Technique for extraction of proteins for enzymatic measurements from sheep muscle and ram spermatozoa

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Keywords: Extraction; protein; muscle; spermatozoa; enzymes.

INTRODUCTION

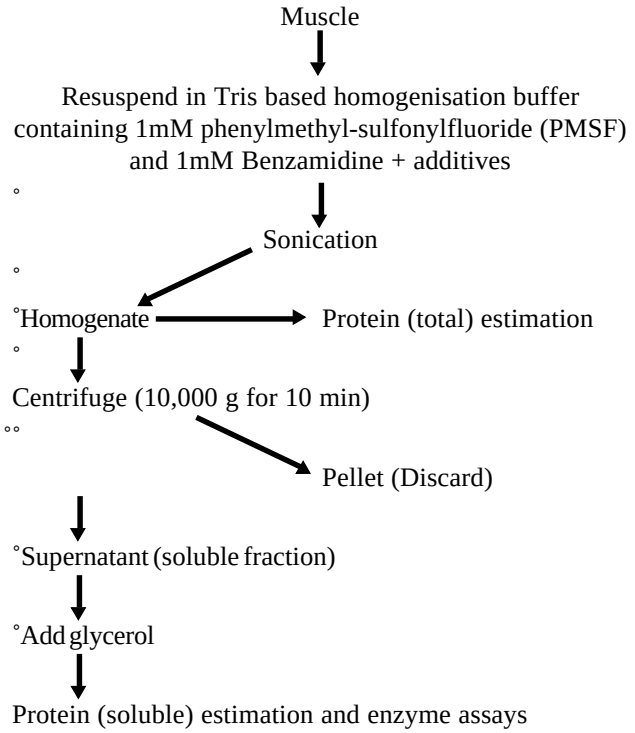
A method for the preparation of enzyme active representative homogenates of muscle is described. This method was also evaluated for spermatozoa and it was observed that the protocol needs to be adapted for various tissue types.

METHODS

Homogenate preparation: Muscle was rinsed in isotonic salt solution containing 1mM benzamidine. It was finely minced with a scalpel blade after trimming visible fat and the mince was processed as outlined in Fig. 1.

Biochemical Measurements: Protein was determined by modified Lowry's assay (1). The activities of lactate dehydrogenase (LDH; 2), nicotinamide adenine dinucleotide phosphate- isocitrate dehydrogenase (NADP-ICDH; 1) and nicotinamide adenine dinucleotide- isocitrate dehydrogenase (NAD-ICDH; 3) were determined spectrophotometrically.

FIG. 1. Preparation of representative homogenates.



RESULTS AND DISCUSSION

The total protein content of the muscle homogenate was used to determine the recovery of proteins in the soluble fraction. The amount of protein in soluble fraction was influenced by the composition of the homogenisation buffer and the duration of sonication. The amount recovered in the presence of 1% sodium dodecyl sulfate (SDS) was ~ 2.5 times greater than in its absence. SDS denatures enzyme proteins and is therefore not suitable for enzymatic studies (1). The hydrophobic and ionic properties of the SDS were mimicked by including non-ionic detergent (e.g. Triton X-100) and/or ionic salt (e.g. sodium citrate) in the homogenisation buffer. This strategy was successful, as the soluble fraction now contained >65% of maximum soluble protein obtained with 1% SDS and the activities of LDH and NADP-ICDH were retained (Table 1). Cold-inactivation of enzyme was observed with NAD-ICDH (3). Addition of glycerol to the soluble fraction (Fig 1) improved the activities of NAD and NADP linked ICDH. The above principles were also applicable to sperm, but substantial differences were found between sperm and muscle in the optimum conditions for processing the homogenates (data not shown).

CONCLUSIONS

It is possible to make representative enzyme active homogenates of biological tissues by using Triton X-100, glycerol and sodium citrate. The component amounts, along with the physical force required for tissue disruption, needs to be optimised for each tissue type and enzymes of interest.

REFERENCES

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TABLE 1: Protein and enzymatic activities in muscle homogenates*

Treatments	Soluble Protein mg /g tissue	LDH Units /g x 10^3	NADP-ICDH Units /g x 10^3
Buffer control	50.9 ± 0.38	186.0 ± 9.46	89.3 ± 9.47
+ SDS	122.6 ± 3.80	0.0	0.0
+Triton X-100 +Citrate	82.1 ± 1.98	182.0 ± 13.25	97.7 ± 13.42

Homogenates were prepared by 3 x 10 second sonic bursts and did not contain glycerol.