Properties and amounts of heat shock proteins in skeletal muscle

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Heat shock proteins (HSP) are considered to be important in protecting and maintaining cellular homeostasis by binding to partially denatured proteins and acting as molecular chaperones. αB-crystallin, HSP25 and HSP72 are thought to protect key components in skeletal muscle such as SERCA pumps or actin. In the present study, we have measured the amounts, diffusibility and activation characteristics of these proteins in fast-twitch (*extensor digitorum longus*) and predominantly slow-twitch (*soleus*) fibres from rat skeletal muscle.

Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of isoflurane with approval of the La Trobe University Animal Ethics Committee, and the *extensor digitorum longus* (EDL) and *soleus* (SOL) muscles were excised. Muscles were either used for obtaining skinned fibre segments or homogenized and the entire muscle homogenate analysed using a quantitative Western blotting technique. To determine the absolute amounts of αB-crystallin, HSP25 and HSP72 in unstressed muscle, known amounts of pure HSP25, HSP72 and αB-crystallin were run on Western blots alongside or with the muscle homogenates samples (Murphy *et al.*, 2009) (see Table). From these measurements αB-crystallin is almost 29 times more expressed compared to HSP72 and ~13 time more than HSP25 in SOL muscle. These measurements of the absolute amounts of HSPs present give insight into the importance of αB-crystallin as well as the binding limitations and physiological function of Hsps in skeletal muscle.

To measure diffusibility, individual fibre segments were mechanically skinned, removing the surface membrane and allowing proteins to diffuse out of the fibre and into the bathing solution. The skinned fibre segment and the matched bathing solution were run on Western Blots and the diffusibility of αB-crystallin, HSP25 and HSP72 could be determined.

In unstressed muscle between 50 – 90% of HSP25, HSP72 and αB-crystallin appeared in the bathing solution within 10 min, indicating these proteins are broadly in rapid equilibrium with the cytoplasm in quiescent fibres. When a muscle was exposed to a potent oxidative stress of 10 mM H_2O_2 whilst pinned at room temperature, or bubbled with 95% O_2 at an elevated temperature (~31°C) for more than 1 hour, the diffusibility of αB-crystallin, HSP25 and HSP72 remained the same as that of an unstressed muscle.

Diffusibility was also investigated after a muscle was heated to 40°C for 30 min whilst pinned under paraffin oil. HSP25 and αB-crystallin became almost completely bound within the fibre, whereas HSP72 showed no change in diffusibility from that of an unstressed muscle. However when the temperature was raised to 45°C, HSP72 was no longer diffusible and became bound within the fibre. When fibre segments from a 40°C heated muscle were washed in the presence of 10 mM DTT, HSP25 and αB-crystallin remained bound and did not became diffusible, indicating the bonds between HSP25 or αB-crystallin and the target site was not a simple disulfide bond.

	Amount (μmol/kg muscle ± SEM)	
	EDL	SOL
HSP72	1.0 ± 0.0	4.3 ± 0.1
HSP25	2.8 ± 0.3	8.9 ± 1.3
αB-crystallin	3.3 ± 0.8	123.9 ± 16.7

Murphy RM, Larkins NT, Mollica JP, Beard NA, Lamb GD. (2009) *Journal of Physiology* **587(2):** 443-60.