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Antioxidant Chemistry of Trypanothione

by

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ABREVIATIONS

CAT	Catalase
DHA	Dehydroascorbate
Gpx	Glutathione peroxidase
GSH	Reduced glutathione
Gsp	Glutathionyl-spermidine
GspS	Glutathionyl-spermidine synthetase
HPLC	High performance liquid chromatography
$T(S)_2$	Oxidized trypanothione
$T(SH)_2$	Reduced trypanothione
TryR	Trypanothione reductase
TryS	Trypanothione synthetase
TXN	Tryparadoxin
TXNPx	Tryparadoxin peroxidase

OUTLINE

i. ABSTRACT

Trypanothione is specialized thiol containing small molecular antioxidant produced by parasites, and is required for them to evade their host's immune response. Its production requires the existence of unique synthesis machinery and a novel reductase requiring NADPH. Trypanothione physically reacts similar to glutathione by directly scavenging free radicals, converting peroxynitrite, and reducing dehydroascorbate; however in many of these functions it surpasses GSH. It is also involved in the thiol dependent metabolism of peroxides by the tryparadoxin peroxide scavenging system. The unique chemical and biochemical reactions of trypanothione are the focus of this review.

1 INTRODUCTION

1.1 Trypanothione is a sulfhydryl containing antioxidant:

Reduced thiols are a ubiquitous source of small molecule antioxidants in cells [1]. They provide reducing potential via reduced sulfur atoms present in their structure. This allows them to be involved in many functions such as the removal of hydrogen peroxide (H_2O_2), and repair of biomolecules [2], a task predominately facilitated out by glutathione (GSH) in many prokaryotic and eukaryotic cells. However, in the past 20 years N^1,N^8-*bis*(glutathionyl)-spermidine, or more commonly trypanothione (TSH_2) after the organisms that it was originally discovered in, has gained attention. Similar to GSH it functions though two reduced sulfhydryl groups and, as it will be described later in this review, is involved in biomolecules repair and scavenging of free radicals (**Figure 1.1**). However, the similarities between these two compounds stop here. Trypanothione has abilities that surpass that of GSH in many facets of free radical scavenging and repair. Trypanosomes and their relatives are parasitic organsisms that cause Chagas' disease, African sleeping sickness, and leishmanias [2,3,4,5]. They infect animals, while skillfully repelling attack from macrophages [6]. Since trypanosomes lack both catalase (CAT) and glutathione peroxidase, (GPx) it was believed that they possess some unique difference in their ability manage oxidative stress that could be used as a target for disease treatment [5,7]. This target was found by Fairlamb and coworkers in 1985 when they discovered trypanothione [1]. While trypanosomes contain GSH, it appears its role as an antioxidant is downplayed, primarily serving in the biosynthesis of trypanothione [8,9].

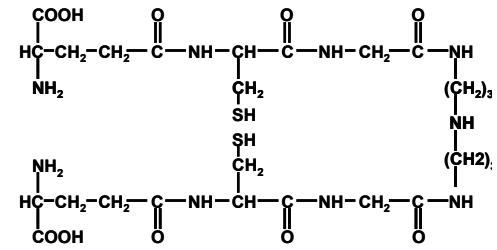

Figure 1.1 Structure of trypanothione

2 DETECTION AND MEASUREMENT

2.1 Detection by HPLC:

One method to both quantitatively and qualitatively measure the presence of trypanothione is though the use of high performance liquid chromatography (HPLC), a method that separates compounds based on their affinity for a column matrix. Daniel Steenkamp has devised a method of direct measurement that requires an initial chemical modification of thiols by 7-Diethylamino-3-(4'-maleimidylphenyl)-4-methylcoumarin prior to ion pair reversed-phase chromatography and detection by fluorescence [8]. After modification, the thiols can be resolved and their identities determined by comparing their retention times with that of synthetically produced standards. Quantitative measurement can be achieved by calculating the area under the peak for each compound. As mentioned earlier the synthesis of trypanothione is not common among eukaryotes, so HPLC provides a means by which organisms can be assayed for their ability to produce trypanothione. Ondarza *et al.* devised a method for this during their characterization of the parasite *Entamoeba histolytica* [10]. Since the *in-vivo* concentration of trypanothione can sometimes be very low, they prepared whole cell extracts and mixed it with trypanothione precursors to produce an abundance of trypanothione to be analyzed by HPLC [10]. Therefore using cell extracts and adding precursors can increase the concentration of trypanothione above detection limits.

3 TRYPANOTHIONE CHEMISTRY

3.1 Synthesis of trypanothione:

Initial chemical analysis of trypanothione revealed it consisted of spermidine, glycine, glutamate, and hemicystine in the ratio of 1:2:2:2 with a molecular weight of 721 g mol^{-1} [1]. Subsequent studies demonstrated that trypanothione is synthesized in two steps, each of which add glutathione to spermidine (**Reactions 3.1 and 3.2)**. These reactions are carried out by one

enzyme in *Trypanosoma cruzi* and *Trypanosoma brucei* called trypanothione synthetase (TryS). Alternatively *Crithidia fasiculata* and *Leishmania spp* have developed two separate enzymes to catalyze them [2,11]. In *C. fasiculata* and *Leishmania spp* the first reaction generates glutathionyl-spermidine (Gsp) (**Reaction 3.1)** via the enzyme glutathionyl-spermidine synthetase (GspS) which catalyzes the addition of one molecule of glutathione to spermidine. Addition of the second glutathione is catalyzed by trypanothione synthetase (TryS), which can easily be mistaken for TryS of *Trypanosoma cruzi*. Currently it is unknown whether these two enzymes form a complex to produce oxidized trypanothione, or reside separately in cells. After synthesis is complete, all trypanothione utilizing organisms reduce it thought the NADPH dependent enzyme trypanothione reductase (TryR) (**Reaction 3.3**). This enzyme is similar in function and structure to glutathione reductase from other organisms. Enzymatic analysis showed it to have a high K_m for both NADPH and trypanothione disulfide (K_m of 5 μM for NADPH and a K_m of 45 μM for trypanothione disulfide) [12]. This enzyme is similar in structure and function to glutathione reductase which is found other organisms [7].

$$\text{GSH} + \text{Spermidine} \xrightarrow{\text{GspS/TryS}} \text{glutathionyl-spermidine (Gsp)} \quad \textbf{3.}$$

$$\text{Gsp} + \text{GSP} \xrightarrow{\text{TryS}} \text{T(S)}_2 \quad \textbf{3.}$$

$$\text{T(S)}_2 \xrightarrow[\text{NADPH}]{\text{TyrR}} \text{T(SH)}_2 \quad \textbf{3.}$$

3.2 Trypanothione vs glutathione: a test of antioxidant ability

Since two glutathione molecules go into the synthesis of trypanothione it could be inferred that the two molecules share many properties. Both are small molecule antioxidants that are activated in their reduced forms. Closer inspection of trypanothione reveals that it has a total positive charge versus the negative charge held by glutathione. This makes trypanothione have different properties, biological functions and antioxidant abilities than glutathione. Awad and colleagues used the protection of DNA after expose to ionizing radiation as an endpoint to

compare how this charge difference might influence the antioxidant abilities of trypanothione and glutathione [3]. In their experiment, oxidized thiols in the presence, or absence of their respective reductases were mixed with transforming DNA and X-irradiated at increasing doses. This allowed for the calculation of a protection factor (PF) for both compounds. Simply put, if a compounds has a higher protection factor for DNA; it can more effectively scavenge free radicals. **Table 3.1** shows the PFs for the different treatments used in their study [3]**.** Notice that at equivalent thiol concentrations trypanothione yielded a higher protection factor over glutathione, and therefore is better antioxidant at removing free radicals that damage DNA. It is also of interest to note that the PF of 50 μM $T(S)_2$ was so high it could not be calculated. They believe that the higher protection by trypanothione can be attributed to its positive charge creating a tighter association with the negatively charged phosphate backbone of DNA, thus putting its reducing potential in close proximity to serve as an "antioxidant shield" for DNA [3]. These findings demonstrate the powerful antioxidant nature of trypanothione, and give insight to how it might work to protect cells.

Table 3.1 Summary of the effects on irradiated t-DNA of various concentrations and combinations of trypanothione, glutathione and their reductses [2].

Agent	Concentration of disulfide added (μmol dm^{-3})	Concentration of reduced thiol (μmol dm^{-3})	Protection Factor
$T(S)_2$	50	-	1.2 ± 0.2
$T(S)_2$	5	-	1.2 ± 0.2
$T(S)_2$	0.5	-	1.0
$T(S)_2$ + TryR	50	103.8 ± 4.5	¥
$T(S)_2$ + TryR	5	9.4 ± 0.4	9.8 ± 0.2
$T(S)_2$ + TryR	0.5	-	3.3 ± 0.9
GSSG	50	-	1.2 ± 0.1
GSSG	5	-	1.1 ± 0.1
GSSG + GR	50	97.6 ± 7.3	3.6 ± 0.7
GSSG + GR	5	10.1 ± 0.6	2.5 ± 0.7

¥ radio protection so great protection factor could not be calculated

3.3 Trypanothione versus peroxynitrite cytotoxicity:

Macrophages dispose of pathogens by producing nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{\bullet -}$) which quickly react to form peroxynitrite anion ($ONOO^-$) [9]. This species rapidly destroys pathogen biomolecules, resulting in their death. Many thiols have been shown to quickly react with, and

hence detoxify peroxynitrite [9]. Thomson and coworkers investigated whether trypanothione's ability to counter peroxynitrite was responsible for *T. cruzi* pathogenicity [9]. In a simple experiment they treated cultured *T. cruzi* with peroxynitrite and measured growth kinetics. The influence of trypanothione in cells was determined by first converting it to an oxidized form using diamide, followed by addition of peroxynitrite and subsequent measurement of growth kinetics. **Figure 3.1** shows that addition of either peroxynitrite or diamide alone slightly decreased *T. cruzi* proliferation [9]. However the combination of both decreased cell growth kinetics by four fold. These data indicate that trypanothione for *T. cruzi* to survive the peroxynitrite produced by macrophages. Therefore a unique antioxidant system that utilizes trypanothione must exist to detoxify peroxynitrite. This pathway is outlined in **Figure 4.2** and will be discussed later.

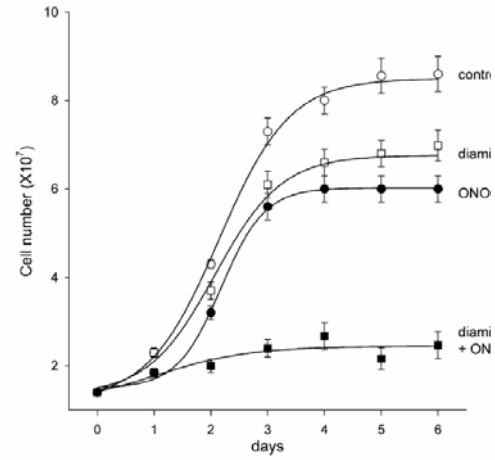

Figure 3.1 Role of intracellular thiols on peroxynitrite-dependent cytotoxicity to *T. cruzi*. 500 μM peroxynitrite was added to cells with or without pretreatment with 5 mM diamide. *T. cruzi* in the absence of any treatment is also shown [9].

3.4 Trypanothione mediated reduction of dehydroascorbate:

Ascorbate is a highly utilized small molecular antioxidant, and the ultimate donator of electrons to free radicals. In other eukaryotes oxidized ascorbate (dehydroascorbate, DHA) can be recycled by glutathione. Luise and colleagues determined that trypanothione could non-enzymatically reduce DHA with a second order rate constant of 22 M^{-1} s^{-1} at pH 6.5 [4]. Additionally they determined that 10 μM of trypanothione could convert DHA 10 times faster than 1 mM GSH under the same conditions [4]. This once again demonstrates the robust nature of trypanothione as an antioxidant by comparing it to well characterized thiol GSH.

4 BIOCHEMICAL ROLES FOR TRYPANOTIONE

4.1 Trypanothione antioxidant repair systems:

As

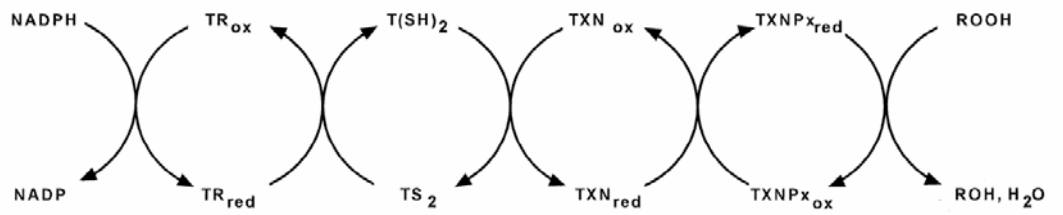

Figure 4.1 Scheme of NADPH-dependent and trypanothione-mediated alkyl-hydroperoxide reduction. TR = trypanothione reductase; $T(SH)_2$ = trypanothione; $T(S)_2$= trypanothione disulphide; TXN = tryparedoxin; TXNPx = tryparedoxin peroxidase;ROOH = alkyl hydroperoxide [5].

mentioned earlier trypanothione containing organisms lack both CAT and Gpx enzymes, yet they find a way counter the oxidative attack of macrophages. Therefore they must have unique system for hydroperoxide metabolism that utilizes trypanothione. Tryparedoxin (TXN) is the first enzyme in this repair pathway, serving as a shuttle for electrons to trypanothione peroxidase (TXNPx). Tryparadoxin has an active site that functions homologous to its distant relative thioredoxin [13]. Trypanothione peroxidase is a peroxiredoxin type enzyme comprising as much as 5% of the total cellular protein; it also functions similarly to thioredoxin [5,9,13]. Overall this system, shown in **Figure 4.1**, works homologous to the GSH system found in other eukaryotes by transferring reducing potential from NADPH to target molecules [5]. In *T. cruzi* this enzyme system has been shown to be quite

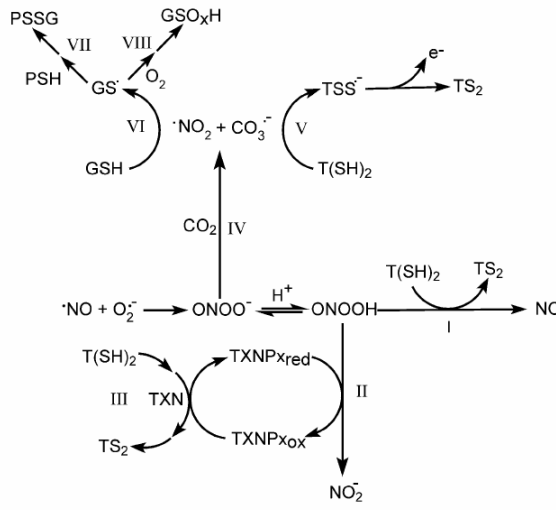

Figure 4.2 Proposed biochemical pathways for the interactions of peroxynitrite with the trypanothione–thiol system in T. cruzi [9].

effective at forming nitrites from peroxynitrite with a second order rate constant of 10^6 M^{-1} s^{-1} [9]. The involvement of these enzymes in this pathway is shown in **Figure 4.2.** This reaction pathway demonstrates the pivotal role trypanothione plays, both directly and enzymatic in the conversion of peroxynitrite. This also sums up how these parasites survive macrophage attack in their hosts.

5 SUMMARY

Trypanothione is a specialized small molecular antioxidant that is used by few organisms which use it to evade their host's immune response. It gains distinctive abilities different from other small antioxidants that use reduced sulfhydryls based on its structure and utilization by a novel enzyme based repair system found in the organisms that produce it [1,9]. While having similar antioxidant abilities as GSH, I have outlined here how trypanothione surpasses it in many functions. Keen examples of this the higher reactivity exhibited by trypanothione towards peroxynitrite and DHA [4,5,9]. Perhaps the most intriguing aspects of trypanothione is the way it is synthesized, and later utilized by cells. This is grounded in the fact that obscure parasites are the only organisms that produce it. Furthermore repair pathways that utilize trypanothione are also unique. Since these systems are so different than other eukaryotes, medical science can explit it as a possible opportunity to cure the diseases caused by the parasites that produce trypanothione and is the subject of ongoing research [11].

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