"AMINO ACID METABOLISM IN BLOODSTREAM TRYPANOSOMA BRUCEI BRUCEI"

By

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D E C L A R A T I O N

I, Jacques Muturi Kabaru, hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with my approval as the University Supervisor.

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SUMMARY

The main purpose of this study was to investigate key enzymes involved in amino acid metabolism in bloodstream Trypanosoma brucei brucei and the leakage of some of these enzymes into the plasma of infected rats.

Using glutamate pyruvate transaminase (GPT) (EC 2.6.1.2) as a cytosolic marker and ∝-glycerolphosphate dehydrogenase (∝GPDH) (EC 1.1.1.8) as a glycosomal marker, the intracellular location of glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1), NAD-linked malate dehydrogenase (NAD-linked MDH) (EC 1.1.1.37), NADP-linked malic enzyme (NADP-linked ME) (EC 1.1.1.40) and glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) in bloodstream T. b. brucei was investigated. This was done by determining the release of these enzymes from isolated bloodstream trypanosomes by increasing cycles of freeze-thawing or increasing concentrations of Triton X-100 and Digitonin.

In one set of experiments, the release of GPT, GOT, NAD-linked MDH and ∝GPDH was studied with 10^8 trypanosomes/ml of PSG. To release maximal activity of GPT, GOT, and NAD^+-linked MDH, 2 cycles of freeze-thawing, 0.04% Triton X-100 or 140 µg Digitonin/10^8 trypano-

somes was required. Six freeze-thawing cycles or 0.07% Triton X-100 released maximal activity of ∝-GPDH. The maximum concentration of Digitonin used (280 µg/10^8 trypanosomes) did not release maximal activity of ∝-GPDH from the trypanosomes. It was concluded that GOT and NAD-linked MDH were cytosolic since they were released in a pattern similar to that of the cytosolic marker GPT.

In another set of experiments, the release of GPT, G6PDH, NADP-linked ME and ∝-GPDH was studied with 5 x 10^8 trypanosomes/ml PSG. To release maximal activity of GPT and G6PDH, 2 cycles of freeze-thawing, 0.04% Triton X-100 or 170 µg Digitonin/5 x 10^8 trypanosomes was required. To release the maximal activity of NADP-linked ME, 2 cycles of freeze-thawing, 0.04% Triton X-100 or 226 µg Digitonin/5 x 10^8 trypanosomes was required. The maximum concentration of Digitonin used (280 µg/5 x 10^8 trypanosomes) did not release maximal activity of ∝-GPDH from the trypanosomes. It was concluded that G6PDH was cytosolic since it was released in a pattern similar to that of the cytosolic marker GPT. The localisation of NADP-linked ME could not be established conclusively.

The plasma levels of GPT, GOT, Pyruvate Kinase (PK) (EC 2.7.1.40) ∝-GPDH and lactate dehydrogenase

(LDH) (EC 1.1.1.27) were measured in healthy and T. b. brucei infected rats. At a parasitemia of approximately 10^8 parasites/ml blood, the plasma level of GPT was elevated 4 - 5 fold; GOT, 2 - 3 fold; PK, 3 fold; ∝GPDH, 2 fold but the level of LDH was not altered. It was proposed that the additional enzyme activity in plasma during T.b. brucei was of trypanosomal origin. To confirm the origin of additional enzyme activities in plasma of T.b. brucei -infected rats, starch gel electrophoresis of healthy and infected rat serum was done. Parallel experiments were set up with parasite lysate. In addition to normal rat serum GPT and GOT, infected rat serum contained trypanosomal GPT and GOT. It was concluded that the elevated levels of these enzymes in serum of rats during infection was due to leakage from trypanosomes.

The activity of GPT in bloodstream T.b. brucei was 0.52 μmoles/min/mg protein. The role of this enzyme in trypanosomes was investigated. Isolated trypanosomes were incubated in PSG containing 10 mM L-glutamate at 25°C. Alanine and ∝-ketoglutarate were produced in equimolar quantities and the amount of alanine produced was a function of glutamate concentration in the incubation medium. Paper chromatography analysis of post-incubation medium showed alanine to be the only amino acid produced

during the incubations. It was proposed that the role of GPT in bloodstream *T. b. brucei* is to convert some of the pyruvate generated in glycolysis to alanine.

The role of GOT in bloodstream *T. b. brucei* was also investigated. Trypanosomal GOT activity was 0.075 µmole/min/mg protein, NAD-linked MDH was 0.140 µmoles/min/mg protein and that of NADP-linked ME was 0.012 µmoles/min/mg protein. Trypanosomal lysates in P_i incubated at $25^{o}C$ with 10 mM L-aspartate, 10 mM ∝-ketoglutarate and 5 mM NADH produced significant amounts of L-malate (0.51 ± 0.005 µmoles L-malate/hr/10^{8} trypanosomes). It was speculated that GOT generates oxaloacetate from aspartate and ∝-keto-glutarate as shown below;

$$\text{Aspartate} + \text{∝-Ketoglutarate} \xrightarrow{\text{GOT}} \text{Glutamate} + \text{Oxaloacetate}$$

The oxaloacetate then acts as a substrate for NAD-MDH generating malate by reduction consuming NADH as shown below;

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{NAD-linked MDH}} \text{NAD}^{+} + \text{Malate}$$

The malate so produced is oxidatively decarboxylated to pyruvate by NADP-linked ME generating NADPH as

shown below;

$$\text{Malate} + \text{NADP}^+ \xrightleftharpoons{\text{NADP-linked ME}} \text{Pyruvate} + \text{NADPH} + CO_2$$

It was further speculated that the most likely role of GOT in bloodstream T. b. brucei is involvement in a series of coupled reactions leading to transhydrogenation of NADH with $NADP^+$. The NADPH generated in this transhydrogenation would supplement that generated by G6PDH. The activity of G6PDH in bloodstream T. b. brucei was 0.012 μmoles/min/mg protein.

The presence of other trypanosomal aminotransferases was investigated using trypanosome lysates in the presence of 10 mM L-amino acid and 10 mM ∝-ketoglutarate at 25°C. The transamination activities obtained with various L-amino acids expressed as μmoles L-glutamate/hr/mg protein were; alanine, 1.44 ± 0.041; aspartate, 0.67 ± 0.012; glutamine, 0.33 ± 0.007; isoleucine, 1.1 ± 0.015; leucine, 1.02 ± 0.028, methionine, 0.65 ± 0.012; phenylalanine, 1.13 ± 0.022; tryptophan, 0.39 ± 0.014; tyrosine, 0.79 ± 0.014 and valine, 1.13 ± 0.013. Two speculations were derived from these results. Firstly was that in addition to the reported trypanosomal transaminases, there are novel non-specific transaminases acting on 10 amino acids with ∝-ketoglutarate

as the amino group acceptor. Secondly, that in addition to the reported trypanosomal transaminases there are at least 5 novel specific transaminases acting on glutamine, isoleucine, leucine, valine and methionine with α-ketoglutarate as the amino group acceptor.

In mammals the transamination and catabolism of methionine may lead to production of very toxic intermediates; methanethiol and hydrogen sulphide. The location of the methionine transaminating enzyme activity in the trypanosomes was investigated. Using GPT as a cytosolic marker, the release of the methionine transaminating enzyme from parasites by freeze-thawing was determined. Maximal activities of both GPT and the methionine transaminating enzyme were released by 2 freeze-thawing cycles. It was concluded that the methionine transaminating enzyme is cytosolic and may leak into the plasma of infected animals contributing to the pathogenicity of trypanosomes.