Abstract

In theoretical part search for new homologs of known UCPs, thus for new members of UCP subfamily was carried out. New UCPs were found in *Drosophila melanogaster* (four UCP species), one in nematode *Caenorhabditis elegans,* one in model organism - soil amoeba belonging lo Protists *(Protista) Dictyostelium discoideum* and one new UCP in plant *Arabidopsis thaliana.*
Constructed phylogenetic tree grouped previously known and newly annotated UCPs into two main groups - "UCP4 cluster" (into which belong besides UCP4 and BMCP/UCP5 all newly uncovered UCP species) and "classical" one comprising UCP1, 2, 3 and PUMPs. Apparent widespread presence of UCP4 type uncoupling proteins throughout the whole animal kingdom might support its role as an ancestor in UCP evolution.
In experimental part of the dissertation importance of particular amino acid residues for proton (and also Cl^-) transport as well as for binding of regulatory NPs was studied. Three UCP1 mutants from the 1st α-helix (D27V, T30A and C24A-D27V-T30A) and two from the second matrix segment (H145L-H147L and R152L) were constructed, expressed and incorporated into proteoliposomes with entrapped H^+ and Cl^- indicator. By the same procedure proteoliposomes with wild type UCP1 were prepared. The transport properties of wt UCP1 were in agreement with the values published.
Saccharomyces cerevisiae/Escherichia coli multicopy shuttle vector pCGS110 was used as the carrier of rat UCP1 cDNA. Method of site-directed mutagenesis used for preparation of altered UCP 1 genes is based on PCR. Parental DNA strand was digested by Dpn I afterwards, so that only genes with codons coding for substituted amino acids were transformed into bacteria. Transformed clones were selected due to ampicilin resistance introduced by shuttle vector. In *E. coli* culture shuttle vector with UCP1 gene was proliferated. Isolated DNA was sequenced to confirm presence of introduced codon exchanges. Selected clones were then electroporated intro ura^- yeast. Expression of UCP1 was stimulated by addition of galactose. Yeast culture grown to OD_{600} = 1 was collected, mitochondria with incorporated heterologously expressed UCP1 were isolated by successive centrifugations at various RCF. UCP1 was released by detergent octypentaoxyethylene and mixture of lipids L-α-phosphatidylcholine, bovine heart cardiolipin and L-α-phosphatidic acid was added to form proteoliposomes. H^+ and Cl^- transport characteristics were measured via quenching of indicator dye SPQ.
All constructed mutant UCP1 species displayed neither any changes (when compared to the wild type) in their Cl^- transport characteristics, nor their nucleotide binding affinity was changed. In mutants D27V, T30A and R152L V_{max} value for H^+ transport was reduced at least to 50%. In mutant C24A-D27V-T30A H^+ transport was impaired in absolute way. The affinity for lauric acid (K_m) was reduced in all mutants (results on K_m for H145L-H147L differ between particular measurements).