A Tandem PDZ Domain Redox Switch in InaD

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***Short Abstract* — In addition to their classic role in pre-organizing signaling complexes, multi-domain scaffolding proteins have recently been shown to play an active role in regulating the efficiency and speed of signal processing in cells. In *Drosophila* photoreceptor neurons, the five-PDZ domain scaffold InaD undergoes light-dependent redox-based cycling between two functionally distinct conformations, a dynamical process that is necessary for fast vision. However, the connection between redox regulation of InaD and changes in scaffolding properties is yet unknown. Here, we show that the minimum unit of redox cycling is not a single PDZ domain but the joint action of two adjacent PDZ domains.**

***Keywords* — allostery, biophysics, signaling, structure, redox, scaffolding, PDZ domain**

I. Background

Scaffolding proteins are multi-domain proteins which bind to multiple components of a signaling pathway. Their modular design suggests that the evolutionary wiring of new signaling pathways may simply require the fusion of generic domains which interact with the desired proteins rather than the re-engineering of protein activities or interfaces [1]. For this idea to be generally true, however, the protein interaction domains must be functionally independent. This assumption of protein domains as "beads on a string" which simply tether signaling components is now known to be violated in many cases. Instead, scaffolds often achieve sophisticated regulation of signaling through the joint action of multiple regions of the scaffold.

The Drosophila phototransduction scaffold InaD contains five PDZ domains which interact with an upstream activator, NorpA; Trp, the output of the pathway; and InaC, which is involved in negative feedback [2]. We found that C606 and C645 of the fifth PDZ domain (PDZ5) of InaD form a disulfide bond which alters the conformation of the binding pocket and is required for the refractory period that follows the visual response [3].

While PDZ5 is thought to bind NorpA *in vivo* and is reduced in the absence of signaling, *in vitro* it does not bind and oxidizes under physiological conditions. There are several examples of the binding and stability of PDZ domains being affected by N- and C-terminal extensions, often other PDZ domains [4]. We hypothesized that PDZ4, which is separated from PDZ5 by a short linker throughout the InaD gene family, may be responsible for these two discrepancies.

II. Results

The PDZ5 redox switch is composed of three potentially coupled processes: (1) binding of NorpA, (2) formation of a disulfide bond in PDZ5, (3) a conformational change in the PDZ5 binding site. We quantitatively measured the effect of PDZ4 on each of these as well as the coupling between each process.

Using FRET competition and redox titration assays, we found that PDZ4 promoted the binding of PDZ5 to NorpA by >6.5 kT and destabilized the disulfide bond by 5.6 kT. The data indicated PDZs 4-5 exist in a slow equilibrium between a PDZ5-like "inactive" state and an "active" state which binds and is less oxidizable. Binding and oxidation are indirectly coupled through their mutual effects on the conformational equilibrium.

We crystallized and solved the structure of PDZs 4-5 in the reduced state. The two domains form an extensive interface, covering 21% of the surface area of PDZ5. There are no direct contacts between PDZ4 and the cysteines or binding site of PDZ5, indicating the effects we observe are due to allostery. Indeed, the interface includes residues implicated in allosteric regulation of other PDZ domains.

III. Conclusion

InaD PDZs 4-5 form an allosteric system which couples binding of NorpA and oxidation of PDZ5 to a conformational equilibrium between an "inactive" and "active" state. As a result, oxidation of PDZ5 triggers unscaffolding of NorpA, which is known to suppress visual signaling. Tandem homologs of PDZs 4-5 exist in many scaffolding protein families, suggesting that the functional coupling of domains might be a generally conserved feature of PDZ scaffolds.

Selected References

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