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Review

Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism

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ABSTRACT

Trypanosomes and leishmania, the causative agents of several tropical diseases, possess a unique redox metabolism which is based on trypanothione. The bis(glutathionyl)spermidine is the central thiol that delivers electrons for the synthesis of DNA precursors, the detoxification of hydroperoxides and other trypanothione-dependent pathways. Many of the reactions are mediated by tryparedoxin, a distant member of the thioredoxin protein family. Trypanothione is kept reduced by the parasite-specific flavoenzyme trypanothione reductase. Since glutathione reductases and thioredoxin reductases are missing, the reaction catalyzed by trypanothione reductase represents the only connection between the NADPH- and the thiol-based redox metabolisms. Thus, cellular thiol redox homeostasis is maintained by the biosynthesis and reduction of trypanothione. Nearly all proteins of the parasite-specific trypanothione metabolism have proved to be essential.

1. Introduction

Trypanosomatids are protozoan organisms of the order *Kinetoplastida* that parasitize a wide variety of invertebrate and vertebrate hosts. The most relevant specimens for human and animal health belong to two genera: *Trypanosoma* and *Leishmania*, which account for over half a million annual human deaths in (sub)tropical regions around the world. In sub-Saharan countries *T. brucei rhodesiense* and *T. b. gambiense* are the causative agents of African sleeping sickness and Nagana cattle disease is caused by *T. b. brucei*, *T. vivax and T. congolense*. In the New World, *T. cruzi* is responsible of Chagas' disease. Different *Leishmania* species occur world-wide and cause *inter alia* black fever, espundia, oriental sore and Kala-Azar. *Crithidia fasciculata* is an apathogenic trypanosomatid that serves as a useful model organism. Trypanosomatids represent one of the earliest branches of eukaryotic evolution with mitochondria and microbodies [1]. The parasites show a large number of biochemical, morphological and genetic peculiarities with the thiol redox metabolism being one of the unique pathways. The genome sequencing projects of *T. brucei* [2], *T. cruzi* [3] and *L. major* [4] have revealed that trypanosomatids lack genes for glutathione reductase (GR) and thioredoxin reductase (TrxR) as well as catalase and selenocysteine-containing glutathione peroxidases. While in most eukaryotic organisms the glutathione (GSH)/GR and thioredoxin (Trx)/TrxR systems maintain the intracellular thiol redox homeostasis, trypanosomatids possess a redox metabolism that is based on the low molecular mass dithiol trypanothione [bis(glutathionyl) spermidine; $T(SH)_2$] [5] and trypanothione reductase (TR) — which keeps it in the reduced form (Table 1) [for reviews see [23,24]]. $T(SH)_2$ and/or TR have also been described in the flagellated green algae *Euglena gracilis* [19] and the amitochondriate pathogenic amoebae *Entamoeba histolytica* [20] and *Naegleria fowleri* [22]. Interestingly, *E. gracilis* and *N. fowleri* were reported to contain both GR as well as TR [19,22]. The absence of the trypanothione system in mammals, the lack of a functional redundancy within the parasite thiol system together with the sensitivity of trypanosomes against oxidative stress render the components of this metabolism attractive drug target molecules [for recent reviews see 24 and 25]. The thorough analysis of the trypanothione metabolism and its control mechanisms will certainly reveal additional unprecedented features and putative new targets for a selective antiparasitic drug development.

2. Thiol redox homeostasis in trypanosomes

In any living organism, the cellular redox homeostasis is affected by an excess of reactive oxygen (ROS) and nitrogen species originating as by-product of aerobic growth or from the environment. Redox-active thiol groups in proteins and low molecular mass compounds play key roles as redox buffers that balance any disturbance of the intracellular redox state [26,27]. Depending on the level and extension of the oxidative stress, the cells may establish short- or long-term

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Table 1
Organisms with documented (trypanosomatids) and putative (non-trypanosomatids) trypanothione metabolism

Organism	Protein[a]			Evidence	Pathology	Reference
	GspS[b]	TryS[c]	TR[d]			
Trypanosoma brucei brucei	Absent	AY155570	X63188	Characterization, RNA-interference and conditional knock-out	Nagana cattle disease	[2, 6–8]
T. b. gambiense	Absent	Tbgamb.1821	Tbgamb.31271	Genome	African sleeping sickness	[9]
T. vivax	Absent	tviv499f03.q1k_27	tviv1287e08.q1k_3	Genome	Nagana cattle disease	[9]
T. congolense	Absent	congo999f08.p1k_9	M21122	Genome and characterization of TR	Nagana cattle disease	[9,10]
T. cruzi	EAN98995	AF311782	M38051	Genome and characterization	Chagas' disease	[3,11,12]
					Leishmaniasis forms:	
Leishmania major	LmjF25.2380 (pseudogene)	AJ311570	CT005244	Genome and characterization	Cutaneous and diffuse	[4,13]
L. donovani	n.r.	AJ430863	Z23135	Genome and characterization of TR	Visceral	[13,14]
L. infantum	AM502243	AM502245	AM502223	Genome	Visceral	[15]
L. amazonensis	n.r.	EF583872	EF583873	cDNA sequence	Diffuse cutaneous	Lin et al. (unpublished)
L. brasiliensis	n.r.	AM494964	AM494942	Genome	Mucocutaneous	[15]
Crithidia fasciculata	U66520	AY603101	CAA78264	Characterization	Insect pathogen	[6,16–18]
Euglena gracilis	n.r.	n.r.	Peptide sequences	Characterization	None	[19]
Entamoeba histolytica	n.r.	n.r.	AF503571	TR activity, $T(SH)_2$ detection and DNA sequence	Dysentery	[20,21]
Naegleria fowleri	n.r.	n.r.	n.r.	TR activity and detection of $T(SH)_2$	Meningoencephalitis	[22]

n. r., not reported;
[a] Accession numbers; [b]Glutathionylspermidine synthetase; [c]Trypanothione synthetase; [d]Trypanothione reductase.

adaptive responses. Kinetoplastids are equipped with a number of unique low molecular mass thiols and redox proteins. The redox homeostasis in these parasites appears to be efficiently regulated since they can successfully withstand the oxidative burst during host infection and perfectly adapt to the different metabolic and environmental conditions imposed by their digenetic life-cycle.

2.1. Low molecular mass thiols

Depending on the species, life stage and growth phase, trypanosomatids contain varying levels of four major low molecular mass thiols: GSH, mono-glutathionylspermidine (Gsp), $T(SH)_2$ and, in some species, ovothiol A (N^1-methyl-4-mercaptohistidine; OvSH; Fig. 1). As in other organisms, GSH is synthesized via two ATP-dependent steps. The first one is catalyzed by γ-glutamylcysteine synthetase (GSH1) [28], an enzyme shown to be essential for *T. brucei* [29], and the second reaction is catalyzed by glutathione synthetase (Fig. 2). Significant amounts of the tripeptide are present as $T(SH)_2$ and, to a lesser extend, as Gsp (Table 2, Section 2.2).

When exponentially growing *C. fasciculata* enter the stationary phase, the $T(SH)_2$ concentration decreases from 1.51 to 0.37 mM and Gsp increases from 0.9 to 2.28 mM [33]. Inoculation of the stationary phase cells into fresh medium is followed by a rapid recovery of $T(SH)_2$ with a concomitant increase in the level of free spermidine [33]. The

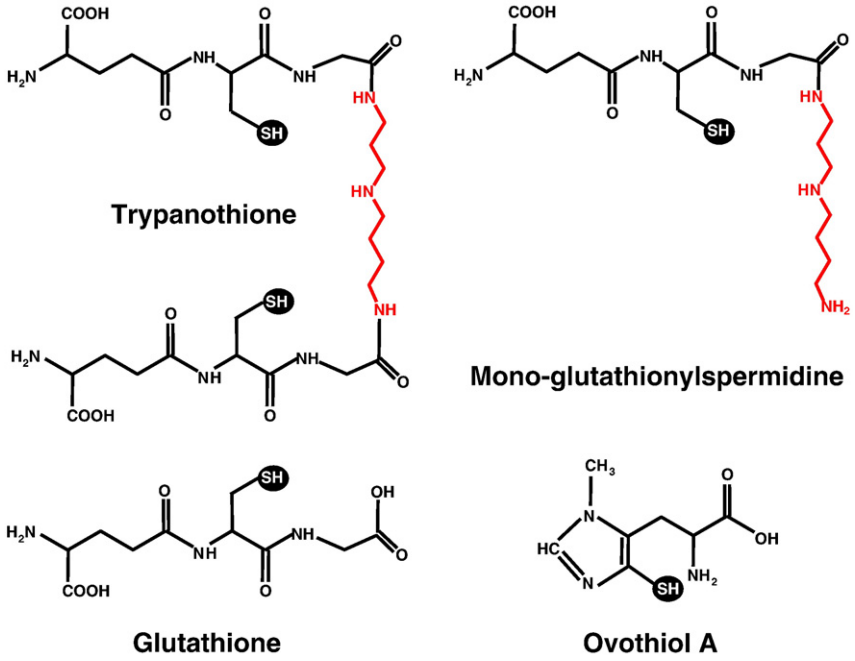

Fig. 1. Low molecular mass thiols occurring in trypanosomatids. The polyamine moiety in the trypanothione [bis(glutathionyl)spermidine] and mono-glutathionylspermidine molecules are depicted in red. Sulfhydryl groups are highlighted by a black background.