

Molecular and biochemical features of three enzymes that may serve as new drug targets in *Cryptosporidium parvum*

Cryptosporidium parvum is a unicellular, obligatory and intracellular parasite belonging to the Phylum Apicomplexa. This parasite can infect both humans and animals, causing an acute diarrhea in immunocompetent persons, and a chronic life threatening infection in immunocompromised individuals. Although many drugs, to combat this parasite, have been empirically tested, there is no completely effective therapy to treat cryptosporidiosis in humans or animals. In recent years, the completion of the genome sequencing projects in *C. parvum* and *C. hominis* along with advances in molecular methods have significantly helped to increase our general understanding of the *C. parvum* metabolic machinery. However, our knowledge concerning many specific pathways and enzymes in *Cryptosporidium* is still limited. Their better understanding in this organism would aid in experimentation of new drugs and new strategy development to treat cryptosporidiosis in humans and animals.

In this dissertation, three essential metabolic enzymes of *C. parvum* have been tested:

Pyruvate:NADP⁺ oxidoreductase (PNO) is a unique, core metabolic enzyme, responsible for converting pyruvate to acetyl-CoA. It is a rare fusion of an N-terminal pyruvate:ferredoxin oxidoreductase domain and a C-terminal cytochrome P-450 reductase domain. PNO is absent in human and animals and it is an essential enzyme for *C. parvum*. This protein was found to target the mitochondria in the distantly related protist *Euglena gracilis*. The determination of PNO subcellular localization either in the cytosol or in the relict mitochondrion of *C. parvum* is critical in assigning the biological role of this enzyme in the parasite therefore, its localization was determined.

CpNARF is a *NARF*-like gene. The name NARF was derivated from human NARF - Nuclear prelamins A recognition factor that shares limited sequence similarity with iron-only hydrogenases but not their hydrogenase activity. The gene from *C. parvum* was cloned and characterized. Domain structure and phylogenetic analyses surprisingly revealed that *CpNARF* resembles more *NARF*-like genes from aerobic protists and higher eukaryotes rather than [Fe]-hydrogenases from other anaerobic protists and bacteria.

S-adenosylhomocysteine hydrolase is an enzyme regulating the S-adenosylhomocysteine metabolic pathway and the regulator of biological transmethylation reactions, which has been considered important in the target-based drug design of antiviral and antiparasitic drugs. Even though, this enzyme is present in human and animal cells, cryptosporidial enzyme differs from them having a plant-like insertion. Therefore, recombinant enzyme was used to evaluate the efficiency of potential inhibitors, leading to studies for elucidation their effect on the *in vitro* growth of *C. parvum*.