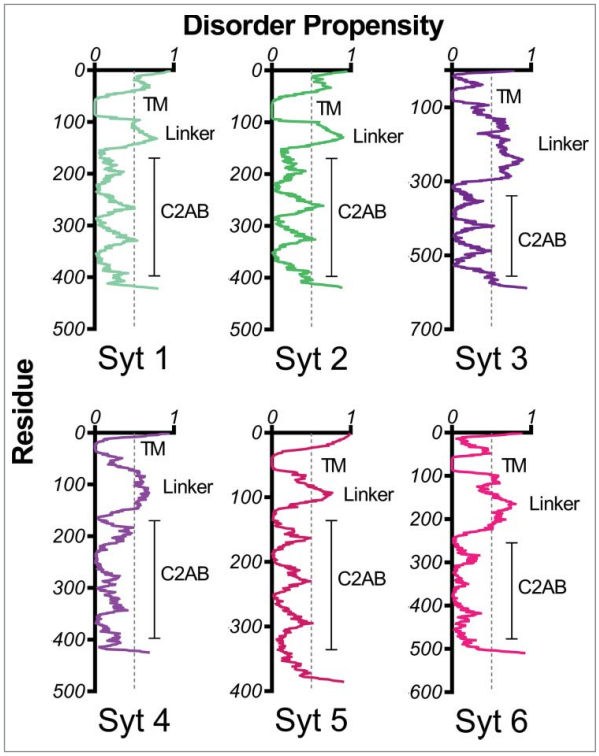

Figure 1. Location of intrinsically disordered regions in first 6 synaptotagmin isoforms. The disorder propensity (low-to-high range, 0–1) of several synaptotagmin isoforms is highest in the region just distal to the transmembrane (TM) helix before the tandem C2 domains (C2AB).

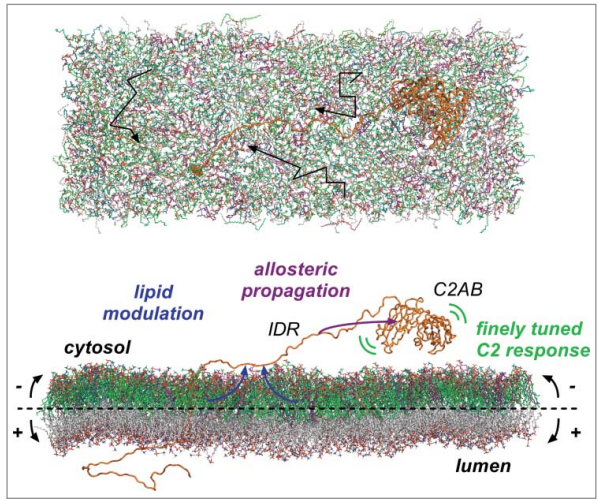

Figure 2. Source of membrane variability for synaptotagmin IDR to sense. (top) The structure of synaptotagmin experiences shifts in the lateral distribution of several distinct lipid species (arrow trajectories) and also (bottom) fluctuations in surface curvature (+ for positive curvature; - for negative curvature). The outer leaflet of the synaptic vesicle membrane depicts a color-coded and proportional distribution of PE (shades of green), PS (shades of purple) and PI (shades of magenta) lipids as described in reference 4. The inner leaflet, which has a distinct set of lipid species, is shown in gray for simplicity. Note that one of the major lipid components, cholesterol, is not shown in the above models. These features are sensed by the synaptotagmin IDR (blue), allosterically propagated to the adjacent C2 domains via local distortions of the IDR's structural ensemble (purple) resulting in fine IDR-membrane modulations to protein function (green).

family. Many synaptotagmins contain a juxta-membrane linker with amino acid compositions that are predicted to be intrinsically disordered (Fig. 1); these regions show characteristically high frequencies of charged, polar and structure-breaking residues while lacking the bulky hydrophobic side chains that promote folding. This means that, in addition to C2 domain divergence among the family, there could also be isoform-specific flavors of intrinsic disorder each of which exerts particular influence over its adjacent C2 domains. Through this lens, intrinsic disorder could simply act to further differentiate isoform functions.

Intrinsic disorder also seems advantageous from the point of view of the complex composition of lipid bilayers. Disordered proteins and protein regions are known to exhibit structural plasticity, interacting with multiple binding partners as part of their regulatory function and often adopting different structural states upon doing so. Synaptic vesicles are densely packed with proteins for potential interactions, but the bilayer surface to which synaptotagmin proteins are anchored provides an even larger diversity of potential interaction partners with an estimated 30 distinct lipid types in an average synaptic organelle.[3] In this regard, the lateral distribution of lipids coupled with surface curvature of the membrane (which changes drastically throughout synaptic vesicle cycling) could present a near infinite number of surfaces to synaptotagmin (Fig. 2). An intrinsically disordered region could capitalize on this surface diversity, possessing the physiochemical characteristics requisite for recognizing each via a multitude of possible conformers. The plethora of membrane-selected ensembles in this IDR could then impact the adjacent C2 domains through modes of allostery, culminating in very fine modulations of function that would then match the protein's complex biological behavior measured in vivo. This pairing of intrinsic disorder and lipid diversity has even further depth when considering the other synaptotagmin isoforms. While all synaptotagmin linker regions are predicted to be disordered, the amino acid compositions constituting each are distinct. This, coupled with the fact that membrane lipid composition is also distinct between the different tissue types each isoform is expressed in, leads to the hypothesis that intrinsic disorder and the vast lipid diversity of different membranes work together to finely and uniquely tune protein response in vesicle trafficking events that are mediated by synaptotagmins.

Recently, our group provided evidence in support of such a mechanism.[4] We found that the IDR of Syt-1 is structurally sensitive to the lipid composition of the membrane, undergoing pronounced changes upon association. While the IDR seems to be mostly disordered when associated with a membrane whose lipid composition mimics that of a synaptic vesicle, its interaction still has a profound impact on the adjacent C2 domain (C2A). This indicates there is allosteric communication between the 2 domains that, furthermore, is tightly coupled to the lipid composition that mimics the protein's native membrane. Further investigations into IDR-membrane interactions in other isoforms may reveal similar functional insights.

All of this points to the high potential of the IDR playing a more nuanced functional role than that of an inert tether. Several groups have acquired evidence in support of flexible tether[5] or oligomerization functions, however, we believe these are just a few of several yet to be identified. Since intrinsically disordered proteins and protein regions are defined in part by functional plasticity, it would not be much of a stretch to ascribe multiple such roles to this disordered domain of synaptotagmin.

Funding

This material is based upon work supported by the National Science Foundation (MCB-1616854) to DDT and AH. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. MEF was supported by NIH (T32 AR007612) to DDT.

ORCID

Michael E. Fealey http://orcid.org/0000-0002-1264-8037
David D. Thomas http://orcid.org/0000-0002-8822-2040
Anne Hinderliter http://orcid.org/0000-0003-4863-8065

References

[1] Perin MS, Brose N, Jahn R, Südhof TC. Domain structure of synaptotagmin (p65). J Biol Chem 1991; 266:623-9; PMID:1985919

[2] van der Lee R, Buljan M, Lang B, Weatheritt RJ, Daughdrill GW, Dunker AK, Fuxreiter M, Gough J, Gsponer J, Jones DT, et al. Classification of intrinsically disordered regions and proteins. Chem Rev 2014; 114:6589-631; PMID:24773235; https://doi.org/10.1021/cr400525m

[3] Takamori S, Holt M, Stenius K, Lemke EA, Grønborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, et al. Molecular anatomy of a trafficking organelle. Cell 2006; 127:831-46; PMID:17110340; https://doi.org/10.1016/j.cell.2006.10.030

[4] Fealey ME, Mahling R, Rice AM, Dunleavy K, Kobany SE, Lohese KJ, Horn B, Hinderliter A. Synaptotagmin I's intrinsically disordered region interacts with synaptic vesicle lipids and exerts allosteric control over C2A. Biochemistry 2016; 55:2914-26; PMID:27191789; https://doi.org/10.1021/acs.biochem.6b00085

[5] Lai Y, Lou X, Jho Y, Yoon TY, Shin YK. The synaptotagmin 1 linker may function as an electrostatic zipper that opens for docking but closes for fusion pore opening. Biochem J 2013; 456:25-33; PMID:24001110; https://doi.org/10.1042/BJ20130949