Molecular & Biochemical Parasitology 206 (2016) 39–45

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Entamoeba thiol-based redox metabolism: A potential target for drug development

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ARTICLE INFO

Article history:
Received 4 November 2015
Received in revised form 8 January 2016
Accepted 9 January 2016
Available online 13 January 2016

Keywords:
Entamoeba histolytica
Thiol
Redox
Oxidative stress
Cysteine
Drug target

ABSTRACT

Amebiasis is an intestinal infection widespread throughout the world caused by the human pathogen *Entamoeba histolytica*. Metronidazole has been a drug of choice against amebiasis for decades despite its low efficacy against asymptomatic cyst carriers and emergence of resistance in other protozoa with similar anaerobic metabolism. Therefore, identification and characterization of specific targets is urgently needed to design new therapeutics for improved treatment against amebiasis. Toward this goal, thiol-dependent redox metabolism is of particular interest. The thiol-dependent redox metabolism in *E. histolytica* consists of proteins including peroxiredoxin, rubrerythrin, Fe-superoxide dismutase, flavodiiron proteins, NADPH: flavin oxidoreductase, and amino acids including L-cysteine, *S*-methyl-L-cysteine, and thioprolines (thiazolidine-4-carboxylic acids). *E. histolytica* completely lacks glutathione and its metabolism, and L-cysteine is the major intracellular low molecular mass thiol. Moreover, this parasite possesses a functional thioredoxin system consisting of thioredoxin and thioredoxin reductase, which is a ubiquitous oxidoreductase system with antioxidant and redox regulatory roles. In this review, we summarize and highlight the thiol-based redox metabolism and its control mechanisms in *E. histolytica,* in particular, the features of the system unique to *E. histolytica,* and its potential use for drug development against amebiasis.

1. Introduction

Entamoeba histolytica is an enteric protist and the causative agent of amebic dysentery and liver abscesses in humans [1]. The World Health Organization estimates that approximately 50 million people worldwide suffer from invasive amebic infection, resulting in 40–70 thousand deaths annually [2]. *E. histolytica* trophozoites usually reside and multiply within the human colon, which constitutes a microaerophilic environment [3] and have high potential for invading and destroying human tissues, leading to disease manifestations [4]. The energy metabolism of this pathogen is believed, despite previous studies indicating that *E. histolytica* is capable of utilizing oxygen [5], to be exclusively fermentative, with phosphorylation taking place only at the substrate level and the transfer of electrons to molecular oxygen is unlikely used for energetic purposes [6,7].

During tissue invasion, *E. histolytica* is exposed to elevated concentrations of exogenous reactive oxygen species (ROS), such as superoxide radical anions ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) [8,9]. These highly toxic ROS cause severe damage to biological macromolecules, leading to metabolic malfunctions. In addition, *E. histolytica* must inactivate ROS produced by essential intrinsic enzymes. In any living organisms, the cellular redox homeostasis is affected by an excess of ROS and reactive nitrogen species (RNS) 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 [10,11]. All forms of life have developed efficient enzymatic systems to resist the oxidative damage generated by ROS and to maintain the intracellular redox balance [12].

Several defense mechanisms exist by which a wide array of enzymatic antioxidative pathways, including superoxide dismutase (SOD), glutathione peroxidase, and glutathione, together with non-enzymatic anti-oxidants, play a pivotal role in survival during oxidative stress [13]. Parasitic organisms in general, and *E. histolytica* in particular, show a remarkable diversity and uniqueness with respect to the nature and functions of their main low

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http://dx.doi.org/10.1016/j.molbiopara.2016.01.004

molecular mass antioxidants [14]. *E. histolytica* lacks most of the typical components of the eukaryotic oxidative stress defense systems including catalase, peroxidase, glutathione, and glutathione recycling enzymes [15,16]. The existence of trypanothione and its metablolism is still in debate (see below). The best studied antioxidative defense system in *E. histolytica* is thioredoxin-dependent system [12,17,18]

In this review, we summarize and highlight the thiol-based redox metabolism and its control mechanisms in *E. histolytica*, unique features of the system in *E. histolytica*, and its potential use for drug development against amebiasis.

2. Thioredoxin-linked metabolism

As described above, *E. histolytica* lacks glutathione reductase enzyme and the ones responsible for synthesis of glutathione, and therefore, relies on the thioredoxin-linked system to prevent the damage caused by oxidative stress [12,17]. The thioredoxin system consists of small dithiol protein thioredoxin (Trx) and NADPH-dependent disulphide oxidoreductase, thioredoxin reductase (TrxR) [19] (Redox-related metabolism in *E. histolytica* is summarized in Fig. 1). The thioredoxin system catalyzes several redox reactions in the cell and Trxs are regarded as general redox messengers that interact with a wide variety of proteins [19] such as ribonucleotide reductase, 3′-phospho-adenylylsulfate reductase, and methionine sulfoxide reductase [20–22]. Trxs contain two cysteine residues within the CXXC motif of the redox-active site [23]. In the oxidized state, these cysteine residues form a disulphide bond, which is in turn reduced by TrxR. In the reduced state, one of the active site cysteines of Trx interacts with target proteins. The thioredoxin system ultimately functions as a donor of reducing equivalents for various biological processes.

Arias et al. first demonstrated the presence of Trx-linked systems in *E. histolytica* [12]. The *E. histolytica* genome (http://amoedb.org/amoeba/) contains a single *TrxR* gene (EHI_155440), which encodes a protein of the low molecular weight TrxR family [12,17]. However, there are 22 *Trx* genes, fourteen of these encode proteins of ~200 amino acids, of which four (EHI_096200; EHI_053840; EHI_004490; EHI_042900) Trx proteins contain the classical Trx consensus sequence motif (CGPC) whereas eight Trxs are larger in size [24]. Only two Trx proteins (EHI_004490; EHI_053840), which possess classical Trx consensus sequence (WCGPC), have been fully characterized [12,18]. The Trx/TrxR redox regulating system is present in the cytoplasm and thus primarily involved in the maintenance of redox balance in the compartment.

TrxR proteins identified in *E. histolytica* also have the coenzyme binding motif (GGGGDAA), and Arg^{183} and Arg^{184} residues predicted to be involved in the interaction with the phosphate moiety of NADPH [18], as well as the conserved active dithiol/disulfide center (CXXC). It has also been demonstrated that *E. histolytica* TrxR also exhibits a H_2O_2-generating NAD(P)H oxidase activity in the presence of O_2 (Fig. 1), and its catalytic mechanism is different from the disulfide reduction [18]. *E. histolytica* TrxR can utilize both NADPH and NADH as reducing equivalent donors for the disulfide reductase activity. Although the affinity of *E. histolytica* TrxR for NADPH is 10-fold higher than that for NADH, the activity exhibited with NADH is noteworthy. TrxRs from other organisms possess restricted specificity toward NADPH [18,25,26]. TrxRs from different organisms such as *Escherichia coli*, *Mycobacterium leprae*, *Plasmodium falciparum*, *Trypanosoma cruzi, Leishmania donovani, E. histolytica, Giardia lamblia* and *Homo sapiens* show diversity in chemical mechanism of thioredoxin reduction [27,28]. This is the basis for attempts to develop specific TrxR inhibitors as drugs against these parasitic diseases (see below).

3. Trypanothione metabolism

There is controversy regarding the presence of trypanothione, a spermidine-containing thiol, in *E. histolytica*. Ondarza et al. [29,30] reported the presence of trypanothione and *in vitro* trypanothione synthetase activity from *E. histolytica* HK-9 [31] trophozoites, a redox metabolite previously thought to be unique to trypanosomatids [32]. However, Ariyanayagam and Fairlamb [33] were unable to confirm the presence of trypanothione in *E. histolytica* HM-1:IMSS, and suggested that trypanothione metabolism is absent or quantitatively insignificant in this organism. Later, Tamayo et al. demonstrated the NADPH-dependent trypanothione reductase activity from *E. histolytica* trophozoite lysates and partially purified the activity, claiming that *E. histolytica* possess trypanothione metabolism [34]. However, the gene encoding trypanothione reductase from *E. histolytica* HK-9 (AF503571), reported by Tamayo et al., apparently has no homologue in the genome of HM-1:IMSS, as well as other *E. histolytica* strains, HM-1:1MSS-A and B, HM-3:1MSS, and KU27, as well as other *Entamoeba* species such as *Entamoeba dispar* SAW760, *Entamoeba* invadens IP1, *Entamoeba* moshkovskii Laredo, and *Entamoeba* nuttalli P19. Therefore, the trypanothione reductase activity demonstrated seems to be attributable to the medium or contaminants. Alternatively, this discrepancy may be interpreted as inter-strain variations of the trypanothione metabolism. Altogether, trypanothione and its metabolism are likely absent in *E. histolytica* in general.

4. Role of L-cysteine/*S*-methyl-L-cysteine metabolism

In most eukaryotes, glutathione is the major thiol, and L-cysteine levels are maintained many fold lower than that of glutathione [35]. However, *E. histolytica* is completely devoid of glutathione and its metabolism, and L-cysteine is the principal low-molecular-weight thiol [16]. It has been shown that L-cysteine is required for the survival, growth, attachment, elongation, motility, gene regulation, and antioxidative stress defense of this organism [16,36]. In general, L-cysteine can be produced either from L-homocysteine via the reverse *trans*-sulfuration pathway or from L-serine by the sufur assimilatory *de novo* biosynthetic pathway [37]. *E. histolytica* is unique in the sense that the forward (L-cysteine to L-methione) and reverse (L-methionine to L-cysteine) *trans*-sulfuration pathways are absent or interrupted, respectively. Thus, L-cysteine is potentially synthesized via the *de novo* biosynthetic pathway, which is typically present in bacteria and plants [38,39]. This pathway consists of two steps that are catalyzed by serine acetyltransferase (SAT, EC 2.3.1.30) and cysteine synthase [CS; OAS (*O*-acetylserine) [thiol] lyase; EC 4.2.99.8] [37]. *E. histolytica* has three isoforms each of SAT and CS [40]. The three different isoform of SAT are feedback inhibited to different extents by the end product L-cysteine, which helps to maintain high L-cysteine levels in *E. histolytica* at all the time [41]. However, it was shown that despite the presence of the *de novo* cysteine biosynthetic pathway, cultivation of axenic trophozoites in the medium lacking supplemented L-cysteine resulted in nearly undetectable intracellular levels of L-cysteine and L-cystine [42]. This suggests that the endogenous biosynthesis is inoperative or insufficient, and the trophozoites greatly depend on L-cysteine uptake from the extracellular milieu [43]. In agreement with this observaton, cultivation of *E. histolytica* requires high concentrations of L-cysteine in the medium, which can be replaced by D-cysteine, L-cystine, or partially L-ascorbic acid, indicating that the extracellular L-cysteine/L-cystine, thiols, or reductants can play an interchangeable role [44].

A previous metabolome study by Husain et al. [42] revealed that under L-cysteine deprivation, OAS and *S*-methylcysteine (SMC) were accumulated in the trophozoites, while the *S*-