Available online at www.sciencedirect.com

SCIENCE DIRECT

Free Radical Biology & Medicine 38 (2005) 175–188

www.elsevier.com/locate/freeradbiomed

Serial Review: The Powerhouse Takes Control of the Cell:
The Role of Mitochondria in Signal Transduction
Serial Review Editor: Victor Darley-Usmar

Mitochondrial proteomics in free radical research ☆

Shannon M. Bailey[a,*], Aimee Landar[b], Victor Darley-Usmar[b]

[a]*Department of Environmental Health Sciences, School of Public Health, University of Alabama at Birmingham, 1530 3rd Avenue South, Ryals Building, Room 623, Birmingham, AL 35294, USA*
[b]*Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA*

Received 23 August 2004; accepted 6 October 2004
Available online 3 November 2004

Abstract

The importance of mitochondrial dysfunction in numerous diseases has long been appreciated. The impact of oxidative and nitrosative stress on mitochondrial function is complex; however, recent progress in the field using proteomics technologies has begun to shed light on the molecular defects responsible for mitochondrial and cellular dysfunction. This review focuses on the state-of-the-art technologies being used and current research endeavors in the field of mitochondrial proteomics with emphasis on those advancements being made in the field of free radical biology to identify the importance of alterations to the mitochondrial proteome in the development of disease.

Keywords: Mitochondria; Proteomics; Posttranslational modifications; Free radical; Oxidative and nitrosative stress; Blue native gel electrophoresis; Alcohol

Contents

Abbreviations: ALDH, aldehyde dehydrogenase; BIAM, biotinylated iodoacetamide; BN-PAGE, blue native polyacrylamide gel electrophoresis; 1(2)-D, one (two)-dimensional; DNPH, 2,4-dinitrophenylhydrazine; 4-HNE, 4-hydroxynonenal; IBTP, (4, iodobutyl) triphenylphosphonium; IEF, isoelectric focusing; LC-MS/MS, liquid chromatography tandem mass spectrometry; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MnSOD, manganese superoxide dismutase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; STZ, streptozotocin.

☆ This article is part of a series of reviews on "The Powerhouse Takes Control of the Cell: The Role of Mitochondria in Signal Transduction." The full list of papers may be found on the home page of the journal.

* Corresponding author. Fax: +1 205 975 6341.
E-mail address: sbailey@uab.edu (S.M. Bailey).

0891-5849/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2004.10.011

Introduction

Mitochondria are recognized as having fundamental roles in many cellular processes including energy metabolism via functioning of the oxidative phosphorylation system, the Kreb's cycle, and β-oxidation of free fatty acids. Mitochondria also house steps critical for heme biosynthesis, ketone body formation, and urea degradation. Mammalian mitochondria are unique in that they contain several copies of their own genome, a circular double-stranded DNA molecule (mtDNA), which encodes 13 essential polypeptides that constitute complexes I, III, IV, and V of the oxidative phosphorylation system, 2 large rRNAs, and 22 tRNAs. As such, the majority of oxidative phosphorylation system proteins and other mitochondrial proteins are encoded by the nuclear genome and imported into mitochondria through a highly regulated system of inner and outer transmembrane import complexes and chaperones [1]. Recently though, interest in mitochondrial physiology has been renewed due to growing evidence implicating mitochondrial involvement in cellular signaling pathways through modulation of intracellular calcium stores, production of reactive species, and the interaction of nitric oxide ($^{\bullet}$NO) on mitochondrial functions such as respiration and biogenesis. Given these important roles of the mitochondrion, it is not surprising that alterations in mitochondrial function are considered to play key roles in development of human disease.

As a source for the formation and target of modifications mediated by reactive oxygen and nitrogen species (ROS/RNS), the mitochondrion is recognized as a site critical in cellular responses to oxidative and nitrosative stress. While numerous mechanisms of oxidant-induced injury have been identified, the impact of oxidants on the overall content of mitochondrial proteins, the "mitochondrial proteome," is only now being investigated. It should also be mentioned that the mitochondrial proteome is not only determined simply by protein levels but also by posttranslational modifications to proteins, which might be significant, particularly in cells undergoing an oxidative or nitrosative stress. Fortunately, several significant developments have been made in the field of proteomics to facilitate the determination of alterations to the mitochondrial proteome under conditions of stress. The purpose of this review is to present an overview of the latest research endeavors in the field of mitochondrial proteomics, with emphasis on those advancements being made in the field of free radical biology, to identify the role of posttranslational modification of mitochondrial proteins in development of disease.

Proteomics approaches applied to mitochondria

Mitochondria have frequently been at the forefront of developing technologies and proteomics is not an exception. In this section we review current approaches but discuss in detail the more "routine" approaches accessible to most research laboratories as outlined in Fig. 1. Two-dimensional (2-D) gel electrophoresis is currently the primary tool used for the separation of proteins for proteomic analysis. This technique combines isoelectric focusing (IEF) in the first dimension where proteins are separated according to differences in net charge (isoelectric point), followed by the separation of proteins based on molecular mass in the second dimension using standard SDS–PAGE (Fig. 1A). This technique is capable of resolving hundreds to thousands of proteins in a complex biological sample on a single 2-D gel, which can then be identified by mass spectrometry. Presently, the mass spectrometry method of choice for identification of proteins separated by 2-D gels is matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry due to its high-throughput nature and relatively low cost. Briefly, protein "spots" are excised from gels and digested with trypsin into peptide fragments, which are then spotted onto a target plate for identification by MALDI-TOF. The peptide masses obtained are then entered into a search engine, e.g., "MASCOT," and a database such as NCBI or SwissProt is queried to match the tryptic peptide fingerprint to a parent polypeptide. These search engines calculate a statistical likelihood that the list of submitted peptides matches that predicted for a protein present in the database. Thus, peptide mass fingerprinting can provide very good preliminary data in identifying proteins of interest. Further confirmation of the identity and amino acid sequence of the peptide is accomplished using more sophisticated mass spectrometry techniques like tandem mass spectrometry. For further information regarding the various mass spectrometry techniques used in proteomics research, please refer to several excellent review articles listed here [2–6].

While conventional 2-D gel electrophoresis is well-suited to identify changes in the levels of the more hydrophilic proteins of the mitochondrion, e.g., matrix proteins, the analysis of membrane proteins is hampered by the fact that many of these proteins precipitate at the basic end of the IEF gels and are thus poorly resolved on conventional 2-D gels [7–9]. To solve this problem alternate protein separation techniques have been used to elucidate the mitochondrial proteome. One approach is the sucrose density gradient fractionation technique developed by Capaldi and colleagues [9–11]. In this technique, mitochondrial extracts are loaded onto a 10–35% step fraction sucrose gradient and centrifuged overnight, and fractions are collected from the