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Review

Methylglyoxal metabolism in trypanosomes and leishmania

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ABSTRACT

Methylglyoxal is a toxic by-product of glycolysis and other metabolic pathways. In mammalian cells, the principal route for detoxification of this reactive metabolite is via the glutathione-dependent glyoxalase pathway forming D-lactate, involving lactoylglutathione lyase (GLO1; EC 4.4.1.5) and hydroxyacylglutathione hydrolase (GLO2; EC 3.2.1.6). In contrast, the equivalent enzymes in the trypanosomatid parasites *Trypanosoma cruzi* and *Leishmania* spp. show >200-fold selectivity for glutathionylspermidine and trypanothione over glutathione and are therefore sensu stricto lactoylglutathionylspermidine lyases (EC 4.4.1.-) and hydroxyacylglutathionylspermidine hydrolases (EC 3.2.1.-). The unique substrate specificity of the parasite glyoxalase enzymes can be directly attributed to their unusual active site architecture. The African trypanosome differs from these parasites in that it lacks GLO1 and converts methylglyoxal to L-lactate rather than D-lactate. Since *Trypanosoma brucei* is the most sensitive of the trypanosomatids to methylglyoxal toxicity, the absence of a complete and functional glyoxalase pathway in these parasites is perplexing. Alternative routes of methylglyoxal detoxification in *T. brucei* are discussed along with the potential of exploiting trypanosomatid glyoxalase enzymes as targets for anti-parasitic chemotherapy.

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1. Introduction

Flagellated protozoa of the family Trypanosomatidae encompass a diverse range of organisms, including the human pathogens *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania* spp., causative agents of sleeping sickness, Chagas' disease and leishmaniasis, respectively. These digenetic parasites undertake complex life cycles, differentiating into a variety of developmental forms while parasitizing both vertebrate and insect vector hosts. Collectively, the diseases are responsible for more than 120,000 fatalities annually and the loss of over 4,600,000 disability adjusted life years (DALYs) [1]. Some of the most socio-economically deprived regions of the world are afflicted by these vector-borne parasites and the accompanying economic burden provides a major obstacle to improving human health [2]. Almost all existing drugs used to treat these diseases suffer from serious problems ranging from severe toxic side effects [3] to acquired drug resistance [4,5]. To further compound these difficulties, treatments often require lengthy periods of hospitalisation and are prohibitively expensive [1]. Therefore, novel drug targets and more effective drug treatments are urgently required for these neglected diseases of poverty.

Metabolic pathways that are absent from, or significantly different to, host pathways are logical starting points for drug

Abbreviations: GLO1, glyoxalase I; GLO2, glyoxalase II; $T[SH]_2$, trypanothione, N^1,N^8-bis(glutathionyl)spermidine; GSH, glutathione; LADH, lactaldehyde dehydrogenase.

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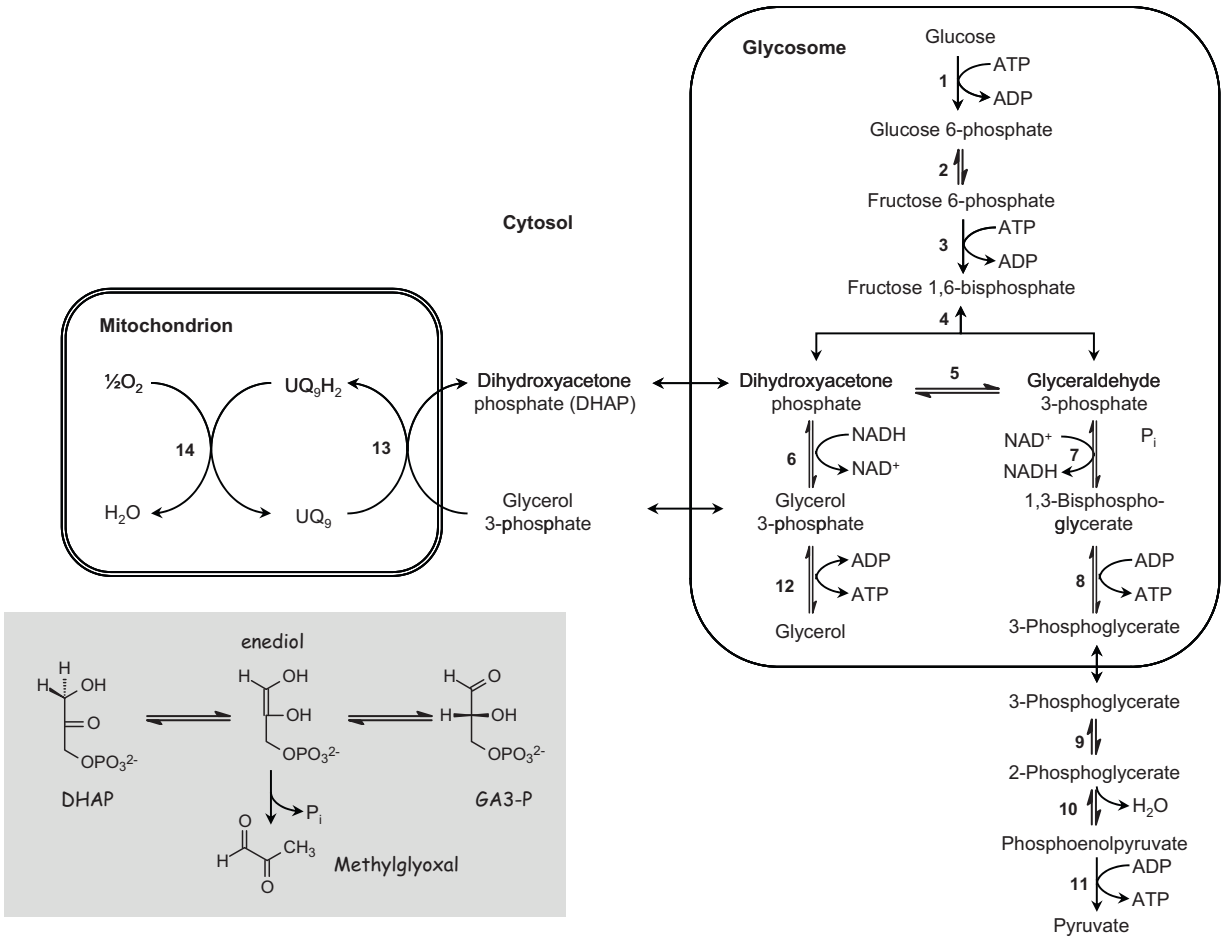

Fig. 1. Glycolytic pathway and metabolic compartments in bloodstream form *T. brucei*. The shaded inset shows the route to methylglyoxal from dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate (GA-3P) via an enediol intermediate. Each mol of NADH generated in step 7 is reoxidised via the glycerophosphate shuttle (steps 6, 13 and 14) where glycerophosphate exits the glycosome in exchange for dihydroxyacetone phosphate. The location of enzymes in the glycosome and mitochondrion are bounded by single or double lines, respectively. Enzyme reaction steps are: 1, hexokinase; 2, glucose 6-phosphate isomerase; 3, phosphofructokinase; 4, aldolase; 5, triose phosphate isomerase; 6, glycerol 3-phosphate dehydrogenase (NAD+); 7, glyceraldehyde 3-phosphate dehydrogenase; 8, phosphoglycerate kinase; 9, phosphoglycerate mutase; 10, enolase; 11, pyruvate kinase; 12, glycerol kinase; 13, glycerol 3-phosphate dehydrogenase (FAD); 14; ubiquinol oxidase (trypanosome alternative oxidase). Other abbreviations: UQ_9 and UQ_9H_2 are ubiquinone and ubiquinol, respectively.

discovery. With this in mind, the glyoxalase pathway, a ubiquitous detoxification pathway that protects against the cellular damage caused by the toxic and mutagenic glycolytic metabolite methylglyoxal [6,7], would seem far from an ideal drug target within these parasites. The glyoxalase pathway comprises glyoxalase I (GLO1) (lactoylglutathione lyase, EC 4.4.1.5) and glyoxalase II (GLO2) (hydroxyacylglutathione hydrolase, EC 3.1.2.6), which act in concert to convert the spontaneously formed hemithioacetal adduct between glutathione and methylglyoxal into D-lactate and glutathione. The universal nature of the glyoxalase pathway emphasises its significance in general cellular function resulting in its conservation throughout evolution. However, quantitative differences in methylglyoxal metabolism of rapidly proliferating cells may be therapeutically exploitable. Elevated levels of GLOI, responsible for the initial step in the detoxification of methylglyoxal, have been found in tumour tissue from human colon, renal and prostate cancers [8] and are believed to be associated with the increased proliferative growth rates of tumours cells. Most significantly, inhibitors of GLO1 have been shown to be selectively toxic, not only to tumour cells [9], but also to other rapidly growing organisms such as the protozoan parasite *Plasmodium falciparum* [10]. These findings have raised the possibility that the glyoxalase pathway may indeed present a viable drug target in the Trypanosomatidae.

The major source of methylglyoxal in cells is a by-product of glycolysis, where the triose phosphate intermediates dihydroxyacetone phosphate and glyceraldehyde 3-phosphate eliminate phosphate via an enediolate intermediate (Fig. 1, inset) [6]. Minor sources of methylglyoxal are from aminoacetone and hydroxyacetone, intermediates generated during catabolism of threonine and acetone [6]. Since *T. cruzi*, *T. brucei* and the *Leishmania* spp. are known to rapidly proliferate, demands for energy within cells are particularly high, resulting in high rates of glycolysis. Indeed, bloodstream-form *T. brucei* maintain respiratory rates approximately two orders of magnitude higher than those seen in mammalian cells [11]. Lacking cytochromes and a functional tricarboxylic acid cycle, this organism is entirely dependent on substrate-level phosphorylation from glycolysis for ATP production. The major end-product of the glycolytic pathway is pyruvate, rather than L-lactate, with net production of 2 mol of ATP per mol glucose consumed (Fig. 1). Lacking a canonical L-lactate dehydrogenase, NADH is oxidised by means of a plant-like glycerophosphate oxidase system that is not coupled to oxidative phosphorylation [12]. Glycolysis is unique in that the initial stages take place within a microbody-like organelle, the glycosome [13] and reducing equivalents from NADH are transferred to the mitochondrial glycerophosphate oxidase via the glycerophosphate/dihydroxyacetone phosphate shuttle. The insect stages of *T. brucei* and all stages of