The effect of amylase treatment on the detection of glycogenin, glycogen debranching enzyme and glycogen phosphorylase proteins in rat fast-twitch skeletal muscle

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Glycogen is an important energy source within contracting skeletal muscle. It has a simple but strictly ordered structure comprising α-1,4-glycosidic bonds with α-1,6-glycosidic bonds at branch points. Glycogen granules are generated on the core protein, glycogenin. Glycogenin is a 37 kDa protein which is described as a self-glycosylating protein that regulates the formation of glycogen. Glycogen debranching enzyme (GDE) and glycogen phosphorylase (GP) are necessary for the energy providing steps of glycogen breakdown. Amylase is an enzyme which breaks down α-1,4-glycosidic bonds of glycogen. In order to determine whether amylase treatment can impact on the amount of the glycogen related proteins that can be detected in skeletal muscle we used western blotting to examine the amount of glycogenin, GDE and GP proteins following incubation with or without amylase. Additionally, we examined glycogenin protein following amylase treatment at varying temperatures.

Male Long-Evans hooded rats (6-8 months old) were sacrificed using a lethal overdose of isofluorane in accordance with the La Trobe University Animal Ethics Committee and the *extensor digitalis longus* (EDL) muscles were excised. Skeletal muscle homogenates were prepared from quiescent fast-twitch EDL rat skeletal muscle and either treated with amylase immediately or 3 h following the removal of the muscle from the animal. In all cases, muscle was kept at room temperature (RT) under paraffin oil. Amylase treatments (0.14 μg/μl) were for 30 min and conducted at 37°C, 30°C or RT. Control muscles were those not treated with amylase.

Very little 37 kDa glycogenin protein was seen in the absence of amylase, however amylase treatment at all temperatures resulted in being able to observe glycogenin, with no difference between the temperatures examined (P>0.05, n=4). Under control conditions, a further band at 55 kDa was observed with the glycogenin antibody and this band disappeared following amylase treatment, suggesting that the 55 kDa is a form of glycogenin, likely glucosylated. There was no difference in the amounts of GDE and GP proteins with or without amylase (P>0.05, n=4-5). Interestingly, there was no difference in the amount of glycogenin, GDE or GP proteins detected in freshly dissected muscle, or muscle that had been kept at RT for 3 h (P>0.05, n=4-5).

The above results indicate that amylase treatment is necessary when glycogenin protein is being examined, however not necessary for examination of GDE or GP. This is likely due to the latter enzymes not being associated with the inner core of glycogen, which is where glycogenin reportedly resides. It is interesting to note, that GDE can be fully released from the glycogen granule without the granule being degraded, suggesting that it might associate with the more outer tiers of glucose moieties.