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The glyoxalase pathway in protozoan parasites

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ABSTRACT

The glyoxalase system is the main catabolic route for methylglyoxal, a non-enzymatic glycolytic byproduct with toxic and mutagenic effects. This pathway includes two enzymes, glyoxalase I and glyoxalase II, which convert methylglyoxal to D-lactate by using glutathione as a catalytic cofactor. In protozoan parasites the glyoxalase system shows marked deviations from this model. For example, the functional replacement of glutathione by trypanothione (a spermidine–glutathione conjugate) is a characteristic of trypanosomatids. Also interesting are the lack of glyoxalase I and the presence of two glyoxalase II enzymes in *Trypanosoma brucei*. In *Plasmodium falciparum* the glyoxalase pathway is glutathione-dependent, and glyoxalase I is an atypical monomeric enzyme with two active sites. Although it is tempting to exploit these differences for their potential therapeutic value, they provide invaluable clues regarding methylglyoxal metabolism and the evolution of protozoan parasites. Glyoxalase enzymes have been characterized in only a few protozoan parasites, namely *Plasmodium falciparum* and the trypanosomatids *Leishmania* and *Trypanosoma*. In this review, we will focus on the key features of the glyoxalase pathway in major human protozoan parasites, with particular emphasis on the characterized systems in *Plasmodium falciparum*, *Trypanosoma brucei*, *Trypanosoma cruzi*, and *Leishmania* spp. We will also search for genes encoding glyoxalase I and II in *Toxoplasma gondii*, *Entamoeba histolytica*, and *Giardia lamblia*.

Introduction

The glyoxalase pathway is the main cellular system responsible for the elimination of methylglyoxal, a toxic compound due to its high reactivity toward proteins and nucleic acid amino groups (Thornalley, 1990). In eukaryotic cells, this 2-oxoaldehyde is mainly formed during glycolysis from the phosphate group β-elimination of the triose phosphates (Lohman and Meyerhof, 1934). Through the sequential action of glyoxalase I (GLO1, lactoylglutathione methylglyoxal-lyase, EC 4.4.1.5) and glyoxalase II (GLO2, hydroxyacylglutathione hydrolase, EC 3.1.2.6), methylglyoxal is converted into D-lactate using glutathione as the cofactor (Thornalley, 1990).

Being ubiquitous and relevant in the cell detoxification of methylglyoxal, the glyoxalase pathway has been studied in some human protozoan parasites as a potential drug target, namely in *Plasmodium falciparum*, *Leishmania* spp. and *Trypanosoma* spp. Hence, differences in glyoxalase I and II enzymes between humans and parasites have been identified (Fig. 1, Table 1). The first enzyme of the glyoxalase pathway, glyoxalase I, is typically a homodimer with the active site located at the interface of the two subunits (Ariza et al., 2006; Barata et al., 2010; Cameron et al., 1997; He et al., 2000; Sukdeo et al., 2004). However, exceptions are found in *P. falciparum* (Iozef et al., 2003), where glyoxalase I is a monomeric enzyme with two active sites. It is allosterically regulated and, as in mammalian cells, depends on glutathione. A difference concerning thiol specificity occurs in trypanosomatids, where reduced trypanothione is used instead of glutathione to convert methylglyoxal into the corresponding thioester (Ariza et al., 2006; Irsch and Krauth-Siegel, 2004; Silva et al., 2008; Sousa Silva et al., 2005). Interestingly, the trypanosomatid *Trypanosoma brucei* does not have a glyoxalase I enzyme but has two glyoxalase II coding genes, raising several questions about the function of the glyoxalase pathway in this parasite (Wendler et al., 2009). Glyoxalase II is the second enzyme of the pathway and hydrolyzes the thioester into D-lactate, regenerating the thiol. In *T. brucei*, only one of these GLO2 enzymes displays a functional glyoxalase II activity (Wendler et al., 2009). *P. falciparum* also has two glyoxalase II enzymes, one located in the cytosol and the other containing a target sequence to the apicoplast (Urscher et al., 2011). However, the existence of a glyoxalase I-like protein (GILP) probably located to the apicoplast, unique in malarial parasites, but inactive with the typical GLO1 substrates is intriguing (Akoachere et al., 2005; Urscher et al., 2011).

Despite some basic features shared between protozoan parasites, several differences are found in their metabolism, and the

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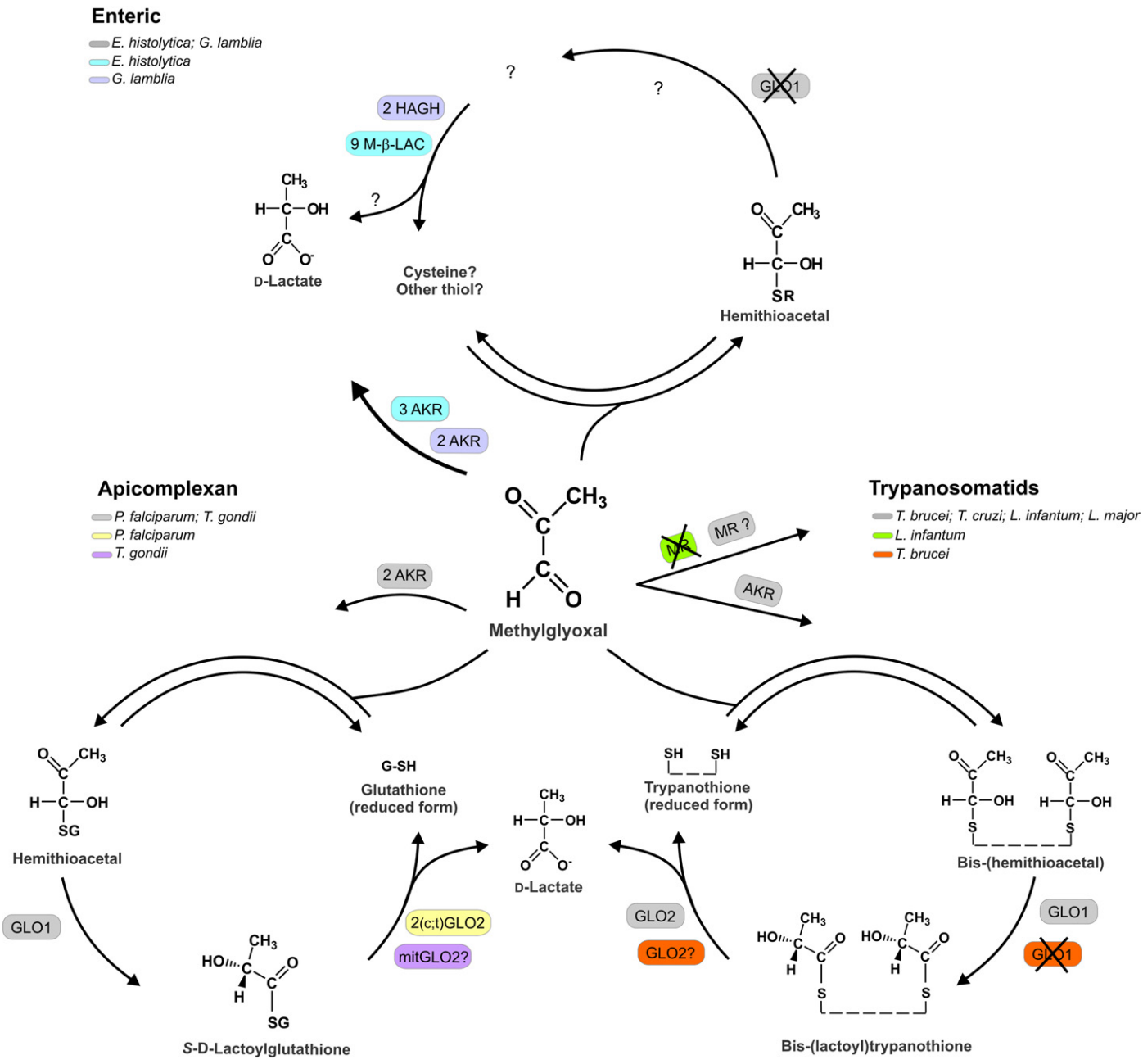

Fig. 1. The glyoxalase pathway in protozoan parasites. Methylglyoxal reacts non-enzymatically with a thiol, either glutathione or trypanothione, forming a hemithioacetal, which is isomerized to the thioester *S*-D-lactoylglutathione or *S*-D-lactoyltrypanothione by glyoxalase I (GLO1). The thioester is then hydrolyzed to D-lactate by glyoxalase II (GLO2), regenerating the thiol. This classic glyoxalase pathway is only found in *Leishmania* spp., *T. cruzi*, and *Plasmodium falciparum*. In all other cases the glyoxalase system seems to be incomplete. *T. brucei* notoriously lacks glyoxalase I as well as the enteric protozoans.

glyoxalase pathway is no exception (Fig. 1). In this review, we present the landscape of knowledge on this thiol-dependent system in protozoan parasites responsible for human diseases: the apicomplexan *P. falciparum* and *Toxoplasma gondii*, the enteric parasites *Entamoeba histolytica* and *Giardia lamblia*, and the trypanosomatids *Leishmania* spp., *T. brucei* and *T. cruzi*.

The apicomplexan parasites *Plasmodium falciparum* and *Toxoplasma gondii*

The presence of an active glyoxalase pathway in an apicomplexan parasite was first reported in *Plasmodium falciparum*-infected erythrocytes (Vander Jagt et al., 1990). Some years later, glyoxalase I and both glyoxalase II enzymes were characterized in this parasite (Akoachere et al., 2005; Iozef et al., 2003). *P. falciparum* has one *GLO1* gene encoding for an active cytosolic glyoxalase I enzyme (Urscher et al., 2010). Its genome also contains a gene coding for a glyoxalase I-like protein (GILP). This enzyme is unique in malarial parasites and seems to be located in the apicoplast but does not exhibit typical GLO1 activity (Akoachere et al., 2005; Urscher et al., 2011). In *P. falciparum* there are two glyoxalase II enzymes, one cytosolic and another containing an apicoplast targeting sequence (Akoachere et al., 2005; Urscher et al., 2010, 2011).

Glyoxalase I enzymes are characteristically homodimers (with 16–18 kDa per monomer), but exceptions are found in *Saccharomyces cerevisiae* (Marmstal et al., 1979) and *Plasmodium falciparum* (Iozef et al., 2003) where GLO1 are monomeric with two active sites. These proteins are most probably the result of