Redox Biology 5 (2015) 267–274

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research Paper

Oxidative proteome alterations during skeletal muscle ageing

Sofia Lourenço dos Santos [a,b,c], Martin A. Baraibar [a,b,c], Staffan Lundberg [d], Orvar Eeg-Olofsson [d], Lars Larsson [e,f], Bertrand Friguet [a,b,c,*]

[a] Sorbonne Universités, UPMC Univ Paris 06, UMR 8256, Biological Adaptation and Ageing-IBPS, Paris F-75005, France
[b] CNRS UMR-8256, Paris F-75005, France
[c] Inserm U1164, Paris F-75005, France
[d] Department of Women's and Children's Health, Uppsala University, Uppsala SE-751 82, Sweden
[e] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm SE-171 77, Sweden
[f] Department of Clinical Neuroscience, Clinical Neurophysiology, Karolinska Institutet, Stockholm SE-171 77, Sweden

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form
21 May 2015
Accepted 29 May 2015
Available online 3 June 2015

Keywords:
Ageing
Human *rectus abdominis* skeletal muscle
Oxidative stress
Protein carbonylation
Proteomics

ABSTRACT

Sarcopenia corresponds to the degenerative loss of skeletal muscle mass, quality, and strength associated with ageing and leads to a progressive impairment of mobility and quality of life. However, the cellular and molecular mechanisms involved in this process are not completely understood. A hallmark of cellular and tissular ageing is the accumulation of oxidatively modified (carbonylated) proteins, leading to a decreased quality of the cellular proteome that could directly impact on normal cellular functions. Although increased oxidative stress has been reported during skeletal muscle ageing, the oxidized protein targets, also referred as to the 'oxi-proteome' or 'carbonylome', have not been characterized yet. To better understand the mechanisms by which these damaged proteins build up and potentially affect muscle function, proteins targeted by these modifications have been identified in human *rectus abdominis* muscle obtained from young and old healthy donors using a bi-dimensional gel electrophoresis-based proteomic approach coupled with immunodetection of carbonylated proteins. Among evidenced protein spots, 17 were found as increased carbonylated in biopsies from old donors comparing to young counterparts. These proteins are involved in key cellular functions such as cellular morphology and transport, muscle contraction and energy metabolism. Importantly, impairment of these pathways has been described in skeletal muscle during ageing. Functional decline of these proteins due to irreversible oxidation may therefore impact directly on the above-mentioned pathways, hence contributing to the generation of the sarcopenic phenotype.

Introduction

Skeletal muscles require dynamic changes in energy supply and oxygen flux for contraction, making it prone to reactive oxygen species (ROS)-mediated damage as a result of an increase in electron flux and leakage from the mitochondrial respiratory chain. Although physiological concentrations of ROS can function as signaling molecules that regulate proliferation, growth, differentiation, and apoptosis [1], when ROS levels overcome the capacity of cellular antioxidant systems, they become toxic, introducing oxidative modifications on cellular macromolecules such as nucleic acids, lipids and, in particular, proteins, inflicting alterations to normal cellular functions. In skeletal muscle, oxidative stress state has negative consequences on action-potential conduction, excitation–contraction coupling, satellite cell differentiation, muscle contraction and mitochondrial respiration [2].

Skeletal muscle ageing is associated with the gradual degenerative loss of skeletal muscle mass, quality, and strength, a condition known as sarcopenia. Oxidative stress contribute at least in part to muscle atrophy [3–6], and previous studies have addressed the extent of protein oxidative damage in the development of sarcopenia in mammalian models [7–10]. Although it is believed that oxidative stress contributes to skeletal muscle dysfunction

Abbreviations: 1D, one-dimensional; 2D, bi-dimensional; ATP, adenosine triphosphate; CK, creatine kinase; DNP, 2–4-dinitrophenylhydrazone; DNPH, 2,4-dinitrophenylhydrazine; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GPD1, Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic; HSP70, Heat shock 70 kDa protein; IEF, Isoelectric focusing; MM-CK, muscle-type creatine kinase; MS, mass spectrometry; MyBPC, myosin-binding protein C; PCr–CK, phosphocreatine–creatine kinase; RA, *rectus abdominis*; RMI, relative modification index; ROS, reactive oxygen species; ZASP, LIM domain-binding protein 3

* Corresponding author at: Sorbonne Universités, UPMC Univ Paris 06, UMR 8256, Biological adaptation and ageing-IBPS, F-75005 Paris, France.
E-mail address: bertrand.friguet@upmc.fr (B. Friguet).

http://dx.doi.org/10.1016/j.redox.2015.05.006

through macromolecular damage, the molecular mechanisms remain elusive. Protein post-translational modifications induced by ROS are important features of oxidative stress [11]. Among them, protein carbonyl content is by far the most commonly used marker of protein oxidation [12,13]. In fact, protein carbonylation occurs when proteins directly react with ROS, leading to the formation carbonyl groups (aldehydes and ketones) for instance on such amino acids side chains as arginine, lysine, threonine and proline. Introduction of carbonyl groups on proteins can also occur through the reaction of aldehydic products of lipid peroxidation and of dicarbonyl compounds upon glycation and glycoxidation. Since it often leads to a loss of protein function and an increased thermosensitivity or hydrophobicity of the targeted protein [14,15], protein carbonylation has been considered as an indicator of protein damage.

In human skeletal muscle, preliminary studies on *vastus lateralis* and external intercostal muscles have shown increased accumulation of protein carbonyls during ageing [16–19]. However, in most cases, the protein targets of these oxidative damages and their functional consequences have not been identified. Indeed, this is an essential step to get a complete view of protein oxidative modifications and to understand the mechanisms by which these oxidized proteins potentially contribute to muscle weakness and dysfunction during ageing.

Therefore, proteomic studies, including the analysis of protein abundance as well as protein carbonylation are expected to provide valuable information to unravel the key molecular pathways implicated. In fact, proteomics and in particular bi-dimensional (2D) gels represent appropriate tools for the detection and identification of specific carbonylated proteins in a complex mixture [2,13,20]. The identification of such oxidatively modified proteins (i.e. the oxi-proteome components), can give some insights into the mechanisms by which these damaged proteins accumulate and potentially affect cellular and/or tissular function during ageing or in disease conditions [21]. In this paper, the occurrence and characterization of carbonylated proteins was studied in human *rectus abdominis* muscle obtained from young and old healthy donors. Although no significant differences in global protein carbonylation was observed at the proteome level, we have used 2D gel electrophoresis based proteomic approaches to improve the resolution of individual proteins for the quantitative analysis of their carbonylation status and further identification of these major skeletal muscle proteins that are targeted by oxidative damage during human *rectus abdominis* skeletal muscle ageing.

Material and methods

Human biopsies

Human *rectus abdominis* muscle biopsies were obtained during surgery. Each biopsy used has the written consent of the volunteer donor. A total of 22 human muscle biopsies were used: 11 from healthy men individuals between 0 to 12 years old (named young samples) and 11 from healthy men individuals between 52 and 76 years old (named old samples) (Table 1). All muscle biopsies had an initial wet weight between 15 and 24 mg (Table 1). The study was approved by the Ethical Committee at the Uppsala University Hospital.

Protein extraction for proteomics analyses

Proteins extracts from skeletal muscle biopsies were obtained by physical disruption of the sample biopsies using a ULTRA-TURRAX® T25 (IKA®) at 4 °C in a lysis buffer containing 10 mM Tris–HCl (pH 7.4), 8 M urea, 2 M thiourea, 4% CHAPS and 20 mM DTT. After incubation on ice for 20 min, soluble proteins were recovered after clarification by centrifugation for 40 min at 21,000 g. Proteins were further precipitated using the 2D clean-up kit (GE Healthcare) and the resulting pellet was re-suspended into the same lysis buffer. Protein concentrations were determined by the Bradford Method [22] using the Bio-Rad Protein Kit Assay (Bio-Rad).

Table 1
Characteristics of the samples biopsies.

Sample no	Donor age (years)	Wet weight (mg)	Protein recovery ratio (w/w in %)[a]
1	0	24.00	7.0
2	1	20.80	4.3
3	5	20.00	7.6
4	9	17.00	9.3
5	6.5	15.00	7.1
6	10.75	21.90	7.6
7	12	22.00	9.1
8	7.1	22.00	9.7
9	76	20.30	9.5
10	70	18.00	8.3
11	74	19.10	7.8
12	66	19.60	8.0
13	74	19.20	8.4
14	65	18.66	5.2
15	65	18.78	6.6
16	71	24.00	10.8
17	12	20.00	15.6
18	3	20.50	3.6
19	9	20.38	11.0
20	56	20.47	7.4
21	61	20.53	17.1
22	52	20.13	27.2

[a] Protein recovery ratio corresponds to the protein amount in mass / biopsy mass.

Protein carbonyl immunodetection after derivatization with DNPH

Carbonylated proteins were derivatized with 2,4-dinitrophenylhydrazine (DNPH) to form 2–4-dinitrophenylhydrazone (DNP) proteins adducts [23]. For total carbonyl quantification, equal quantities of proteins were loaded and separated by SDS-PAGE 12% (v/v). Chemicals for SDS-PAGE were purchased from Bio-Rad. All other chemicals were of analytical grade and obtained from Sigma-Aldrich. For the detection of carbonylated proteins, gels were electrotransferred onto Hybond-C nitrocellulose membranes (GE Healthcare) and incubated with anti-DNP antibodies (1:5000, Sigma-Aldrich). Carbonylated proteins were revealed by a fluorescent anti-rabbit IgG 800CW (1:15,000) polyclonal antibody (LI-COR). Densitometry analyses were performed using NIH ImageJ software and the data are expressed as % volume in pixels.

For 2D gel electrophoresis, derivatization of proteins carbonyls was achieved on IPG strips after isoelectric focusing (IEF), with a 10 mM DNPH, 2 M HCl solution at room temperature (RT) as described previously [24]. 500 μg of protein were diluted on a rehydratation buffer (7 M urea, 2 M thiourea, 1% Amberlite, 4% CHAPS, 1.2% Destreak (GE Healthcare), 0.5% Pharmalyte pH 3–10 (GE Healthcare)) and loaded into 13 cm IPG strips pH 3–10 NL (GE Healthcare). Gel rehydration of the IPG strips was done overnight at RT in an Immobiline DryStrip rewelling tray. IEF was performed using an Ettan™ IPGphor™ 3 Isoelectric Focusing System (GE Healthcare) at 20 °C using the following electrical profile: step, 150 V for 11 h; grad, 1000 V for 3 h; grad, 8000 V for 2 h; step, 8000 V for 1 h. Neutralization step after derivatization with DNPH was performed with 2 M Trizma-Base containing 30% of glycerol (GE Healthcare). Before SDS-PAGE, all IPG strips were equilibrated with a 6 M urea, 10% SDS and 30% Glycerol (GE Healthcare), 0.5 M Tris–HCl (pH 8.8) solution for 2 steps of 15 min: the first one with