

Abstract

Naegleria gruberi is a free-living non-pathogenic amoeba. It is a close relative to *Naegleria fowleri*, a pathogen that causes primary amoebic meningoencephalitis in humans (PAM). As a free-living organism, *Naegleria gruberi* is adapted to aerobic lifestyle but also possesses remarkable traits of anaerobic organism such as Fe-Fe hydrogenase capable of hydrogen production.

This work focuses on three types of iron-containing proteins interacting with oxygen and its reactive species (ROS) that were uncovered in the genome of *N. gruberi* - hemerythrin, protoglobin and rubrerythrin. Studied proteins have been isolated and purified as recombinant proteins and antibodies have been produced against all three of them. We found a single homolog of rubrerythrin in the genome of *N. gruberi* and successfully localised it in the mitochondrion in contrast to hemerythrin and protoglobin that exhibit cytosolic localisation. *In vitro* characterization of these recombinant proteins included mainly size-exclusion chromatography and UV-vis spectrophotometry. Ability to bind oxygen was shown by spectral changes of recombinant hemerythrin purified under anaerobic conditions and recombinant protoglobin isolated aerobically. Western blot analysis revealed changes in expression levels of these proteins in *N. gruberi* cultivated with ROS-inducing compounds or under conditions of different copper and iron concentrations. Our results indicate the role of these proteins in the protection against oxidative stress and/or in the homeostasis of metals which are mechanisms crucial for cell survival.