

Abstract

Advances in DNA sequencing led to a technological breakthrough, that allowed analysis of complete genomes including those of parasitic protists *Trichomonas vaginalis* and *Giardia intestinalis*. These organisms are studied not only for their clinical importance, but also from the evolutionary point of view for their adaptation to anaerobic environment. Genome sequencing and annotations of predicted proteins alone did not bring detail view into functioning of their mitochondrion related organelles - in *G. intestinalis* mitosomes, not-participating in energetic metabolism, in *T. vaginalis* hydrogenosomes, producing molecular hydrogen and ATP by means of substrate phosphorylation. Traditional methods based on a fractionation by ultracentrifuging in density gradient and subsequent biochemical and enzymological analyzes were extended by one- and two-dimensional electrophoresis with subsequent identification of proteins by mass spectrometry.

Methods of multidimensional separation of peptides produced by specific proteolysis of a complex mixture and subsequent tandem mass spectrometry were used to identify subproteome of respective organelle.

Isobaric reagents iTRAQ were used in mitosomal proteome of *G. intestinalis* analysis to probe distribution of respective proteins in two gradient ultracentrifugation fractions most enriched with mitosomes. This approach allowed reduction of numbers of candidate proteins for further bioinformatic and localising experiments. Mitosomal localization of twenty proteins was confirmed.

Hydrogenosomal membrane proteins from *T. vaginalis* were purified by Triton X-114 phase separation. SDS-PAGE and in-gel digestion was followed by LC-MALDI. Information on proteins that do not have number of copies comparable to metabolic enzymes and that cannot be easily separated by conventional isoelectric focusing was gained. Proteins engaged in organelle biogenesis and membrane transport were identified by this approach.

Relative quantitation with tagging by iTRAQ reagent was used to study hydrogenosomal protein expression in trichomonads cultivated in iron restricted conditions. 179 proteins were identified, of which 58 were strongly influenced by availability of iron.