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Selenoproteins of African trypanosomes are dispensable for parasite survival in a mammalian host

Mariana Bonilla[a], Erika Krull[a], Florencia Irigoín[b,d], Gustavo Salinas[c,e,*], Marcelo A. Comini[a,**]

[a] Redox Biology of Trypanosomes Laboratory, Institut Pasteur de Montevideo, Uruguay
[b] Molecular Human Genetics Laboratory, Institut Pasteur de Montevideo, Uruguay
[c] Worm Biology Laboratory, Institut Pasteur de Montevideo, Uruguay
[d] Departamento de Histología y Embriología, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay
[e] Cátedra de Inmunología, Departamento de Biociencias, Facultad de Química, Universidad de la República, Montevideo, Uruguay

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ABSTRACT

The trace element selenium is found in polypeptides as selenocysteine, the 21st amino acid that is co-translationally inserted into proteins at a UGA codon. In proteins, selenocysteine usually plays a role as an efficient redox catalyst. Trypanosomatids previously examined harbor a full set of genes encoding the machinery needed for selenocysteine biosynthesis and incorporation into three selenoproteins: SelK, SelT and, the parasite-specific, Seltryp. We investigated the selenoproteome of kinetoplastid species in recently sequenced genomes and assessed the *in vivo* relevance of selenoproteins for African trypanosomes. Database mining revealed that SelK, SelT and Seltryp genes are present in most kinetoplastids, including the free-living species *Bodo saltans*, and Seltryp was lost in the subgenus *Viannia* from the New World *Leishmania*. Homology and sinteny with bacterial sulfur dioxygenases and sulfur transferases suggest a putative role for Seltryp in sulfur metabolism. A *Trypanosoma brucei* selenocysteine synthase (SepSecS) null-mutant, in which selenoprotein synthesis is abolished, displayed similar sensitivity to oxidative stress induced by a short-term exposure to high concentrations of methylglyoxal or H_2O_2 to that of the parental wild-type cell line. Importantly, the infectivity of the SepSecS knockout cell line was not impaired when tested in a mouse infection model and compensatory effects *via* up-regulation of proteins involved in thiol-redox metabolism were not observed. Collectively, our data show that selenoproteins are not required for survival of African trypanosomes in a mammalian host and exclude a role for selenoproteins in parasite antioxidant defense and/or virulence. On this basis, selenoproteins can be disregarded as drug target candidates.

1. Introduction

Selenium is an essential trace element for diverse organisms, including several eukaryotic lineages [1,2]. The essential role of selenium in eukarya is due to the function of selenoproteins, which contain the genetically encoded 21st amino acid, selenocysteine (Sec, three letter code or U, one letter code) [2,3]. Most selenoproteins are redox enzymes containing a catalytic Sec residue. The Sec incorporation machinery includes several genes dedicated to Sec biosynthesis and decoding of UGA^{Sec} codons present in selenoprotein mRNAs [2,3]. The selenoproteomes of several protozoan lineages have been elucidated, but its functional relevance is not understood [4–6]. Among the protozoa kingdom, kinetoplastids are early-diverging eukaryotes that, in general, present complex life cycles and evolved several unique metabolic features such as a thiol-redox system based on a small polyamine-based

Abbreviations: GLO2, glyoxalase II or hydroxyacylglutathione hydrolase; Grx1, monothiol glutaredoxin 1; Grx3, monothiol glutaredoxin 3; MG, methylglyoxal; TXN, tryparedoxin; TXN-Px, tryparedoxin peroxidase; PDO, persulfide dioxygenase; PSTK, phosphoseryl-tRNASec kinase; SDO, sulfur dioxygenase; Sec, selenocysteine; SepSecS, Sec-tRNA synthase; SPS2, selenophosphate syntethase; SQR, sulfide quinone oxidoreductase; STS, sulfurtransferase; TR, trypanothione reductase.

* Corresponding author at: Worm Biology Laboratory, Institut Pasteur de Montevideo, Mataojo 2020, CP–11400 Montevideo, Uruguay.
** Corresponding author at: Redox Biology of Trypanosomes Laboratory, Institut Pasteur de Montevideo, Mataojo 2020, CP-11400 Montevideo, Uruguay.
E-mail addresses: gsalin@fq.edu.uy (G. Salinas), mcomini@pasteur.edu.uy (M.A. Comini).

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dithiol and devoid of selenocysteine-containing peroxidases [7]. Nonetheless, as most Sec incorporating organisms, the kinetoplastida lineage possesses the entire Sec incorporation machinery dedicated to a small selenoproteome [5]. The "TriTryps" selenoproteome consists of a trypanosomatid specific selenoprotein (Seltryp) and two selenoproteins present in other lineages: SelT and SelK, which are predicted to contain transmembrane helices associated to the endoplasmic reticulum [8]. Seltryp possesses two rhodanese domains and an N-terminal Sec-containing redox motif. SelT possesses an N-terminal CXXU motif within a discontinuous thioredoxin (Trx) domain. In mammals, SelT has been implicated in neuroendocrine regulation, but its specific function has not been elucidated [9]. SelK possesses a C-terminal Sec residue and has been found to modulate the activity of palmitoyltransferase DHHC6 thereby regulating cellular localization of proteins [10]. The function of selenoproteins in kinetoplastida is not known. Both insect- and bloodstream stage *Trypanosoma brucei* cells were sensitive to nanomolar concentrations of auranofin, an inhibitor of some selenoproteins [5]. This led to the proposal that selenoproteins or the pathway of Sec incorporation might constitute a new pharmacological target. However, this concept was later challenged since genetic evidence with *T. brucei* null mutants on phosphoseryl-tRNASec kinase (PSTK, EC 2.7.1.164, the enzyme that catalyzes the phosphorylation the L-seryl-tRNASec to form O-phosphoseryl tRNASec) and Sec-tRNA synthase (SepSecS, EC 2.9.1.2, the enzyme that catalyzes the conversion of O-phosphoseryl tRNASec to Sec-tRNA^{Sec}) indicated that the Sec incorporation pathway is dispensable for both life stages of *T. brucei* cultivated *in vitro* in non-stressed conditions [6]. In a later study, it was shown that both wild-type and the null mutant in SepSecS *T. brucei* strains were equally sensitive to auranofin and thus the trypanocidal action was not connected to selenoproteins [11]. Furthermore, this study also showed that the absence of selenoproteins did not increase sensitivity to H_2O_2-induced oxidative stress. On the other hand, *T. brucei* selenophosphate synthetase [12] (SPS2, EC 2.7.9.3, the enzyme that catalyzes the synthesis of selenophosphate, the selenium donor for the Sec incorporation pathway) knockdown by RNAi severely hampers the parasite survival in the presence of an oxidizing environment, and led to propose that selenoproteins are involved in long-term oxidative protection of trypanosoma cells [13]. So far, there is no report on the relevance of Sec incorporation or selenoproteins during infection *in vivo*. It is worth to note that several trypanosomatid species are causative agents of highly disabling and fatal diseases in human and livestock for which research on drug target validation is of upmost importance to pave the way for the urgently needed new therapies. In this article we provide conclusive evidence that selenocysteine synthesis does not affect *T. brucei* infection rate *in vivo*. In addition, we provide *in silico* clues regarding a putative function of Seltryp in a specific mitochondrial transsulfuration pathway in the kinetoplastida lineage.

2. Materials and methods

2.1. Database search and software for identification of Sec decoding- and seleno-proteins in kinetoplastida

Data mining of Sec decoding genes and selenoprotein genes in the kinetoplastid lineage was carried out using as queries PSTK and SepSec (gene signature of Sec incorporation into proteins in eukarya), and the amino acid sequences of *T. brucei* selenoprotein T, selenoprotein K and Seltryp [5], respectively. The amino acid sequences of the query proteins were used in tblastn or blastp searches against kinetoplastid genomes and proteomes deposited in GeneDB [14] and in TriTrypDB [15] data bases. In the case of *Leishmania panamensis* and *Leishmania brasiliensis* tblastn searches were also performed on transcripts, to have additional evidence for the absence of the gene encoding Seltryp. For all selenoprotein genes identified, the presence of an in frame UGA codon encoding a Sec residue at the correct place was examined.

Blastp and psi-blast homology searches were performed using the full-length or sequence domains of Seltryp as a query against eukaryotic and prokaryotic genomes deposited at NCBI and in the integrated microbial genomes data base [16], respectively. The identified sequences were retroblasted against kinetoplastid genomes to obtain the best reciprocal hits.

2.2. Materials

The polyclonal rabbit antibodies against *T. brucei* tryparedoxin peroxidase (TXN-Px), tryparedoxin (TXN) and the polyclonal guinea pig antibodies against monothiol glutaredoxin 1 (Grx1) and monothiol glutaredoxin 3 (Grx3) were generated previously [17,18]. The rabbit serum against *T. brucei* aldolase was provided by Dr Christine Clayton (Zentrum für Molekulare Biologie der Universität Heidelberg, Germany) [19]. Methyl glyoxal and the supplements of the HMI-9 medium were purchased from Sigma. Iscove's Modified Dulbecco's Medium (IMDM), fetal bovine serum (FBS) and the HRP-conjugated goat anti-rabbit and rabbit anti-guinea pig antibodies were purchased from GE Healthcare and Invitrogen, respectively.

2.3. Cultivation of bloodstream T. brucei

NYSM wild-type (WT) [20] and SepSecS knockout (SepSecS-KO) [11] cell lines of bloodstream *T. brucei* strain 427 were a gift of Dr. André Schneider (University of Berne, Swiss). Both cell lines were cultivated aerobically in a humidified incubator at 37 °C with 5% CO_2 in HMI-9 medium supplemented with 10% FBS, 50 U/mL penicillin, 50 μg/mL streptomycin.

2.4. Short-term treatment of T. brucei with methylglyoxal or hydrogen peroxide

Exponentially growing cultures of the WT and SepSecS-KO *T. brucei* cell lines were harvested by centrifugation at 2000*g* for 10 min at room temperature. Cell suspensions in PBS-glucose 1% (PBS-G) containing 2×10^6 cells/mL were prepared for each strain and dispensed in a 96 well culture plate (200 μL cell suspension/well). Cells were incubated for 2 h at 37 °C and 5% CO_2 with 1, 2.5, 5 and 10 mM methylglyoxal (MG) or 10 mM H_2O_2. Non-treated cells were included as control and all conditions were assayed in duplicate with a lag time of 20 min between treatments for each cell line. Immediately after incubation cells were transferred to a U-bottomed 96 well plate and propidium iodide was added to a final concentration of 2 μg/mL, prior to analysis by flow cytometry. After homogenization by pippeting, 50 μL of each sample were acquired on a BD Accuri C6 Flow Cytometer (BD Biosciences, USA) at fast flow rate and using a FSC-H threshold of 30,000. Viable cells were defined as propidium iodide negative events excluding the cell debris. The percentage of viability for each condition was calculated relative to the non-treated controls set as 100% viability.

2.5. Mice infection experiments

The animal protocols used in this work were evaluated and approved by the Animal Use and Ethic Committee (CEUA) of the Institut Pasteur de Montevideo (Protocol 2009_1_3284). They are in accordance with FELASA guidelines and the National law for Laboratory Animal Experimentation (Law no. 18.611).

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