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Review

Trypanothione: A unique bis-glutathionyl derivative in trypanosomatids ☆

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ABSTRACT

Background: Trypanosomatids are early-diverging eukaryotes devoid of the major disulfide reductases – glutathione reductase and thioredoxin reductase – that control thiol-redox homeostasis in most organisms. These protozoans have evolved a unique thiol-redox system centered on trypanothione, a bis-glutathionyl conjugate of spermidine. Notably, the trypanothione system is capable to sustain several cellular functions mediated by thiol-dependent (redox) processes.

Scope of review: This review provides a summary of some historical and evolutionary aspects related to the discovery and appearance of trypanothione in trypanosomatids. It also addresses trypanothione's biosynthesis, physicochemical properties and reactivity towards biologically-relevant oxidants as well as its participation as a cofactor for metal binding. In addition, the role of the second most abundant thiol of trypanosomatids, glutathione, is revisited in light of the putative glutathione-dependent activities identified in these organisms.

Major conclusions: Based on biochemical and genome data, the occurrence of a thiol-redox system that is strictly dependent on trypanothione appears to be a feature unique to the order Kinetoplastida. The properties of trypanothione, a dithiol, are the basis for its unique reactivity towards a wide diversity of oxidized and/or electrophilic moieties in proteins and low molecular weight compounds from endogenous or exogenous sources. Novel functions have emerged for trypanothione as a potential cofactor in iron metabolism.

General significance: The minimalist thiol-redox system, developed by trypanosomatids, is an example of metabolic fitness driven by the remarkable physicochemical properties of a glutathione derivative. From a pharmacological point of view, such specialization is the Achilles' heel of these ancient and deadly parasites. This article is part of a Special Issue entitled Cellular functions of glutathione.

Abbreviations: 1-C-Grx, monothiol glutaredoxin; 2-C-Grx, dithiol glutaredoxin; CBS, cystathionine-β-synthase; CGL, cystathionine-γ-lyase; CHAP, cysteine histidine-dependent amidohydrolases/peptidases (protein domain); CS, cysteine synthase; DMPO, 5,5′-dimethyl-l-pyrroline-N-oxide; DNIC, dinitrosyl iron complex; DNTIC, dinitrosyl-trypanothione iron complex; eEF1B, eukaryotic elongation factor 1B; EPR, electron paramagnetic resonance; Fe–S, iron–sulfur cluster on a protein; GPx, glutathione peroxidase; Grx, glutaredoxin; GSH, reduced glutathione; GshA, γ-glutamyl-cysteine synthetase; GshB, glutathione synthetase; GSNO, nitrosoglutathione; Gsp, mono-glutathionylspermidine; GspS, mono-glutathionyl spermidine synthetase; GSSG, glutathione disulfide; GST, glutathione-S-transferase; HED, hydroxyethyl disulfide; ISC, iron sulfur cluster in general, related to the biosynthetic machinery; LIP, "labile" or chelatable iron pool; LMW, low molecular weight; MSR, methionine sulfoxide reductase; MST, 3-mercaptopyruvate sulfurtransferase; ODC, ornithine decarboxylase; Prx, peroxiredoxin; Pxs, peroxidases (general term); PxIII, glutathione-type tryparedoxin-dependent peroxidase III; RnR, ribonucleotide reductase; RTS, reverse transsulfuration; SAT, serine acetyltransferase; Spd, spermidine; SpS, spermidine synthase; $T(SH)_2$, dihydrotrypanothione; TDR1, thiol-dependent reductase 1; Trx, thioredoxin; TrxR, thioredoxin reductase; TryR, trypanothione reductase; TryS, trypanothione synthetase; TS_2, trypanothione disulfide; TXN, tryparedoxin; UMSBP, universal minicircle sequence binding protein

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1. Introduction

The identification of glutathione (γ-glutamylcysteinylglycine, GSH) can be traced back to the late 19th century, when several organic molecules were discovered in the protein-free extract of "protoplasm" from different cell types. Among them, those reacting with nitroprusside salt ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) called the attention to chemical physiologists, leading to the isolation and identification of biological cornerstones like cysteine and glutathione [1]. Glutathione was originally reported in 1888 by Joseph De Rey-Pailhade as a substance widely distributed in nature that received the name of "philothion" ("sulfur loving") due to its capacity to reduce sulfur to hydrogen sulfide. In 1921, Frederick G. Hopkins "rediscovered" this molecule providing preliminary evidence about its physiological significance and chemical composition (e.g. glutamic acid and cysteine) from which the name "glutathione" stem [2]. Controversies around the precise structure of GSH [3] were finally solved few years later [4]. Research on the biological role of GSH gained new impetus in the late 60s upon the development of oxidizing molecules able to penetrate cell membranes (see references [1] and [5] for historical details). Almost contemporarily, the GSH-dependent antioxidant enzyme GPx was discovered in red blood cells [6,7], highlighting the role of this low molecular weight (LMW) thiol in the redox balance and antioxidant defenses of biological systems. Since then, the number of studies referring to GSH has been growing exponentially and revealed novel functions for this monothiol and/or its derivatives as chelating or signaling molecules.

The appearance of GSH in organisms is largely associated to the development of an aerobic life style that demanded a careful handling of oxygen and iron [8–10]. In fact, most cells have evolved antioxidant systems and regulatory mechanisms of protein function based on the utilization of GSH as thiol-redox cofactor. The GSH system consist of glutathione reductase (GR, EC 1.8.1.7), which regenerates the reduced form of GSH, glutathione peroxidases (GPx, EC 1.11.1.9), which reduce peroxides at the expense of GSH, and glutaredoxins (Grx), which allow for an efficient electron shuttle from GSH to different protein targets. Operating in a similar fashion, the thioredoxin system includes thioredoxin reductases (TrxR, EC 1.8.1.9), thioredoxins (Trx, EC 1.8.1.8) and the Trx-dependent peroxidases peroxiredoxins (Prx, EC 1.11.1.15). Both systems work in a concerted and complementary manner to sustain cellular redox homeostasis [10–12]. Some organisms have evolved specific systems based on GSH derivatives, such as the phytochelatins (GSH polymers [13]) in plants or polyamine conjugates of GSH in certain protozoans (e.g. trypanosomatids). In this article we will focus in some aspects of the unique biology of the trypanosomatid-specific GSH-polyamine conjugates.

1.1. Glutathione and derivatives in Trypanosomatids: historical and evolutionary scenario

Trypanosomatids are monoflagellated unicellular protists that belong to the phylum *Euglenozoa*, one of the oldest lineages in eukaryotic evolution. They are obligated heterotrophs adapted to survive either as free-living organisms or as endo-parasites in a diverse range of organisms (e.g. plants, insects, fish, amphibian, mammals) [14–18]. Several members of the genus *Trypanosoma* and *Leishmania* are responsible for causing human diseases with high mortality and morbidity rates, e.g. African sleeping sickness (*T. brucei gambiense* and *T. b. rhodesiense*), Chagas disease (*T. cruzi*) and leishmaniasis (*L. infantum, L. donovani*, *L. major*) [19]. Other trypanosomatid species like *T. b. brucei*, *T. vivax*, *T. congolense* and *T. equiperdum* infect livestock producing important economical loses in endemic countries [20]. The life cycle of the human pathogenic species involve the adaptation of the parasite to live inside the insect vector or the mammalian host. To cope with the host's different environments these organisms have evolved adaptive mechanisms based on exceptional biochemical and ultrastructural features [21–24]. The adaptive responses are masterly synchronized during differentiation to intermediate life cycle stages and involve a tight control of gene expression mostly at post-transcriptional level [25] but, as recently reported, also at transcriptional level [26]. Indeed, one of the key pathways that are modulated during differentiation includes components of the unique thiol-dependent redox metabolism [27–29].

Owing to the clinical relevance of trypanosomiasis and leishmaniasis, research on this area was, and continues to be, dominated by the role of the parasite's redox systems in antioxidant and xenobiotic defenses. In this respect, early studies addressed the essentiality of the thiol metabolism in trypanosomes using arsenical compounds [30] and inhibitors of GSH biosynthesis [31,32]. Almost simultaneously, the biochemist Alberto Boveris reported for the first time the occurrence of GSH in a trypanosomatid [33]. This work provided two intriguing results: i) the insect stage of *T. cruzi* has "one-tenth of the glutathione content compared to rat liver cells" and ii) a GSH-dependent peroxidase (GPx) activity was not detected in these organisms, from which the authors concluded that trypanosomes were deficient in anti-oxidative defenses [33]. In subsequent years, the search for the components of classical redox systems like GR or TrxR was intensified but render unsuccessful, leading to the proposal that the redox metabolism of the parasites may significantly differ from those present in most organisms. Finally, it took a decade to elucidate the enigmatic redox system of trypanosomatids that turned out to be composed by: i) the unusual N^1, N^8-bis-glutathionylspermidine (trypanothione[2]) derivative as the main LMW thiol [34,35], ii) the NADPH-dependent trypanothione reductase (TryR, EC 1.8.1.12), which recycles trypanothione disulfide (TS_2) back to dihydrotrypanothione ($T(SH)_2$) [36], and iii) the Trx-like oxidoreductase tryparedoxin (TXN), which catalyzes the electron transfer from $T(SH)_2$ to different protein targets [37,38]. The biosynthetic pathway for trypanothione has been determined for *T. brucei*, *L. major* and, partly, for *T. cruzi* (see Section 3 [39–45]). It is interesting to note that several components of this system are not exclusive from Trypanosomatids and have been also identified in Euglenids [46] and Bodonids[3] [47], pointing to an ancient and common phylogenetic origin of this metabolic specialization in the evolutionary path from which extant trypanosomes evolved (Fig. 1). On the other hand, genome sequencing of the major trypanosomatids' species corroborated the earlier evidences that pointed to the absence of GR and TrxR in Kinetoplastids [48–51].[4] In line with the lack of thiol-redox back-up systems, genetic manipulation of parasites revealed the indispensability of the trypanothione metabolism for cell viability and virulence [27,52–59]. Notably, even trypanosomal Grx – a highly conserved protein family characterized by using GSH as a reducing cofactor in organisms as diverse as viruses, bacteria and humans [60,61] – evolved a surprising specificity for $T(SH)_2$ [62–64] (see Section 5.2). As a whole, the trypanothione-dependent system appears as a condensed redox hub of the more ubiquitous GR/GSH/Grx and TrxR/Trx systems, where the reducing power of a LMW dithiol is combined with the action of devoted multipurpose oxidoreductases (TXN, Grx) that funnel electrons to different target proteins. In this regard, $T(SH)_2$ participates in a plethora of important cellular functions that involve the elimination of endogenously or exogenously produced toxic metabolites or chemicals, the regulation of key metabolic or signaling pathways and metal homeostasis (see Sections 4 and 5; reviewed in [39,65,66]).

Since the discovery of trypanothione, together with the overwhelming evidences on its biological significance, cysteine and glutathione were relegated as mere biosynthetic precursors of the parasite specific dithiol. However, cysteine uptake or production, GSH biosynthesis and GSH-dependent proteins are strongly related to trypanothione

[2] Trypanothione refers to the pool of reduced and oxidized forms of the compound; dihydrotrypanothione indicates the reduced ($T(SH)_2$) and trypanothione disulfide, the oxidized (TS_2) form of trypanothione, respectively.

[3] The class Kinetoplastida, together with Diplonemida (deep-sea organisms) and Euglenida (non-obligated photosynthetic organisms) form the phylum Euglenozoa [15]. Kinetoplastida is divided into the orders Bodonida and Trypanosomatida. Bodonids are auxothrophic biflagellates with a free-living or parasitic life style from which the Trypanosomatids are considered to originate [15,47,48].

[4] http://www.sanger.ac.uk/resources/downloads/protozoa/bodo-saltans.html.