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Proteome profile of functional mitochondria from human skeletal muscle using one-dimensional gel electrophoresis and HPLC-ESI-MS/MS

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ABSTRACT

Mitochondria can be isolated from skeletal muscle in a manner that preserves tightly coupled bioenergetic function *in vitro*. The purpose of this study was to characterize the composition of such preparations using a proteomics approach. Mitochondria isolated from human *vastus lateralis* biopsies were functional as evidenced by their response to carbohydrate and fat-derived fuels. Using one-dimensional gel electrophoresis and HPLC-ESI-MS/MS, 823 unique proteins were detected, and 487 of these were assigned to the mitochondrion, including the newly characterized *SIRT5*, *MitoNEET* and *RDH13*. Proteins detected included 9 of the 13 mitochondrial DNA-encoded proteins and 86 of 104 electron transport chain (ETC) and ETC-related proteins. In addition, 59 of 78 proteins of the 55S mitoribosome, several TIM and TOM proteins and cell death proteins were present. This study presents an efficient method for future qualitative assessments of proteins from functional isolated mitochondria from small samples of healthy and diseased skeletal muscle.

1. Introduction

Amitochondriate cells, such as erythrocytes, are viable, but are highly dependent on glycolysis [1]. The symbiosis of the mitochondrion with the anaerobic eukaryotic cell has provided the host with a number of the organelle's functions, the most important being aerobic metabolism which provides ATP as a source of chemical energy. A proportion of these mitochondrial functions are ubiquitous to all tissues: ion and metabolite transport, fuel oxidation, ATP production, regulation of oxidative stress, heme biosynthesis, cell survival and death [2]. However, tissue-specific functions (urea metabolism in liver, γ-aminobutyric acid metabolism in brain, and dominance of oxidative phosphorylation in heart) of mitochondria correlate with increased expression of proteins that are involved in the upregulated pathway [3–5] although the consequences of post-

Abbreviations: FA, formic acid; OXPHOS, oxidative phosphorylation; GO, Gene Ontology annotation; FFA, free fatty acid; TIM, inner membrane translocase; TOM, outer membrane translocase; MRP, mitochondrial ribosomal protein.

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translational modifications on the activity of mitochondrial proteins cannot be disregarded, particularly OXPHOS subunits, which all can be phosphorylated [6]. The vast majority, about 99%, of mitochondrial proteins are coded by the nuclear genome (13 are coded by mitochondrial DNA (mtDNA)), with estimates for the mammalian mitoproteome ranging from 1000–4200 proteins [7–9], and estimate of the human mitochondrial proteome nearing 1500 proteins [7].

Even though there are considerable challenges in the solubilization of hydrophobic peripheral and integral membrane subunits, which characterizes mitochondrial proteins (ex: electron transport chain (ETC) subunits) [10], interest in the mammalian mitochondrial proteome is flourishing. This stems from an increased acknowledgment of the involvement of this organelle in disease, whereby one or more of its functions are compromised by mutations in mtDNA or nuclear-encoded mitochondrial genes, changes in the metabolic milieu, allosteric regulators and cell signaling. Mitochondrial dysfunction is thought to be implicated in the development and/or progression of neurodegenerative diseases, such as Parkinson's and Alzheimer's disease, cancer, aging, encephalomyopathies and cardiomyopathies (for review see [11]). The role of mitochondrial dysfunction in type 2 diabetes mellitus remains a matter for investigation [12–15]. Several attempts to define the mammalian mitochondrial proteome have proven to be successful [4,5,16–20], although few studies have focused on human tissue [7,21–23], and none on fully functional human mitochondria. To date, the largest human mitochondria proteome description stems from Taylor et al. [7] and Gaucher et al. [23], who identified 615 and 680 proteins, respectively, from a single human heart mitochondrial preparation.

Isolation of mitochondria from skeletal muscle that can be used for functional analysis *in vitro* is useful for studies of mechanisms regulating normal physiology as well as pathophysiological states [13,22,24–29]. The present study was undertaken to characterize the proteome of preparations of highly coupled, fully functional, human skeletal muscle mitochondria. Analysis of functional human skeletal muscle mitochondria by HPLC-ESI-MS/MS identified a total of 823 proteins: 487 known mitochondria proteins (388 assigned by Gene Ontology (GO) to mitochondria and 99 manually annotated to mitochondria using published experimental evidence) resulting in a list of 336 mitochondria-associated proteins. We demonstrate, for the first time, that both functional and proteomic assessments can be done on metabolically-active mitochondria isolated from a small (100 mg) sample of human skeletal muscle. These data hold promise for global analysis of functional and quantitative changes in the mitochondrial proteome of patients affected by disorders involving skeletal muscle and potentially assist in interpreting functional assays using these mitochondria.

2. Materials and methods

2.1. Subjects

A percutaneous needle muscle biopsy was taken under local anesthesia from the *vastus lateralis* muscle of healthy volunteers (males, 40±10 years old (range: 26–59), body mass index: 24±1 kg/m^2; percent body fat: 21±2%) with normal glucose tolerance, with no family history of type 2 diabetes and not taking medication known to affect glucose metabolism. The muscle biopsy was taken after the subject had consumed nothing but water overnight, and while the subject rested in a supine position. The subject had been informed to maintain a normal diet and not to engage in vigorous physical activity for 3 days prior to the study. The purpose, nature and potential risks of the study were explained to the participant, and written consent was obtained before participation. The protocol was approved by the Institutional Review Board of Arizona State University.

2.2. Small-scale isolation of mitochondria

Following the biopsy, the muscle specimen was trimmed of visible fat, immediately weighed, minced and subjected to a protease (Type XXIV, Sigma Chemical Co., St-Louis, MO) digestion for 7 min. Mitochondria were isolated by a modified method of Makinen and Lee [30] using a protease (Sigma, P-8038) treatment to liberate both subsarcolemmal and intermyofibrillar mitochondria. Briefly, the muscle biopsy (~100 mg) was treated with protease for 7 min, followed by a glass to glass homogenization using a Con-Torque homogenizer (10 passes). The crude homogenate was centrifuged at 800 × *g* (10 min, 4 °C) to rid the homogenate of cellular debris and nuclei. The supernatant was then centrifuged at 14,000 × *g* (10 min, 4 °C). The pellet, containing the mitochondrial fraction, was resuspended in SOLUTION II (in mM: 100 KCl, 40 Tris–HCl, 10 Tris–Base, 1 $MgCl_2$, 0.1 EDTA, 0.2 ATP, 1.5% FFA free BSA, pH 7.5 at 4 °C). Following centrifugation at 7000 × *g* (10 min, 4 °C), the pellet was resuspended in SOLUTION III (in mM: 100 KCl, 40 Tris–HCl, 10 Tris–Base, 1 $MgCl_2$, 0.1 EDTA, 0.2 ATP, pH 7.5 at 4 °C). A final centrifugation at 3500 × *g* (10 min, 4 °C) yielded a mitochondrial pellet in which minimal endogeneous cytosolic fuel substrates remain. The pellet was resuspended in 100 μl mannitol sucrose buffer (in mM: 220 mannitol, 70 sucrose, 10 Tris–HCl, 1 EGTA) and kept on ice for the duration of the respiration studies.

2.3. Immunoblotting

Twenty micrograms of mitochondrial lysate and whole muscle lysate (from the same individual) were resolved by 12.5% SDS-PAGE, transferred to a nitrocellulose membrane and analyzed by western blot. Blot was blocked with 5% non-fat milk in 1× Tris-buffered saline (Bio-Rad, Hercules, CA) and 0.2% Tween 20, incubated with primary antibody overnight at 4 °C (in 5% non-fat milk, ATP synthase beta subunit; 1:2500, Molecular Probes, Invitrogen, Carlsbad, CA), and then incubated with a secondary horseradish-conjugated antibody for 1 h at room temperature (anti-mouse IgG, Santa Cruz, CA). Immunoreactive bands were visualized by enhanced chemiluminescence (Western Lightning Plus, Perkin-Elmer, CT).

2.4. Mitochondrial function assessment

To ensure that the mitochondrial preparations were of high quality, mitochondrial coupling, represented by the ADP/O ratio (ADP/O = 125 nmol ADP/(natom O_2 consumed during state