Phosphorylation of small heat shock proteins in response to heat stress

N.T. Larkins, R.M. Murphy and G.D. Lamb, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

Small heat shock proteins (sHSP), HSP25 and αB-crystallin are thought to protect key components in skeletal muscle such as the myofibrils. Both sHSP can be reversibly phosphorylated and this may be important in regulating the mechanisms that can cause conformational changes to the structure of the protein and as a consequence modify its function. The present study examined the level of phosphorylation at Serine 59 on αB-crystallin (pSer59-αB-crystallin) and Serine 82 HSP25 (pSer82-HSP25) in control and heat-treated muscles. Additionally, this study also investigated whether the phosphorylation influences the diffusibility of αB-crystallin and HSP25 respectively, in particular whether or not the sHSPs are phosphorylated when they bind to sites within the muscle fibre.

Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of isoflurane (4% volume: volume) with approval of the La Trobe University Animal Ethics Committee, and the *soleus* muscle was excised. Muscles were either used as a control muscle (unheated) or heated at 40°C for 30 min under paraffin oil. The effect of the heat stress was investigated in unfractionated muscle homogenates as well as in individual fibre segments, both of which were analysed using a quantitative Western blotting technique. To determine the level of phosphorylation of HSP25 Ser82 and αB-crystallin Ser59, very small aliquots of whole muscle homogenate were run on a given gel, together with a range of amounts of a positive for phosphorylated HSP25 Ser82. Heating a *soleus* muscle to 40°C resulted in ~3 fold increase in the level of phosphorylation at the Ser82 site of HSP25 and ~12 fold increase in the level of phosphorylation at the Ser59 site of αB-crystallin (n=4). The extent of phosphorylation of HSP25 at Ser82 was further quantified using a calibration curve generated by plotting the density of the phosphorylated HSP25 Ser82 *versus* the density of HSP25 for each loaded sample. The slope of each curve was calculated and compared against the slope of the positive for HSP25 Ser82, which was assumed to be 100% phosphorylated and run on the same gel. From this, the mean percentage of phosphorylation at Ser82 on HSP25 was found to be ~9 % in the rested (control) muscle and to increase to ~35% after the heating (n=4). In summary, the above results demonstrate that the extent of phosphorylation of both sHSPs was increased when *soleus* muscles were given the 40°C heat treatment.

In order to investigate whether phosphorylation influences sHSP diffusibility, individual fibre segments were isolated from control and heat-treated muscles and mechanically skinned, whereby the surface membrane is removed, allowing proteins to diffuse out of the fibre and into the bathing solution. Each skinned fibre segment and corresponding bathing solution were run in adjacent lanes on Western blots, allowing assessment of the diffusibility of phosphorylated and total αB-crystallin, and phosphorylated and total HSP25. In unstressed muscle fibres, 22 ± 13% (mean ± S.D.) of the total HSP25 and 26 ± 13% of pSer82-HSP25 remained within the fibre after a 10 min bathing time (n=20). Similarly in those same fibres for αB-crystallin, 17 ± 12% of the total and 30 ± 10% of the phosphorylated pSer59 form remained within the fibre. Following 40°C heat treatment, 85 ± 12% and 90 ± 8% of the phosphorylated and total HSP25 remained within the fibre after the heat treatment (n=12). Likewise in those same fibres, 87 ± 8% and 85 ± 10% of the phosphorylated and total αB-crystallin remained within the fibre after the heat treatment. Linear regressions showed no significant relationship between the amounts of phosphorylated sHSP and total sHSP remaining in the fibre, in either the 40°C heat treated or unstressed conditions. These results indicate that phosphorylation at the Ser59 site of αB-crystallin, or at the Ser82 site of HSP25, do not noticeably influence the diffusibility of these sHSPs.