Quantification of calsequestrin-1 and calsequestrin-2 in rat slow- and fast-twitch skeletal muscle

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Calsequestrin is a low-affinity, high-capacity Ca^{2+} binding protein. In muscle, there are two known calsequestrin isoforms, CSQ1 and CSQ2, which are found inside the sarcoplasmic reticulum (SR). CSQ1 is highly expressed in skeletal muscle and can bind a theoretical maximum of ~80 Ca^{2+} per molecule, though probably only ~50 Ca^{2+} per molecule at the maximum free [Ca^{2+}] likely attainable within the SR. CSQ2 is highly expressed in cardiac muscle and can bind up to ~35 Ca^{2+} per molecule at attainable SR [Ca^{2+}]. The relative amounts of CSQ1 and CSQ2 in different fibre types in skeletal muscle are unknown and in the present study we have assessed the relative and absolute amounts of CSQ1 and CSQ2 in rat skeletal muscle. Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of fluothane in accordance with the La Trobe University Ethics Committee and the *extensor digitalis longus* (EDL) and *soleus* (SOL) muscles were excised. For comparisons of EDL and SOL muscles, portions of muscles were homogenized and equal amounts of muscle were analysed by Western blotting using antibodies specific for either CSQ1 or CSQ2. The amount of CSQ1 was greater in EDL muscle homogenates, with SOL muscle homogenates having only about one-third as much CSQ1. There was very little CSQ2 in EDL muscle homogenates and this equated to ~2 % of the CSQ2 found in SOL muscle homogenates. Since SOL muscle is made up of both type I (~85%) and type IIa (~15%) fibres, we further examined the amounts of CSQ1 and CSQ2 in segments of individual fibres dissected from either EDL or SOL muscles. CSQ1 was expressed in all EDL fibres examined and on average SOL fibres expressed ~20% of this amount of CSQ1. CSQ2 was very rarely found in EDL fibres (1 fibre out of 48 fibres examined expressed a similar amount of CSQ2 as in SOL fibres), with there typically being no CSQ2 in EDL fibres and CSQ2 being seen in most SOL fibres. When a SOL fibre did not express CSQ2, it was likely that it was a type IIa fibre. To investigate this further, a number of SOL fibres were mechanically-skinned and individually mounted onto a force transducer and the force responses recorded in Sr^{2+}-based activation solutions at pSr^{2+}(= $-\log_{10}[Sr^{2+}]$) 5.3 and pSr^{2+} 4 (maximum force). It is known that type I fibres exhibit close to maximal force at pSr^{2+} 5.3, whereas type IIa fibres show little or no response at pSr^{2+} 5.3. Out of the 7 SOL fibres examined, 2 fibres showed no force response in pSr^{2+} 5.3, and hence were type IIa fibres. When the same fibres were examined by Western blotting, they were found to express only CSQ1, whereas the other 5 type I SOL fibres all expressed both CSQ1 and CSQ2. To determine the absolute amounts of CSQ1 and CSQ2 in fibres, known amounts of pure CSQ1 and CSQ2 were run on Western blots alongside individual fibres. The amount of CSQ1 was found to be ~100 μM in EDL fibres (n = 6) and ~30 μM in SOL fibres (n = 19) (Note: mM denotes mmol per litre total fibre volume). The amount of CSQ2 in SOL type I fibres was determined to be ~8 μM (n = 9). These concentrations of CSQ1 and CSQ2 fit well with the total SR Ca^{2+} capacities of fibres found previously, which were ~3.6 mM in EDL fibres, ~1.3 mM in SOL type I fibres and likely intermediate in SOL type IIa fibres. It also suggests that at the normal endogenous levels of SR Ca^{2+} (which is ~1.0 to 1.1 mM in all fibre types), in EDL fibres CSQ1 is loaded at only ~20% of the maximal attainable capacity, whereas in SOL type I fibres the CSQ1 and CSQ2 are likely to be loaded at closer to their maximal attainable capacities.

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