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# A glutathione-specific aldose reductase of *Leishmania donovani* and its potential implications for methylglyoxal detoxification pathway

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ABSTRACT

Methylglyoxal is mainly catabolized by two major enzymatic pathways. The first is the ubiquitous detoxification pathway, the glyoxalase pathway. In addition to the glyoxalase pathway, aldose reductase pathway also plays a crucial role in lowering the levels of methylglyoxal. The gene encoding aldose reductase (ALR) has been cloned from *Leishmania donovani*, a protozoan parasite causing visceral leishmaniasis. DNA sequence analysis revealed an open reading frame (ORF) of ~855 bp encoding a putative protein of 284 amino acids with a calculated molecular mass of 31.7 kDa and a predicted isoelectric point of 5.85. The sequence identity between *L. donovani* ALR (LdALR) and mammals and plants is only 36–44%. The ORF is a single copy gene. A protein with a molecular mass that matched the estimated ~74 kDa according to the amino acid composition of LdALR with a maltose binding tag present at its N-terminal end was induced by heterologous expression of LdALR in *Escherichia coli*. In the presence of glutathione, recombinant LdALR reduced methylglyoxal with a $K_m$ of ~112 μM. Comparative structural analysis of the human ALR structure with LdALR model suggests that the active site anchoring the N-terminal end of the glutathione is highly conserved. However, the C-terminal end of the glutathione backbone is expected to be exposed in LdALR, as the residues anchoring the C-terminal end of the glutathione backbone come from the three loop regions in human, which are apparently shortened in the LdALR structure. Thus, the computational analysis provides clues about the expected mode of glutathione binding and its interactions with the protein. This is the first report of the role of an ALR in the metabolic disposal of methylglyoxal in *L. donovani* and of thiol binding to a kinetoplastid aldose reductase.



## 1. Introduction

*Leishmania donovani*, a flagellated protozoan parasite, is the causative agent of visceral leishmaniasis. Sandflies transmit promastigote forms of the parasite to the mammalian host, where they invade macrophages and transform into amastigotes (Pulvertaft and Hoyle, 1960). These parasites along with the trypanosomes belong to the order 'kinetoplastidae'. A unique feature of these parasitic protozoa is the presence of a unique dithiol trypanothione ($N^1$, $N^8$-bis(glutathionyl) spermidine) and the flavoenzyme trypanothione reductase (Muller et al., 2003; Krauth-Siegel et al., 2005). This is in contrast to human and other eukaryotes, which contain ubiquitous glutathione/glutathione reductase system. The major role of trypanothione and trypanothione-dependent enzymes is to protect cells against oxidative damage (Thornalley, 1990). Another important function of thiols is to protect cells from toxic metabolite by-products such as methylglyoxal, a reactive 2-oxoaldehyde. Methylglyoxal is a mutagenic and cytotoxic compound mainly formed as a by-product of glycolysis. It is also produced by catabolism of amino acids (Thornalley, 1996). It reacts with both proteins and nucleic acids resulting in glycation end products which are implicated in diseases like diabetes and related clinical complications (Lo et al., 1994; Vaca et al., 1994).

Methylglyoxal is mainly catabolized by two major enzymatic pathways (Fig. 1). The first is the ubiquitous detoxification pathway, the glyoxalase pathway (Thornalley, 1990, 1996). A typical glyoxalase system consists of two enzymes, glyoxalase I (lactoylglutathione methylglyoxal lyase, EC.4.4.1.5) and glyoxalase II (hydroxyacyl glutathione hydrolase, EC.3.1.2.1). Both the enzymes require thiol cofactor for their function. The glyoxalase system has been shown to be dependent on glutathione in mammals and other eukaryotes (Yadav et al., 2005a,b). The glyoxalase system is dependent on the dithiol trypanothione, in trypanosomes and *leishmania* (Vickers et al., 2004; Ariza et al., 2006; Greig et al., 2006). Alternate pathway involves aldose reductase (aldehyde reductase, EC.1.1.1.21) that converts methylglyoxal into acetol in a NADPH dependent two step reaction. In addition to

*Abbreviations:* ALR, aldose reductase; dNTP, deoxynucleotide triphosphate; LdALR, *L. donovani* aldose reductase; ORF, open reading frame; PCR, Polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; MBP, Maltose-binding protein; TbPGFS, *Trypanosoma brucei*, prostaglandin f2-alpha synthase.

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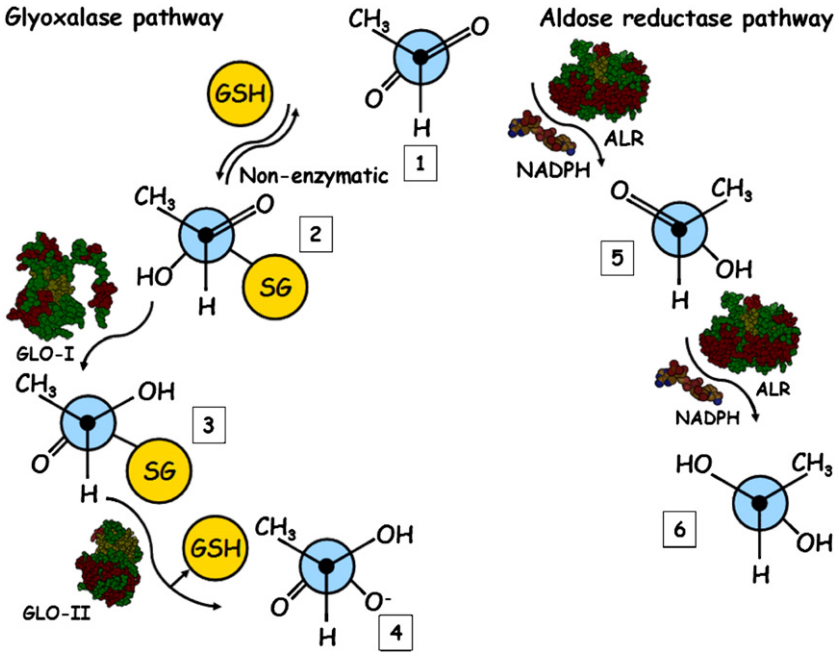


**Fig. 1.** Catabolism of methylglyoxal by the glyoxalase pathway involves two enzymes for the conversion of methylglyoxal to D-lactate and the alternative pathway involves aldose reductase converting methylglyoxal to Propane-1,2 diol in human. (1) Methylglyoxal (2) Hemithioacetal (3) *S*-D-Lactoylglutathione (4) D-Lactate (5) Acetol (6) Propane-1,2 diol. GSH: glutathione, reduced form; ALR: aldose reductase: GLO-I: glyoxalase I; GLO-II: glyoxalase II; NADPH: nicotinamide adenine dinucleotide phosphate, reduced form; SG: glutathione part of the molecule.

these two detoxification pathways, 2-oxoaldehyde dehydrogenase and betaine aldehyde dehydrogenases convert methylglyoxal into pyruvate. Except glyoxalases, all the other enzymes have been shown to act on free methylglyoxal and require NADPH as a cofactor for their activity (Vander Jagt and Hunsaker, 2003).

However, studies have shown that glutathiolated aldehydes enhance the catalytic activity of aldose reductase in human and yeast (Cagen and Pisano, 1979; Ramana et al., 2000; Dixit et al., 2000; Doorn et al., 2003). Aldose reductase has broad substrate specificity. It has been shown that in yeast, during oxidative stress conditions, the glutathione levels are depleted and the glyoxalase enzymes are inactive, aldose reductase then detoxifies about 40% of the methylglyoxal in the cell (Ponces et al., 2003; Gomes et al., 2005). In liver, where the glutathione levels are high, glutathione dependent glyoxalase pathway is preferred over the NADPH dependent aldose reductase pathway. In the presence of physiological concentrations of glutathione, methylglyoxal also spontaneously conjugates with glutathione to form hemithioacetal, which is the actual substrate of glyoxalase I. Jagt et al. (2001) have studied the relative importance of glyoxalase-I and aldose reductase in the metabolism of methylglyoxal in a glutathione dependent pathway.

The aldo-keto reductase enzymes comprise a functionally diverse gene family, which catalyze the NADPH-dependent reduction of a variety of carbonyl compounds. Searches in the genomic data of *Leishmania major* and *Leishmania infantum* identified putative aldose reductase genes (http://www.ebi.ac.uk/parasites/LGN/). There are five aldose reductase family sequences identified in *L. major* and *L. infantum* genome. In the present study we for the first time report the role of an ALR in the metabolic disposal of methylglyoxal in *L. donovani* and of thiol binding to a kinetoplastid aldose reductase.

## 2. Materials and methods

### 2.1. Materials

Trypanothione disulphide was obtained from Bachem. Restriction enzymes and *Taq DNA* Polymerase were obtained from MBI Fermentas. All other chemicals were of analytical grade and were available commercially.

### 2.2. Parasite and culture conditions

*L. donovani* Indian isolate 2001-S promastigotes were cultured at 22 °C in modified M199 medium (Sigma) supplemented with 100 U/ml penicillin (Sigma), 100 μg/ml streptomycin (Sigma) and 10% heat inactivated foetal bovine serum (Hyclone).

### 2.3. Cloning of ALR gene from L. donovani and sequence analysis

A ~855-bp DNA fragment was amplified from genomic DNA, using a sense primer with a flanking BamHI site (underlined), 5′-CGGGATCCATGGCTGAC GTTGGTAAG-3′, that coded for the amino acid sequence MADVGK at positions 1–18 and the antisense primer with a flanking HindIII site (underlined), 5′-CCCAAGCTTT-TAGAACTGCGCCTCATC-3′, which corresponded to amino acid residues DEAQF including the stop codon, at positions 838–855. PCR was performed in 50 μl reaction volumes containing 100 ng of genomic DNA, 25 pmol each of the gene-specific forward and reverse primers, 200 μM of each dNTP, 2 mM $MgCl_2$ and 5 U of *Taq DNA* polymerase. The conditions for PCR were as follows: 95 °C for 10 min, then 35 cycles of 95 °C for 30 s, 59 °C for 30 s and 72 °C for 1 min. Final extension was carried out for 10 min at 72 °C. A single 855-bp PCR product was obtained and subcloned in to pGEM-T vector (Promega) and subjected to automated sequencing. Sequence analysis was performed by DNAstar whereas comparison with other sequences of the database were performed using the search algorithm BLAST [23]. The amplified DNA fragment of ~855 bp (LdALR) was cloned into the BamHI/HindIII site of pMAL-c2 vector (New England Biolabs, Inc.). The recombinant construct was transformed into *BL21* (*DE3*) strain of *Escherichia coli*. Multiple sequence alignment of amino acid sequences was performed using the T-Coffee alignment program. The phylogenetic tree was constructed using PHYLIP which used an output of T-Coffee program.