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Allostery and instability in the functional plasticity of synaptotagmin I

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Synaptotagmin I (Syt I) is the calcium ion sensor for regulated release of neurotransmitter. How Syt I mediates this cellular event has been a question of extensive study for decades and yet, a clear understanding of the protein's diverse functionality has remained elusive. Using tools of thermodynamics, we have identified two intrinsic properties that may account for Syt I's functional plasticity: marginal stability and negative coupling. These two intrinsic properties have the potential to provide great conformational flexibility and suggest that Syt I's functional plasticity stems in part from subtle rearrangements in the protein's conformational ensemble. This model for Syt I function is discussed within the context of the nervous system's overall plasticity.

Keywords: synaptotagmin I, negative coupling, denaturation, stability, plasticity

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Neural Plasticity as a Model for Synaptotagmin I Function

The nervous system is known for its plasticity, both in development when synapses are being directed and connected and in life, as occurs with learning, memory and emotion.[1-3] This plasticity of the nervous system is part of what enables great human diversity in response to stimuli. With this type of global behavior, it might be expected that the component parts of the nervous system function in analogous ways. For the neuronal cell membrane, this is likely true.[4] The weak interaction energies between membrane lipids create a dynamic surface, but also allow for the possibility of protein-induce reorganization, a potential mechanism for mediating several cellular signaling events through domain formation.[5-7] Weak energetics, in this case, are what enable membrane plasticity and environmental responsiveness.[8] In addition to the membrane, there may also be plasticity in the proteins that mediate neuronal activity. Synaptotagmin I (Syt I), a key regulatory protein responsible for sensing the calcium ion (Ca^{2+}) influx that triggers neurotransmitter release,[9-11] may be one such example.

Like the immensely diverse neuronal networks that develop from a limited set of neuronal genes, the origins of Syt I's diverse in vivo functionality is incompletely understood.[12,13] How Syt I is able to mediate vesicle docking, regulate SNARE complex function, enhance membrane disruption, facilitate vesicle and plasma membrane fusion and participate in the reuptake of synaptic vesicles is at odds with our current view of neuronal protein function.[13-16] Recent experimental evidence may, however, shed light on how Syt I accomplishes these numerous but related cellular tasks.[17,18] Much like the proposed selective stabilization of neuronal connections in developing networks[12,19] and the refinement of neural circuits with environmental input,[1,3,20] Syt I function may be the result of a molecular-level stabilization and refinement. In this neural plasticity analogy, Syt I's "primary connectivity" stems from the genetic code which provides a primary amino acid sequence that ultimately gives rise to a protein structure of low thermodynamic stability (and, consequently, a wide range of possible conformations).[21,22] Protein conformers imparted with important biological functions are selectively stabilized (or induced) by ligand and their function further refined by additional binding partners in the immediate cellular microenvironment.

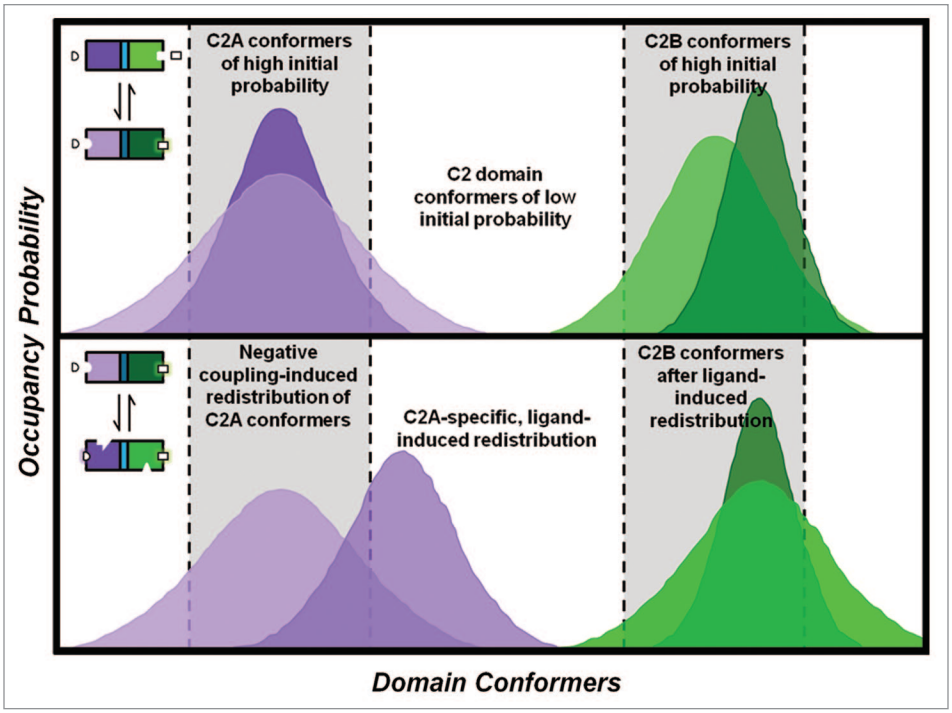

Figure 1. Conceptual representation of negative coupling- and ligand-induced redistribution of C2 domain conformers. Top panel: Initially, C2A (purple) and C2B (green) have basal level stability and corresponding distributions of conformers (note: colors correspond to models in upper left corner of diagram). Upon binding of a C2B-specific ligand or binding partner, both conformer distributions change (dark purple to light; light green to dark). The resultant change weights conformers in each domain's ensemble differently, allowing for C2A conformers that are initially less populated to become more significantly populated. Bottom panel: The more accessible conformers in the destabilized C2A domain, if binding-competent, can be selectively stabilized by ligand (or other binding partners) resulting in a ligand-induced redistribution of conformers (light purple to dark). This selective stabilization of C2A conformers simultaneously drives ensemble broadening in the adjacent C2B domain (dark green to light) through negative coupling. The end result of the negative coupling- and ligand-induced redistribution inter-play is different combinations of domain conformer subsets for mediating different molecular events of neurotransmitter release.

Allostery and Instability: Possible Origins of Syt I Plasticity

The basis for the above analogy stems from recent experimental observations of Syt I; namely, that the protein's two C2 domains (C2A and C2B) are marginally stable and negatively coupled.[17,18] By discussing how each domain's conformation changes as a result of both intrinsic properties during two theoretical binding events, a possible origin for Syt I's functional plasticity (that is physiologically consistent with the gross and microscopic features of the nervous system) can be seen. The discussion starts within a Monod-Wyman-Changeux (MWC) context of allostery.[23-25]

In their unbound form, both C2 domains have some basal level stability. When the first domain-specific ligand or binding partner binds to the C2B domain, for example, its free energy of stability is increased. Because the domains are negatively coupled, however, the opposite effect is seen in C2A; C2A's free energy decreases and gives the domain a higher degree of conformational flexibility. When the stability of the C2A domain is lower, the energetic barrier between different conformations is lower. This allows the domain to more easily access conformers in the ensemble that were originally occupied with low probability (**Fig. 1**, top panel). If the now more readily accessible conformers are binding competent, their higher probability of being occupied will facilitate ligand binding (higher probability of binding-competent conformers is indicated in **Figure 1** by the appearance of sites in the protein model depicted in the upper left corner of each panel. Note that the appearance of a binding site in this MWC context serves only to indicate the increased probability of a binding-competent conformer; though not initially shown in the model, those binding-competent conformers would always be present in the ensemble). As ligand binds, the binding-competent conformer is removed from the conformational equilibrium. To replenish this depleted conformer, the equilibrium of the ensemble shifts and more of the binding-competent conformer becomes available for additional ligation. The cycle of binding, conformer removal and equilibrium shifting repeats ultimately resulting in the re-weighting of all conformers in the ensemble. Conformers that were of low initial probability may become more populated.

Because Syt I's C2 domains have interlinked ensembles, however, the ligand-induced redistribution in C2A not only stabilizes a subset of its own domain conformers, it also lowers the energetic barrier between the conformers of C2B through negative coupling (**Fig. 1**, bottom panel). Since the first binding event redistributed C2B's ensemble, the C2A-induced destabilization allows for a different subset of conformers (which may have different molecular ramifications) to become more

accessible compared with C2B's basal state ensemble.

Alternatively, these two intrinsic properties can also be discussed within a Koshland-Némethy-Filmer (KNF) context of allostery.[26] Since C2A and C2B have marginal stability, the ease with which ligands or binding partners cause a structural change in Syt I increases. When the first ligand or binding partner binds to C2B, it causes a local conformational change in that domain. This change in local structure of C2B is subsequently communicated to C2A through the C2 domain interface. Because the interaction between the two domains is destabilizing, C2B causes C2A to become weaker allowing the C2A domain to take on new conformations not initially possible with basal stability (referring back to the bottom panel of **Fig. 1**, this time in a KNF context, the appearance of binding sites in the Syt I model results from ligand- and/or binding partner-induced conformational changes of each domain). Because the C2A domain can now adopt these new conformations, it can bind to an additional ligand or binding partner. When this ligand or binding partner binds C2A, however, another local conformational change occurs. This conformational change is not only communicated to the C2B domain through the destabilizing interaction that is negative coupling, but may also induce new binding sites within the C2A domain itself.

In either case of allostery, the end result of these two intrinsic protein properties is combinations of domain conformers that vary continuously with each ligand and binding partner. Just as weak energetics underlies the plasticity and responsiveness of the membrane, similar features seem to be present in Syt I.

Dynamic Shifting of Conformations and Diversity of Function

The illustrative example of shifting conformer distributions (MWC model) and changing conformations (KNF model) described above starts to show the conformational plasticity of Syt I with two theoretical binding events. When considering the number of ligands (Ca^{2+}, phosphatidylserine, phosphatidylinositol) and binding partners (SNAP-25, syntaxin, synaptobrevin, complexin) known to interact with each C2 domain and that do so differentially, the complexity of conformational change increases. When expanding the scope further still to incorporate influence from other lipid and protein species present in both vesicle and plasma membranes as well as the cytosol, the intricacy of conformational changes are staggering. If within each C2 domain's conformational repertoire there are distinct conformer subsets that mediate different molecular events of neurotransmission,[27] then ligand and binding partner-induced selections of domain conformer combinations (with subtle refinements from other constituents of the protein's microenvironment) may permit the nuanced functions of Syt I observed in vivo.[13-15] It is in this molecular stabilization and subtle refinement of conformers, enabled by allostery and instability, that Syt I mimics nervous system plasticity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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