Properties and proteolytic activity of m-calpain in rat skeletal muscle

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m-Calpain is a ubiquitously expressed Ca^{2+}-dependent protease with diverse functionality in skeletal muscle including, but not limited to, roles in cell migration, fusion and membrane repair. It is believed to require >100 μM free [Ca^{2+}] for activation (Cong *et al.*, 1989; Elce *et al.*, 1997), although this requirement may be dependent on phosphorylation status and/or phospholipid binding (Goll *et al.*, 2003). Given the peak tetanic [Ca^{2+}] within skeletal muscle fibres normally reaches only 2-20μM (Baylor & Hollingworth, 2003), this raises the question of how m-calpain fulfills its role as a protease in skeletal muscle.

EDL and *soleus* muscles were dissected from male Long-Evans hooded rats sacrificed by anaesthetic overdose (4% v: v halothane) with approval of the La Trobe University Animal Ethics Committee. Western blotting was used to quantify the absolute amount of m-calpain by comparing known concentrations of pure rat recombinant m-calpain to whole skeletal muscle homogenates. The total amount of m-calpain was found to be ~1.0 μmol/kg muscle mass in predominantly slow-twitch soleus muscle and ~0.3 μmol/kg muscle mass in fast-twitch *extensor digitorum longus* muscle. Experiments in which mechanically skinned fibre segments were washed in aqueous solutions for set times showed that ~75% of the total m-calpain is freely diffusible within a quiescent fibre.

The proteolytic activity of m-calpain was also assessed using mechanically-skinned single fibres. Once skinned, the fibre segment was stretched to approximately twice its resting length so that no force-producing cross-bridges could be formed, with the resulting passive force being due to extension of titin, a large elastic sarcomeric protein that is a known substrate for m-calpain. Proteolysis of titin was gauged from the decline in passive force when a stretched fibre segment was exposed to 1 μM rat recombinant m-calpain over a range of elevated free [Ca^{2+}]. Proteolytic activity of m-calpain was observed even with free [Ca^{2+}] as low as 4 μM, and the rate of decline of passive force reached ~17% / min at 20 μM free Ca^{2+}. The rate of passive force decline was even greater at higher free [Ca^{2+}], reaching ~250% / min at 500 μM Ca^{2+}. In the presence of 20 μM free [Ca^{2+}], porcine-derived native m-calpain added exogenously at 1 μM resulted in proteolysis of titin at 9% / min, approximately half the rate observed with the rat recombinant mcalpain under the same conditions. Passive force decline over the physiological range of free [Ca^{2+}] was also measured both with and without ATP present in the solution and proteolytic activity was found to be the same in both cases. With both native and recombinant m-calpain, proteolytic activity could always be rapidly stopped by lowering the free [Ca^{2+}] to <10 nM. Furthermore, the proteolytic activity of mcalpain at 2 μM free Ca^{2+} was unchanged irrespective of whether or not the m-calpain had been activated at higher [Ca^{2+}] beforehand.

In conclusion, these findings demonstrate that m-calpain displays considerable proteolytic activity at physiological Ca^{2+} conditions occurring in muscle fibres. Furthermore, the findings distinguish its regulation from that of the other ubiquitous calpain, μ-calpain, which becomes more Ca^{2+}-sensitive following exposure to elevated [Ca^{2+}], suggestive that the ubiquitous calpains likely have quite different roles in skeletal muscle.

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