New and Notable

Dystrophin's Tandem Calponin-Homology Domains: Is the Case Closed?

Neville R. Kallenbach[†*] and Roberto Dominguez[‡]
[†]Department of Chemistry, New York University, New York, New York; and [‡]Department of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Actin plays a central role in the eukaryotic cytoskeleton and interacts with a large number of proteins (1). The actin-binding domains in these proteins have been classified into different groups based on structural similarity. A repeat of two calponin-homology domains (designated CH1-CH2) constitutes one major class of low-affinity actin-binding domains (ABDs) (2,3). Despite the abundance and importance of this domain, the mechanism by which it binds F-actin is intensely debated. There are two competing structural models, closed (or compact) and open (or extended), referring to the relative position of the two CH domains upon binding to filamentous actin (F-actin). All the structures of isolated ABDs show the two CH domains tightly packed against one another, i.e., in a closed conformation. However, in two of the structures (utrophin and dystrophin), the closed conformation results from domain swapping between two monomers in the crystal; this is considered to be a crystallization artifact because both ABDs appear to be monomeric in solution.

The prevalence of the closed conformation in crystal structures and the fact that hydrophobic amino acids are buried at the interface between CH domains has led to the notion that this conformation is stable and may be preserved upon weak-affinity binding to F-actin. However, a debate has emerged as a result of conflicting electron-microscopic studies, centering on whether the ABD is closed or open when bound to actin. Furthermore, the domain-swapped structures of utrophin and dystrophin indicate that these two ABDs can be at least transiently open. Because of such discrepancies, independent methods are needed to characterize the conformation of tandem CH domains in solution and in their F-actin-bound form.

An article in this issue of *Biophysical Journal* by Singh and Mallela (4) brings the technique of pyrene excimer fluorescence to bear on the question of the solution conformation of the tandem CH domain in dystrophin. The technique is simple in principle. When two pyrene molecules are close in space, their aromatic rings stack against one another resulting in the formation of an excimer and the appearance of a new fluorescence band (5). Labeling cysteines in the CH domains with pyrene can then determine whether or not the two positions are proximal from the observation of excimer fluorescence band. Taking advantage of two endogenous cysteine residues in the dystrophin ABD sequence, one in each CH domain, the authors label each with pyrene, and look for the signature pyrene excimer fluorescence band. They conclude that contrary to the domain-swapped crystal structure (6), dystrophin's ABD is monomeric in solution and adopts a closed conformation affording significant interactions between the individual CH domains. Excimer fluorescence is also observed in the F-actin-bound form, implying that the ABD remains in a close conformation when bound to F-actin. This technique thus elegantly demonstrates that the free and F-actin bound conformation of dystrophin's ABD is closed.

The study raises interesting questions concerning the conformational preferences of dystrophin versus utrophin. Deficiency of dystrophin is associated with Duchenne's muscular dystrophy, whereas deficiency of utrophin has less severe consequences. Utrophin biosynthesis is elevated in Duchenne patients. Contrary to the tandem CH domains in dystrophin, those of utrophin appear to bind F-actin in an open form, as revealed by an incisive spin-spin double-labeling experiment (7). Because the CH domains in the two proteins share 82% sequence similarity and 73% identity, understanding how the two proteins achieve such different conformational preferences is thus of interest in elucidating their biological functions.

Do these conformational preferences distinguish the actin-binding surfaces of the two proteins? The relatively low-resolution pyrene excimer experiment used by the authors does not provide quantitative distance information. More quantitative measurements, using, for instance, fluorescence resonance energy transfer and spin-labeling (i.e., DEER), can be used in the future to address this question and to investigate mutations of the actin-binding domain of dystrophin that are associated with disease (8). The results could shed needed light on the longer-term possibility of designing smaller stable proteins that might offer some treatment for muscular dystrophy (9). The question of open-versus-closed conformations is of interest for other CH domains, and the results of Singh and Mallela contribute importantly to the ongoing debate.

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*Correspondence: neville.kallenbach@nyu.edu

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