Mutation within the C-terminus of skeletal calsequestrin disrupts calcium binding

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Depolarisation of the sarcolemma triggers Ca^{2+} release through ryanodine receptor (RyR) calcium release channels in the sarcoplasmic reticulum (SR) of skeletal muscle. Calsequestrin (CSQ) is a highly acidic glycoprotein found in the sarcoplasmic reticulum of cardiac and skeletal muscle. CSQ is the major Ca^{2+} binding protein within the SR and also functions as an regulator of the RyR, by forming a quaternary complex with the RyR, triadin and junctin. CSQ binds Ca^{2+} with a high capacity and moderate affinity, with a major putative Ca^{2+} binding motive occurring in the negatively charged residue-rich C-terminal tail (CSQ_{CTT}, residues 354-367). The CSQ_{CTT} is also thought to be responsible for CSQ's interaction with triadin and junctin.

To examine the role of the CSQ_{CTT} in Ca^{2+} binding and the formation of the quaternary complex, point mutations within this region of CSQ were produced. After subcloning rabbit skeletal wildtype (WT) CSQ into a pGEX-5X-1 vector (containing a glutathione-*S*-transferase tag), two mutants were generated by PCR, E354A and E354A/D356A. Mutants and WT rabbit skeletal CSQ PCR products were transformed and expressed in *Escherichia coli* BL21(DE3).

We found that the Ca^{2+} binding capacity of CSQ was reduced as the numbers of negatively charged residues within the CSQ_{CTT} are decreased. Compared with WT CSQ (100%), maximal Ca^{2+} binding capacity of E354A, and E354A/D356A was 92% and 87 %, respectively. WT and mutant CSQ interactions with triadin and junctin were analysed utilizing a glutathione-*S*-transferase affinity column. Initial results suggest that like WT CSQ, E354A and E354A/D356A CSQ interact with both triadin and junctin under physiological conditions (150 mM salt, 1 mM Ca^{2+}_{free}).

Our results suggest that the CSQ_{CTT} forms a major Ca^{2+} binding motive in rabbit skeletal muscle, and that both resides E354 and D356 are required both for Ca^{2+} binding and for stabilizing the Ca^{2+} binding motif, but are less important residues for associated protein binding.