Identifying the skeletal muscle ryanodine receptor activation domain of triadin

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Muscle function is influenced by Ca^{2+} handling proteins in the sarcoplasmic reticulum (SR) of muscle fibres. One such protein is the skeletal muscle ryanodine receptor type 1 (RyR1), a ligand gated Ca^{2+} release channel. This channel is regulated by a variety of SR luminal proteins including the Ca^{2+} binding protein calsequestrin (CSQ1) and two associated transmembrane proteins, triadin and junctin. While triadin was initially believed to be an anchoring protein which tethered CSQ1 to the RyR1, it has recently become apparent that it plays a larger role in RyR1 regulation. Triadin's luminal interaction with the RyR1 leads to an enhancement of channel activity, and three discrete regions of its luminal tail have been found to bind to RyR1 (Caswell *et al.*, 1999; Lee *et al.*, 2004). To identify whether one of these regions alone can regulate RyR1, we synthesized a peptide containing amino acids 200-232 of rabbit skeletal muscle triadin. We then determined its physical and functional interactions with purified RyR1s isolated from the skeletal muscle of euthanized rabbits (Wei *et al.*, 2009).

Using affinity chromatography, we confirmed that the 200-232 triadin peptide bound to RyR1s in solution. Functional studies confirmed that the peptide had more than just a physical interaction with RyR1. This was shown through [^{3}H]ryanodine binding assays, where the amount of [^{3}H]ryanodine binding to RyR1 is directly proportional to channel activity. Incubation of RyR1 with the peptide increased [^{3}H]ryanodine binding ~1.7-fold, indicating that RyR1 activity is increased in the presence of the peptide. A similar effect was observed in lipid bilayer experiments where the current passing across a single channel is monitored in real-time. Purified RyR1s reconstituted in artificial lipid bilayers showed a significant ~1.8-fold increase in open probability when their luminal sides were exposed to peptide, relative to before peptide addition. As the effect of the peptide mimics the increased RyR1 activity induced by full-length triadin (Goonasekera *et al.*, 2007; Wei *et al.*, 2009), it appears that residues 200-232 of triadin are sufficient to replicate RyR1 regulation by full-length triadin.

The triadin sequence of this peptide was postulated to align as a β-strand *in vivo* to facilitate the RyR1-triadin interaction (Kobayashi *et al.*, 2000). To discover whether this structural element was important for RyR1 regulation and whether all residues in the peptide were necessary for RyR1 regulation, the experiments were repeated with a series of mutant peptides. When eight residues predicted to line up along one side of the putative β-strand were mutated to alanines, the peptide did not bind to or activate the RyR1. The mutations caused little change to the already disordered structure of the WT peptide, suggesting the residues themselves, not their structure, were essential for RyR1 regulation. As the RyR1 residues critical for Trisk95 binding are acidic (Lee *et al.*, 2004; Goonasekera *et al.*, 2007), it seemed likely that the basic residues on triadin would form charged-pair interactions with the RyR1. When only five basic residues in this region were mutated, the peptide similarly did not bind to RyR1 or regulate the channel. Therefore all or several of these five basic residues are critical for allowing Trisk95 to modulate RyR1 activity.

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