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Glucose metabolism in *Trypanosoma cruzi*

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Abstract

The causative agent of Chagas disease, *Trypanosoma cruzi*, metabolizes glucose through two major pathways: glycolysis and the pentose phosphate pathway. Glucose is taken up via one facilitated transporter and its catabolism by the glycolytic pathway leads to the excretion of reduced products, succinate and L-alanine, even in the presence of oxygen; the first six enzymes are located in a peroxisome-like organelle, the glycosome, and the lack of regulatory controls in hexokinase and phosphofructokinase results in the lack of the Pasteur effect. All of the enzymes of the pentose phosphate pathway are present in the four major stages of the parasite's life cycle, and some of them are possible targets for chemotherapy. The gluconeogenic enzymes phosphoenolpyruvate carboxykinase and fructose-1,6-bisphosphatase are present, but there is no reserve polysaccharide.

Introduction

Trypanosoma cruzi, a flagellated protozoan parasite, is the causative agent of the American trypanosomiasis Chagas disease. The infection is endemic in Latin America, where its prevalence is estimated at 10–12 million cases,

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and has been classified by the WHO (World Health Organization) as one of the most neglected diseases. No vaccines have been developed so far, and the low effectiveness of the chemotherapeutic agents available, together with their undesirable side effects, makes the treatment of Chagas disease difficult. Therefore there is an urgent need for the identification of novel drug targets to improve the treatment of this disease [1].

The parasite has a complex life cycle, involving two forms present in the gut of the insect vector, the replicative epimastigote and the infective metacyclic trypomastigote, and two forms present in the infected mammal, the replicative intracellular amastigote and the bloodstream trypomastigote, released from infected cells into the blood. Most biochemical studies on this parasite have been performed with epimastigotes, which can be readily grown in axenic culture; some studies have been conducted with the other parasite stages, which can also be obtained, in smaller amounts, in culture (amastigotes and bloodstream-like trypomastigotes in mammalian cell cultures; metacyclic trypomastigotes by the differentiation of culture epimastigotes) [2]. The genome project of *T. cruzi* [3] has facilitated studies on the enzyme pathways operating in the parasite; functional studies have been hampered, however, by the fact that forward and reverse genetic approaches with this parasite have been difficult. For example, as happens with most *Leishmania* spp., and at variance with *Trypanosoma brucei*, it lacks the machinery for RNAi (RNA interference) down-regulation of protein expression [4]. Therefore it is not surprising that most biochemical studies nowadays are being performed with *T. brucei*.

Glucose uptake

Only one gene encoding a glucose transporter has been identified in the *T. cruzi* genome, and it is present as a cluster of multiple copies in tandem. The ORF (open reading frame) encodes a protein with 12 predicted transmembrane domains, characteristic of the superfamily of sugar facilitated transporters that passively transport glucose with high affinity, and shares with other kinetoplastid hexose transporters the ability to transport D-fructose [5]. Glucose transport has been thoroughly studied in both epimastigotes, which thrive in the insect's gut, and bloodstream trypomastigotes, which obviously live in a glucose-rich medium; intracellular amastigotes, however, live in the cytosol of mammalian cells, where free glucose cannot be abundant. In good agreement, it has been shown recently that amastigotes do not take up glucose from the medium, and the expression of the transporter has not been found, either at the mRNA or protein levels [6]. This fact indicates a metabolic feature very important in all trypanosomatids: since the different stages in their complex life cycles live in very different environments, they must have adapted to these environments by changing their metabolism when they are confronted with a new medium. Epimastigotes, although normally living in a medium in which glucose is supposed to be scarce (the insect's

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gut), prefer glucose to amino acids, even though L-proline and other amino acids are readily transported and consumed; indeed, even the intracellular epimastigote, an intermediate stage in the differentiation of amastigotes to trypomastigotes, expresses the transporter, albeit at a lower level compared with trypomastigotes [6]. It is supposed nowadays that the amastigotes obtain their energy mostly from amino acids, although the oxidation of fatty acids cannot be excluded [6].

Aerobic fermentation of glucose

Trypanosomatids are among the cells that have a higher rate of glucose consumption; this is associated with the highly unusual fact that they produce and excrete into the medium fermentative, namely still-reduced, compounds from glucose catabolism, even under aerobic conditions, instead of oxidizing glucose completely to CO_2 and water (see [7] for a review). This peculiar metabolism was called 'aerobic fermentation of glucose' by Theodor von Brand, the pioneer of parasite biochemistry. This name emphasizes that their production is normally performed by other organisms under anaerobic conditions; it is shared with a number of other parasites, both protozoa and helminths [8].

In the case of *T. cruzi*, the major products of aerobic glucose catabolism by epimastigotes, as shown by NMR using [^{13}C]glucose, are succinate and L-alanine [9]. Acetate, which is a product detected by enzymatic analysis of the culture medium [10], is probably a minor catabolite of glucose, originating mostly from amino acids and, perhaps, fatty acids, since its production from glucose followed by [^{13}C]glucose NMR has been reported to be very low [9] or zero [11]. The balance between succinate and L-alanine shifts, depending on whether CO_2 is present at a high concentration {which favours succinate production by PEPCK (phosphoenolpyruvate carboxykinase); see below and [9]} or if the latter enzyme is inhibited, favouring L-alanine production [11].

Another characteristic feature of glucose catabolism in trypanosomatids is the lack of the Pasteur effect. This effect means that in most organisms, from baker's yeast to human muscle, the transition from anaerobiosis to aerobiosis is accompanied by a rapid and considerable decrease in glucose utilization, due to the tight regulation of the glycolytic pathway and the tricarboxylic acid cycle. Trypanosomatids do not display the Pasteur effect, and they show, in the most thoroughly studied cases, even a 'reverse Pasteur effect', meaning that glucose consumption may be even lower under anaerobic conditions [7]. This odd behaviour is related to the lack of the major controls on the glycolytic pathway, as described in the next section.

Glycolytic pathway

The glycolytic pathway in trypanosomatids presents a so far unique subcellular compartmentation: the first six enzymes involved are placed in a peroxisome-like organelle, which for this reason was called the 'glycosome'

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by its discoverers, Fred Opperdoes and Piet Borst, in 1977. This organelle contains, in addition to these glycolytic enzymes, which in the bloodstream forms of *T. brucei* can account for 90% of the total organelle protein, enzymes belonging to other metabolic pathways, such as the PPP (pentose phosphate pathway), the β-oxidation of fatty acids, purine salvage, and biosynthetic pathways for pyrimidines, other lipids and squalene (see [12] and references therein).

All of the glycolytic enzymes are encoded by genes identified in the *T. cruzi* genome project, and in most cases there is also proteomic evidence for their expression (Table 1). Figure 1 summarizes our present knowledge of glycolysis and its connection with the tricarboxylic acid cycle in the parasite; the metabolic pathways are similar to those thoroughly studied in the procyclic form of *T. brucei*, but in the case of *T. cruzi* there is much less information on their actual function. Besides, there is no evidence in *T. cruzi* of the presence of some of the enzymes related to these pathways in the procyclic form. For instance, a cMDH [cytosolic MDH (malate dehydrogenase)] is not present in *T. cruzi*, having mutated to an L-α-aromatic hydroxyacid dehydrogenase that is unable to reduce oxaloacetate [13]. The enzymes converting glucose into 3-phosphoglycerate are present inside the glycosome in *T. cruzi*, whereas the other enzymes of the pathway are cytosolic (Figure 1). The first reaction, the phosphorylation of glucose to glucose 6-phosphate, is catalysed in *T. cruzi* by two different enzymes: HK (hexokinase) [14] and GK (glucokinase) [15]; the three-dimensional structure of the latter has been determined [16]. A relevant feature of both enzymes is that, in contrast with the HKs from most organisms, they lack the regulatory inhibition by glucose 6-phosphate. The affinity of HK for glucose was 10-fold higher than in the case of GK (apparent K_m values of 60 μM and 1 mM respectively); both enzymes are specific for glucose and are located in the glycosomes [14,15], in good agreement with the presence of peroxisomal targeting signals. Cáceres et al. [14] reported the presence of a third, cytosolic, HK activity, which seems to also have fructokinase activity.

T. cruzi epimastigotes contain two isoforms of phosphoglucose isomerase, one cytosolic and the other glycosomal; both were inhibited by erythrose 4-phosphate, 6-phosphogluconate and mannose 6-phosphate [17].

PFK (phosphofructokinase), the major regulatory enzyme of glycolysis in many organisms, like the similar enzyme from *T. brucei* [18], is not significantly affected by effectors such as ATP, citrate or P_i [19]. The *T. cruzi* PFK presented Michaelian kinetics for fructose 6-phosphate and was activated only by 5′-AMP [19]. In addition, like the similar enzyme from other trypanosomatids, *T. cruzi* PFK shows the highest sequence similarity with PP_i-dependent PFK, despite being ATP-dependent, and is glycosomal [20].

T. cruzi has three genes encoding phosphoglycerate kinase, named *PGKA*, *PGKB* and *PGKC*. The three isoforms are expressed in the epimastigotes. PGKB accounts for ~80% of the total activity and is cytosolic, whereas

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Table 1. Genomic and proteomic data on the enzymes mentioned in the present chapter, detected in the CL Brener done, as described in the Tritryps database (http://www.tritrypdb.org/tritrypdb/)

The parasite stages where peptides have been identified by tandem MS are: A, amastigote; E, epimastigote; M, metacyclic trypomastigote; T, bloodstream trypomastigote.

Enzyme	Gene ID	Stage
Glycolysis		
HK	Tc00.1047053508951.20	A, E, M, T
GK	Tc00.1047053510187.100	E, M
Glucose-6-phosphate isomerase, glycosomal	Tc00.1047053506529.508	A, E, M
6-Phospho-1-fructokinase	Tc00.1047053508153.340	A, E, M
Fructose-bisphosphate aldolase	Tc00.1047053504163.40	A, E, M, T
Triose-phosphate isomerase	Tc00.1047053508647.200	M, T
Glyceraldehyde-3-phosphate dehydrogenase	Tc00.1047053506943.50	A, E, M, T
Phosphoglycerate kinase	Tc00.1047053511419.40	A, E, M, T
Phosphoglycerate mutase	Tc00.1047053507811.44	Without peptides
2,3-Bisphosphoglycerate-independent phosphoglycerate mutase	Tc00.1047053506247.330	A, E, M, T
Enolase	Tc00.1047053504105.140	A, E, M, T
PK2	Tc00.1047053507993.390	E, M, T
Pyruvate dehydrogenase		
Pyruvate dehydrogenase E1 component subunit α	Tc00.1047053507831.70	A, E, M
Pyruvate dehydrogenase E1 component subunit β	Tc00.1047053510091.80	A, E, M, T
Dihydrolipoyl transacetylase (E2)	None identified	
Dihydrolipoyl dehydrogenase (E3)	Tc00.1047053507089.270	A, E, M, T
Tricarboxylic acid cycle		
Citrate synthase	Tc00.1047053509801.30	A, M, T
Aconitase	Tc00.1047053511277.290	A, E, M, T
Isocitrate dehydrogenase	Tc00.1047053506925.319	A, E, M, T
Isocitrate dehydrogenase, mitochondrial precursor	Tc00.1047053511575.60	A, M, T
2-Oxoglutarate dehydrogenase, E1 component	Tc00.1047053510717.30	A, E, M, T
2-Oxoglutarate dehydrogenase, E2 component	Tc00.1047053503935.20	A, M
Succinyl-CoA synthetase α subunit	Tc00.1047053508479.340	Without peptides
Succinate dehydrogenase flavoprotein	Tc00.1047053511909.40	A, E, M, T
Fumarate hydratase	Tc00.1047053509879.40	A, M
MDH, mitochondrial	Tc00.1047053507883.109	A, E, M, T

(*Continued*)

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Table 1. (continued)

Enzyme	Gene ID	Stage
Glycolytic auxiliary enzymes		
PEPCK, glycosomal	Tc00.1047053507547.90	A, E, M, T
MDH, glycosomal	Tc00.1047053506503.69	A, E, M, T
Pyruvate phosphate dikinase	Tc00.1047053510101.140	A, E, M, T
PPP		
G6PDH	Tc00.1047053509287.50	A
6-PGL	Tc00.1047053503713.30	Without peptides
6PGDH	Tc00.1047053510737.10	A, E, M, T
RPI	Tc00.1047053508601.119	E, M, T
RPE	Tc00.1047053509213.70	Without peptides
TKT	Tc00.1047053511067.30	E, M
TAL	Tc00.1047053503477.20	A, E, M, T
Other enzymes		
Fructose-1,6-bisphosphatase, cytosolic	Tc00.1047053506649.70	Without peptides
Phosphoglucomutase	Tc00.1047053511911.130	Without peptides
Acetyl:succinate CoA-transferase*	Tc00.1047053504153.360	A, E, M, T
UDP-galactose 4-epimerase	Tc00.1047053506303.50	E, M
L-Galactonolactone oxidase	Tc00.1047053507047.150	Without peptides

*Annotated as succinylCoA:3-ketoacid-CoA transferase, mitochondrial precursor, putative.

PGKA and PGKC are both glycosomal, the first associated with the glycosomal membrane and the second, the least abundant of the three, located in the glycosomal matrix [21].

The PK (pyruvate kinase) from *T. cruzi* epimastigotes, like the similar enzymes from *T. brucei* and *Leishmania major*, is strongly activated by fructose 2,6-bisphosphate, which was able to counteract, at micromolar concentrations, the inhibition by millimolar concentrations of ATP and P_i [22].

As a result of the lack of effect of the normal inhibitors of HK and PFK, the operation of glycolysis in *T. cruzi*, and in other trypanosomatids as well, is not influenced appreciably by the presence or absence of oxygen, resulting in the lack of the Pasteur effect [7]. The lack of the classical regulation of these enzymes has been proposed to be compensated by the compartmentation of the first six glycolytic enzymes in the glycosome [23].

Genes encoding pyruvate dehydrogenase and all of the tricarboxylic acid cycle enzymes are present in the CL Brener clone genome (Table 1), with the exception of that encoding the NAD-linked ICDH (isocitrate dehydrogenase); there are, however, genes encoding two NADP-linked ICDHs, which we have recently shown to be mitochondrial and cytosolic [23a]. The latter had been assumed to be glycosomal, due to the presence of a PTS_1 (peroxisomal targeting

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signal 1) glycosomal targeting signal. It is possible that the mitochondrial isoform plays the role of the absent NAD-linked ICDH. Functionality of the cycle in the parasite has been assumed from experiments looking at the oxidation of ^{14}C-labelled glucose and acetate, by normal and dyskinetoplastic (i.e. lacking the mitochondrial genome) epimastigotes [24]. More recently, experiments with the procyclic stage of *T. brucei* suggest that this parasite does not use the tricarboxylic acid cycle activity for energy generation [25], but that different parts of the cycle have different functions [26]. Similar experiments have not been conducted on *T. cruzi*.

T. cruzi has a functional respiratory chain [27], and at least phosphorylation sites 2 and 3 are operative; the presence of a NADH oxidase linked to phosphorylation (complex I) has been long debated. Recently, Carranza et al. [28] have compared oxygen consumption and respiratory control ratios in the presence of NADH-linked substrates or succinate in wild-type *T. cruzi* and natural mutants with important deletions in the genes encoding the ND4, ND5 and ND7 subunits of complex I. The lack of significant differences led them to conclude that complex I has a limited function in the parasite. Therefore it is possible that reducing equivalents enter the respiratory chain as succinate, through the action of a NADH-dependent fumarate reductase [29,30].

Considering all of these facts, a big question remains: what is the reason for the production and excretion of still-reduced catabolites during aerobic glycolysis? One possibility is that, since the respiratory chain may be designed to oxidize succinate as its main fuel, and since it is rather deficient in cytochrome contents, the excretion of succinate might represent an overflow of reduced catabolite that the chain is not able to cope with [31]. If this is true, then other systems must be used to ensure the re-oxidation of glycolytic NADH. The more prevalent opinion nowadays is that the generation of fermentation products is necessary to keep the glycosome in both redox and energetic balance. During glycolysis, two ATP molecules are consumed in the reactions catalysed by HK and PFK, and two NADH molecules are produced in the reaction catalysed by glyceraldehyde-3-phosphate dehydrogenase. There are two glycosomal isoforms of PGK in *T. cruzi*; however, they would not be enough to keep the ATP concentration balanced to ensure that glycolysis would proceed. PEP (phosphoenolpyruvate), obtained from 3-phosphoglycerate in the cytosol, can produce one ATP molecule in the PK reaction. However, it is generally accepted that part of the PEP will enter the glycosome, and there it will suffer one of two possible fates, both yielding ATP: to be carboxylated to oxaloacetate by the ADP-dependent PEPCK [32], or to be converted into pyruvate by the PP_i-dependent PPDK (pyruvate-phosphate dikinase) [33] (Figure 1). Both reactions are catalysed by glycosomal enzymes and compete for the substrate PEP. If the PEPCK reaction is favoured, the oxaloacetate produced will be reduced to L-malate by glycosomal MDH [34], L-malate is dehydrated to fumarate by fumarate hydratase, and fumarate is reduced to succinate by the glycosomal NAD-dependent fumarate reductase [35]. Genes encoding all of these

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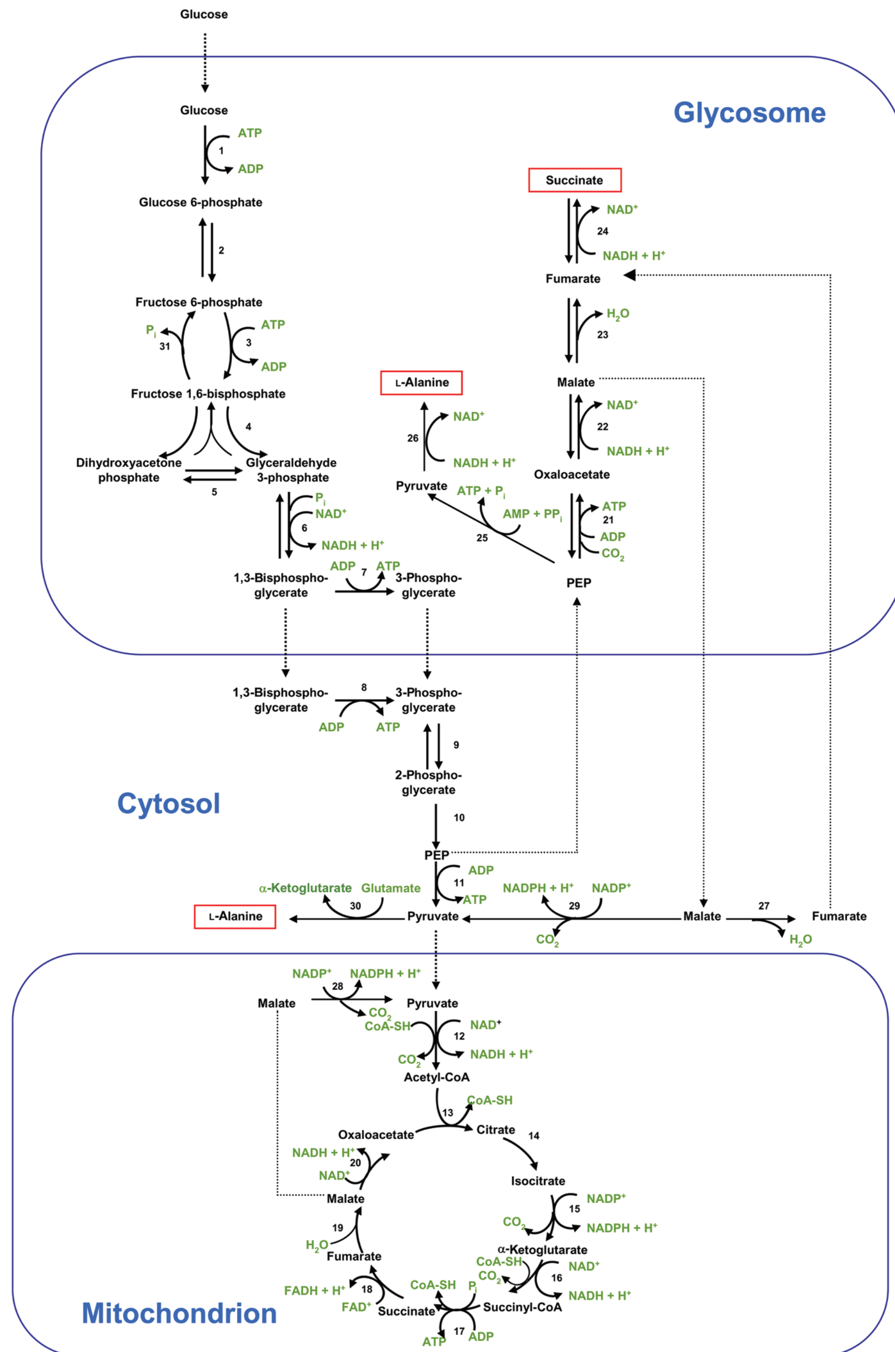

Figure 1. Schematic representation of glycolysis and the tricarboxylic acid cycle in *T. cruzi*
The enzymes described are: 1, HK/GK; 2, phosphoglucose isomerase; 3, PFK; 4, aldolase; 5, triose phosphate isomerase; 6, glyceraldehyde-3-phosphate dehydrogenase; 7, phosphoglycerate kinase (glycosomal); 8, phosphoglycerate kinase (cytosolic) 9, phosphoglycerate mutase; 10, enolase; 11, PK; 12, pyruvate dehydrogenase complex; 13, citrate synthase; 14,

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enzymes have been identified in the CL Brener clone genome (Table 1); the second fumarate hydratase gene present predicts a cryptic PTS_1 glycosomal targeting motif at the C-terminus, as in the case of *T. brucei* [36]. This isoform, however, might be cytosolic, as proposed for *T. brucei* [36], and still participate in glycosomal succinate production by transfer of L-malate to the cytosol and then of fumarate to the glycosome (Figure 1). When PP_i accumulates due to the biosynthetic reactions operating inside the glycosome, PPDK would act as a PP_i scavenger taking the place of the absent inorganic pyrophosphatase, thereby allowing these biosynthetic pathways to proceed [33]. NADH re-oxidation under these circumstances would be provided by ADH (L-alanine dehydrogenase), which has been reported as a glycosomal activity in *T. cruzi* [33]. This role of PEP as a triple crossroads in glucose catabolism would help to explain the reciprocal behaviour of succinate and L-alanine as final products, as mentioned earlier.

The production of acetate, detected by enzymatic analysis [10] may be mediated by acetyl:succinate CoA-transferase coupled with succinyl-CoA synthetase, generating ATP by substrate-level phosphorylation in the mitochondrion, as has been proposed for procyclic forms of *T. brucei* [37]. Genes encoding both enzymes are present in the *T. cruzi* genome (Table 1). However, as pointed out earlier, it is most likely that the bulk of the acetate produced by the parasite does not originate from glucose, at least in the epimastigote stage.

PPP

The PPP (Figure 2), starting as glycolysis from glucose 6-phosphate, is involved in the production of the ribose 5-phosphate required for nucleotide synthesis and of reducing power in the form of NADPH. The reduced coenzyme is essential for biosynthetic pathways and also for protection against oxidative stress, due to ROS (reactive oxygen species). Throughout its life cycle this parasite is exposed to oxidative stress imposed by ROS derived from its own aerobic metabolism and from the host immune response [38]. To detoxify hydroperoxides, *T. cruzi* possesses several pathways making an intricate network converging to reduced trypanothione, $T[SH]_2$, which is maintained in its reduced form by trypanothione reductase, with the utilization of NADPH [38]. Therefore it is absolutely essential for the parasite to have reliable pathways for the maintenance of a suitable pool of this reduced coenzyme.

Figure 1 (continued). aconitase; 15, NADP-linked ICDH; 16, α-ketoglutarate dehydrogenase complex; 17, succinate thiokinase; 18, succinate dehydrogenase; 19, fumarate hydratase (mitochondrial); 20, MDH (mitochondrial); 21, PEPCK; 22, MDH (glycosomal); 23; fumarate hydratase (glycosomal); 24, NAD-linked fumarate reductase; 25, pyruvate phosphate dikinase; 26, ADH; 27, fumarate hydratase (cytosolic); 28, NADP-linked malic enzyme (mitochondrial); 29, NADP-linked malic enzyme (cytosolic); 30, alanine aminotransferase; 31, fructose-1,6-bisphosphatase. The aerobic fermentation products are shown in boxes. L-Alanine can be produced both in the glycosome and in the cytosol, which fits in well with the detection of two different pools in experiments with [^{13}C]glucose [9]. Succinate can also be produced in the cytosol through the action of the cytosolic dihydro-orotate dehydrogenase (not shown), which uses fumarate as the electron acceptor [30].

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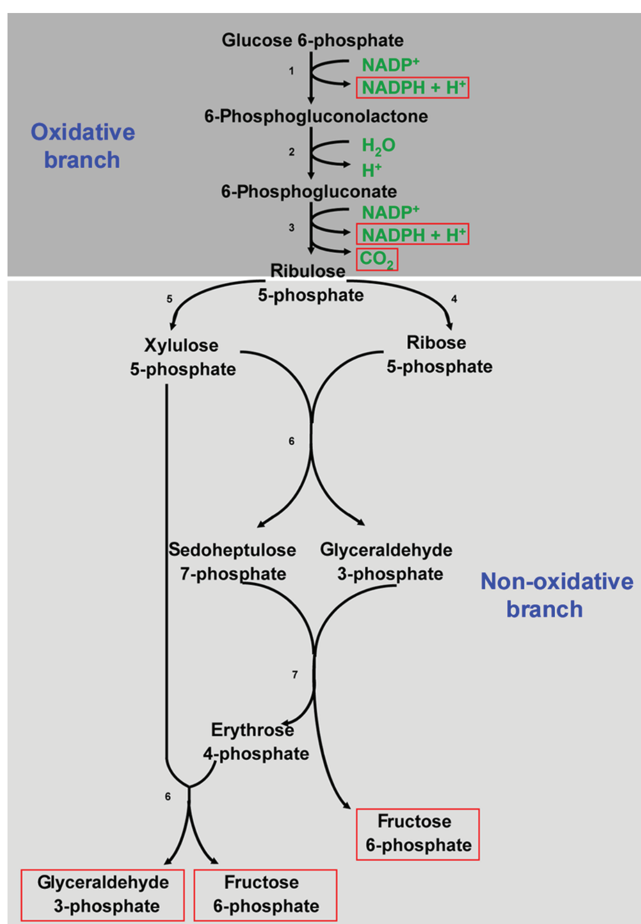

Figure 2. Schematic representation of the PPP in *T. cruzi*
Enzymes are: 1, G6PDH; 2, 6-PGL; 3, 6PGDH; 4, RPI; 5, RPE; 6, TKT; 7, TAL. All reactions of the non-oxidative branch are reversible. The products of the pathway are shown in boxes.

The PPP consists of two branches: an oxidative branch involving G6PDH (glucose-6-phosphate dehydrogenase), 6-PGL (6-phosphogluconolactonase) and 6PGDH (6-phosphogluconate dehydrogenase), and a non-oxidative, or sugar interconversion, branch involving RPI (ribose-5-phosphate isomerase), RPE (ribulose-5-phosphate epimerase), TAL (transaldolase) and TKT (transketolase) (Figure 2). The PPP has also been known as the pentose phosphate cycle, since the fructose 6-phosphate and glyceraldehyde 3-phosphate formed can be converted back into glucose 6-phosphate, entering the oxidative branch again. However, it is not necessary for the PPP to act as a cycle, and the different enzymatic reactions will be operative according to the cell's needs.

The functionality of the PPP in *T. cruzi* was shown using glucose labelled with ^{14}C at C1 or C6. In normal conditions, 10% of the glucose metabolized

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goes through the PPP [39]. In the presence of 0.2 mM Methylene Blue, a permeant scavenger of NADPH, the glucose flux in epimastigotes through the PPP doubled, from 10 to 20% of the total glucose utilization [39].

All seven enzymes of the PPP are present in the four major stages of the biological cycle of *T. cruzi* [39]. With the only exception of RPE, the other enzymes presented their highest activities in metacyclic trypomastigotes**.** In *T. brucei*, on the other hand, all enzymes of the PPP seemed to be present in procyclic trypomastigotes, but some of them could not be detected in the bloodstream form of the parasite [40]. In epimastigotes from *T. cruzi* all of the enzymes seem to present multiple subcellular localizations [39]; the bulk of the enzyme activities was cytosolic, with only the exception of RPE, both in digitonin extraction and in subcellular fractionation experiments, although probably all of them have minor localizations in the glycosomes.

G6PDH is encoded in *T. cruzi* CL Brener clone by several genes located in three of the parasite chromosomes [41]. The *T. cruzi* enzyme has, when compared with the human enzyme, a 37-residue N-terminal extension, which may be involved in regulation by the redox state of the parasite. The relevance of G6PDH in the defence mechanisms of *T. cruzi* against oxidative stress was demonstrated by incubating metacyclic trypomastigotes, a form of the parasite naturally exposed to ROS, with H_2O_2. After a 6 h incubation in the presence of the oxidizing agent at 70 μM, there was a 46-fold increase in G6PDH protein level and specific activity. This did not happen in the epimastigote, a parasite stage that is not supposed to be exposed to oxidative stress [41].

The gene encoding *T. cruzi* 6-PGL is present in the CL Brener clone as a single copy per haploid genome; its sequence predicts a possible internal PTS_1 glycosomal targeting signal. The recombinant 6-PGL behaves as a monomeric enzyme, with a molecular mass of 29 kDa [42].

The 6PGDH from *T. cruzi* is encoded by a single-copy gene per haploid genome in the CL Brener clone [43]. Deletion of 6PGDH is lethal for all cells studied, since accumulation of 6-phosphogluconate inhibits phosphoglucose isomerase, thus blocking glycolysis in addition to the PPP. Therefore specific inhibitors for the parasite's 6PGDH could become good lead compounds for the development of new drugs. Some of the inhibitors developed by Dardonville et al. [44] for the *T. brucei* 6PGDH were toxic to *T. cruzi* amastigotes at concentrations lower than 10 μM.

The haploid genome of the CL Brener clone of *T. cruzi* contains one gene coding for a Type B RPI, absent in higher eukaryote genomes, but not for Type A RPIs, most frequent in eukaryotes. Since Type A and B RPIs are totally unrelated, this enzyme might therefore be a good target for chemotherapy. Site-directed mutagenesis allowed the identification of the essential amino acid residues at the RPI B active site [45].

The genome of the CL Brener clone of *T. cruzi* contains two genes encoding RPEs [42]. One of them predicts a PTS_1 glycosomal targeting signal (SHL) at the C-terminus; however, digitonin extraction studies showed that RPE

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activity is extracted from the epimastigotes by very low concentrations of the detergent, even lower than those required to extract the PK used as a cytosolic marker [39]. This suggests the possibility of the existence of a new, highly digitonin-accessible, subcellular compartment.

The gene encoding TAL from the CL Brener clone of *T. cruzi*, which is present as a single copy per haploid genome, has been expressed in *Escherichia coli* as an active enzyme [42]. As in higher animals, TAL presents several isoforms in different stages of the parasite.

The genome of the CL Brener clone contains a single gene encoding TKT [42], which has 67% identity with the TKT from *Leishmania mexicana* [46]. The enzyme bears a C-terminal PTS_1, suggesting a dual localization, cytosolic and glycosomal, as shown for *Leishmania* promastigotes [45].

These results indicate that the PPP in *T. cruzi* presents several interesting targets for the chemotherapy of Chagas disease, which are at present under evaluation.

Other aspects of glucose metabolism

T. cruzi contains galactosylated glycoconjugates, which require the generation of UDP-Gal (UDP-galactose). Since the hexose transporter of the parasite does not transport D-galactose, this sugar must originate from glucose. The parasite contains phosphoglucomutase, which interconverts glucose 6-phosphate and glucose 1-phosphate, required for the synthesis of UDP-Glc (UDP-glucose); the enzyme is encoded by a single gene [47]. UDP-Glc can be epimerized by a UDP-Glc 4′-epimerase [48], producing the UDP-Gal required for glycoconjugate synthesis; the fact that the enzyme is not able to epimerize UDP-GlcNAc (UDP-*N*-acetylglucosamine) to UDP-GalNAc (UDP-*N*-acetylgalactosamine) may explain why *O*-glycosylation of the parasite's mucins is initiated via GlcNAc linked to serine or threonine, instead of the more usual GalNAc [48]. The enzyme seems to be essential, since null mutants could not be obtained, and two single-allele knockout clones displayed aberrant morphology [49]. The epimastigotes have been shown to contain, in addition to UDP-Glc, UDP-GlcNAc and UDP-Gal (pyranose form), UDP-galactofuranose, UDP-rhamnopyranose, UDP-glucuronic acid, UDP-xylose, GDP-mannose and GDP-L-fucose [50].

The vitamin C biosynthetic pathway is present in *T. cruzi*, which cannot incorporate ascorbate from the medium [51]; the last enzyme of the pathway, galactonolactone oxidase, which can utilize D-arabinono-γ-lactone in addition to L-galactono-γ-lactone, has been characterized using the recombinant enzyme [51].

Gluconeogenesis in the parasite has not been properly studied, but its genome contains the gene encoding fructose-1,6-bisphosphatase (Table 1) and the enzyme activity has been determined in extracts of the parasite [52]. The presence of the freely reversible PEPCK indicates that gluconeogenesis from several amino acids is very likely to be operative. The parasite also lacks a

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reserve polysaccharide, in contrast with *Leishmania* spp., which contain a mannose polymer, mannogen (previously known as mannan), which is a virulence factor in the parasite [53].

Conclusions

The present review covers only the most essential aspects of the metabolic pathways in which glucose is involved in *Trypanosoma cruzi*, emphasizing the major differences with higher eukaryotes. Most of the studies performed have, as their ultimate goal, the exploitation of these differences for the development of new effective drugs for the treatment of diseases. Much more is known of the energy metabolism in the African trypanosome, *T. brucei*, but space limitations have prevented us from analysing in detail those results for comparison. The thorough functional studies performed on the procyclic stage of *T. brucei* are difficult to duplicate in *T. cruzi* epimastigotes, essentially due to the lack of efficient genetic approaches; these approaches will have to be developed in order to obtain the necessary further insight into the energy metabolism of *T. cruzi*.

Summary

- T. cruzi *takes up glucose via a facilitated transporter, which seems to be absent in the intracellular amastigote stage.*
- *Glycolysis is partially compartmentalized in the glycosome; even in the presence of oxygen, reduced products (succinate and alanine) are excreted into the medium (aerobic fermentation of glucose).*
- *The lack of regulatory controls of HK and PFK results in the absence of the Pasteur effect. The tricarboxylic acid cycle is probably operative, as is the respiratory chain, although there are doubts about the functionality of complex I.*
- *The PPP is operative in the parasite. Some of the enzymes can be considered promising targets for chemotherapy.*
- *The two essential enzymes for gluconeogenesis are present, but there is no reserve polysaccharide.*

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