**Comparison of protein extraction methods in tendon for mass spectrometry analysis**

Y. A. Kharaz1, D. Zamboulis1, Karen Sander, E. Comerford1, P. Clegg1, M. J. Peffers1

1Comparative Musculoskeletal Research Group, Department of Musculoskeletal Biology, Institute of Ageing and Chronic Disease, Leahurst, University of Liverpool, CH64 7TE, UK.

Introduction

Tendons play a fundamental role in musculoskeletal system and locomotion by transferring forces generated from muscles to the skeleton. Tendon injuries can occur due to sports related incidents, as result of trauma to overuse or during disease or ageing. Using proteomic techniques tendon protein profiles and pattern could be defined under different conditions. However, little attempt has been made to identify the optimum protein extraction technique to increase the coverage of tendon proteome. The aim of this study was to optimise tendon sample preparations technique for mass spectrometry analysis, which will be valuable to future tendon research studies.

Method

Tendon samples were collected from equine superficial digital extensor tendons (SDFT) that were between 11-13 years old. All samples were deglycolysated using chondroitinase (ABC) for 6 hour at 37°C. The samples were divided in four parts and subjected to 4 M guanidine- HCL, 0.1% rapigest, combination of 4M guanidine-HCL and 0.1% rapigest or 4M guanidine-HCL followed by 0.1% rapigest extraction. Soluble extracts were reduced, alkylated, trypsin digested and desalted. The proteolysed products were identified using LC-MS/MS analysis using an Ultimate 3000 Nano system coupled to Q- Exactive. Proteins were identified using PEAK®7 (version 7, Bioinformatics Solutions, Canada) against the equine protein sequence database. Progenesis LC-MS software (Nonlinear Dynamic) was used for label-free quantification. Proteins were classified using protein analysis through evolutionary relationships (PANTHER) classification system.

Results

A total of 230 proteins were identified using guanidine extraction, 116 proteins with rapigest extraction, 143 proteins using combination of guanidine and rapigest extraction and 206 proteins with guanidine followed by rapigest extraction method. The least variability in protein recovery and in number of identified peptides and proteins was found in guanidine followed by rapigest method. In addition guanidine followed by rapigest extraction method also had the lowest variability in miss cleavage and scan rate compared to the other extraction methods.

Label free comparison identified significantly more cellular associated proteins in guanidine method, whilst extractions with rapigest resulted in significantly more extracellular proteins such as collagen type I, III, IV and V. This data suggest that rapigest mainly functions to solubilise the insoluble collagen proteins, however it may not be the best method in terms of extracting the cellular proteins. Principle component analysis demonstrated that samples with guanidine followed by rapigest extraction method were more grouped together, which validates that this method has the least variability in comparison to the other extraction methods.

Significance: The ability to produce robust and effective guidelines in sample preparation will be invaluable for future studies in tendon research. This study identified the optimum method with least variability for protein extraction to increase the coverage of the tendon proteome.