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Original article

Hydrogen peroxide resistance in *Strigomonas culicis*: Effects on mitochondrial functionality and *Aedes aegypti* interaction

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ABSTRACT

Reactive oxygen species (ROS) are toxic molecules involved in several biological processes such as cellular signaling, proliferation, differentiation and cell death. Adaptations to oxidative environments are crucial for the success of the colonization of insects by protozoa. *Strigomonas culicis* is a monoxenic trypanosomatid found in the midgut of mosquitoes and presenting a life cycle restricted to the epimastigote form. Among *S. culicis* peculiarities, there is an endosymbiotic bacterium in the cytoplasm, which completes essential biosynthetic routes of the host cell and may represent an intermediary evolutive step in organelle origin, thus constituting an interesting model for evolutive researches. In this work, we induced ROS resistance in wild type *S. culicis* epimastigotes by the incubation with increasing concentrations of hydrogen peroxide (H_2O_2), and compared the oxidative and energetic metabolisms among wild type, wild type-H_2O_2 resistant and aposymbiotic strains. Resistant protozoa were less sensitive to the oxidative challenge and more dependent on oxidative phosphorylation, which was demonstrated by higher oxygen consumption and mitochondrial membrane potential, increased activity of complexes II-III and IV, increased complex II gene expression and higher ATP production. Furthermore, the wild type-H_2O_2 resistant strain produced reduced ROS levels and showed lower lipid peroxidation, as well as an increase in gene expression of antioxidant enzymes and thiol-dependent peroxidase activity. On the other hand, the aposymbiotic strain showed impaired mitochondrial function, higher H_2O_2 production and deficient antioxidant response. The induction of H_2O_2 resistance also led to a remarkable increase in *Aedes aegypti* midgut binding *in vitro* and colonization *in vivo*, indicating that both the pro-oxidant environment in the mosquito gut and the oxidative stress susceptibility regulate *S. culicis* population in invertebrates.

1. Introduction

Trypanosomatidae family (Euglenozoa: kinetoplastida) comprises protozoa which are divided into two groups of organisms, classified according to their life cycle: the dixenous trypanosomatids whose life cycle involves invertebrate (especially insects) and vertebrate (including man) hosts or plants, and the monoxenous organisms, which live mainly in invertebrates [1]. Monoxenous trypanosomatids from the genera *Strigomonas*, *Angomonas* and *Kentomonas* present a single endosymbiont bacterium in their cytosol, which divides in synchrony with other host-cell structures and before the segregation of the protozoan kinetoplast and nucleus [2,3]. The trypanosomatids endosymbiosis is a result from a monophyletic event, with the symbiont being an obligate β-proteobacterium phylogenetically close to species from the genera *Bordetella*, *Achromobacter* and *Tayrolella* [4–7]. Although the biochemical role of the endosymbiotic bacteria is still not completely elucidated, previous research has revealed that the bacterial presence promotes morphological and biochemical adaptations in the protozoa, constituting a true mutualistic relationship [8]. Since endosymbiont-free strains (aposymbiotic) generated in laboratory by antibiotic treatment are available for side-by-side comparisons with the wild type strain, several studies regarding the relation between the symbiont and host

Abbreviations: SHTs, symbiont harboring trypanosomatids; ROS, reactive oxygen species; H_2O_2, hydrogen peroxide; $O_2^{\bullet-}$, superoxide anion; TNX, tryparedoxin; GSH, glutathione; TSH_2, trypanothione; ETC, electron transport chain; OXPHOS, oxidative phosphorylation; ROX, residual oxygen consumption

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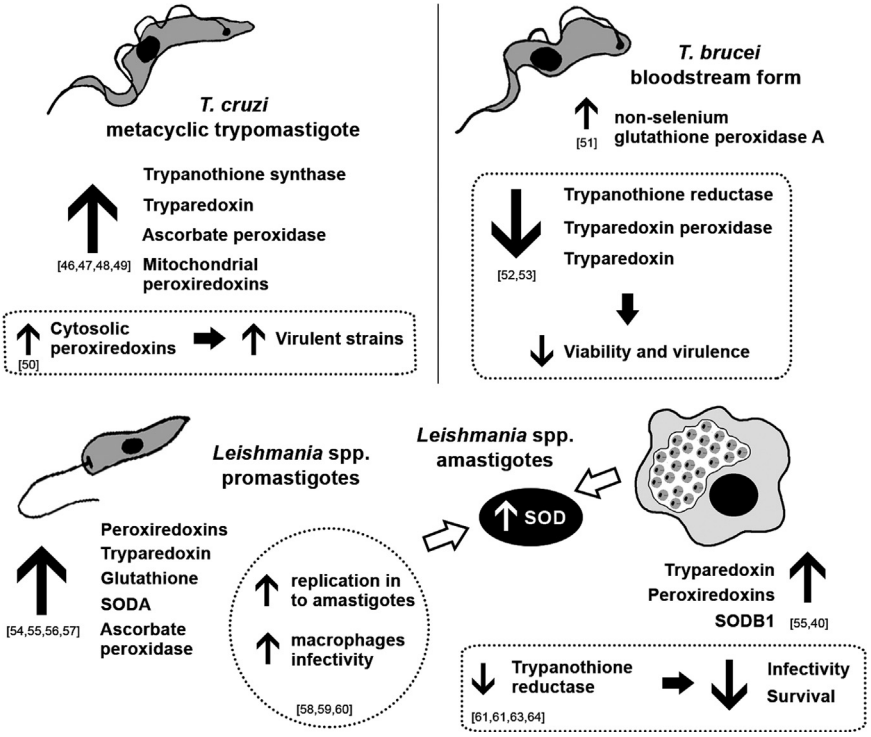

Fig. 1. The diagram shows the role of trypanosomatids antioxidant enzymes in the interaction with their hosts. SOD, superoxide dismutase isoforms [46–64].

interaction have been performed. The main contributions of symbiont are associated with the complementation of metabolic routes with enzymes not presented in the host, such as those required for amino acids (ornithine, lysine and riboflavin), vitamins and heme synthesis [9–13]. Specifically in the heme biosynthetic pathway, the endosymbiont provides seven out of ten enzymes necessary for the production of this ferriprotoporphyrin-IX [11,12].

Heme is vital for all aerobic organisms, due to its participation in many metabolic processes, including cellular respiration, through the formation of cytochromes that compose the mitochondrial electron transport chain (ETC) [14,15]. The mitochondrion of trypanosomatids is unique and branched, presenting a circular interlocked DNA which forms a network named kinetoplast (kDNA) [16,17]. In trypanosomatids, although the oxidative phosphorylation (OXPHOS) is the main energetic source as in other eukaryotic cells, the mitochondrial physiology is quite different. ETC complex I presents no apparent functionality, being the protozoa respiration completely dependent of complex II [18–21]. In *Angomonas deanei* syn. *Crithidia deanei* [6], the endosymbiont presence leads to an increase in O_2 consumption, which was not observed in the isolated symbiont [22]. Recently, it was demonstrated that wild type *Strigomonas culicis* syn. *Blastocrithidia culicis* [6] wild type consumed more O_2 than the aposymbiotic strain [23].

In aerobic organisms, more than 90% of the consumed O_2 is directly reduced to H_2O by ETC [24], however the residual oxygen can be partially reduced by ETC electron leakage, which accredits the mitochondrion as one of the main producers of reactive oxygen species (ROS) in eukaryotic cells [25]. The ROS amount generated depends on cellular physiological state, with alterations in respiratory machinery directly affecting the redox balance and the ROS production [26,27]. *Trypanosoma cruzi* epimastigotes present an active mitochondrial metabolism and produce less ROS than bloodstream trypomastigotes that show an uncoupled ETC [28]. Differences in *Trypanosoma brucei* mitochondria were also observed, with the bloodstream forms showing low mitochondrial activity (cytochromes and other molecules are not expressed) and the insect forms presenting a much more functional mitochondrion [29,30]. Besides that, ROS also participate in signaling pathways. Depending on the concentration, these reactive species, which are induced by physiological molecules, like heme, favors the increase of *T. cruzi* epimastigotes proliferation mediated by calmodulin kinase II activation. On the other hand, parasite proliferation is impaired and induction of metacyclogenesis occurs in the presence of antioxidants [31,32]. In contrast to studies carried out in dixenous trypanosomatids, the knowledge about the oxidative metabolism in monoxenous trypanosomatids is limited, with few studies focusing the energy metabolism and mitochondrial functionality [33].

The antioxidant defenses of trypanosomatids are based in the presence of a specific redox system dependent of trypanothione/trypanothione reductase, which acts as an alternative system to glutathione/glutathione reductase that is present in almost all eukaryotes [34]. The trypanothione [$T(SH)_2$] is a conjugate of two glutathione (GSH) and one spermidine molecules that participate in numerous physiological pathways, providing reducing equivalents and generating trypanothione disulfide (TS_2). The levels of reduced trypanothione are regulated by the activity of trypanothione reductase (TR), an enzyme present in all Kinetoplastida representatives [35–39]. Three different classes of trypanothione-dependent peroxidases have been identified in these protozoa, with different intracellular locations and electron donor substrates: tryparedoxin peroxidases, non-selenium glutathione peroxidases and ascorbate peroxidase. All these enzymes are not directly reduced by TR, thus the electron transfer between the flavoprotein and the reducing molecules is mediated by $T(SH)_2$ [40]. Tryparedoxin peroxidase uses tryparedoxin (TNX) as a source of reducing electrons during the removal of hydroperoxide, while the non-selenium glutathione peroxidases-like enzymes mainly use the thioredoxin as reducing molecules, although also can get electrons to GSH [41–44]. Moreover, all the trypanosomatids have four superoxide dismutase (SOD) genes and, differently of mammalian enzyme, these SOD are dependent of iron as cofactor. These enzymes are responsible to the dismutation of superoxide anion ($O_2^{\bullet-}$) in hydrogen peroxide (H_2O_2) and O_2 [45]. The role of these antioxidant enzymes during trypanosomatids life cycle and host interaction was showed in many studies (Fig. 1), however, the detoxification mechanisms of the different antioxidants in trypanosomatids are still under investigation [35,65]. Despite the presence of $T(SH)_2$/TR and peroxidases systems in monoxenous trypanosomatids, little is known about their role in the interaction between these protozoa and their hosts.