Characteristics of MMP-2 protein in rat skeletal muscle

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In skeletal muscle, matrix metalloproteinases (MMPs) are zinc and calcium-dependent enzymes that are implicated in maintaining integrity and homeostasis of the muscle extracellular matrix (ECM) (Vu & Werb, 2000). MMP-2, or gelatinase A, is a proteolytic enzyme. Previous studies mainly focused on its ECM functions, such as those involved in motility, differentiation, and regeneration of skeletal muscle fibres through processing extracellular substrates (Hadler-Olsen *et al.*, 2013). As for MMP-2 function in the intracellular milieu, in cardiac muscle one group has demonstrated that MMP-2 plays crucial intracellular roles in cardiomuscular damage caused by oxidative stress such as ischemia reperfusion injury (Wang *et al.*, 2002). Wang *et al.* also localized MMP2 in thin and thick filaments and targeted on contractile proteins such as troponin I in cardiomyocytes (Wang *et al.*, 2002). However, in skeletal muscle the intracellular function of MMP-2 remains obscure. In this study, we examined the quantification, localization, chemical activation and gelatinolytic activity of MMP-2 in resting rat skeletal muscles.

Soleus (SOL) muscle was excised from male Sprague Dawley rats (4-6 months old) which had been sacrificed by lethal overdose of isoflurane in accordance with the La Trobe University Ethics Committee. To determine absolute amounts of MMP-2 in skeletal muscle, known amounts of pro- and active MMP-2 pure protein were detected alongside rat muscle homogenates on western blotting. MMP-2 localisation inside skeletal muscle fibres was examined by comparing intact and skinned muscle fibres (where the sarcolemma had been peeled away) and also using Triton X-100 for membrane solubilisation. All of the intact skeletal muscle fibres, skinned fibres and Triton wash solutions were analysed by western blotting. APMA (4-aminophenylmercuric acetate), a chemical reagent, was used to activate pro-MMP-2 to its active form, which was detected by western blotting, with gelatinolytic activity detected by zymography.

The absolute amount of MMP-2 in SOL is ~10 nmol/kg wet weight muscle (n=7 rats). Most of MMP-2 seems to be located intracellularly, as the amount of MMP-2 detected in skinned fibres was similar to that in intact fibres (n=7 groups fibres from 3 rats). The results of MMP-2 localization examined in skinned muscle fibres indicated that about 60% of pro-MMP-2 was readily washed out, very little pro-MMP-2 was released by a subsequent Triton X-100 treatment, and about 40% of pro-MMP-2 remained in the fibre (n=7 group fibres from 3 rats), indicating that the majority of pro-MMP-2 is freely diffusible in cytosol, very little is associated with membranous compartments, and the remainder is bound within the fibre. Western blotting data revealed that after APMA treatment, most or all of the pro-MMP-2 (72 kDa) was activated to active MMP2 (64 kDa). In line with this, when gelatinolytic activity was examined with zymography, APMA treatment resulted in decreased activity of the pro-MMP-2 band and increased activity of the active MMP-2 band. This work highlights that MMP-2 localisation in resting healthy skeletal muscle is quite different from that generally assumed. Future work will ascertain these properties in diseased muscle.

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