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Intermediate Metabolism in *Trypanosoma cruzi*

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Epimastigotes of *Trypanosoma cruzi*, the causative agent of Chagas disease, catabolize proteins and amino acids with production of NH_3, and glucose with production of reduced catabolites, chiefly succinate and L-alanine, even under aerobic conditions. This "aerobic fermentation of glucose" is probably due to both the presence of low levels of some cytochromes, causing a relative inefficiency of the respiratory chain for NADH reoxidation during active glucose catabolism, and the lack of NADH dehydrogenase and phosphorylation site I, resulting in the entry of reduction equivalents into the chain mostly as succinate. Phosphoenol pyruvate carboxykinase and pyruvate kinase may play an essential role in diverting glucose carbon to succinate or L-alanine, and L-malate seems to be the major metabolite for the transport of glucose carbon and reduction equivalents between glycosome and mitochondrion. The parasite contains proteinase and peptidase activities. The major lysosomal cysteine proteinase, cruzipain, has been characterized in considerable detail, and might be involved in the host/parasite relationship, in addition to its obvious role in parasite nutrition. Among the enzymes of amino acid catabolism, two glutamate dehydrogenases (one NADP- and the other NAD-linked), alanine aminotransferase, and the major enzymes of aromatic amino acid catabolism (tyrosine aminotransferase and aromatic α-hydroxy acid dehydrogenase), have been characterized and proposed to be involved in the reoxidation of glycolytic NADH.

KEY WORDS: *Trypanosoma cruzi*; aerobic fermentation of glucose; cruzipain; transaminases; glutamate dehydrogenases; aromatic amino acid catabolism.

INTRODUCTION

Trypanosoma cruzi, the causative agent of the American Trypanosomiasis, Chagas disease, is a parasitic flagellate which has a complex life cycle, involving a replicative form, the amastigote, and a nonreplicative form, th bloodstream trypomastigote, present in the mammalian host, and also a replicative form, the epimastigote, and a nonreplicative form, the infective metacyclic trypomastigote, in the insect vector (Brener, 1973). Although at present all these developmental stages can be obtained either in axenic culture (Contreras *et al.*, 1985), or in mammalian cell culture (Andrews and Colli, 1982), or from infected mammals (Gutteridge *et al.*, 1978), most metabolic and enzymological studies so far have been performed with axenic culture epimastigotes, presumably identical to those naturally present in the insect vector. Comparative studies using epimastigotes, amastigotes, and bloodstream trypomastigotes (Gutteridge and Rogerson, 1979) suggest that, at variance with African trypanosomes (Opperdoes, 1987), the different developmental stages of *T. cruzi* have qualitatively similar metabolic characteristics.

This review deals with carbohydrate, protein, and amino acid catabolism in *T. cruzi*, stressing their possible interrelationships.

TRANSPORT AND UTILIZATION OF CARBOHYDRATE, PROTEINS AND AMINO ACIDS

It has been known for many years that epimasti-

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gotes of *T. cruzi* are able to take up and consume both carbohydrates and amino acids, excreting into the medium some reduced glucose catabolites, chiefly succinate, and NH_3, respectively (von Brand, 1979). Glucose is preferred over amino acids and consumed first when both are present (Cannata and Cazzulo, 1984; Cazzulo *et al.*, 1985).

Both glucose and amino acids are actively taken up from the culture medium by the epimastigotes (for review, see Cannata and Cazzulo, 1984; Cazzulo, 1984); proteins are incorporated into the parasites by pynocytosis, mostly at the level of the flagellar pocket (De Souza, 1984), and they seem to be able to cover the energy requirements of the parasite (Avila *et al.*, 1983). Protein and amino acids may be an energy reserve for the parasite, which at least under some circumstances, such as the epimastigote–metacyclic trypomastigote differentiation, seems to be able to use as fuel a significant proportion of its own protein (Rodriguez *et al.*, 1990). Moreover, the reservosomes, organelles specific of the epimastigote stage, which contain ingested protein and the major cysteine proteinase, cruzipain, disappear during differentiation to metacyclics (Soares *et al.*, 1992).

There is a large amino acid pool, which can be oxidized by the parasite; almost half of it is L-alanine (Williamson and Desowitz, 1961); proline, glycine, and glutamic acid are also abundant (O'Daly *et al.*, 1983). Indeed, it is necessary to starve the epimastigotes for 24 h in order to detect a clear stimulation of respiration by glucose or amino acids (Sylvester and Krassner, 1976).

Glucose is consumed at a fairly high rate [0.7 μmol min^{-1} g (wet weight)$^{-1}$] by the epimastigotes; this rate is nevertheless about 5-fold lower than that shown by other Trypanosomatids, like *Crithidia fasciculata* or *Trypanosoma brucei* (Cazzulo *et al.*, 1988a).

CARBOHYDRATE CATABOLISM: THE AEROBIC FERMENTATION OF GLUCOSE

This subject has been reviewed recently *in extenso* (Cannata and Cazzulo, 1984; Cazzulo, 1992a,b; Urbina, 1993). Therefore, only a brief description will be given here, and the reader is referred to these reviews for further information. The production of organic acids by *T. cruzi* epimastigotes during glucose catabolism has been known for about 40 years. Recently this question has been re-examined using two different approaches: (a) specific enzymatic assay of catabolites in culture medium and cell suspension supernatants; (b) the noninvasive technique of ^{13}C-nuclear magnetic resonance (NMR), which allows one to follow glucose catabolism by living cells. Both techniques indicate that succinate and L-alanine are the major catabolites produced (Cazzulo *et al.*, 1985; Engel *et al.*, 1987; Frydman *et al.*, 1990; Urbina *et al.*, 1990); only a little of the enzymatically detected acetate (Cazzulo *et al.*, 1985; Engel *et al.*, 1987) actually originates from glucose (Frydman *et al.*, 1990), most of it probably arising from lipid and amino acid catabolism. The NMR evidence suggests that there are two different alanine pools, with different access to deuterium from the medium; one of them is excreted, whereas the other is retained within the cell (Frydman *et al.*, 1990). Glycerol and ethanol, which are produced by other Trypanosomatids, are not produced at all by *T. cruzi* epimastigotes (Cazzulo *et al.*, 1988a); similarly negative results were obtained for other catabolites reported by earlier authors, such as lactate, pyruvate, and malate (Cannata and Cazzulo, 1984).

Evidence obtained in several laboratories indicates that both glycolysis and the pentose phosphate shunt are operative in epimastigotes of *T. cruzi* (Cannata and Cazzulo, 1984). Glycolysis presents two distinctive characters as compared with the mammalian host: (a) most of the glycolytic enzymes are placed in a microbody, which for this reason was named "glycosome" by Opperdoes and Borst (1977), who discovered it in *T. Brucei*; (b) hexokinase and phosphofructokinase, key regulatory enzymes in most glycolytic systems, from bacteria to mammals, are little or not affected by most common effectors (Urbina and Crespo, 1984; Racagni and Machado de Domenech, 1983; Aguilar and Urbina, 1986; Taylor and Gutteridge, 1986). The only control found so far for a glycolytic enzyme in Trypanosomatids is the strong activation of pyruvate kinase by very low concentrations of fructose 2,6-bisphosphate (van Schafftingen *et al.*, 1985). The pyruvate kinase from *T. cruzi* epimastigotes is strongly activated by fructose 2,6-bis phosphate, which is able to counteract (at μM concentrations) the inhibitory effects of mM concentrations of P_i and ATP (Cazzulo *et al.*, 1989). As a result of the missing controls, the operation of glycolysis is not influenced appreciably by the presence or absence of oxygen; this is seen as a complete lack of the Pasteur effect (Cazzulo *et al.*, 1988a), or even as a negative Pasteur effect (Cazzulo, 1992b).

The early demonstration of the aerobic fermentation of glucose raised the question of whether the

tricarboxylic acid cycle and the respiratory chain were operative in *T. cruzi*. The present evidence (Cazzulo, 1992a,b) indicates that both pathways are actually functional, and all the cycle enzymes, with the only exception of α-oxoglutarate dehydrogenase, have been detected in cell-free extracts.

All glycolytic systems must have, in order to remain functional, an efficient system for the reoxidation of the NADH generated in the reaction of glyceraldehyde-3-phosphate dehydrogenase. This system is usually the respiratory chain under aerobic conditions, and several possible fermentative pathways under anaerobic conditions.. *T. cruzi* has several possible ways for NADH reoxidation, all of which seem to be operative under aerobic conditions. These are, in addition to reoxidation in the respiratory chain, production of succinate and probably also of L-alanine. The production of succinate was shown by Bowman *et al.*, (1963) to depend on CO_2 fixation, which seems to be mediated by phosphoenolpyruvate carboxykinase (PEPCK) (Cazzulo, 1992b). Recent ^{13}C-NMR evidence (De los Santos *et al.*, 1985) has shown that the CO_2-fixing enzyme in *C. fasciculata* is unmistakably PEPCK. The labelling pattern in similar experiments performed with *T. cruzi* is also compatible with this possibility (Frydman *et al.*, 1990), as is the fact that, in both Trypanosomatids, the relative proportion of succinate and the other major product (ethanol for *C. fasciculata*, L-alanine for *T. cruzi*) changes with the addition of bicarbonate to the incubation medium (De los Santos *et al.*, 1987; Frydman *et al.*, 1990). Moreover, recently Urbina *et al.* (1990) have shown *in vivo*, in ^{13}C-NMR experiments, that addition to the cells of an inhibitor of PEPCK, 3-mercaptopicolinic acid, results in a decrease in succinate production, with a concomitant increase in L-alanine production. PEPCK is ADP-linked, as those from yeast and bacteria, and at variance with the PEPCK of higher animals, which is specific for GDP of IDP (Cataldi de Flombaum *et al.*, 1977), and has been recently purified and studied by Urbina (1987), who showed a strong inhibition of oxaloacetate decarboxylation by ATP which was proposed to be relevant for metabolic regulation *in vivo* (Urbina, 1993). For succinate production the oxaloacetate synthesized in the PEPCK reaction must be reduced to L-malate by malate dehydrogenase, L-malate then dehydrated to fumarate by fumarase, and fumarate finally reduced to succinate by a fumarate reductase (Boveris *et al.*, 1986). Reduction to L-malate would be enough for the reoxidation of all glycolytic NADH if both PEP molecules were converted into succinate, with no energy loss, since ATP is synthesized by PEPCK as well as by pyruvate kinase. The reason for succinate production, and its excretion, which probably takes place when the respiratory chain is not able to cope with the input of reduction equivalents, seems to be due to the fact that the respiratory chain of *T. cruzi* is adapted to the oxidation of succinate instead of NADH, NADH dehydrogenase and the first phosphorylation site being absent (Denicola-Seoane *et al.*, 1992).

The other major product of glucose catabolism by *T. cruzi* is L-alanine. We have recently proposed (Duschak and Cazzulo, 1991) that its production might be linked to the reoxidation of glycolytic NADH, through the participation of either NAD- or NADP-linked glutamate dehydrogenases, malic enzyme, and alanine aminotransferase. The subcellular localization of the enzymes involved is compatible with the possible function of this pathway *in vivo* (Duschak and Cazzulo, 1991). The pathway involving the NADP-linked glutamate dehydrogenase and malic enzyme has the advantage that it does not require the existence of a cytosolic pool of glycolytic NADH, which is produced inside the glycosome. The reduction equivalents would be transported into the cytosol as L-malate. A decrease in L-alanine production, accompanied by an increase in succinate production, in the presence of added $NaHCO_3$ has been observed, and makes sense either if excess CO_2 shifts the balance toward the PEPCK reaction, or if both products originate in the same intermediate, L-malate. In the latter case, the effect of HCO_3^- might be due to product inhibition of the malic enzyme reaction.

In addition to the fermentation pathways, under aerobic conditions at least part of the glycolytic NADH in *T. cruzi* is reoxidized through a respiratory chain consisting of cytochromes b, c_{558}, and probably three alternative terminal oxidases, cytochrome oxidase $(a + a_3)$, cytochrome o, and cytochrome d (Stoppani *et al.*, 1980; Carneiro and Caldas, 1982; Affranchino *et al.*, 1986). The amounts of cytochromes b and $a + a_3$ present in epimastigotes are significantly lower than those found in mammalian mitochondria (Boiso *et al.*, 1979), and the resulting relative inefficiency of the respiratory chain might be one reason for the existence of the aerobic fermentation, as a complementary pathway for NADH oxidation, when glucose is being massively utilized (Cazzulo, 1992b; Urbina, 1993). Oxidation of substrates in the respiratory chain of *T. cruzi* is coupled to phosphorylation (Stoppani and Boiso, 1973; Affranchino *et al.*, 1985). Submitochondrial particles

were able to oxidize several substrates, with respiratory control values in the range of 2.8–2.0. The operation of coupling sites II and III was suggested. NADH oxidation was not controlled by ADP, nor inhibited by antimycin or cyanide; the apparent absence of phosphorylation site I might be a common property of Trypanosomatids, since it was previously reported for two species of *Crithidia* (Kusel and Storey, 1972; Toner and Weber, 1972; Opperdoes *et al.*, 1976). As in all other systems known, either prokaryotic or eukaryotic, oxidative phosphorylation is mediated in *T. cruzi* by a mitochondrial oligomycin sensitive Mg^{2+} ATPase (De Sastre and Stoppani, 1973), which was solubilized from the mitochondrial membranes (Frasch *et al.*, 1978) and purified to electrophoretic homogeneity (Cataldi de Flombaum *et al.*, 1979).

As described above, inhibitory controls usually present in most organisms, and which are responsible for the Pasteur effect, are lacking in *T. cruzi*, which seems to be adapted to consume as much glucose as is able to enter the cell. Even if lack of controls is indifferent, or even useful, for the parasite, which has no obvious reason to save its host's glucose, some kind of regulatory mechanism is necessary to prevent futile cycles, which might interfere with NADH reoxidation. In this sense, the reaction catalyzed by malic enzyme must be controlled, to avoid recycling of C_4 dicarboxylic acid, synthesized by PEPCK from the C_3 PEP, back to the C_3 pyruvate. The present evidence suggests that subcellular compartmentation is the major factor involved in the regulation of glycolysis in Trypanosomatids. The high rate of glucose consumption is probably due to the very high concentration of both, glycolytic enzymes and intermediates, inside the glycosome, which contains the glycolytic enzymes from hexokinase to glyceraldehyde 3-phosphate dehydrogenase (Taylor and Gutteridge, 1987). PEPCK and one isoenzyme of malate dehydrogenase are glycosomal,

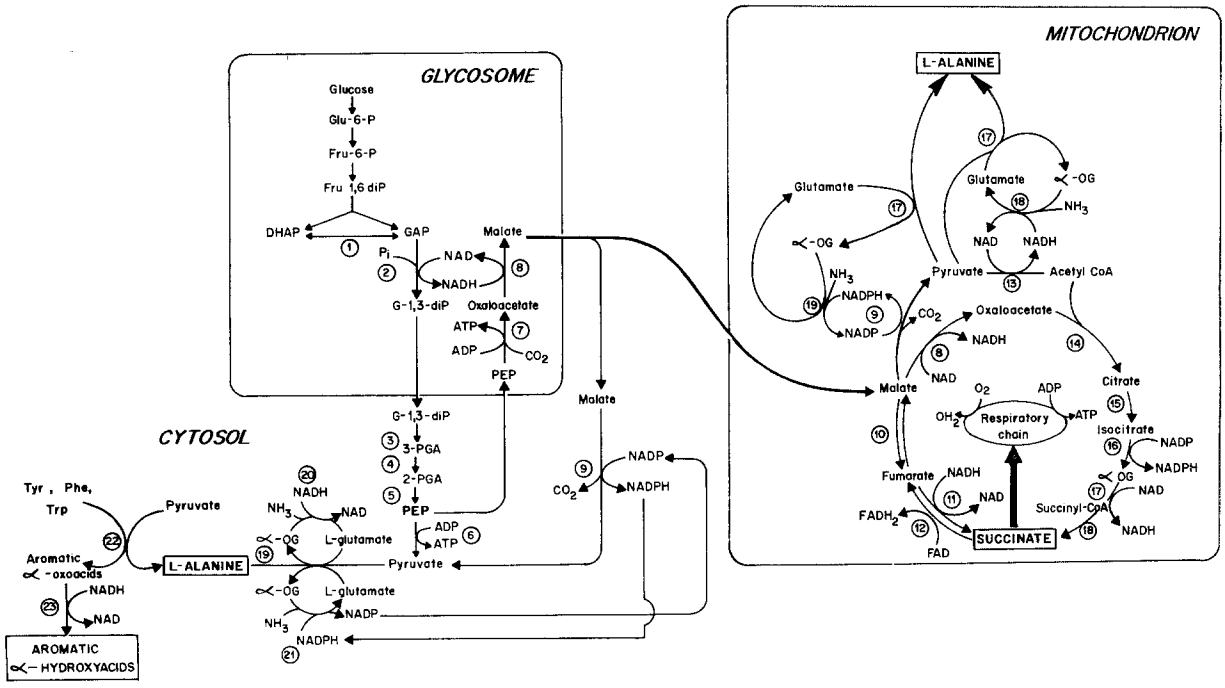

Fig. 1. Enzyme pathways for glucose catabolism and its possible connections with amino acid metabolism in *Trypanosoma cruzi*. Glu-6-P, glucose-6-phosphate; Fru-6-P, fructose-6-phosphate; Fru-1,6-diP, fructose 1,6-bisphosphate; GAP, glyceraldehyde-3-phosphate; DHAP, dihydroxyacetone phosphate; G-1,3-diP, 1,3-diphosphoglycerate; 3-PGA, 3-phosphoglycerate; 2-PGA, 2-phosphoglycerate; PEP, phosphoenolpyruvate; α-OG, α-oxoglutarate. The enzymes involved are the following: (1) triosephosphate isomerase; (2) glyceraldehyde-3-phosphate dehydrogenase; (3) phosphoglycerokinase; (4) phosphoglyceromutase; (5) enolase; (6) pyruvate kinase; (7) phosphoenolpyruvate carboxykinase; (8) malate dehydrogenase; (9) malic enzyme; (10) fumarase; (11) fumarate reductase; (12) succinate dehydrogenase; (13) pyruvate dehydrogenase; (14) citrate synthase; (15) aconitase; (16) isocitrate dehydrogenase; (17) α-oxogluterate dehydrogenase; (18) succinate thiokinase; (19) alanine aminotransferase; (20) NAD-linked glutamate dehydrogenase; (21) NADP-linked glutamate dehydrogenase; (22) tyrosine aminotransferase; (23) aromatic α-hydroxyacid dehydrogenase. Products excreted into the culture medium are boxed.

whereas malic enzyme has two isoenzymes, one mitochondrial and the other cytosolic (Cannata and Cazzulo, 1984). Figure 1 summarizes the data on subcellular compartmentation of enzymes in *T. cruzi*, as well as the interconnections between glycolysis and the tricarboxylic acid cycle and the proposed pathways for NADH oxidation. PEP is produced in the cytosol, and can then follow two different pathways: (a) it can be taken up by the glycosome, and carboxylated to oxaloacetate by PEPCK; (b) it can be converted into pyruvate by the cytosolic pyruvate kinase. Glycosomal oxaloacetate may be converted into L-malate by the glycosomal malate dehydrogenase, whereas cytosolic pyruvate may be transaminated to L-alanine, or transported into the mitochondrion for oxidation in the Krebs cycle. In addition, pyruvate can also be produced in the cytosol by the reaction of malic enzyme II, or in the mitochondrion by the reaction of malic enzyme I; this raises the possibility of two different pools of pyruvate, which might explain the presence of two different L-alanine pools, recently detected in epimastigotes by ^{13}C-NMR (Frydman *et al.*, 1990). Production of L-malate inside the glycosome will leave this organelle in redox balance, if both molecules of PEP are taken up by the glycosome and converted into exaloacetate and afterwards into L-malate. L-Malate will probably be transferred then to the mitochondrion via cytosol; part of it may be decarboxylated in the cytosol to pyruvate by malic enzyme, producing NADPH for cytosolic biosynthetic processes. When L-malate reached the mitochondrial matrix, its fate will depend on whether the Krebs cycle is actively operating or not. In *T. cruzi* epimastigotes under anaerobic conditions, or if for some reason the operation of the cycle is low, some oxaloacetate obtained by the malate dehydrogenase reaction will accumulate, and it will be able to inhibit the highly sensitive (K_i for oxaloacetate 9 μM) malic enzyme I (Cannata and Cazzulo, 1984), thus blocking L-malate decarboxylation, and diverting most of the L-malate to succinate via fumarate. On the other hand, if the cycle is working actively, the oxaloacetate concentration will be kept low by the citrate synthase reaction, malic enzyme I will be active, and part of the L-malate will be decarboxylated to pyruvate and enter the cycle as acetyl-CoA to be oxidized. Activity of malic enzyme I during operation of the tricarboxylic acid cycle is suggested by the production of labelled L-alanine during oxidation of ^{14}C-acetate by the epimastigotes (Cannata and Cazzulo, 1984), although participation of PEPCK acting as a decarboxylating enzyme under these circumstances is also possible (Urbina *et al.*, 1990; Urbina, 1993).

The present evidence suggests a central role for L-malate in the aerobic fermentation process, and a key position of malic enzyme in the regulation of the pathway.

PROTEIN AND AMINO ACID CATABOLISM

Production of Ammonia

Proteins and amino acids are consumed by epimastigotes in culture, with production of ammonia (von Brand, 1979), which is liberated into the medium; *T. cruzi*, at variance with other Trypanosomatids, is strictly ammonotelic (Yoshida and Camargo, 1978) and lacks all the enzymes of the urea cycle (Camargo *et al.*, 1978).

Epimastigotes of *T. cruzi* have a considerable intracellular acid pool of free amino acids, which can be used for the synthesis of proteins or some derived substances, or for energy production. These amino acids are likely to be produced by proteolysis of exogenous or endogenous proteins, or to be taken up from the culture medium. Production of L-alanine as a product of glucose catabolism has already been discussed.

Proteolytic Activities

A number of proteolytic activities have been described in *T. cruzi* epimastigotes, starting with the work of Itow and Camargo (1977) and Avila *et al.* (1979); it is difficult to tell whether all the activities described belong to different enzymes, since these early studies were performed with crude extracts (Cazzulo, 1984). Some proteinases, all of them belonging to the group of the cysteine proteinases, have been purified to homogeneity from epimastigotes. One of them has a high molecular mass (about 200 kDa), and hydrolyzes efficiently benzoyl-arginine-*p*-nitroanilide (BAPA), in addition to several proteins (Bongertz and Hungerer, 1977). This proteinase might be the same enzyme recently reported and studied in further detail by Ashall (1990). Two enzymes have been purified and reported to have molecular mass values of about 60 kDa (Rangel *et al.*, 1981; Bontempi *et al.*, 1984); they might be the same enzyme, except for some differences in substrate and inhibitor specificity (Cazzulo, 1984). The molecular mass of this cysteine proteinase, for which the

name "cruzipain" has been proposed (Cazzulo *et al.*, 1990b), is about 40 kDa according to sequence, but apparent values ranging from 60 to 35 kDa have been reported, depending on the electrophoretic conditions (Martínez and Cazzulo, 1992). This developmentally regulated enzyme, which seems to be the major cysteine proteinase of the parasite (Campetella *et al.*, 1990), is a high-mannose type glycoprotein (Cazzulo *et al.*, 1990a) placed in the lysosomes (Bontempi *et al.*, 1989). The enzyme is encoded by a large number of genes (up to 130 in the Tul 2 stock of the parasite), placed in tandem in two or four chromosomes, depending on the parasite strain (Campetella *et al.*, 1992). The mature enzyme seems to be made up of two different domains, a catalytic domain, which presents high homology with cathepsin L and papain (Campetella *et al.*, 1992; Eakin *et al.*, 1992), as well as with the cysteine proteinase recently cloned and sequenced in *T. brucei* (Mottram *et al.*, 1989), and a C-terminal domain, which has a lower degree of homology with similar extensions predicted, from *c*DNA sequences, for the enzymes from *T. brucei* (Mottram *et al.*, 1989) and *Leishmania mexicana* (Souza *et al.*, 1992). The latter domain is responsible for the high antigenicity of cruzipain in chronic Chagas disease (Martínez *et al.*, 1991, 1993; Cazzulo and Frasch, 1992). Recent studies with permeant cysteine proteinase inhibitors indicate that some enzymes of this class, most probably some of the cruzipain isoforms, are essential for mammalian cell infection and parasite development (Meirelles *et al.*, 1992; Harth *et al.*, 1993; Franke de Cazzulo *et al.*, 1993).

In addition to cysteine proteinases, which are by far the best studied in this parasite, recently evidence has been presented (by DNA sequencing) for the presence of a serine proteinase (Sakanari *et al.*, 1989), and also there is a recent report of a metalloproteinase, which might have some resemblance to the *Leishmania* gp63 (Greig and Ashall, 1990). There is no evidence for the possible presence of aspartil proteinases in *T. cruzi*. Several peptidases have been recently detected in cell-free extracts of epimastigotes (Healy *et al.*, 1992). One cysteine peptidase, which might be one of them, has been recently purified to homogeneity (Santana *et al.*, 1992).

Amino Acid Catabolism

T. cruzi epimastigotes are able to oxidize a number of amino acids, presumably through the tricarboxylic acid cycle (Sylvester and Krassner, 1976). The catabolic pathways for the carbon chain of the amino acids have not been examined in detail, and none of the enzymes involved has been demonstrated. Serine hydroxymethyl transferase, involved in the serine–glycine interconversion, has been characterized (Nosei and Avila, 1985). On the other hand, the enzymes responsible for the exchange and disposal of amino nitrogen have been studied in much greater detail. The enzymes involved in the liberation of the amino group of the different amino acids as ammonia are the transaminases, many of which transfer the amino group to α-oxoglutarate to give L-glutamate, and the glutamate dehydrogenases, which liberate the $-NH_2$ group as NH_3. In *T. cruzi* epimastigotes,, a number of amino acids are able to transaminate with α-oxoglutarate or pyruvate, to yield L-glutamate or L-alanine, respectively (Zeledón, 1960). The enzymes responsible, aspartate aminotransferase (ASAT, Cazzulo *et al.*, 1983) and alanine aminotransferase (ALAT, Barros and Caldas, 1983), have been partially purified from epimastigotes, and characterized; they have molecular masses in the vicinity of 100 kDa, and seem not to differ significantly from their mammalian counterparts. Both enzymes have at least two isoenzymes in most *T. cruzi* isolates, and are among the six enzyme systems proposed by Miles *et al.*, (1978, 1980) for the classification of isolates of the parasite into zymodemes. Leucine aminotransferase has also been detected in *T. cruzi* epimastigotes (Montamat *et al.*, 1987). Recently the two enzymes responsible for aromatic amino acid catabolism, tyrosine aminotransferase (TAT) and an aromatic L-α-hydroxyacid dehydrogenase (AHADH), have been detected in epimastigotes of *T. cruzi* (Nowicki *et al.*, 1992), and purified to homogeneity (Montemartini *et al.*, 1993; Cazzulo *et al.*, 1993). The subcellular localization of ALAT, ASAT, TAT, and AHADH has been determined (Duschak and Cazzulo, 1991; Nowicki *et al.*, 1992). All these enzymes are present in two compartments, the cytosol and the mitochondrial matrix, but whereas ASAT is about 90% mitochondrial (Duschak and Cazzulo, 1991), TAT and AHADH are about 90% cytosolic. One of the ALAT activities seems to belong to the TAT molecule (Montemartini *et al.*, 1993). We have recently detected phenyl lactate and *p*-hydroxyphenyl lactate in epimastigote culture supernatants (Montemartini *et al.*, unpublished results); although they are minor products, compared with succinate and L-alanine, it is possible that their production might be linked to the reoxidation of cytosolic NADH (Fig. 1).

T. cruzi epimastigotes contain two different glutamate dehydrogenases, one NADP-linked and one NAD-linked (Cazzulo *et al.*, 1977; 1979; Walter and Ebert, 1979). The parasite therefore resembles bacteria, fungi and plants in this respect, and clearly differs from higher animals, which possess only one, conenzyme-unspecific, glutamate dehydrogenase. Both enzymes have been purified to homogenity (Juan *et al.*, 1978; Walter and Ebert, 1979), and their properties studied in some detail (Cazzulo, 1984; Urbina and Azavache, 1984). The NADP-linked enzyme seems to be very similar to the enzyme from *Escherichia coli* in terms of amino acid composition and of N-terminal sequence (Cazzulo *et al.*, 1988b). The NADP-linked glutamate dehydrogenase might be biosynthetic (Carneiro and Caldas, 1983), whereas the NAD-linked enzyme might have a catabolic role, since its activity increases in the late exponential phase of growth, when amino acids are certainly being consumed and NH_3 is produced (Cazzulo *et al.*, 1985), and is strongly inhibited by ATP (Cazzulo *et al.*, 1979; Walter and Ebert, 1979; Urbina and Azavache, 1984), and also by acetyl-CoA (Urbina and Azavache, 1984). We have recently proposed (Duschak and Cazzulo, 1991) that the glutamate dehydrogenases, which are present both in the mitochondrion and the cytosol, may be involved in the reoxidation of glycolytic NADH through L-alanine production and excretion (Fig. 1).

OXIDATION OF AMINO ACID CARBON CHAINS AND RELATIONSHIP TO CARBOHYDRATE CATABOLISM

Epimastigotes of *T. cruzi* are able to actively catabolize L-proline, as well as other amino acids (Sylvester and Krassner, 1976). The oxidation in the cycle of intermediates of the cycle itself, such as oxaloacetate and α-oxoglutarate, arising from amino acid catabolism, requires their previous conversion into acetyl-CoA. This can be accomplished through the action of malic enzyme on L-malate, or through the action of an oxaloacetate decarboxylase on oxaloacetate; the latter role can be fulfilled by the concerted action of PEPCK and pyruvate kinase just as well. Urbina (1993) has suggested that the preferential use of glucose before amino acids by *T. cruzi* epimastigotes is due to the tight regulation of NAD-linked glutamate dehydrogenase and PEPCK, and the lack of regulation of the glycolytic pathway; the former enzymes would be kept inhibited during active glycolysis by the high ATP concentration, whereas they would become fully active after glucose is exhausted and the ATP contents decrease. However, the experimental evidence of PEPCK participation in succinate excretion mentioned above indicates that under conditions of active glycolysis the enzyme is acting as an auxiliary to this pathway, despite the inhibition of the carboxylation reaction by ATP reported by Urbina. According to this author, amino acid catabolism by *T. cruzi* epimastigotes would be mediated by transport of L-malate from the mitochondrion to the glycosome, formation of oxaloacetate by malate dehydrogenase, decarboxylation of this intermediate to PEP by PEPCK, transport of PEP to the cytosol, formation of pyruvate by pyruvate kinase, and transport of pyruvate into the mitochondrion, where it would be oxidized to acetyl-CoA and incorporated into the cycle through the citrate synthase reaction. This pathway would be essentially a reversal of most of the pathway for carbohydrate catabolism depicted in Fig. 1. In support of their proposal, Urbina *et al.* (1990) report some inhibition of proline oxidation by 3-mercaptopicolinic acid. Although this pathway is feasible, since the reaction catalyzed by PEPCK is freely reversible, the possibility that mitochondrial malic enzyme I is involved instead of (or in addition to) PEPCK is also likely. The conversion of Krebs cycle intermediates into acetyl-CoA mediated by malic enzyme would take place in only subcellular compartment, the mitochondrion, instead of requiring three (mitochondrion, glycosome, cytosol, mitochondrion again), as in Urbina's proposal. As pointed out above, the strong inhibition of malic enzyme I by oxaloacetate suggests that it will be active only when the concentration of this intermediate is kept low by citrate synthase, and the cycle is fully active. The fact that the total malic enzyme activity was inhibited only by 6% by 3-mercaptopicolinic acid (Urbina *et al.*, 1990) has been considered as proof for the lack of participation of malic enzyme I in amino acid catabolism (Urbina, 1993). However, under Urbina's experimental conditions (10 mM L-malate as substrate) both malic enzymes I and II are assayed together, and therefore the actual contribution of malic enzyme I cannot be assessed.

There can be little doubt that PEPCK in *T. cruzi*, and in other Trypanosomatids as well, has a catabolic function, as suggested by its lack of the glucose repression characteristic of PEPCKs having a glyconeogenic role (Cazzulo *et al.*, 1985). This applies to any of its proposed roles in the parasite's catabolism.

The role played by PEPCK in carbohydrate catabolism by *T. cruzi* is clear; a demonstration of its exclusive participation in amino acid catabolism in the parasite, as opposed to malic enzyme I, will require more experimental evidence.

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