

The occurrence and distribution of α -hydroxy-acid dehydrogenase in some members of the order Kinetoplastida

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LDH-X is the isoenzyme of lactate dehydrogenase found in mammalian spermatozoa, occurring in cytosolic and mitochondrial locations. Gossypol strongly inhibits it, and the spermicidal action of this compound is attributed to the disruption of a reducing shuttle. The flagellated protozoan, *Trypanosoma cruzi*, contains an enzyme activity similar to LDH-X, called α -hydroxy-acid dehydrogenase, which is here shown to possess cytosolic and glycosomal components. The glycosome is a microbody-like organelle containing the early glycolytic enzymes. We postulate that the inhibition of replication of *T. cruzi* by gossypol derives from interference with glycosomal reducing shuttles. *T. lewisi* resembles *T. cruzi* in this respect.

Lactate dehydrogenase Gossypol Microbody Glycosome

1. INTRODUCTION

Chagas' disease is a major human health problem in Latin America; there is no effective chemotherapy [1]. Gossypol, an empirically discovered male antifertility agent [2], inhibits motility and replication of the flagellated protozoan *Trypanosoma cruzi* [3] which causes this disease.

Gossypol is a potent inhibitor of LDH-X, the isoenzyme of lactate dehydrogenase found only in spermatozoa and spermatogenic cells [4]. It occurs in the cytosol and mitochondrial matrix [5] and it is suggested [6] that it forms a reducing shuttle.

T. cruzi possesses a similar activity, α -hydroxy-acid dehydrogenase (aHAdeHase), which will also

reduce a range of straight and branched-chain α -ketoacids. Two isoenzymes with differing substrate specificities have been purified [7]. Gossypol is a potent inhibitor of aHAdeHase, malate dehydrogenase and glutamate dehydrogenase from *T. cruzi* (K_i values 0.21–10.3 μ M) [3].

In members of the order Kinetoplastida, the early glycolytic enzymes are contained within a microbody-like organelle, the glycosome. This was first described in *T. brucei* [8] and later demonstrated in *T. cruzi* [9,10]. This paper indicates that, of those members of the order examined, aHAdeHase is only found in the stercorarian section of the genus *Trypanosoma*, and that it is found in cytosolic and glycosomal compartments.

2. MATERIALS AND METHODS

Media components were obtained from Difco, chemicals were supplied by Fisons and BDH. Biochemicals were purchased from Sigma, purified enzymes were from the same source or from Boehringer.

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Epimastigote *T. cruzi* (Sonya strain) and promastigote *Leishmania tarentolae* (strain LV 414) were grown in axenic liquid culture [11]. *Crithidia fasciculata* (strain ATCC 11, 745) was maintained in a similar way [12]. Bloodstream trypomastigote *T. brucei* (strain 14/2/164) and *T. lewisi* were obtained and purified as described [13]. Organisms were washed in Krebs-Ringer glucose solution, then broken by grinding with silicon carbide [14] in a buffered sucrose medium of composition 0.25 M sucrose, 1 mM disodium edetate, 25 mM Tris-HCl, pH 7.6. The abrasive was removed by a low-speed centrifugation. Subcellular fractions were then prepared using an MSE PrepSpin 50 ultracentrifuge.

High-speed supernatant and pellet fractions were obtained by centrifuging the lysate at $140\,000 \times g$ for 60 min. For isopycnic analysis, the lysate

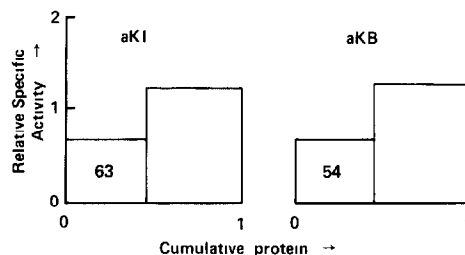


Fig.1. Distribution of aHadeHase between soluble and high-speed pellet fractions prepared from an epimastigote *T. cruzi* lysate. The block on the left of each histogram represents the particulate activity [20]; the figures within each block represent the percentage latency of that fraction. aKI, the distribution of aHadeHase with α -ketoisocaproate as substrate; aKB, that with α -ketobutyrate.

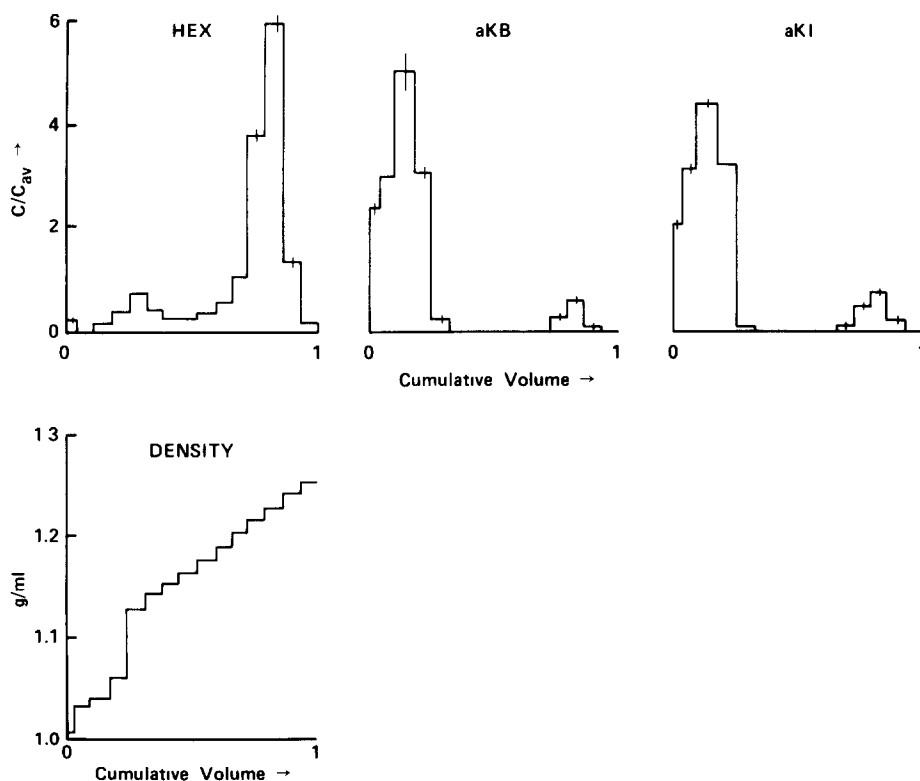


Fig.2. The distribution of hexokinase (HEX) and aHadeHase determined by isopycnic centrifugation of an epimastigote *T. cruzi* lysate. Each fraction was assayed thrice for each activity. Vertical bars represent 1 SD. Results are presented as described [20]. Recoveries (%): HEX, 103; aHadeHase with α -ketobutyrate as substrate (aKB), 111; aHadeHase with α -ketoisocaproate (aKI), 107.

was first centrifuged at $5000 \times g$ for 10 min, to remove flagellar complexes [8], and then the supernatant was layered on to a linear sucrose gradient, 0.8–2.0 M, volume 20 ml. The gradient was centrifuged at $130\,000 \times g$ for 180 min, and finally fractionated and characterised by refractrometry.

All assays were performed on a Unicam SP8000 double-beam recording spectrophotometer with the cuvette holder maintained at 28°C . Latency was determined by the addition of Triton X-100 to a final concentration of 0.1%, w/v. All sucrose gradient fractions were assayed with this addition.

Assays were as described: aHAdHase [7], hexokinase, phosphoglycerate kinase and L-amino acid- α -ketoglutarate aminotransferases [15], cystathione γ -lyase [16], and serine and threonine dehydratases [17]. L-Amino acid oxidase was assayed by measuring the production of α -ketoacids by a colorimetric method [15].

3. RESULTS

3.1. *Epimastigote T. cruzi*

The distribution of aHAdHase between soluble and high-speed pellet fractions is shown in fig.1. With both substrates, about a third of the total activity was found in the pellet fraction and showed substantial latency.

Isopycnic centrifugation of a lysate gave the results in fig.2. With either substrate, the particulate aHAdHase activity co-equilibrated with that of hexokinase at a modal density of 1.23 g/ml.

Various sources of α -ketoacid substrates for aHAdHase were sought. In whole cell lysates, no L-amino acid oxidase could be detected with L-alanine, -leucine, -valine or -isoleucine as substrates. There was no latent activity and no endogenous inhibitor of a commercial source was present in lysates. Cystathionine γ -lyase, serine

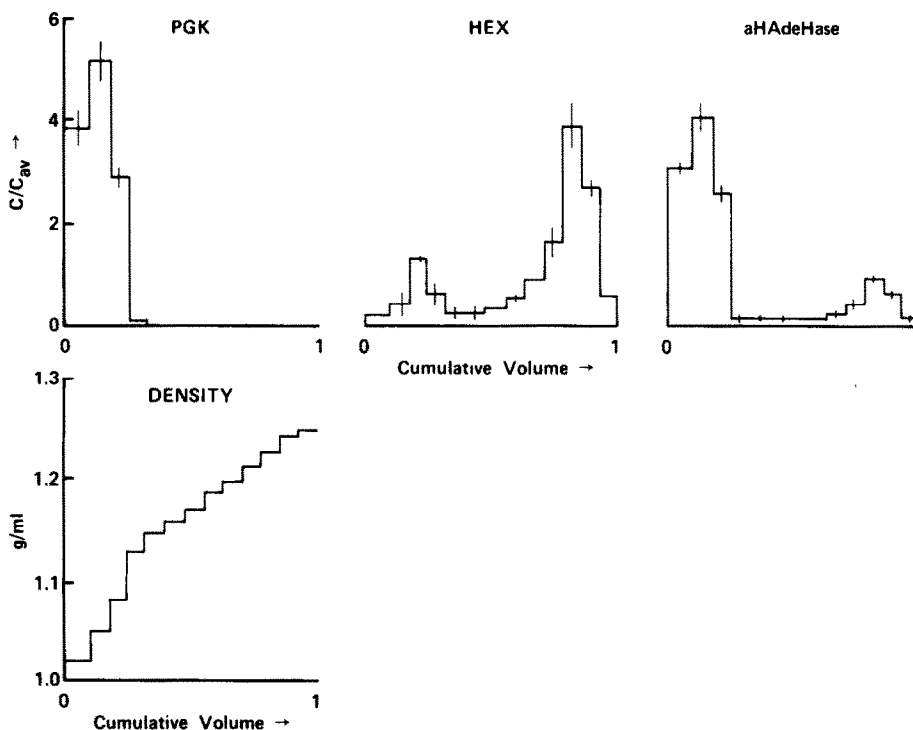


Fig.3. The distribution of hexokinase (HEX), phosphoglycerate kinase (PGK) and aHAdHase determined by isopycnic centrifugation of a bloodstream trypomastigote *T. lewisi* lysate. Results are portrayed as described in fig.2. Recoveries (%): PGK, 105; HEX, 113; aHAdHase with α -ketoisocaproate as substrate, 77.

and threonine dehydratases were also not detectable.

Aminotransferase activities were detectable with several amino acid substrates. Isopycnic analysis demonstrated that, in each case, most of the activity was retained above the gradient, suggesting a cytosolic localisation. Virtually no activity co-equilibrated with hexokinase (not shown).

3.2. Other members of the order Kinetoplastida

Cell lysates were assayed for aHAdHase with α -ketoisocaproate as substrate. No activity was demonstrable in *L. tarentolae*, *T. brucei* or *C. fasciculata*. There was no latent activity in any lysate, nor was any endogenous inhibitor demonstrable. Substantial activity was found in a *T. lewisi* lysate.

Isopycnic centrifugation of *T. lewisi* lysates showed (fig.3) that phosphoglycerate kinase is retained above the gradient; the bulk of the hexokinase activity is found in dense fractions with a modal density 1.23 g/ml; and aHAdHase shows components co-equilibrating with both these markers.

Early reports of LDH activity in *T. rhodesiense* were fallacious: the activity originated in contaminating platelets [18]. However, using α -ketoisocaproate as substrate no activity was detectable in freeze-thaw lysed uninfected rat blood. All aHAdHase assays on *T. lewisi* were performed using this substrate.

4. DISCUSSION

In epimastigote *T. cruzi*, about a third of the aHAdHase activity is sedimentable and shows latency. Isopycnic centrifugation shows that the activity co-equilibrates with the glycosomal marker, hexokinase. The two isoenzymes of aHAdHase have different substrate specificities - I shows greatest activity with α -ketobutyrate, II with α -ketoisocaproate [7]. Similar subcellular distributions were seen with either substrate.

Several potential sources of α -ketoacids were sought in *T. cruzi* lysates. Only aminotransferase activities were detected; no significant glycosomal activities were detected. The small particulate component had a distribution suggestive of a mitochondrial location, and the majority of the activity was soluble. We therefore suggest that α -ketoacid

substrates of aHAdHase are produced by cytosolic aminotransferases.

When bloodstream trypomastigote *T. brucei* transforms to the procyclic (culture) form, glycosomal malate dehydrogenase (MDH) appears; it is speculated [19] that this forms part of a malate oxaloacetate reducing shuttle, providing NAD⁺ for glycosomal glyceraldehyde-3-phosphate dehydrogenase. MDH has a similar distribution in epimastigote *T. cruzi* [21]. MDH and aHAdHase may both function as reducing shuttles in *T. cruzi*; both are potently inhibited by gossypol [3.]

aHAdHase activity was also detected in *T. lewisi*. Use of α -ketoisocaproate as substrate demonstrated clearly that this activity was of protozoal rather than mammalian origin. Its microbody-like subcellular distribution is the first demonstration of the apparent presence of glycosomes in this species of trypanosome.

aHAdHase could not be detected in other members of the order Kinetoplastida examined. Both *T. cruzi* and *T. lewisi* are members of the stercorarian section of the genus *Trypanosoma*. The general metabolic similarities between the mammalian (bloodstream trypomastigote and tissue amastigote) and culture (epimastigote) forms of *T. cruzi*, together with the demonstration of glycosomal aHAdHase in trypomastigote *T. lewisi*, suggest strongly that similar shuttles exist in these mammalian stages.

Gossypol, or a compound derived from it, may find a role in the chemotherapy of Chagas' disease. We suggest that it would act by disruption of glycosomal reducing shuttles.

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