ABSTRACT

Located in the mitochondrial inner membrane, uncoupling proteins (UCP) dissipate the proton electrochemical gradient across the membrane, resulting in the reduction of ATP synthesis. Abundantly expressed in the brown adipose tissue, UCP1 transport protons to the mitochondrial matrix and plays an important role in thermogenesis. It has been suggested that neuronal UCP homologs (UCP2, UCP4, and UCP5) have crucial roles in the function and protection of the central nervous system. However, with the exception of thermogenesis, no definite physiological role has been assigned to UCPs, and their structure and specific functions are poorly understood. The main goal of this study is to explore the structure and functional properties of mammalian UCPs, with a focus on the less known neuronal UCPs. Using recombinant protein expression techniques, all UCPs were produced in good yields in bacteria, purified to high purities, and reconstituted into liposomes for structural and functional studies. Conformational analysis of UCPs was performed by circular dichroism and fluorescence spectroscopies. To assess the ion transport activity of the proteins, ion transport assays (both proton and chloride) were developed for reconstituted UCPs using the anion-sensitive fluorescent probe SPQ. Three specific objectives, in the form of three separate but interrelated research projects, were targeted in this study. In the first project, the ion transport activity of neuronal UCPs was examined in vitro for the first time. The comparative conformational and ion transport study of neuronal UCPs provided fundamental information on their structure and function. In the second project, to gain molecular insight into UCP2’s mechanism of ion transport, potential key amino acid residues, involved in ion transport, were mutated using site-directed mutagenesis. The results of this study revealed the essential role of positively charged amino acid residues in TM2 in the protein’s chloride transport pathway. This study also offers new insights in the structure-function relationship of UCP2 and other UCPs. In the third and last project, optimized folding of recombinant UCP was reported using the pET26 vector containing a bacterial periplasmic targeting sequence. The incorporation of this leading sequence allowed UCP1, UCP2, UCP4, and UCP5 to be expressed in the membrane of E. coli. The proteins were then purified using different mild detergents. All proteins exhibited helical structures in detergents and phospholipid bilayers and displayed proton transport function. Moreover, self-associated functional forms of UCPs in lipid membranes were observed and characterized for the first time. This latter discovery can lead to new insights in the structure-function relationships of UCPs. Overall, these studies have important implications in understanding the structure and proton transport mechanism of UCPs in the mitochondria.

EXAMINATION COMMITTEE:
Dr. Hermann Eberl, CHAIR (Department of Mathematics and Statistics)
Dr. Masoud Jelokhani-Niaraki, ADVISORY COMMITTEE (Wilfied Laurier University-Department of Chemistry and Biochemistry)
Dr. Leonid Brown, ADVISORY COMMITTEE (Department of Physics)
Dr. George Harauz, EXAMINATION COMMITTEE (Department of Molecular & Cellular Biology)
Dr. Heiko Heerklotz, EXTERNAL EXAMINER (University of Freiburg, Germany - via Skype)