Structural and functional characterisation of the interaction of the dihydropyridine receptor II-III loop with the ryanodine receptor

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In skeletal muscle, the dihydropyridine and ryanodine receptors (DHPR & RyR) are two membrane proteins that play a central role in excitation-contraction coupling. It is now widely accepted that an interaction between these two proteins is involved in triggering the release of calcium *via* the RyR into the SR. Recent attention has focused on the exact site of interaction and the loop between the second and third repeats of the skeletal DHPR α1 subunit (II-III loop) has been shown to be a critical region interaction site. In an attempt to correlate the structure of this loop with its function our group has previously determined the structure of several functionally active peptides derived from the II-III loop, however structural data for the whole II-III loop at a molecular level has remained elusive.

In this study we focus on the structure/function relationship of the full DHPR II-III loop. This protein has been fully expressed, purified and fully assigned by multidimensional NMR techniques. The conformation of the protein exists as a series of helical elements and turns arranged in an open type structure. The location of the binding site on the RyR has been identified and this fragment has been expressed, purified and refolded. We show by fluorescence experiments that these proteins interact with micromolar affinity. We highlight the regions of the II-III loop that are important for interaction with the RyR.