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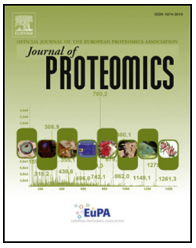

Synaptic mitochondria: A brain mitochondria cluster with a specific proteome

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ABSTRACT

The synapse is a particularly important compartment of neurons. To reveal its molecular characteristics we isolated whole brain synaptic (sMito) and non-synaptic mitochondria (nsMito) from the mouse brain with purity validated by electron microscopy and fluorescence activated cell analysis and sorting. Two-dimensional differential gel electrophoresis and mass spectrometry based proteomics revealed 22 proteins with significantly higher and 34 proteins with significantly lower levels in sMito compared to nsMito. Expression differences in some oxidative stress related proteins, such as superoxide dismutase [Mn] (Sod2) and complement component 1Q subcomponent-binding protein (C1qbp), as well as some tricarboxylic acid cycle proteins, including isocitrate dehydrogenase subunit alpha (Idh3a) and ATP-forming β subunit of succinyl-CoA ligase (SuclA2), were verified by Western blot, the latter two also by immunohistochemistry. The data suggest altered tricarboxylic acid metabolism in energy supply of synapse while the marked differences in Sod2 and C1qbp support high sensitivity of synapses to oxidative stress. Further functional clustering demonstrated that proteins with higher synaptic levels are involved in synaptic transmission, lactate and glutathione metabolism. In contrast, mitochondrial proteins associated with glucose, lipid, ketone metabolism, signal transduction, morphogenesis, protein synthesis and transcription were enriched in nsMito. Altogether, the results suggest a specifically tuned composition of synaptic mitochondria.

Biological significance
Neurons communicate with each other through synapse, a compartment metabolically isolated from the cell body. Mitochondria are concentrated in presynaptic terminals by

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active transport to provide energy supply for information transfer. Mitochondrial composition in the synapse may be different than in the cell body as some examples have demonstrated altered mitochondrial composition with cell type and cellular function in the muscle, heart and liver. Therefore, we posed the question whether protein composition of synaptic mitochondria reflects its specific functions. The determined protein difference pattern was in accordance with known functional specialties of high demand synaptic mitochondria. The data also suggest specifically tuned metabolic fluxes for energy production by means of interaction with glial cells surrounding the synapse. These findings provide possible mechanisms for dynamically adapting synaptic mitochondrial output to actual demand. In turn, an increased vulnerability of synaptic mitochondria to oxidative stress is implied by the data. This is important from theoretical but potentially also from therapeutic aspects. Mitochondria are known to be affected in some neurodegenerative and psychiatric disorders, and proteins with elevated level in synaptic mitochondria, e.g. C1qbp represent targets for future drug development, by which synaptic and non-synaptic mitochondria can be differentially affected.

1. Introduction

Neuronal synapses are cell compartments highly separated from the cell body in functional and metabolic sense [1]. The energy consumption of synapses is extremely high if normalized to volume in order to keep the membrane potential [2], and support neurotransmission [3] and local protein synthesis [4–6]. To meet the metabolic demand, synapses are surrounded by glial elements, which support synaptic metabolism [7]. In turn, sMito possess characteristics of highly intensive and dynamic ATP production and intensive oxygen metabolism [8–10]. Functionally, synapse is extremely sensitive to oxidative stress and selectively vulnerable in several diseases [11]. While a compartmentalization of metabolism between neuronal and glial cells exists for the cell bodies, too, there are specific differences for the synapse. Transporters and enzymes localized in presynaptic terminals and adjacent glial processes allow the uptake of amino acid neurotransmitters glutamate and GABA into astrocytes and the constant supply of glutamine from astrocytes to the terminals in exchange [12,13]. The synaptic transmission related functional differences suggest that constitutional differences exist between sMito and nsMito. In addition, an increasing number of studies support the pivotal role of mitochondria in pathological changes in synaptic function in neurodegenerative diseases [14–18]. Despite its significance, however, our knowledge on the protein levels in sMito is seriously limited. So a systematic and reliable proteomics study on sMito is a real need for better understanding of synaptic functions in health and disease. The key for the investigation of sMito by proteomics is a reliable and validated separation of synaptic mitochondria from the rest of the mitochondria. Thus, we used independent methods for the validation of sMito and nsMito in the present study.

Intracellular positioning dependent alterations in mitochondrial protein composition have been revealed in the muscle, heart and liver where basic functions of mitochondria (e.g. energy metabolism and the biosynthesis of metabolites) are fine-tuned according to local requirements [19]. Thus, the mitochondria of a certain tissue can be clustered by function and oxygen consumption [20]. The mechanisms of protein network tuning of mitochondria are the transport of different amounts of proteins into the mitochondria and the altered profile of mitochondrial gene expression and local protein synthesis [21]. In synapses, beside the mitochondrial protein synthesis, presynaptic nerve terminals also contain a local cytosolic protein synthesis system transcribing a heterogeneous population of mRNAs supplied by mRNA transport system [22–25]. Synaptic translation of mRNAs is known to play a critical role in the functional regulation of sMito [26–29]. Thus, the non-nuclear protein synthesis and synaptic protein sorting already revealed in the synapse can provide the basis of creating specific protein composition in sMito. However, it is unknown what kinds of metabolic pathways are particularly the characteristic of the synapse. Therefore, in the present study, we addressed the difference in the protein content between sMito and nsMito using two-dimensional differential gel electrophoresis. We identified 103 protein spots whose levels differed between sMito and nsMito and identified the proteins with mass spectrometry. Since the preparation of mitochondria is critically important, we checked the purity and integrity of the mitochondria using electron microscopy and fluorescence activated cell analysis and sorting (FACS). Furthermore, some of the highest protein changes including ATP-forming β subunit of succinyl-CoA ligase (SuclA2), mitochondrial isocitrate dehydrogenase subunit alpha (Idh3a), superoxide dismutase [Mn] (Sod2), and complement component 1Q subcomponent-binding protein (C1qbp) were confirmed by Western blot and the former 2 also by immunohistochemistry.

2. Materials and methods

2.1. Animals

Six three-month-old Balb/c mice were used for analytical and another twelve for preparative proteomic experiments, five for electron microscopy, five for light-microscopic immunohistochemistry and two for electron microscopic immunolabeling. Animals were kept under a 12 h-light–dark cycle (light was on from 08.00 AM to 08.00 PM) and food and water were supplied *ad libitum*. The handling and experimentation of all animals conformed to The Code of Ethics of the World Medical

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