A STUDY OF TRANSAMINASES IN BLOODSTREAM *TRYPANOSOMA BRUCEI BRUCEI*

BY

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A thesis submitted in partial fulfilment for the degree of Master of Science in the University of Nairobi.

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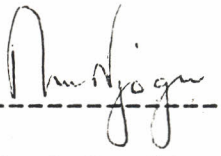
DECLARATION

I, Stephen Mwaniki Njogu hereby declare that this is my original work and has not been presented for award of a degree in any other University.

Stephen Mwaniki Njogu
CANDIDATE

This thesis has been submitted for examination with our approval as University Supervisors.



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A-C-K-N-O-W-L-E-D-G-E-M-E-N-T-S

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

ADP	Adenosine 5'-diphosphate
ADH	Alanine dehydrogenase
ATP	Adenosine 5'-triphosphate
CO_2	Carbon dioxide
DEAE-Cellulose	Diethylaminoethyl cellulose
EDTA	Ethylaminotetracetic acid
GLDH	Glutamate dehydrogenase
α -GPDH	α-glycerol phosphate dehydrogenase
GPO	Glycerol Phosphate Oxidase
GPT	Glutamate Pyruvate Transaminase
I.U	International unit
α-KG	α-Ketoglutarate
LDH	Lactate dehydrogenase
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
OAA	Oxaloacetic acid
SHAM	Salicylhydroxamic acid
Tris	Tris (hydroxy methyl) amino methane
Wks	Weeks

LIST OF SCHEMES

UNIT ABBREVIATIONS

cm	Centimetre
gm	Gram
M	Molar concentration
ml	Millilitre
mM	Millimolar
nm	Nanometer
pH	-Log H^+ concentration
^oC	degrees Celcius
µg	Microgram
µl	Microlitre

S-U-M-M-A-R-Y

With glutamate pyruvate transaminase as a cytosolic marker and α-glycerol phosphate dehydrogenase as a glycosomal marker the intracellular location of transaminases utilising leucine, isoleucine, valine, phenylalanine, tyrosine and tryptophan as substrates was investigated. The transaminase activities were released from the trypanosomes by increasing the concentrations of Triton X-100 ranging from 0 to 0.02% v/v. To release maximal activities of GPT, leucine: α-ketoglutarate transaminase, isoleucine: α-ketoglutarate transaminase, valine: α-ketoglutarate transaminase, phenylalanine: α-ketoglutarate transaminase, tyrosine: α-ketoglutarate transaminase and tryptophan: α-ketoglutarate transaminase, 0.01% (v/v) Triton X-100 was required. The concentration of Triton X-100 required to release maximum activity of α-GPDH was 0.015% (v/v). It was concluded that these transaminases are localized within the cytosol since their pattern of release corresponded to that of GPT and was different from that ofα-GPDH.

The specificity of the transaminases for the acceptor of the amino group from L-valine, L-leucine L-isoleucine, L-Glutamine, L-methionine, L-aspartate, L-phenylalanine, L-tyrosine and L-tryptophan was investigated using α-ketoglutarate and pyruvate. The rate of transamination with pyruvate as the acceptor of α-amino group was lower than when

α-ketoglutarate was the amino group acceptor. Branched chain amino acids were transaminated at approximately the same rate around 1.10 ± 0.030 μmoles glutamate/hr/mg protein with α-ketoglutarate and 0.30 ± 0.01 μmoles alanine/hr/mg protein with pyruvate as α-amino group acceptor. Methionine gave the highest rate of transamination with pyruvate as the α-amino group acceptor with 0.414 ± 0.012 μmoles L-alanine formed/hr/mg protein. It was concluded that both α-ketoglutarate and pyruvate could be acceptors of the -amino group during transamination although α-ketoglutarate is the preferred substrate.

The effect of pH on the enzymes catalysing the transamination of L-leucine, L-isoleucine, L-valine, L-phenylalanine, L-tyrosine and L-tryptophan with α-ketoglutarate as α-amino group acceptor was investigated within pH values ranging from 6.5 to 9.0. The highest transaminating activity was observed at the pH ranging between 7.8-8.5, beyond which the activity of the enzymes decreased. It was therefore concluded that the optimum pH for the transaminases present in bloodstream T.b. brucei lie between 7.8 to 8.5 pH range.

Results obtained on the stability of these transaminases when stored at 4°C and 25°C over 48 hours period indicated a gradual decrease in activity with time. About 50-80% of the original transaminase activity was lost at 4°C within 48 hours. Little or no activity remained at 25°C over the same period. It was observed that the rate at

which transaminase activity for the branched chain amino acids; leucine, isoleucine and valine was lost at 4°C and 25°C was approximately the same. However, transaminase activity for the aromatic amino acids was lost at differing rates at 4°C and 25°C. There was no significant loss of transaminase activity for both branched chain and aromatic amino acids in the presence of dithiothreitol.

Results from this study suggest that, branched chain amino acid are catalysed by either very closely similar or common transaminases whereas the aromatic amino acids are transaminated by different enzymes.

C-H-A-P-T-E-R--1

INTRODUCTION

1.1 THE TRYPANOSOME

Trypanosomes are parasitic protozoa that cause serious diseases of humans and domestic animals in Tropical Africa and South America. They belong to the order kinetoplastidae and the family Trypanosomatidae (Hoare, 1972; Vickerman, 1976). Depending on the mode of transmission by the vector, the genus Trypanosoma is generally subdivided into two main groups; the stercorarian and the salivarian.

The stercorarian parasite Trypanosoma cruzi causes Chagas disease and infects an estimated 10 million people in South America (Fairlamb, 1982). The salivarian trypanosomes comprises of Trypanosoma brucei brucei, T.b. rhodesiense, T.b gambiense, T. vivax, T. evansi and T. congolense. T.b. rhodesiense and T.b. gambiense cause the sleeping sickness in humans. T.b. rhodesiense is more virulent than T.b gambiense. T.b. brucei, T.vivax and T. congolense cause nagana in cattle whereas T. evansi cause surra in horses and camels (Hoare, 1972; Fairlamb, 1982).

Animal trypanosomiasis causes a serious nutritional and economic problem in Africa. Over 3 million cattle die each year due to various forms of nagana and rearing

of cattle, sheep and goats is not possible in 10 million square kilometres of land in Africa due to infestation with tsetse flies which transmit the disease (Fairlamb, 1982).

Morphologically T.b. brucei, T.b. rhodesiense and T.b. gambiense are indistiguishable, differing only in their infectivity to man (Vickerman, 1965). They are often thought to be subspecies of the older T. brucei species. They are designated T. brucei brucei, T. brucei gambiense and T. brucei rhodesiense (Vickerman, 1965; Hoare, 1972). This group of trypanosomes comprises the T brucei subgroup.

Trypanosomes belonging to the T. brucei subgroup are digenic. They spend part of their life cycle in the insect (invertebrate host) and part in mammalian (vertebrate host). The insect vector involved in their transmission is tsetse fly of the genus Glossina within which they undergo a series of developmental stages (Vickerman, 1976). The tsetse fly ingests parasites when taking a bloodmeal from an infected animal. Within the midgut of the insect stumpy forms differentiate into procyclic forms which do not possess the variable surface glycoprotein and are noninfective to mammalian host (Barry and Vickerman, 1979). After about three weeks, the procyclic forms migrate from the insect's midgut to its salivary glands where they develop into metacyclic forms which have a variable surface

glycoprotein and capable of infecting the mammalian host (Vickerman, 1969; Le Ray *et al*, 1978). The infective metacyclics are injected by the feeding tsetse fly into the host bloodstream where they develop into long slender forms which develop quickly by binary fission and undergo the characteristic antigenic variation (McNeillage, *et al*, 1969; Barry and Hadjuk, 1978).

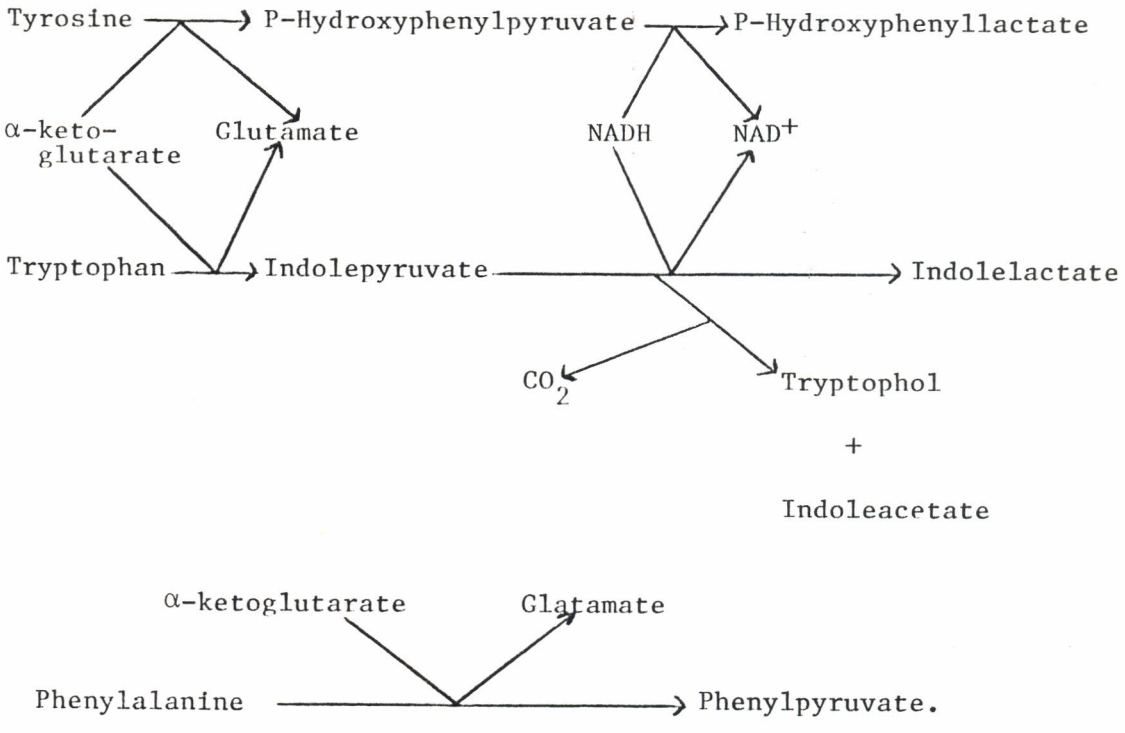
1.2 COMPARTMENTATION OF ENZYMES INVOLVED WITH ENERGY METABOLISM IN BLOODSTREAM FORMS

The bloodstream forms of *Trypanosoma brucei brucei* depend entirely on glycolysis for their energy production (Ryley, 1956; 1962; Fulton and Spooner, 1959). The first part of this pathway is catalysed in a subcellular organelle called a glycosome (Opperdoes *et al*. 1977). This highly specialized microbody contains all the enzymes required for the conversion of glucose and glycerol into 3-phosphoglyceric acid (i.e. hexokinase, glucose phosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, Glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, glycerol-3-phosphate dehydrogenasae, and glycerol kinase). Pyruvate kinase that catalyses the reaction from 2-phosphoenolpyruvate to pyruvate is localized in the cytosol. One mole of glucose is oxidized to produce two moles of pyruvate and a net two moles of ATP in aerobic conditions (Visser

and Opperdoes, 1980). Reoxidation of NADH generated during glycolysis is effected by means of a Glycerol phosphate oxidase system which involves a glycosomal NAD^+-dependent glycerol phosphate dehydrogenase and a mitochondrial glycerol phosphate oxidase (Grant and Sargent, 1960; Bowman and Flynn, 1976; Opperdoes et al, 1976). Pyruvate is the only end product in aerobic glycolysis in bloodstream T.b. brucei and maybe transaminated to alanine by alanine aminotransferase within the cytosol (Fulton et al, 1957; Chappel et al, 1972).

SCHEME I

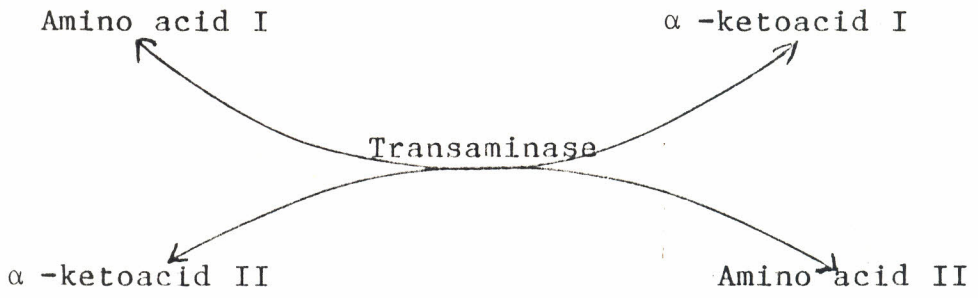
CATABOLISM OF AROMATIC AMINO ACIDS BY BLOODSTREAM FORMS OF T.B. GAMBIENSE (Gutteridge and Coombs, 1977)



1.3 TRANSAMINASES

There is widespread transaminase activity in living organisms (Rudman and Meister, 1953; Stenman et al, 1974; Tachiki and Tochikura, 1975; Patel et al, 1977; Paris and Magasanik, 1981). Approximately sixty transaminase activities are known (Christen and Metzler, 1985).

A typical transamination reaction is shown in the following reaction:



1.3.1 Occurrence of transaminase activities in bloodstream forms of T. brucei brucei, T.b rhodesiense and T.b. gambiense

Tryptophan and tyrosine aminotransferase activities have been reported in bloodstream T.b. gambiense (Stibbs and Seed, 1973,; 1975; Hall et al, 1981). These two enzymes are involved in the catabolism of the aromatic amino acids. The reactions they catalyse are illustrated in Scheme I. The presence of glutamate oxaloacetate transaminase in trypanosomes has also been reported in T.b. gambiense and T.b.

rhodesiense (Godfrey and Kilgour, 1976) and in T.b. brucei (Visser and Opperdoes, 1980; Steiger et al, 1980; Godfrey and Kilgour, 1973; 1976). Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase catalyse the following two reactions respectively (Green et al, 1945):

(i) Alanine + α-ketoglutarate $\rightleftharpoons$ glutamate + pyruvate

(ii) Aspartate + α-ketoglutarate $\rightleftharpoons$ glutamate + oxaloacetate

1.4 SURVEY OF TRANSAMINASES ACTIVITY IN BLOODSTREAM T.B. BRUCEI.

A survey of transaminase activity in bloodstream forms of Trypanosoma b. brucei has recently been carried out (Kabaru and Njogu, 1988). T.b. brucei lysates transaminated phenylalanine, tryptophan, tyrosine, leucine, isoleucine, valine, alanine, aspartate, glutamine and methionine. In these transaminations α -ketoglutarate was used as the amino group acceptor. Among the aromatic amino acids, the highest transaminase activity was given by phenylalanine followed by tyrosine and tryptophan. The branched chain amino acids; valine, leucine and isoleucine were transaminated at similar rates. Results of these transaminase activities are summarised in Table I.

No detailed information is available regarding

TABLE 1 - Title : TRANSAMINATION OF SOME AMINO ACIDS BY TRYPANOSOMA BRUCEI BRUCEI (Kabaru and Njogu, 1988)

	L-AMINO ACID	μ moles L-glutamate formed/hr/mg protein
Branched amino acids	Leucine	1.022 ± 0.027(4)
	Isoleucine	1.092 ± 0.015(4)
	Valine	1.132 ± 0.013(4)
Aromatic amino acids	Phenylalanine	1.132 ± 0.002(4)
	Tyrosine	0.789 ± 0.013(4)
	Tryptophan	0.385 ± 0.014(4)
Other amino acids	Alanine	1.444 ± 0.041(4)
	Aspartate	0.667 ± 0.012(4)
	Glutamine	0.330 ± 0.007(4)
	Methionine	0.645 ± 0.011(4)

their localization, optimum pH, stability and substrate specificity.

1.5 INTRACELLULAR LOCALIZATION OF ENZYMES

GPT and GOT are localized in the cytosol of bloodstream forms of T.b. brucei (Opperdoes et al, 1977). In bloodstream forms of these organisms there are three major compartments. These are the cytosol, the glycosome and the mitochondrion with their markers being GPT, α-GPDH and oligomycin sensitive ATPase respectively. The intracellular localization of enzymes in trypanosomes can be determined by comparing their latency with that of the markers for the various cell compartments. The use of freeze-thawing and detergents such as Triton X-100 and digitonin has been employed by others to determine the differences in localization of enzymes within the cell (Steiger et al, 1974; Visser and Opperdoes, 1980). These procedures disrupt the integrity of the biological membrane making it permeable to molecules non-selectively. If an enzyme is enclosed by more than one membrane, it would require higher detergent concentrations or more cycles of freeze-thawing to release maximum activity than one enclosed by only one membrane. Kabaru (1987) investigated intracellular localization of glucose 6-phosphate dehydrogenase, NAD-linked malate dehydrogenase and NADP-linked malic enzyme in bloodstream T.b. brucei by using

Triton X-100, digitonin and freeze-thawing. He found these enzymes to be localized in the cytosol of these organisms. Intracellular localization of transaminase activities present in bloodstream T.b. brucei has not been investigated.

1.6 SUBSTRATE SPECIFICITY OF TRANSAMINASES

Several studies have been carried out on substrate specificity of transaminases in various organisms and tissues.

In Escherichia coli three transaminases designated A, B and C were observed (Rudman and Meister, 1953). Transaminase A was specific for the amino acids leucine, phenylalanine and tyrosine with α-ketoglutarate as the acceptor of the amino group. Transaminase B was found to be specific for the branched chain amino acids leucine, isoleucine and valine. It was also able to transaminate phenylalanine and tyrosine. On the other hand transaminase C was specific for valine only among the branched amino acids with pyruvate as the acceptor of the amino group.

In rat tissues three transaminases I, II and III specific for the branched chain amino acids have been shown (Ichihara, 1975). Transaminase I and II acted equally well on valine, leucine and isoleucine. The best amino group acceptor was found to be α-ketoglutarate.

TABLE II - Title : AMINO GROUP SPECIFICITY OF L-LEUCINE PYRUVATE TRANSAMINASE UTILISING PYRUVATE, α-KETOGLUTARATE AND OXALOACETATE AS THE ACCEPTORS OF THE AMINO GROUP (Tachiki, et al. 1975)

Amino group acceptor	Relative activity (%)
Pyruvate	100
α-Ketoglutaric acid (KGA)	59
Oxaloacetate (OAA)	10

Amino group acceptors utilising L-leucine pyruvate transaminase activity in Acetobacter suboxydans are shown in Table II. The table shows that α-ketoglutarate gave approximately 60% of the activity obtained with pyruvate whereas it was 10% when oxaloacetate was used.

The specificity of rat liver glutamine transaminase in respect to α-ketoacid has also been studied (Cooper and Meister, 1972). Broad α-ketoacid specificty was observed suggesting that glutamine may offer a physiological mechanism for amination of many α-ketoacids to provide corresponding amino acid for protein synthesis.

In case of glutamine transaminase from brain tissue, the most active ketoacid amino group acceptor was found to be phenylpyruvate (Leuven, 1975; 1976). Pyruvate and α-ketoglutarate exhibited 28 and 7% relative activity compared with that of phenylpyruvate respectively.

1.7 EFFECT OF pH ON THE ACTIVITY OF TRANSAMINASES

Table III is a summary of the optimum pH of several transaminases derived from different sources. The optimum pH of L-leucine-pyruvate transaminase from Acetobacter suboxydans was found to be 5.0 (Tachiki and Tochikura, 1975). The optimum pH for GPT and GOT derived from bloodstream stage of T.b. brucei have been found to be pH 8.0 (Steiger et al, 1974). Glutamine

TABLE III - Title : OPTIMUM pH OF SOME TRANSAMINASES FROM DIFFERENT SOURCES

NAME OF TRANSAMINASE	SOURCE	ASSAY BUFFER	OPTIMUM pH	REFERENCE
Glutamine : α-ketoglutarate transaminase	Rat brain	Borate buffer	9.0 - 9.2	Leuven, (1975)
Glutamine: α-ketoglutarate transaminase	Rat kidney	Tris-HCl buffer	8.4	Cooper and Meister, (1974)
Leucine: (Methionine) transaminase	Rat liver mitochondria	Potassium phosphate buffer	7.8	Ikeda and Ichihara, (1976)
Glutamate: Pyruvate transaminase	Bloodstream *T.b. brucei*	Tris	8.0	Steiger and Krassner, (1974)
Glutamate: oxaloacetate transaminase	Bloodstream *T.b. brucei*	Tris	8.0	Steiger and Krassner, (1974)
Glutamate: Pyruvate transaminase	Culture forms of *T.b. brucei*	Tris	6.2 & 7.2	Steiger and Krassner, (1974)
Glutamate: Oxaloacetate transaminase	Culture forms of *T.b. brucei*	Tris	6.0	Steiger and Krassner, (1974)
Leucine: Pyruvate transaminase	Acetobacter suboxydans	Acetate and potassium phosphate	5.0	Tachiki *et al*, (1975)

transaminase from rat brain and kidney was found to have different optimum pH values (Leuven, 1975, Cooper and Meister, 1974). Leucine (Methionine) transaminase from rat liver mitochondria had an optimum pH of 7.8 (Ikeda and Ichihara, 1976). GOT from the culture stage of T.b. brucei had an optimum pH of 6.0 different from that of GOT from the bloodstream stage. Except for GPT and GOT from bloodstream forms of T.b. brucei; the effect of pH on the activity of the other transaminases present in these organisms has not been investigated.

1.8 THE STABILITY OF TRANSAMINASES

Several studies have shown that transaminases are unstable. As early as 1945, Green et al showed that glutamic oxaloacetate transaminase was unstable in solution and in presence of ammonium ions. Cammarata and Cohen, (1950) carried out a study of several transaminase activities from various tissues of pig. It was shown that transaminase activities were unstable when kept in cold and in an atmosphere of nitrogen. Some of the transaminases lost upto 80% of their activity after a period of eight weeks. It was apparent that even in crude prepararions the enzymes deteriorated with storage in lyophilized state, in vacuo and in cold. The results are shown in Table IV. Steiger et al. (1974) have shown GPT and GOT from trypanosomes to be unstable losing their activity at a rate of 10% per

TABLE IV - Title : EFFECT OF STORAGE OF LYOPHILIZED MUSCLE AND LIVER EXTRACTS AT 4°C (Camarata and Cohen, 1950)

	AGE OF PREPARATION		INACTIVATION
	2 WKS	8WKS	
	LYOPHILIZED MUSCLE EXTRACT		
	μ l CO_2	μ l CO_2	Per cent
Methionine	57	50	0
Valine	237	64	73
Isoleucine	208	84	60
Phenylalanine	67	71	0
Tryptophan	65	60	0
	LYOPHILIZED LIVER EXTRACT		
Cysteine	46	8	82
Glycine	57	9	84
Arginine	147	119	19
Tyrosine	144	78	46
Leucine	99	37	63

day. It is possible that transaminase activity may be unstable requiring use of stablizing agents to prevent loss of activity with time. Research has not been carried out to show the extent to which transaminases present in bloodstream forms of T.b. brucei are unstable.

1.9 AIMS OF STUDY

The aims of this study were:

Firstly, to establish the intracellular location of leucine, valine, isoleucine, phenylalanine, tyrosine and tryptophan transaminase activities in bloodstream forms of T.b. brucei.

Secondly, to establish the substrate specificity of these transaminases for α-ketoglutarate or pyruvate as the amino group acceptors.

Thirdly, to determine the optimum pH for the assay mixture of the activity of these transaminases in trypanosome lysates.

Fourthly, to determine the stability of transaminases at different storage conditions.

C-H-A-P-T-E-R---2

MATERIALS AND METHODS

Most chemicals used were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Others were from BDH Chemicals, Poole, Dorset, U.K. All reagents were of analar grade.

2.1 ORGANISMS

Trypanosoma brucei EATRO 1969 was used throughout the study. It was originally isolated from a female hyena in Tanzania in 1970. It was inoculated into rats and mice, collected and frozen as a stabilate. Early in 1978, it was cloned at the International Centre for Insect Physiology and Ecology (ICIPE), Kenya and proven to be infective to tsetse flies (Glossina morsitans morsitans) and rodents.

2.2 IN VIVO GROWTH OF PARASITES

The parasites were maitained in the laboratory by serial passage in Sprague-Dawley rats of body weight 200-300 g. The rats were housed and fed on rat pellets.

2.3 BUFFERS

(i) Phosphate Saline Glucose (PSG). This was prepared according to Taylor et al, (1974). It consisted of

56 mM D-glucose, 44 mM NaCl, 2 mM NaH_2PO_4 and 56 mM Na_2HPO_4 (pH was adjusted to 8.0 with NaOH).

(ii) Phosphate buffered saline (PS) consisting of 57 mM Na_2HPO_4, 3.36 mM NaH_2PO_4 and 73.7 mM NaCl with pH adjusted to 8.0. Unless otherwise stated in the text, the above two buffers were routinely used for the manipulation of the trypanosomes.

2.4 MAINTENANCE OF PARASITES

Rats were infected intraperitoneally with 10^6-10^7 trypanosomes using 1 ml disposable syringe fitted with a 21 gauge hypodermic needle. This produced an acute infection resulting to death after 72-96 hours after inoculation. The parasites were harvested when the parasitaemia was above 1 x 10^8/ml of the blood, while the animal was under diethyl ether anaesthesia. First, the thorax was opened to expose the heart. Then the exposed heart was sprayed with 5%(w/v) trisodium citrate in phosphate saline glucose (PSG) buffer as anticoagulant. The heart was then punctured with a needle fitted to a 10 ml plastic syringe and as much blood as possible was collected. Usually about 10 ml of blood were obtained from a 250 g rat. The trypanosomes were counted on an improved Neubaeur Hemocytometer.

2.4.1 Separation of the trypanosomes from infected blood by the hypotonic lysis method.

Infected rat blood was immediately centrifuged at 1000 x g for 10 minutes at 4°C. The trypanosomes were collected from the interface with a Pasteur Pipette. Care was taken to avoid much contamination by the erythrocytes. There remained some 20-30% contamination of the trypanosomes collected, by erythrocytes. The contaminating erthrocytes were removed by hypotonic shock technique developed by Njogu and Kiaira (1982). This method was preferred to the diethylaminoethyl cellulose (DEAE-cellulose) anion exchange (Lanham and Godfrey, 1970) because it was quick enabling the preparation of large numbers of trypanosomes within relatively short periods, which are sufficiently pure for metabolic studies. It is also inexpensive. The trypanosome rich layer (buffy coat) was suspended in 100 volumes of 1 mM sodium phosphate buffer of pH 7.5 containing 0.3% (w/v) NaCl, and incubated at 37°C for 3 minutes in a Dubnoff Metabolic Shaking Incubator. The treatment was stopped by addition of 0.1 volumes of ten-fold concentrated phosphate saline glucose (pH 8.0).

The trypanosome suspension was centrifuged at 1000 x g for 10 minutes at 4°C to obtain a trypanosome pellet. The pellet was washed by gentle resuspension in fresh PSG and recentrifuged at 1000 x g for 10 minutes at 4°C. The final suspension of clean trypanosomes was

kept in ice ready for use. The trypanosomes in this medium retained motility for at least 1.5 - 2 hours.

2.5 TREATMENT OF TRYPANOSOMES WITH TRITON X-100

Stock solution of 1% (v/v) Triton x-100 was prepared in water. Appropriate volumes of the detergent were added to final concentrations ranging between 0-0.02% (v/v). The trypanosome suspensions containing the detergent were agitated gently to expose the trypanosomes to the same concentration of Triton x-100 and incubated for 10 minutes at 25°C. The incubations were terminated by centrifugation at 1000 x g for 10 minutes at 4°C. The enzyme activities in the supernatant were determined.

2.6 METABOLITE AND ENZYME ASSAYS

When the end products of transamination were to be determined, incubations were carried out in a Dubnoff Metabolic Shaking Incubator in 10 ml plastic centrifuge tubes (at 25°C) with 1 ml final volume. Incubations were terminated as described by Bergemeyer (1978). An equal volume of 14% (v/v) perchloric acid was added to the incubation mixture to give a final concentration of 7% (v/v). The samples were centrifuged at 1000 x g for 10 minutes at 4°C. The deproteinized samples were neutralized with 20% (w/v) K_2HPO_4 after which they were centrifuged at 1000 x g for 10 minutes at 4°C. The

supernatants were used for determination of L-glutamate or L-alanine formed.

All metabolites or enzyme activities were determined at 25°C on a Perkin Elmer Spectrophotometer or a Unicam SP 1800 spectrophotometer coupled to a Pye Unicam recorder using cuvettes of 1 ml capacity and 1 cm light path.

An extinction coefficient of 6.23×10^{-6} per mole at 340 nm was used in all determinations (Bergemeyer, 1974). The concentration of the metabolites in each sample assayed was estimated using the following formula:

$$C = \frac{\Delta E}{6.23} \times \frac{V}{v} \times \text{Dilution}$$

where C = concentration of respective metabolite in μmoles/ml sample.

ΔE = change in absorbance during the assay due to the presence of volume v of sample in a cuvette containing final volume V of assay mixture.

2.6.1 Determination of the trypanosomal transaminase activity

Neutralized α-ketoglutarate or pyruvate and L-amino acids were added to supernatants obtained after treatment of the trypanosomes with Triton X-100 to a

final concentration of 10 mM. This mixture was incubated at 25°C for 1 hour in a Dubnoff Metabolic Shaking Incubator. Incubations were terminated as described above and the metabolites determined.

2.6.1.1 Transamination reactions with α-ketoglutarate as the acceptor of the α-mino group

2.6.1.1.1 Leucine: α-ketoglutarate transaminase activity

L-Leucine + α-ketoglutarate ⇌ L-glutamate + α-ketoisocaproate

2.6.1.1.2 Valine: α-ketoglutarate transaminase activity

L-valine + α-ketoglutarate ⇌ L-glutamate + ketoisovalerate

2.6.1.1.3 Isoleucine: α-ketoglutarate transaminase activty

L-Isoleucine + α-ketoglutarate ⇌ L-glutamate + ketomethylvalerate

2.6.1.1.4 Phenylalanine: α-ketoglutarate transaminase activity

L-phenylalanine + α-ketoglutarate ⇌ L-glutamate + phenylpyruvate

2.6.1.1.5 Tryptophan; α-ketoglutarate transaminase activity

L-tryptophan + α-ketoglutarate ⇌ L-glutamate + indolepyruvate

2.6.1.1.6 Methionine: α-ketoglutarate transaminase activity

L-methionine + α ketoglutarate ⇌ L-glutamate + 4-methylthio-ketobutyrate

2.6.1.1.7 Glutamine: α -ketoglutarate transaminase activity

L-glutamine + α-ketoglutarate ⇌ L-glutamate + α-ketoglutamate

2.6.1.1.8 Aspartate: α-ketoglutarate transaminase activity

L-aspartate + α-ketoglutarate ⇌ L-glutamate + oxaloacetate

2.6.1.1.9 Tyrosine: α-ketoglutarate transaminase activity

L-tyrosine + α-ketoglutarate ⇌ L-glutamate + β-hydroxyphenylpyruvate

2.6.1.2 Reactions with pyruvate as the amino group acceptor

Pyruvate was added as amino group acceptor instead

of ∝-ketoglutarate. Alanine was formed in all cases and the corresponding α-ketoacids as given in 2.6.1.1.1 to 2.6.1.1.9.

2.6.1.3 Glutamate pyruvate transaminase

This enzyme was assayed as described by Bergemeyer (1974). The assay mixture contained 80 mM L-alanine, 0.18 mM NADH and 3.7 I.U of lactate dehydrogenase/ml. The reaction was started with 18 mM α-ketoglutarate.

2.6.2 α-Glycerol phosphate dehydrogenase

This enzyme was assayed as described by Opperdoes et al (1977). The assay mixture contained 50 mM sodium phosphate buffer (pH 7.2), 1 mM EDTA and 0.15 NADH. The reaction was started by addition of 0.5 mM dihydroxyacetone phosphate.

2.6.3 L-Glutamate

L-glutamate was assayed in a system containing 290 mM glycine - 232 mM hydrazine buffer (pH 9), 0.97 mM ADP and 1.6 mM NAD^+. The reaction was started by adding 4..5 I.U of glutamate dehydrogenase (GLDH) per ml free from ammonium ions. The reaction mixture was incubated for 60 minutes at 25°C. The assay was based on the reduction of NAD^+ at 340 nM.

$$\text{L-glutamate} + NAD^+ + H_2O \underset{\text{GLDH}}{\overset{\text{ADP}}{\rightleftharpoons}} \alpha\text{-ketoglutarate} + NH_4^+ + NADH + H^+$$

2.6.4 L-Alanine

L-alanine was the end product of transamination when the trypanosome lysates were incubated with pyruvate. It was assayed as described by Olembo (1980). The assay system contained 92 mM glycine, 770 mM hydrazine buffer (pH 10), and 0.74 mM NAD^+. The reaction was started by adding 0.38 I.U. alanine dehydrogenase (ADH) per ml. The ADH used was free from ammonium ions. It was a suspension in glycerol. The reaction mixture was incubated for one hour at 25°C.

$$\text{L-alanine} + NAD^+ H_2O \overset{\text{ADP}}{\rightleftharpoons} \text{pyruvate} + NADH + NH_4^+$$

2.7 EFFECT OF pH ON THE TRYPANOSOMAL TRANSAMINASE ACTIVITIES

Phosphate buffered saline was prepared with pH ranging between 6.5 - 9.0. The concentrations of Na_2HPO_4 and NaH_2PO_4 were adjusted for proper buffering. Trypanosome pellets were washed several times with buffer having the appropriate pH. Transaminases

activity was released by 0.02% (v/v) Triton X-100, and centrifuged at 1000 x g for 10 minutes at 4°C. The supernatant obtained were used for the determination of transaminase activity.

2.8 DETERMINATION OF STABILITY OF TRYPANOSOMAL TRANSAMINASES

Transaminase activities in $1x10^8$-$2x10^8$ trypanosomes were released by 0.02% (v/v) of Triton X-100. The solubilized trypanosomes were centrifuged at 1000 x g for 10 minutes at 4°C. The supernatant were kept at 25°C and 4°C. After intervals of 12 hrs, aliquots were taken and assayed for the transaminase activity.

2.9 PROTEIN DETERMINATION

Protein was determined by the method of Lowry *et al*, (1951) using Bovine Serum Albumin (BSA) as a standard. The standard curve was constructed using a serum albumin concentration ranging from 0-40 µg. To each protein sample 1.15 mls of solution A (prepared freshly by mixing 1 ml 1% $CuSO_4$, 50 ml of 2% Na-K-tartarate and 2% Na_2CO_3 in 0.1 M NaOH) was added. The solution was allowed to stand for 10 minutes, after which 0.11 mls of solution B (Folin-Ciocalteau reagent diluted 1:2 with water) was then added and thoroughly mixed. The solutions were left to stand for 30 minutes at room temperature. The optical density was then read

at 578 nm in a Perkin Elmer Spectrophotometer. Blanks were prepared with distilled water in place of the BSA and the same process was repeated. Sample aliquots were also treated in the same way and the protein concentration was read on the standard curve.

CHAPTER 3

3.1 LOCALIZATION OF SOME TRYPANOSOMAL ENZYMES BY USE OF TRITON X-100

The pattern of release of glutamate : pyruvate transaminase, α-glycerophosphate dehydrogenase, leucine : α-ketoglutarate transaminase, isoleucine: α-ketoglutarate transaminase, valine: α-ketoglutarate transaminase, phenylalanine: α-ketoglutarate transaminase, tyrosine: α-ketoglutarate transaminase and tryptophan: α-ketoglutarate transaminase from isolated bloodstream forms of Trypanosoma brucei brucei was investigated using increasing Triton x-100 concentrations. The aim of performing these experiments was to establish the intracellular localization of these enzymes in the trypanosomes. α-glycerophosphate dehydrogenase was used as a marker for the glycosome and glutamate pyruvate transaminase as a marker for the cytosol.

3.1.1 Release of glutamate pyruvate transaminase and α-glycerol phosphate dehydrogenase

Results in Figure 1 show the release of glutamate pyruvate transaminase and α-glycerol phosphate dehydrogenase as a function of increasing concentrations of Triton X-100. These results show that there was gradual increase in enzyme activity with increase in

FIGURE 1

RELEASE OF GLUTAMATE PYRUVATE TRANSAMINASE AND α-GLYCEROL PHOSPHATE DEHYDROGENASE FROM BLOODSTREAM FORMS OF TRYPANOSOMA BRUCEI BRUCEI BY VARIOUS CONCENTRATIONS OF TRITON X-100.

Intact trypanosomes (cell density 2 x 10^8/ml) in PSG were incubated at 25°C for 10 minutes with Triton X-100 concentrations ranging from 0 to 0.02% (v/v). Incubations were terminated by centrifugation at 1000 x g for 10 minutes at 4°C and enzyme assayed in the supernatants. The results shown are mean values from four individual experiments. Total enzyme activity was released by 0.02% Triton X-100.

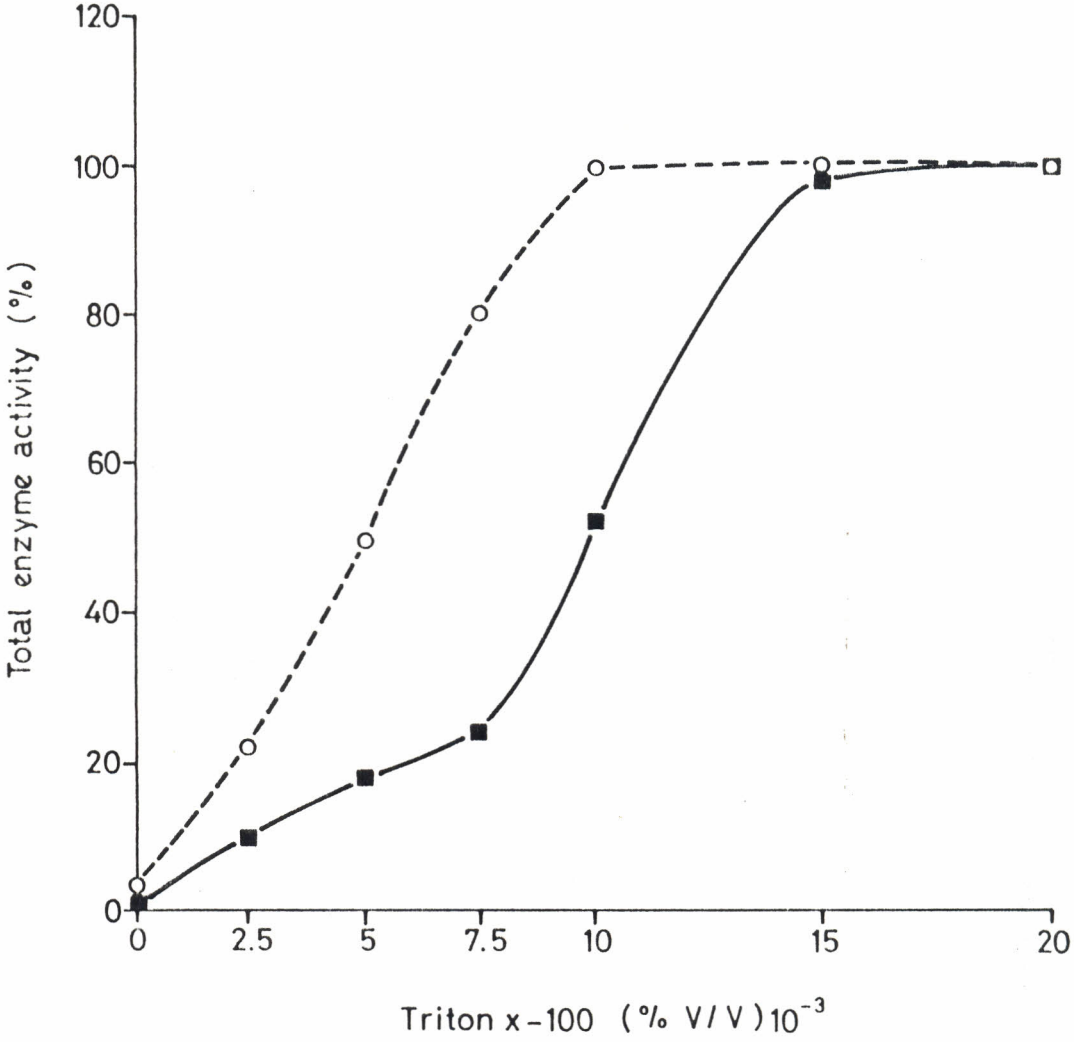
Total enzyme activities;

GPT 2.952 ± 0.002 μmoles/min/mg protein

α-DPDH 3.150 ± 0.007 μmoles/min/mg protein

O------O GPT

■——■ α-GPDH


120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x-100 (% V/V) 10^{-3}

Triton X-100 concentration. The release of α-glycerol phosphate dehydrogenase activity required higher concentration of Triton X-100 than glutamate pyruvate transaminase. Figure 1 shows that 0.01% (v/v) Triton X-100 was required to release maximum activity of glutamate pyruvate transaminase whereas α-glycerol phosphate dehydrogenase required about 0.015% v/v. The pattern of release of α-glycerol phosphate dehydrogenase was different from that of glutamate pyruvate transaminase. The maximum enzyme activities released were; glutamate pyruvate transminase 2.952 ± 0.002 and α-glycerol phosphate dehydrogenase, 3.150 ± 0.007 μmoles/hr/mg protein respectively.

There was about 3% glutamate pyruvate transaminase activity in the medium in the absence of Triton X-100. This activity was probably due to the presence of dead trypanosomes. However, in case of α-glycerol phosphate dehydrogenase no measureable activity in the absence of Triton X-100 was observed.

It was concluded that glutamate pyruvate transaminase occurs in a compartment different from that occupied by α-glycerol phosphate dehydrogenase. It has been shown in bloodstream form of T.b. brucei that glutamate pyruvate transaminase is cytosolic ((Steiger et al. 1980; Visser and Opperdoes, 1980) whereas

α-glycerophosphate dehydrogenase is glycosomal (Opperdoes et al, 1977; 1984).

3.1.2 Release of leucine, isoleucine and valine transaminase from bloodstream forms of T.b. brucei by Triton X-100.

Results presented in Figure 2 show the release of leucine: α -ketoglutarate transaminase from bloodstream trypanosomes suspended in PSG in the presence of increasing concentrations of Triton x-100.

There was gradual increase in activity of the enzyme with increase in Triton X-100 concentrations. The amount of detergent required to release maximum activity of leucine : α-ketoglutarate transaminase was 0.01% (v/v). The pattern of release of leucine : α-ketoglutarate transaminase was the same as that of glutamate pyruvate transaminase and was different from that of α -glycerol phosphate dehydrogenase (Figure 1). It was concluded that leucine : α -ketoglutarate transaminase activity is located in the same compartment with glutamate pyruvate transaminase but different from that of α- glycerol phosphate dehydrogenase.

There was measurable activity of leucine: α -ketoglutarate transaminase in the absence of Triton x-100. This activity was 2% of the maximum activity released. The maximum enzyme activity released was 1.113 ± 0.008, μ moles L-glutamate formed/hour/mg protein.

Release of isoleucine: α-ketoglutarate transaminase from the trypanosomes is shown in Figure 3.

There was a gradual release of the enzyme activity with increasing Triton X-100. In the absence of the detergent about 2% of the maximum activity released was observed. The maximum activity of isoleucine: α -ketoglutarate transaminase released was 1.134 ± 0.005, μmoles L-glutamate formed/hour/mg protein.

The pattern of release of isoleucine: α -ketoglutarate transaminase resembles that of glutamate pyruvate transaminase but is different from that of α-glycerol phosphate dehydrogenase (Figure 1). It was therefore concluded that isoleucine: α -ketoglutarate transaminase activity is located in the same compartment as glutamate pyruvate transaminase.

The pattern of release of valine: α -ketoglutarate transaminase is shown in Figure 4. These results show resemblance to those in Figures 2 and 3. The pattern of release of valine α-ketoglutarate transaminase also resembles that of glutamate pyruvate transaminase (cytosolic marker) as shown in Figure 1. The maximum activity of valine α-ketoglutarate transaminase released was 1.125 ± 0.004 μmoles L-glutamate formed/hr/mg protein. The percentage of maximum activity obtained without Triton X-100, attributed to leakage in PSG alone was 3%.

Results shown in Figures 2, 3 and 4 closely resemble each other. It is possible that the enzyme(s)

FIGURE 2

RELEASE OF LEUCINE : α -KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF T.B. BRUCEI BY TRITON X-100.

Trypanosomes (cell density 2 x 10^8/ml) in PSG were incubated at 25°C for 10 minutes with Triton X-100 concentrations ranging from 0 to 0.02% (v/v). Incubations were terminated by centrifugation at 1000 x g for 10 minutes at 4°C. The supernatants obtained were used for the determination of enzyme activity as described in the Materials and Methods. The results shown are mean values of three separate experiments. Total enzyme activity was released by 0.02% Triton X-100.

Total enzyme activity was;
1.113 ± 0.008 μ moles L-Glutamate/hr/mg protein.

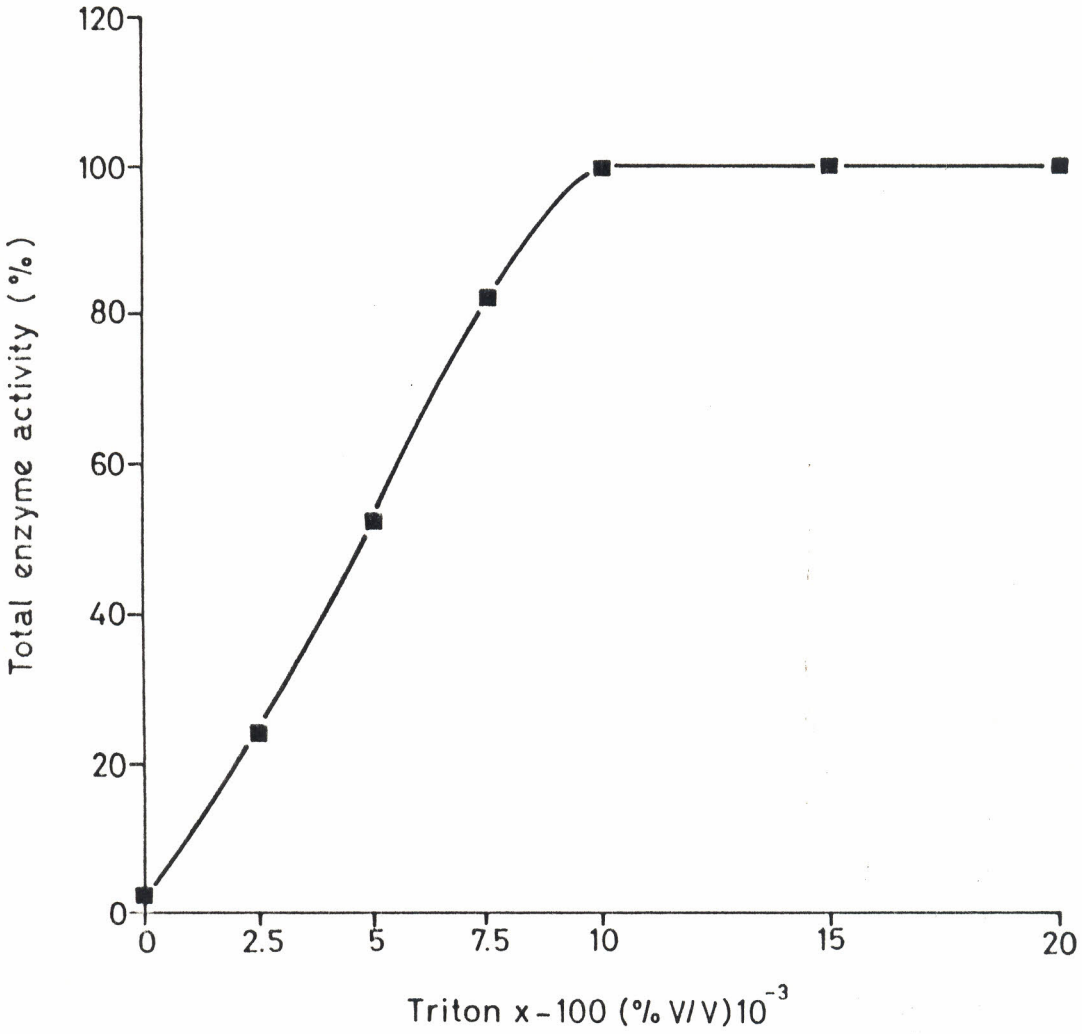

120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x-100 (% V/V) 10^{-3}

FIGURE 3

RELEASE OF ISOLEUCINE : α-KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF T.B. BRUCEI BY TRITON X-100.

The experimental details for the determination of enzyme activity are as described in the legend to Figure 2.

Total enzyme activity was;

1.134 ± 0.005 μmoles L-glutamate formed/hr/mg protein.

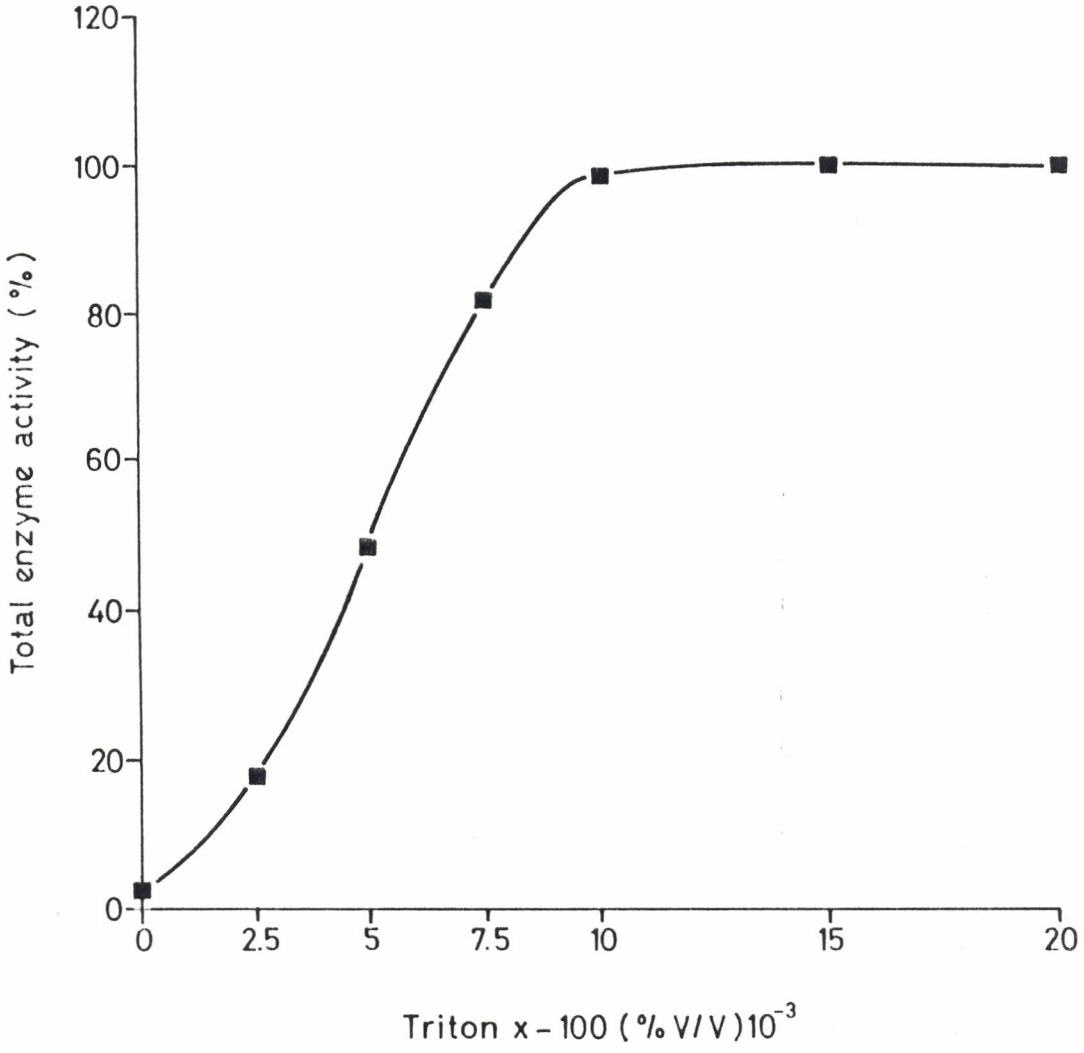

120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x-100 (% V/V)10^{-3}

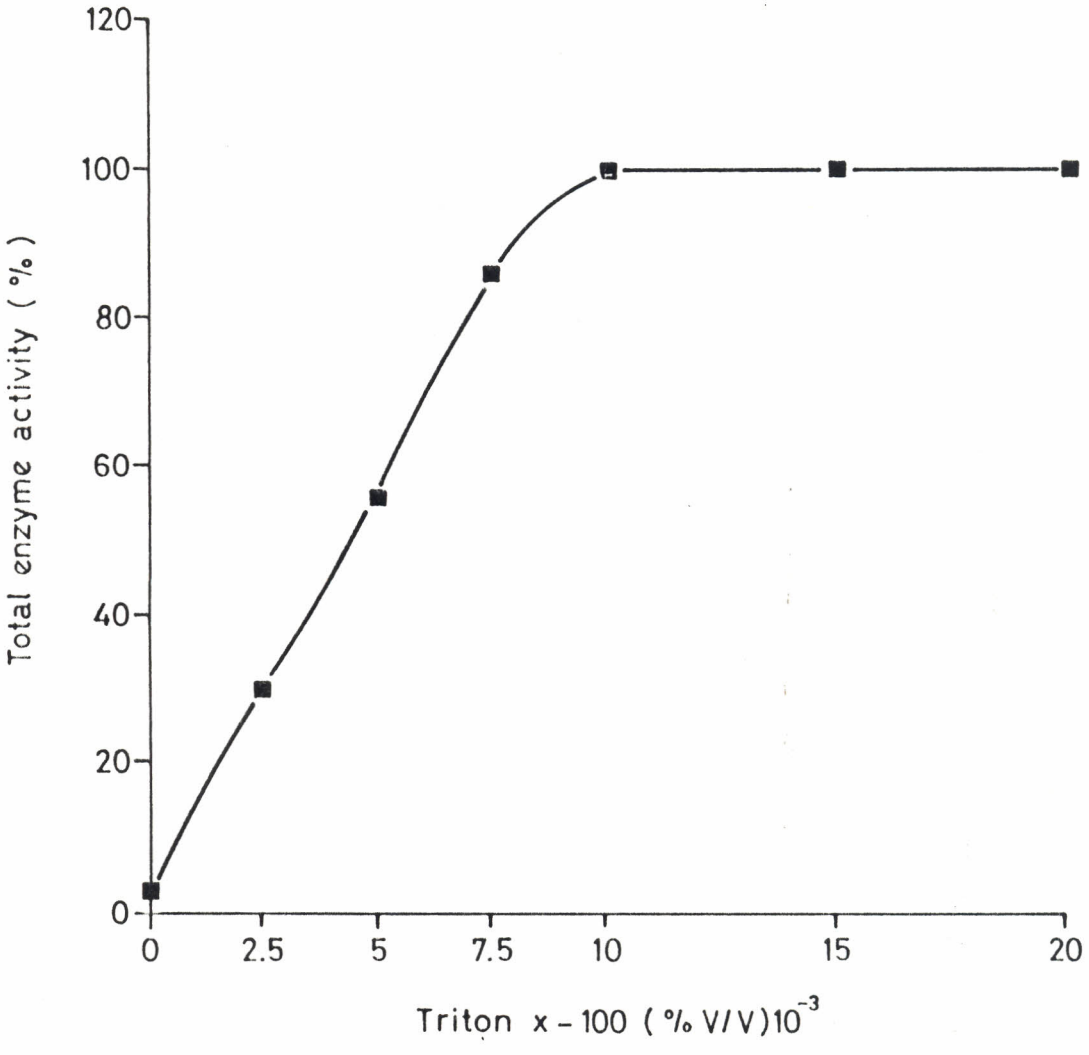
FIGURE 4

RELEASE OF VALINE : α-KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM T.B. BRUCEI BY VARIOUS CONCENTRATION OF TRITON X-100.

The experimental details for the determination of enzyme activity are as described in the legend to Figure 2.

Total enzymes activity was;

1.125 ± 0.004 μmoles L-glutamate formed/hour/ mg protein.


120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x - 100 (% V/V)10^{-3}

catalysing the transamination of the branched chain amino acids; leucine, isoleucine and valine are located within the cytosol because the pattern of release was identical to that of glutamate pyruvate transaminase activity which is known to be in the cytosol.

3.1.3 Release of phenylalanine, tyrosine and tryptophan transaminase from bloodstream forms of T.b. brucei by Triton X-100

Figure 5 shows that phenylalanine: α -ketoglutarate transaminase activity from bloodstream forms of T.b. brucei was gradually released by increasing concentrations of Triton X-100. Maximum activity was attained at about 0.010%(v/v) of the detergent. There was some activity before the addition of Triton X-100. This activity was about 2% of the maximum activity released. The pattern of release of phenylalanine: α -ketoglutarate transaminase resembled that of glutamate pyruvate transaminase shown in Figure 1. The maximum activity released by Triton X-100 was 1.141 ± 0.02 μmoles L-glutamate formed/hr/min.

The release of tyrosine α -ketoglutarate transaminase from trypanosomes by varying concentrations of Triton X-100 is shown in Figure 6. The pattern of release of this enzyme closely resembles that of phenylalanine: α-ketoglutarate transaminase. It is also similar to that of glutamate -pyruvate transaminase

FIGURE 5

RELEASE OF PHENYLALANINE : α-KETOGLUTARATE TRANSAMINASE ACTIVITY FROM BLOODSTREAM FORMS OF T.B. BRUCEI BY VARIOUS CONCENTRATIONS OF TRITON X-100.

The experimantal details for the determination of the enzyme activity are as described in the legend to figure 2.

Total enzyme activity was;

1.141 ± 0.020 μ moles L-glutamate formed/hour/mg protein.

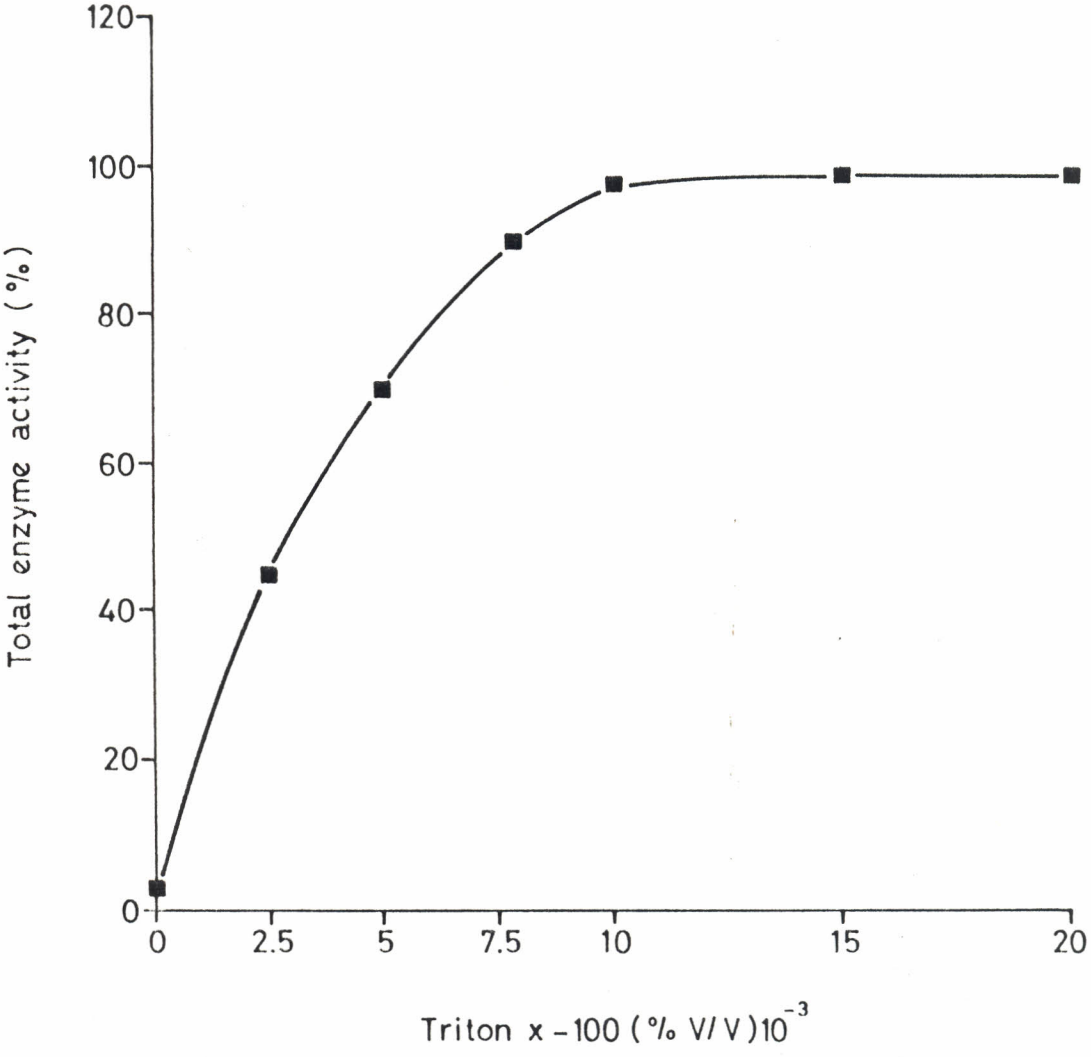

120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x-100 (% V/V)10^{-3}

FIGURE 6

RELEASE OF TYROSINE : α -KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORM OF T.B. BRUCEI BY VARIOUS CONCENTRATIONS OF TRITON X-100.

Experimental details for determination of transaminase activity are as decribed in the legend to Figure 2.

Total enzyme activity was;

0.792 ± 0.015 μ moles L-glutamate/formed/hour

mg protein.

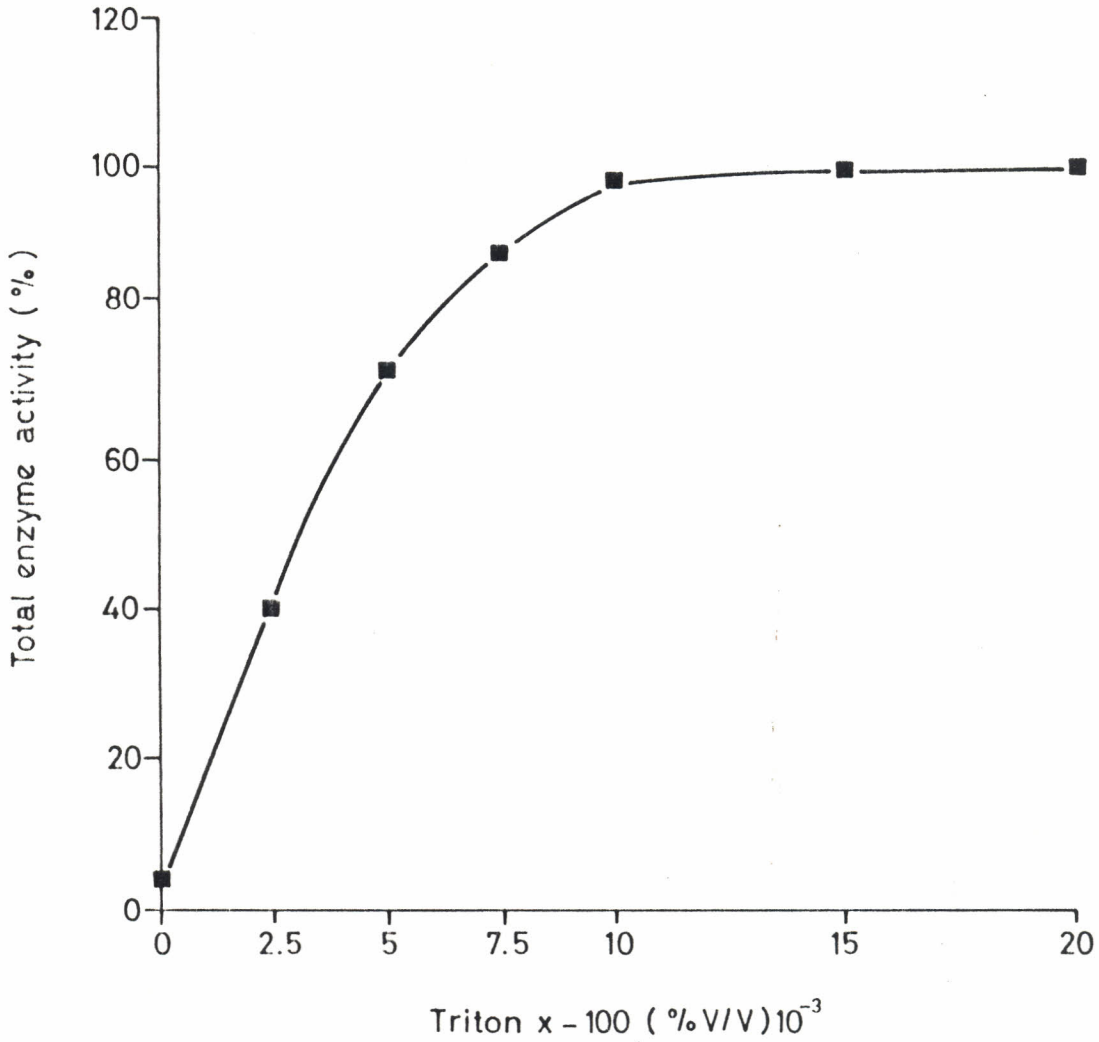

120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x - 100 (%V/V)10^{-3}

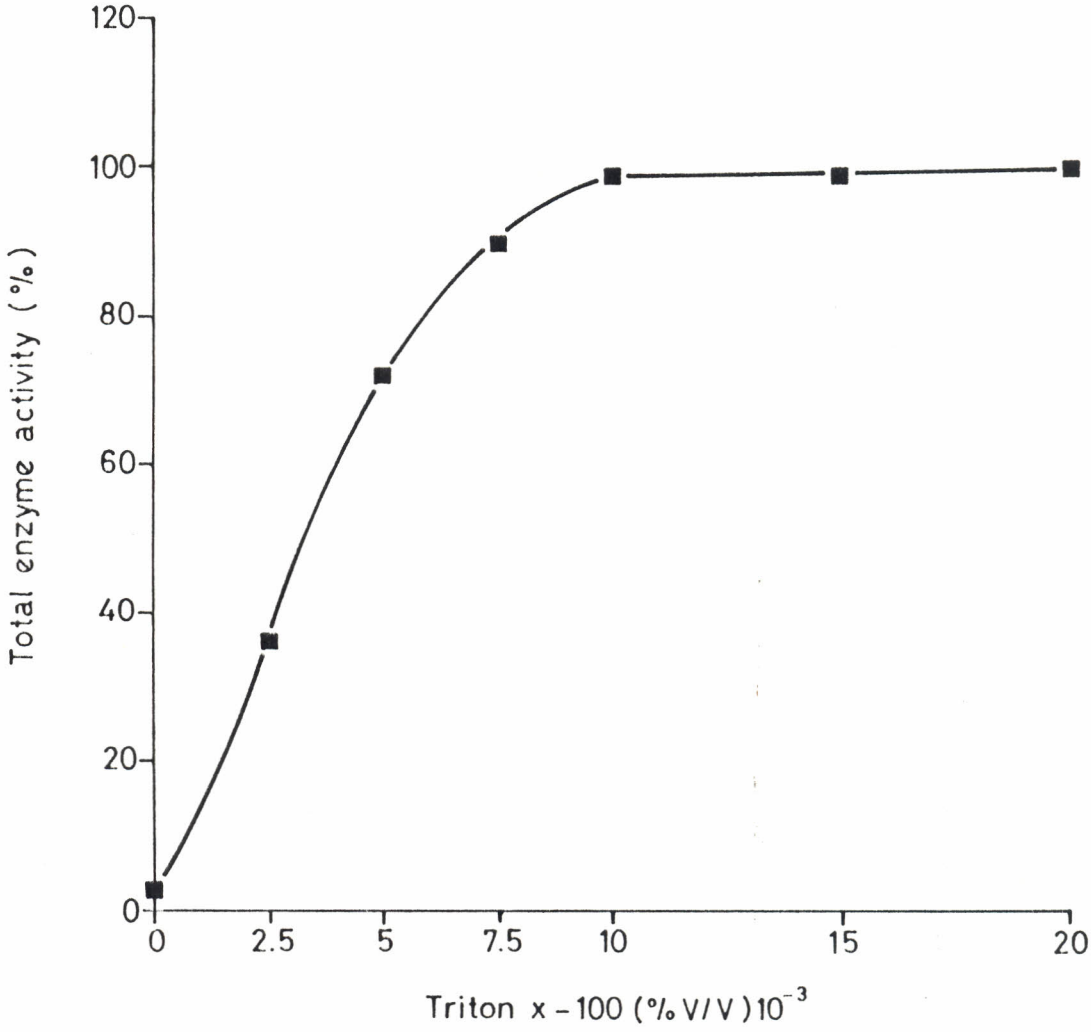
FIGURE 7

RELEASE OF TRYPTOPHAN : α -KETOGLUTARATE TRANSAMINASE ACTIVITY FROM BLOODSTREAM FROM T.B. BRUCEI BY VARIOUS CONCENTRATIONS OF TRITON X-100.

Experimental details are as outlined in the legend to Figure 2.

Total enzyme activity was;

0.348 ± 0.03 μ moles L-glutamate formed/hour/mg protein.


120
100
80
60
40
20
0
Total enzyme activity (%)
0
2.5
5
7.5
10
15
20
Triton x-100 (% V/V) 10^{-3}

(Fig. 1) suggesting the same localization. Maximum activity was attained at 0.010% (v/v) of Triton X-100. Of the maximum activity released 4% was attributed to leakage in the absence of Triton X-100. The maximum activity released was 0.792 ± 0.015 μmoles L-glutamate formed/hr/mg protein.

The release of tryptophan: α-ketoglutarate transaminase activity (Figure 7) was similar to phenylalanine and tyrosine transaminases. Its pattern of release by increasing concentrations of Triton X-100 is also similar to that of glutamate pyruvate transaminase (the cytosolic marker) shown in Figure 1. Maximum activity released was 0.348 ± 0.03 μ moles L-glutamate formed/hr/mg protein. By comparison to the maximum activity obtained with Triton X-100, the percentage of enzyme activity attributed to leakage in PSG alone was 2%.

Results presented show that enzymes catalysing the transamination of the branched and aromatic amino acids in bloodstream forms of T.b. brucei are located within the cytosol and not in the glycosomes.

3.2 COMPARISON OF TRYPANOSOMA BRUCEI BRUCEI TRANSAMINASES SPECIFICITY FOR PYRUVATE AND -KETOGLUTARATE AS AMINO GROUP ACCEPTORS

Specificity of several transaminases activities in bloodstream forms of T.b. brucei for either α-ketoglutarate or pyruvate as the acceptor of the

α-amino groups was investigated. These experiments were carried out to determine which of the two ketoacids is the preferred substrate during transamination. The results are presented in Tables V,VI and VII.

Table V shows the production of alanine and glutamate by lysates of bloodstream T.b. brucei after incubation with the branched chain amino acids (leucine, isoleucine and valine) and either α-ketoglutarate or pyruvate. The results show that the rate at which glutamate was formed was approximately four times higher than that of alanine in all cases. These results suggest that α-ketoglutarate is preferred to pyruvate during transamination.

Results presented in Table VI show that alanine and glutamate were produced when the parasite lysates were incubated with the aromatic amino acids; phenylalanine, tyrosine and tryptophan. Among the three amino acids phenylalanine was transaminated at the highest rate to give 1.378 ± 0.113 μmoles L-glutamate hr/mg protein and 0.193 ± 0.012 μmoles L-alanine/ hr/mg protein respectively. It is apparent that the rate of glutamate production was higher than that of alanine with phenylalanine, tyrosine and tryptophan as α-amino group donors. The rate of glutamate production with tyrosine was about 50% of that obtained with phenylalanine. The lowest rate of glutamate production was observed when the trypanosomal lysates were incubated with tryptophan.

TABLE V

Figures are means ± standard deviations for the number of determinations shown in the parenthesis. Bloodstream forms of T.b. brucei lysates were used for each determination. Incubation time was for 1 hour at 25°C. The concentration of substrate used were 10 mM α-ketoglutarate or pyruvate, 10 mM L-amino acid and T.b. brucei lysates to a final volume of 1 ml. L-glutamate and L-alanine formed were determined.

GLUTAMATE AND ALANINE PRODUCTION BY BLOODSTREAM T.B BRUCEI LYSATES AFTER INCUBATION WITH BRANCHED CHAIN AMINO ACIDS

L-AMINO ACID	μmoles L-Glutamate formed/hr/mg protein	μmoles L-Alanine formed/hr/mg protein
Leucine	1.113 ± 0.07(4)	0.301 ± 0.020(4)
Isoleucine	1.121 ± 0.041(4)	0.283 ± 0.011(4)
Valine	1.109 ± 0.014(4)	0.280 ± 0.002(4)

TABLE VI

Figures are mean ± standard deviations for the number of determinations shown, in the parenthesis. Aliquots of trypanosomal lysates were used for each determination. Incubation mixture contained 10 mM α-ketoglutarate, or pyruvate, 10 mM L-amino acid and T.b. brucei lysates to a final volume of 1 ml. The reactions were carried out at 25°C and stopped after 1 hour L-glutamate or L-alanine formed were determined.

GLUTAMATE AND ALANINE PRODUCTION BY BLOODSTREAM T.B. BRUCEI LYSATES AFTER INCUBATION WITH THE AROMATIC AMINO ACIDS

L-AMINO ACID	μ moles L-Gluatamate formed/hr/mg protein	μ moles L-Alanine formed/hr/mg protein
Phenylalanine	1.378 ± 0.113(4)	0.193 ± 0.012(4)
Tyrosine	0.782 ± 0.050(4)	0.162 ± 0.006(4)
Tryptophan	0.371 ± 0.068(4)	0.145 ± 0.005(4)

TABLE VII

Aliquots of cell lysates were incubated in a system containing 10 mM α-ketoglutarate or pyruvate and 10 mM L-amino acid. The final incubation volume was 1 ml. Incubations were carried out at 25°C for 1 hour after which the amount of L-glutamate and L-alanine were determined. Figures are mean ± standard deviations for the number of determinations shown in the parenthesis.

GLUTAMATE AND ALANINE PRODUCTION BY BLOODSTREAM T.B. BRUCEI LYSATES AFTER INCUBATION WITH ASPARTATE GLUTAMINE AND METHIONINE

L-AMINO ACID	µmoles L-Glutamate formed/hr/mg protein	µmoles L-Alanine formed/hr/mg protein
Aspartate	0.528 ± 0.01(4)	0.184 ± 0.022(4)
Glutamine	0.334 ± 0.003(4)	0.245 ± 0.007(4)
Methionine	0.890 ± 0.079(4)	0.414 ± 0.012(4)

These results also suggest that α-ketoglutarate is the preferred amino group acceptor during transamination of the aromatic amino acids.

Other amino acids that led to glutamate and alanine production as a result of incubation of the trypanosomes lysates with either α-ketoglutarate and pyruvate were aspartate, methionine and glutamine. Methionine gave the highest rate of transamination producing 0.890 ± 0.079 μmoles L-glutamate per hr. per mg. protein and 0.414 ± 0.012 μmoles L-alanine per hr. per mg. protein with α-ketoglutarate and pyruvate respectively. Glutamine gave the least rate of transamination with α-ketoglutarate. The rate of transamination with α-ketoglutarate was observed to be higher with all the three amino acids than with pyruvate suggesting that the former is the preferred substrate.

Results in Tables V, VI and VII seem to suggest that the transaminases present in the bloodstream forms of Trypanosomal brucei brucei prefer α-ketoglutarate to pyruvate as the acceptor of the α-amino groups during transamination.

3.3 CHARACTERIZATION OF SOME TRANSAMINASES FROM BLOODSTREAM FORMS OF T.B. BRUCEI

Results in the previous sections show that several transaminases exist in bloodstream forms of T.b brucei. No information is available in regard to the properties of these enzymes. Experiments were carried out to

investigate the effect of pH on the activity of enzymes catalysing the transamination of the branched chain and aromatic amino acids. The stability of these enzymes was also investigated over a 48 hour period. The results on these two aspects are presented in the following section.

3.3.1 Effect of pH on Trypanosoma brucei brucei transaminases

The activity of the transaminases was examined in a series of phosphate buffers over the pH range 6.5 - 9.0. Figure 8 shows the effect of pH on leucine: α - ketoglutarate transaminase. The activity of the enzyme rose with increase in pH from 0.56 µmoles L-glutamate formed/hr/mg protein at pH 6.5 to 1.12 µmoles L-glutamate formed/hr/mg protein at pH 8.0. Maximum activity was observed in the pH range of 7. 8 to 8.5. Further increase in pH led to decrease in the activity of the enzyme. The same pattern was also observed on the effect of pH on the activities of isoleucine and valine: α-ketoglutarate transaminases Figures 9 and 10, with the highest activity observed between pH 7.8 and 8.5.

Results presented in Figure 11 show the effect of pH on the activity of phenylalanine: α-ketoglutarate transaminase. The activity of the enzyme rose with increase in pH from 0.7 µmoles L-glutaramate formed/hr/mg protein at pH 6.5 to 1.2 µmoles L-

FIGURE 8

EFFECT OF pH ON THE ACTIVITY OF LEUCINE α-KETOGLUTARATE TRANSAMINASE IN BLOODSTREAM FORMS OF TRYPANOSOMA BRUCEI BRUCEI.

Aliquots of trypanosomes lysates (cell density 2×10^8/ml) were incubated in phosphate buffer of pH ranging between 6.5 - 9.0. The incubation mixture contained α-ketoglutarate and L-amino acid to a final concentration of 10 mM contained in a total volume of 1 ml. This concentration was saturating at all pH levels. The length of incubations was 1 hour at $25^{\circ}C$. The amount of glutamate formed was determined as described in the materials and methods. Results presented are an average of three separate experiments.

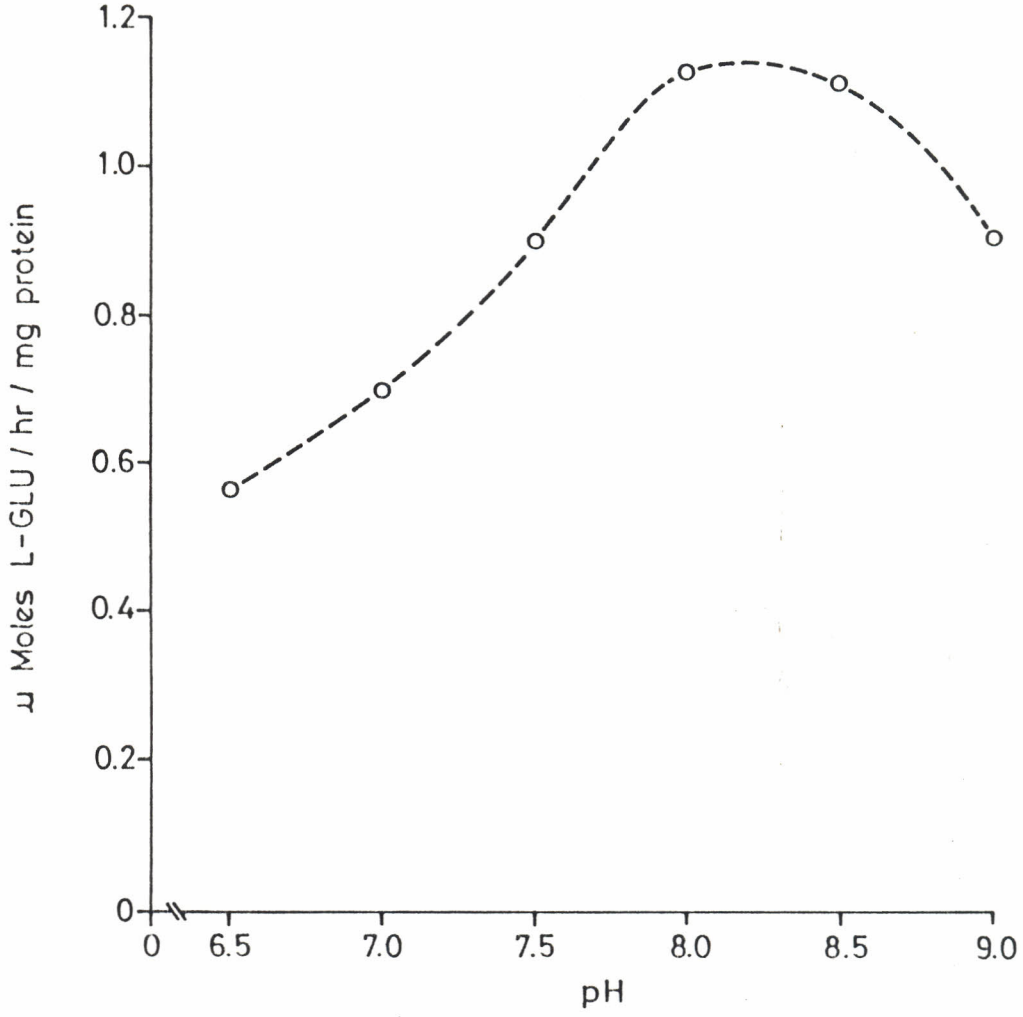

1.2
1.0
0.8
0.6
0.4
0.2
0
μ Moles L-GLU / hr / mg protein
0
6.5
7.0
7.5
8.0
8.5
9.0
pH

FIGURE 9

EFFECT OF pH ON THE ACTIVITY OF ISOLEUCINE α-KETOGLUTARATE TRANSAMINASE IN BLOODSTREAM FORMS OF T.B. BRUCEI.

Aliquots of trypanosome lysates containing 2×10^8 trypanosomes were incubated in a total volume of 1 ml in potassium phosphate buffer of pH ranging from 6.5 - 9.0. In the incubation mixture were α-ketoglutarate and L-amino acid to a final concentration of 1 mM. Each incubation was stopped after 1 hour and the amount of glutamate was determined. The rate of L-glutamate was plotted as a function of pH.

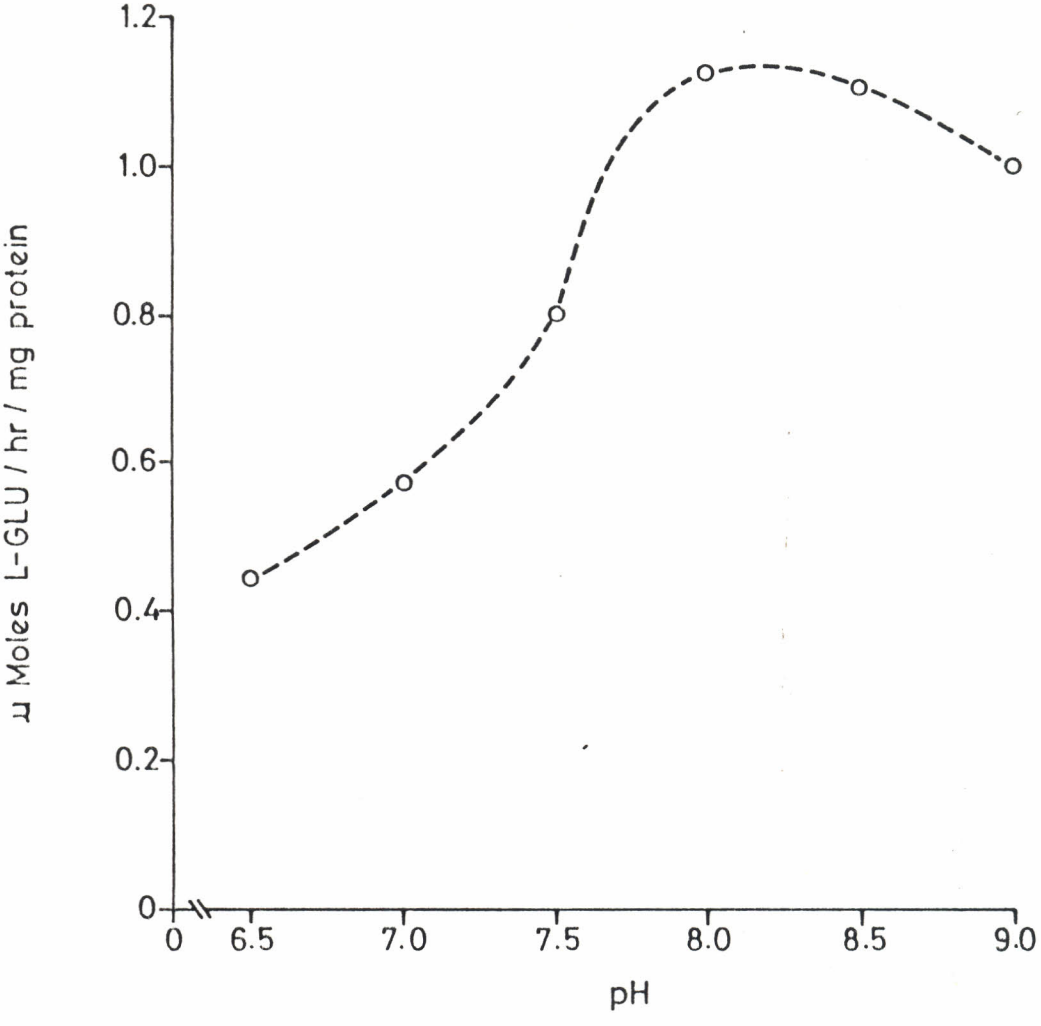

1.2
1.0
0.8
0.6
0.4
0.2
0
μ Moles L-GLU / hr / mg protein
0
6.5
7.0
7.5
8.0
8.5
9.0
pH

FIGURE 10

EFFECT OF pH ON THE ACTIVITY OF VALINE α-KETOGLUTARATE TRANSAMINASE IN BLOODSTREAM FORMS OF T.B. BRUCEI

Aliquots of trypanosome lysates containing 2 x 10^8 trypanosomes were incubated with 10 mM α-ketoglutarate and L-amino acid in potassium phosphate buffer of pH ranging between 6.5 - 9.0. The final incubation volume was 1 ml. The reaction was stopped after 1 hour and the amount of L-glutamate was determined. The rate of L-glutamate formed as a function of pH was plotted.

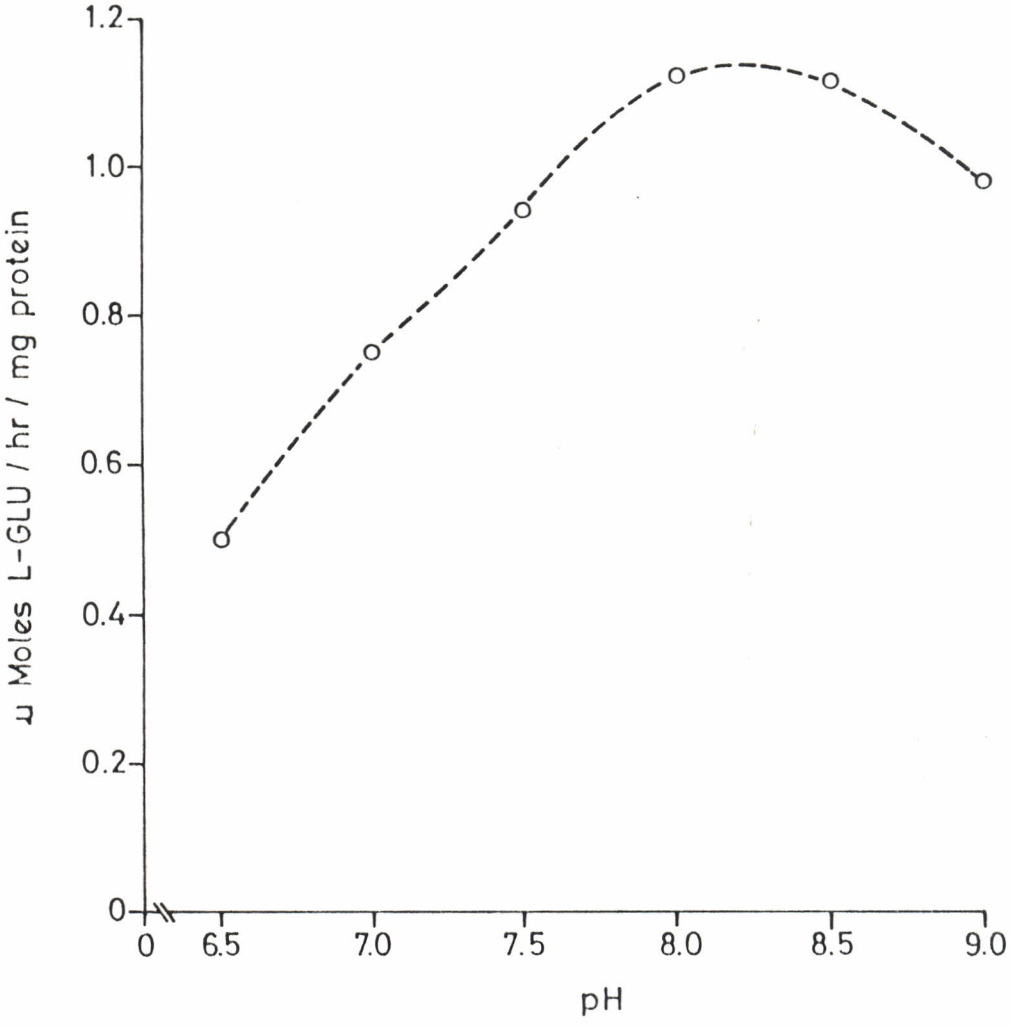

1.2
1.0
0.8
0.6
0.4
0.2
0
μ Moles L-GLU / hr / mg protein
0
6.5
7.0
7.5
8.0
8.5
9.0
pH

FIGURE 11

EFFECT OF pH ON THE ACTIVITY OF PHENYLALANINE:α-KETOGLUTARATE TRANSAMINASE IN BLOODSTREAM FORMS OF T.B. BRUCEI

Aliquots of trypanosome lysates containing 2×10^8 trypanosomes were incubated in potassium hosphate buffer of pH ranging between 6.5 - 9.0 and containing 10 mM α-ketoglutarate and 10 mM L-phenylalanine. Incubations were carried out for 1 hour after which the amount of L-glutamate was determined. The rate of L-glutamate formed is plotted as a function of pH.

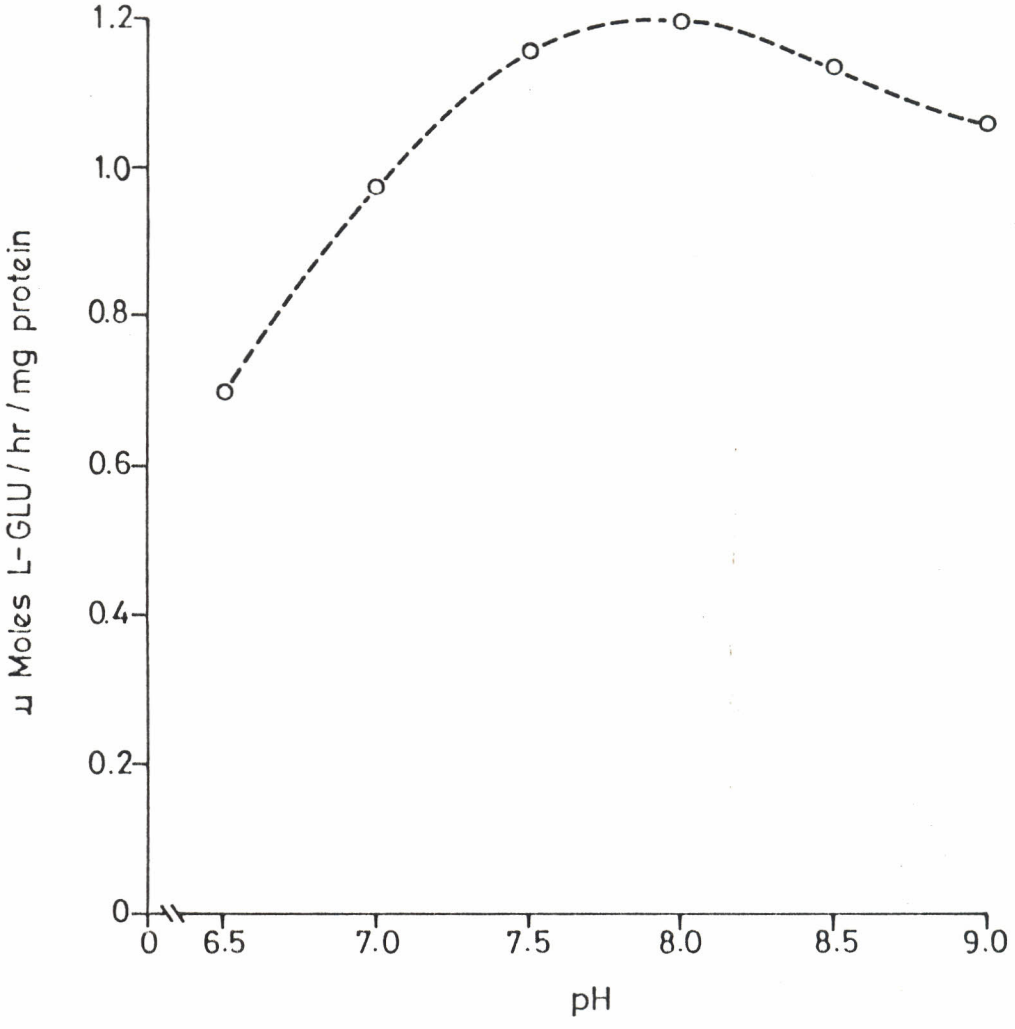

1.2
1.0
0.8
0.6
0.4
0.2
0
μ Moles L-GLU / hr / mg protein
0
6.5
7.0
7.5
8.0
8.5
9.0
pH

FIGURE 12

EFFECT OF pH ON THE ACTIVITY OF TYROSINE: α-KETOGLUTARATE TRANSAMINASE IN BLOODSTREAM FORMS OF T.B. BRUCEI

Aliquots of trypanosome lysates (2×10^8) were incubated in potassium phosphate buffer pH 6.5 - 9.0 containing 10 mM α -ketoglutarate and 10 mM L-tyrosine. Metabolism was terminated after 1 hour and then L-glutamate was determined. The amount of L-glutamate formed is plotted as a function of pH.

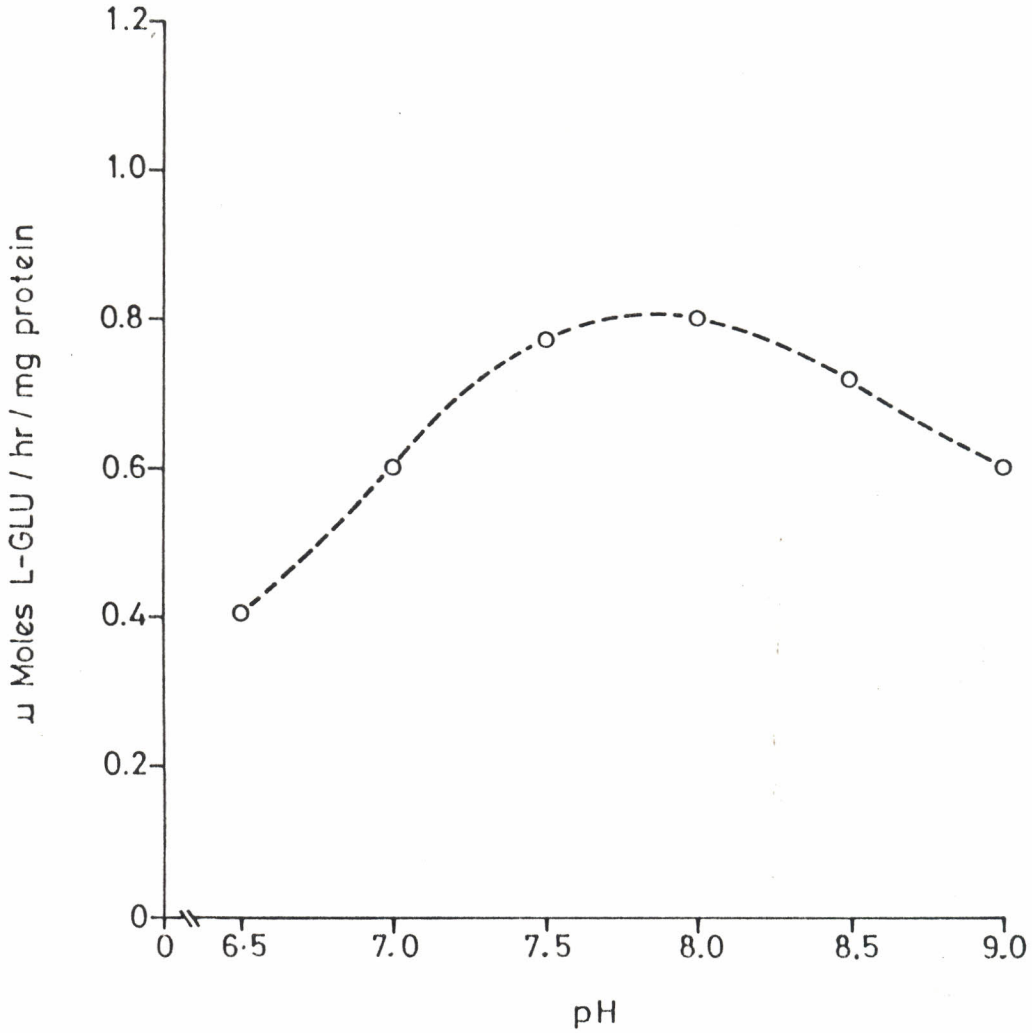
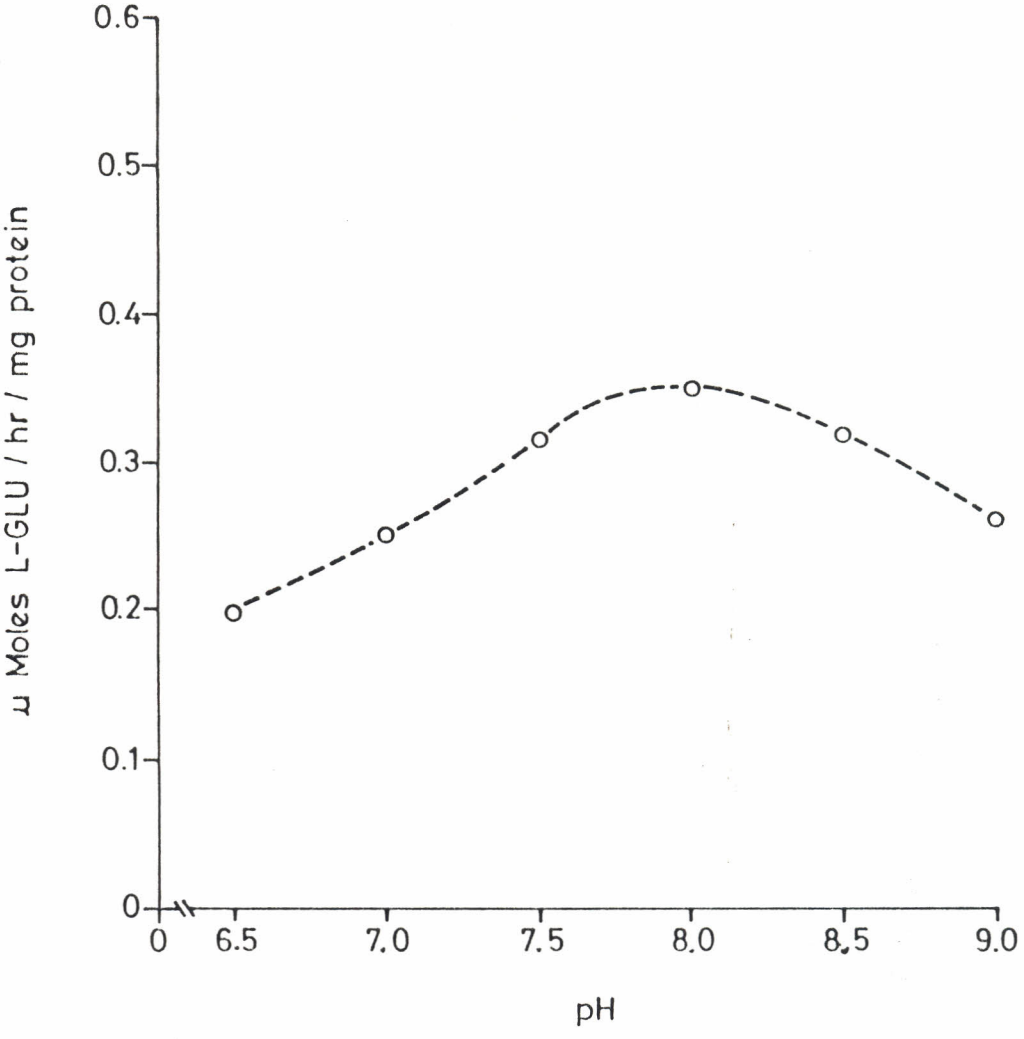

1.2
1.0
0.8
0.6
0.4
0.2
0
μ Moles L-GLU / hr / mg protein
0
6·5
7.0
7.5
8.0
8.5
9.0
pH

FIGURE 13

EFFECT OF pH ON THE ACTIVITY OF TRYPTOPHAN α-KETOGLUTARATE TRANSAMINASE IN BLOODSTREAM FORMS OF T.B. BRUCEI

Aliquots of trypanosome lysates containing 2 x 10^8 trypanosomes were incubated in potassium phosphate buffer pH 6.5 - 9.0. Present in the incubation mixture were stopped after 1 hour and L-glutamate was determined. Results are plotted as L-glutamate formed versus pH.


0.6
0.5
0.4
0.3
0.2
0.1
0
μ Moles L-GLU / hr / mg protein
0
6.5
7.0
7.5
8.0
8.5
9.0
pH

glutamate/hr/mg protein at pH 8.0. The optimal pH range was between 7.5 and 8.5. Figures 12 and 13 show the effect of pH on the activities of the enzymes catalysing the transamination of tyrosine and tryptophan. Increase in pH led to higher transaminase activity upto pH 8.2 upon which further increase in pH led to a decrease in their activity. Optimum pH range for the aromatic amino acids transaminases (pH 7.5-8.5) was slightly broader than that observed with the enzymes catalysing the transamination of the branched chain amino acids.

3.3.2 Stability of transaminases present in bloodstream Trypanosoma brucei brucei

Figure 14 shows decrease in activity of leucine: α-ketoglutarate transaminase at $4^{o}C$ and $25^{o}C$ respectively. At $4^{o}C$ the activity of the enzyme after 48 hours was 50% of the original. At $25^{o}C$, after 48 hours no activity was observed.

Figure 15 shows the decrease in isoleucine: α-ketoglutarate transaminase. As observed with leucine: -ketoglutarate transaminase no activity remained after 48 hours when stored at $25^{o}C$. Of the original acitivity 50% remained after 48 hours when the trypanosomes were stored at $4^{o}C$.

Results for the decrease in activity of valine: α-ketoglutarate transaminase are shown in Figure 16. Whereas no activity remained when the trypanosomes were

FIGURE 14

STABILITY OF LEUCINE α -KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF TRYPANOSOMA BRUCEI BRUCEI

Samples of trypanosome lysates containing 2×10^8 cells/ml were kept at 4^oC and 25^oC. After every twelve hours aliquots were taken and incubated in a total volume of 1 ml in potassium phosphate buffer pH 8.0 containing 10 mM L-leucine and 10 mM α-ketoglutarate. Incubations were carried out at 25^oC for one hour. The amount of L-glutamate formed was determined as outlined in section.

Results are expressed as relative activity (%) versus time (hours). Total enzyme activity released by 0.1% (v/v) of Triton x-100 was 1.123 ± 0.125 μmoles L-glutamate formed/hr/mg protein. Results shown are mean values of three individual experiments.

O- - - - O 4^oC

■——■ 25^oC

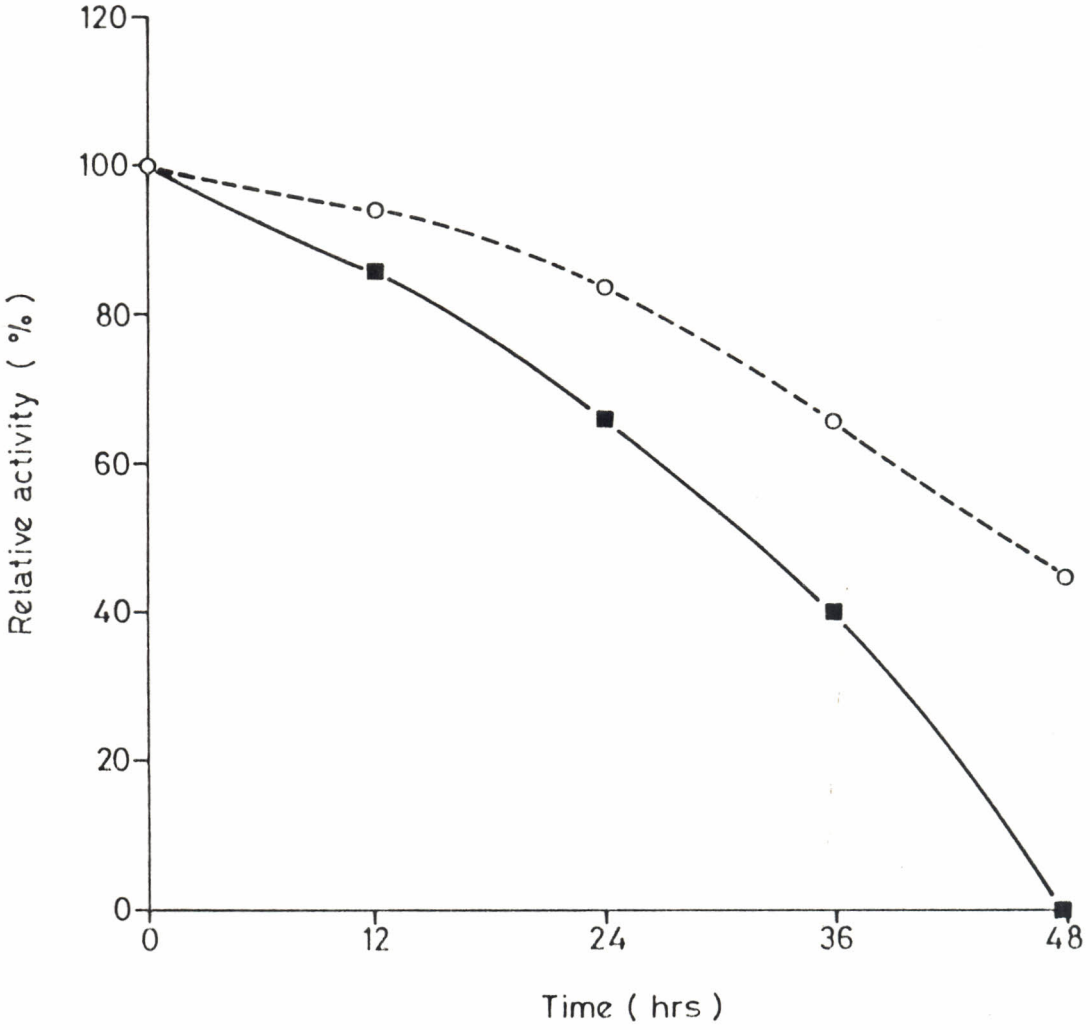

120
100
80
60
40
20
0
Relative activity (%)
0
12
24
36
48
Time (hrs)

FIGURE 15

STABILITY OF ISOLEUCINE: α -KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF T.B. BRUCEI

Experimental details are as outlined in the legend to Figure 14. Results shown are expressed as relative activity (%) versus time (hours).

Total enzyme activity was 1.132 ± 0.031 µmoles L-glutamate formed/hr/mg protein. Results shown are mean values of three individual experiments.

O------O $4^{o}C$

■——■ $25^{o}C$

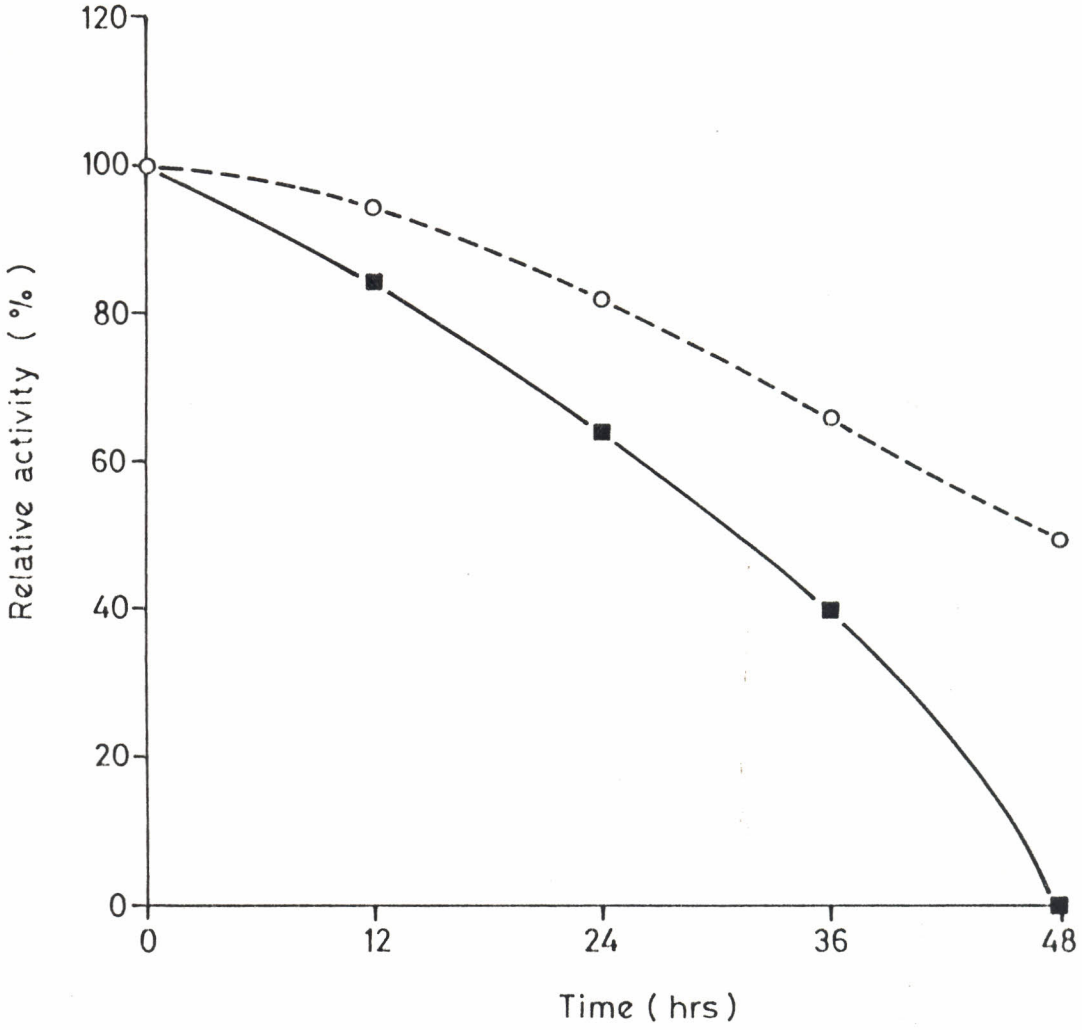

120
100
80
60
40
20
0
Relative activity (%)
0
12
24
36
48
Time (hrs)

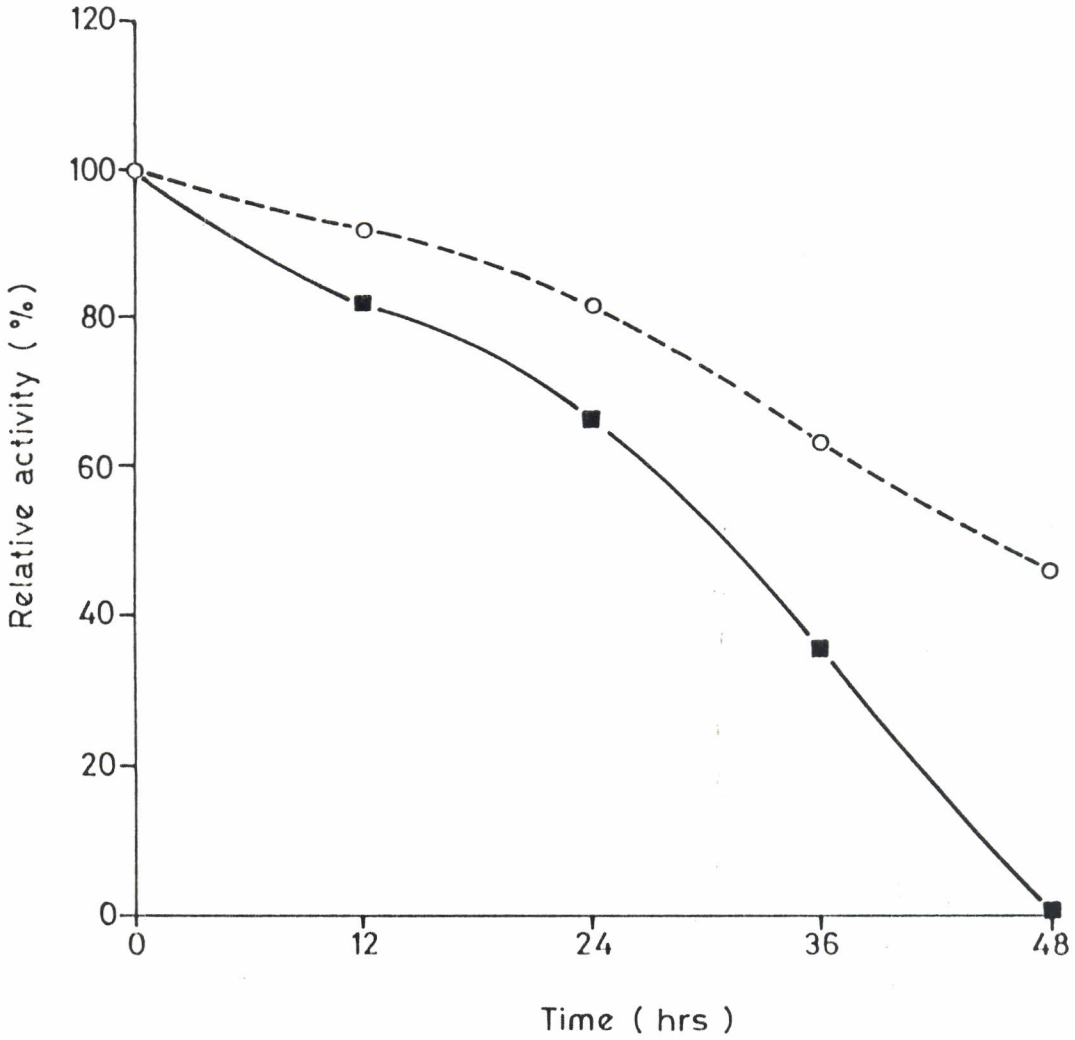
FIGURE 16

STABILITY OF VALINE: α-KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF T.B. BRUCEI

Experimental details are as outlined in the legend to Figure 14. Results are expressed as relative activity (%) versus time (hours). Total enzyme activity was 1.125 ± 0.025 μmoles L-glutamate formed/hr/mg protein. Results are shown as mean values of three individual experiments.

O---O $4^{o}C$

●—● $25^{o}C$.


120
100
80
60
40
20
0
Relative activity (%)
0
12
24
36
48
Time (hrs)

stored at 25°C after 48 hours, about 48% of the original activity remained after 48 hours when stored at 4°C.

Results in Figures 14, 15 and 16 show that the enzymes catalysing the transamination of leucine, isoleucine and valine lost their activity in the same pattern.

Figure 17 shows the decrease in phenylalanine: α-ketoglutarate transaminase activity. The enzyme activity decreased with storage at 25°C and 4°C. However, the rate of decrease in activity was higher at 25°C than at 4°C. The activity that remained when the enzyme was stored at 25°C after 48 hours was 5% of the original activity. At 4°C the amount of enzyme activity that remained after 48 hours was 46% of the original.

There was decrease in activity of tyrosine: α-ketoglutarate (Fig. 18) when stored at both 25°C and 4°C, however the rate of decrease was higher at 25°C. Of the original activity only 4% remained after 48 hours when the enzyme was stored at 4°C. At 25°C the activity was 40% of the original after 25°C.

Results for the decrease in activity of tryptophan: α-ketoglutarate transaminase stored at 25°C are shown in Figure 19. Tryptophan α-ketoglutarate transaminase exhibited the highest rate of decrease among the transaminases investigated when stored at 4°C. Only 26% of the original activity remained after 48 hours.

FIGURE 17

STABILITY OF PHENYLALANINE : α-KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS T.B. BRUCEI

Experimental details are as outlined in the legend to Figure 14. Results are expressed as relative activity (%) versus time (hours). Total enzyme activity was 1.139 ± 0.009 μmoles L-glutamate formed/hr/mg protein. Results are shown as mean values of three individual experiments.

O- - - - -O $4^{o}C$

■———■ $25^{o}C$

FIGURE 18

STABILITY OF TYROSINE : α-KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF T.B BRUCEI.

The experiments were carried out in the same way as outlined in the legends to Figure 14. Results are expressed as relative activity (%) versus time (hours).

Total enzyme activity was 0.745 ± 0.01 μ moles L-glutamate formed/hr/mg protein. Results are mean values of three individual experiments.

O------O $4^{o}C$

●——● $25^{o}C$

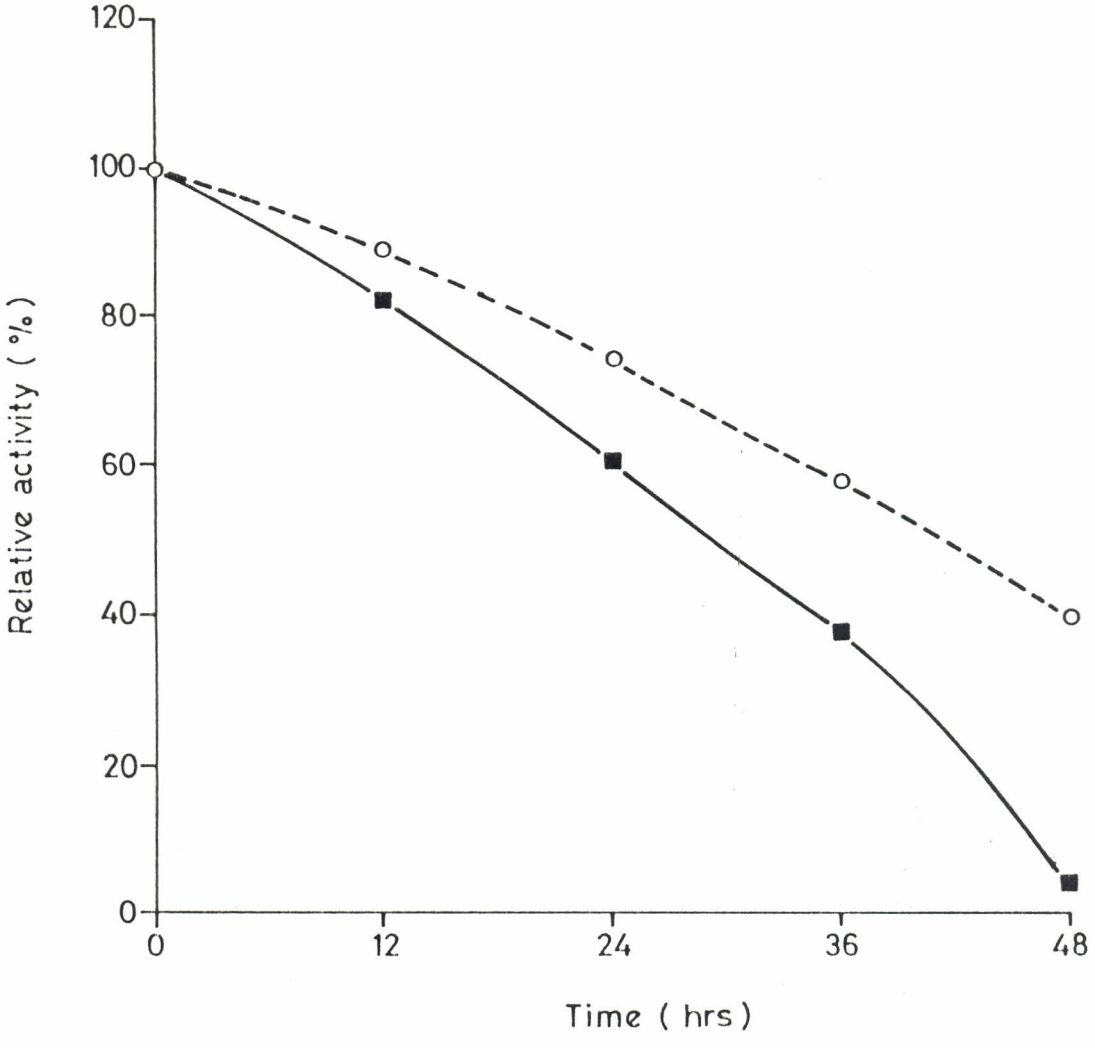

120
100
80
60
40
20
0
Relative activity (%)
0
12
24
36
48
Time (hrs)

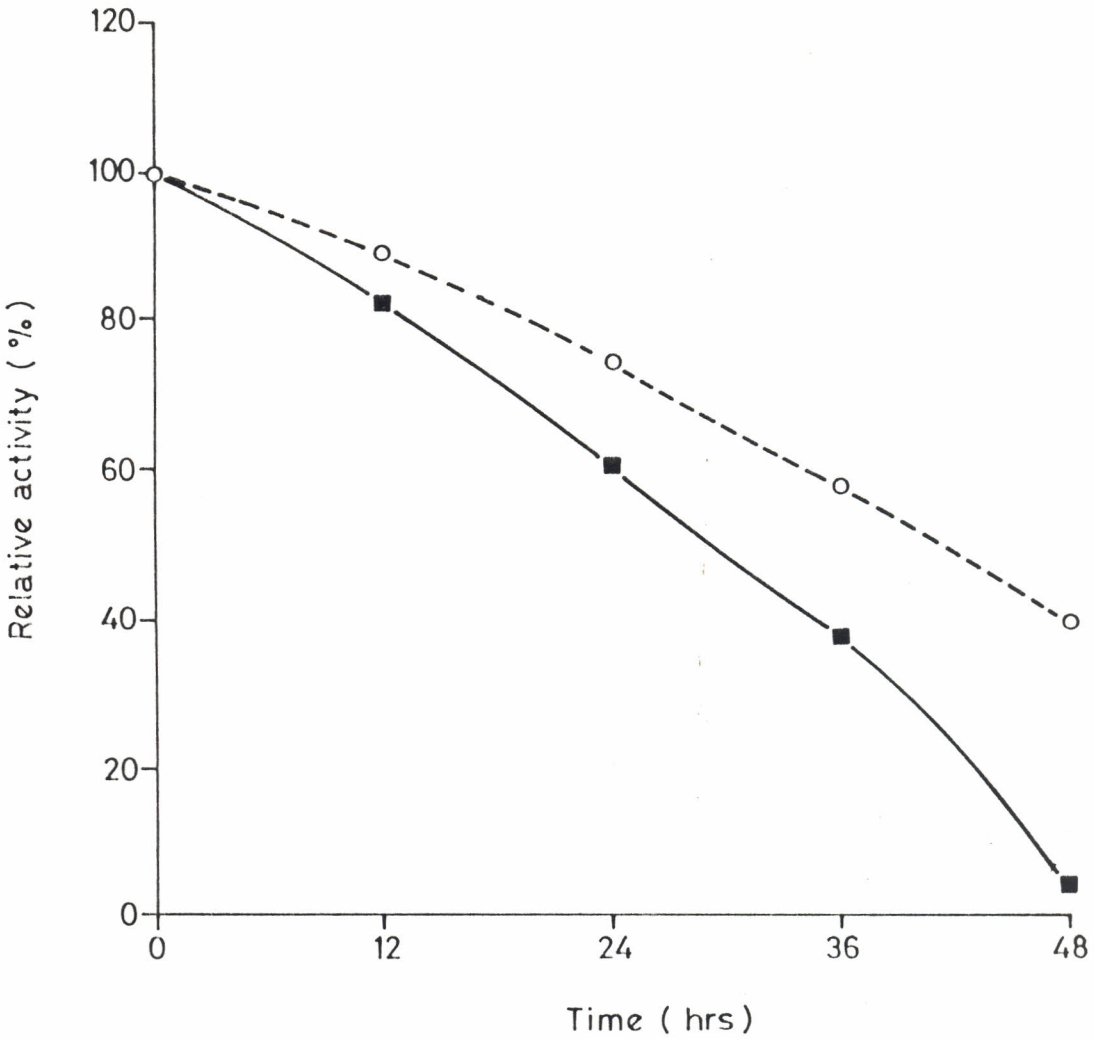
FIGURE 19

STABILITY OF TRYPTOPHAN α-KETOGLUTARATE TRANSAMINASE FROM BLOODSTREAM FORMS OF T.B. BRUCEI

Experimental details are as outlined in the legend to Figure 14. Results are expressed as relative activity (%) versus time (hours). Total enzyme activity was 0.340 ± 0.036 μmoles L-glutamate formed/hr/mg protein. Results are mean values of three individual experiments.

O----O $4^{o}C$

■——■ $25^{o}C$


120
100
80
60
40
20
0
Relative activity (%)
0
12
24
36
48
Time (hrs)

Whereas no activity remained for the enzymes catalysing the transamination of branched chain amino acids kept at 25°C after 48 hours; little activity was observed with aromatic amino acid transaminases.

Table VIII shows results obtained when the enzymes catalysing the transamination of the branched and aromatic amino acids were stored at 4°C in the presence of dithiothreitol. Results show that there were insignificant loss of transaminases activity over 48 hour period. This suggests that the presence of dithiothreitol helped to preserve the activity of these enzymes significantly as compared with results obtained in the absence of the compound.

TABLE VIII

Aliquots of trypanosome lysates containing 2 x 10^8 trypanosomes were incubated in phosphate buffer (pH 8.0) containing 10 mM α-ketoglutarate, 10 mM L-amino acid and 1 mM dithiothreitol. The total incubation volume was 1 ml. Incubations were carried out at 25^{o}C for 1 hr, after which L-glutamate formed was determined. Figures are means $\pm$ standard deviations for the number of determinations shown in the parenthesis.

TABLE VIII

STABILITY OF TRANSAMINASES IN BLOODSTREAM T.B. BRUCEI LYSATES STORED AT 4°C WITH 1 mM DITHIOTHREITOL OVER 48 HOUR PERIOD

AMINO ACID	ACTIVITY AT 0 TIME (μmoles L-Glutamate formed/hr/mg protein)	ACTIVITY AFTER 48 HOURS (μmoles L-Glutamate formed/hr/mg protein)
L-leucine	1.126 ± 0.005(4)	1.119 ± 0.01(4)
L-isoleucine	1.140 ± 0.045(4)	1.135 ± 0.051(4)
L-valine	1.127 ± 0.002(4)	1.126 ± 0.003(4)
L-phenylalanine	1.138 ± 0.004(4)	1.137 ± 0.003(4)
L-Tyrosine	0.748 ± 0.045(4)	0.745 ± 0.045(4)
L-tryptophan	0.374 ± 0.005(4)	0.373 ± 0.004(4)

C.H.A.P.T.E.R...4

DISCUSSION

4.1 LOCALIZATION OF SOME TRYPANOSOMAL TRANSAMINASES BY TRITON X-100

Biological membranes are physical barriers that separate the aqueous compartments with different solute composition. They are composed of protein and lipids. The lipid component consists of phospholipids and cholesterol. Phospholipid tails contribute a continous non-polar hydrocarbon core to the membrane (Singer and Nicolson, 1972) Polar substances usually cross the biological membranes by help of specific carrier proteins situated within the membrane. It is possible to abolish the selective permeability of the biological membranes artificially by disrupting the arrangement of the membrane components.

Triton X-100 a non-ionic detergent solubilizes the hydrophobic moeities in biological membranes, making it permeable to molecules non-selectively. An enzyme enclosed by more than one membrane in an organism would require higher concentrations of detergent to release than one enclosed by only one membrane.

In bloodstream *T.b. brucei* the glycosomal membrane is more resistant to membrane disruption methods than the plasma membrane possibly due to the small size of

the organelle (Hayashi *et al.*, 1971; Muller, 1973). Triton X-100 has been used to release several enzyme activities localized within the trypanosomes (Visser and Opperdoes, 1980; Opperdoes, Borst and Spits, 1977; Oduro *et al*, 1980). In bloodstream form of *T.b. brucei* α-glycerolphosphate dehydrogenase is glycosomal (Opperdoes *et al.* 1977; 1980; 1984) and glutamate pyruvate transaminase is cytosolic (Steiger *et al.*, 1980; Visser and Opperdoes, 1980).

In this investigation Triton X-100 treatment was used to disrupt the integrity of *T.b. brucei* membranes in an effort to localize some transaminases. The release of the transaminases by Triton X-100 in trypanosomes is evidenced by increase in their activity as the concentration of the detergent was increased. Increasing concentrations of Triton X-100 led to the release of leucine: α-ketoglutarate transaminase, isoleucine: α-ketoglutarate transaminase, valine: α-ketoglutarate transaminase, phenylalanine: α-ketoglutarate transaminase, tyrosine: α-ketoglutarate transaminase and tryptophan: α-ketoglutarate transaminase (Figures 2-7) until maximum activity was achieved. About 0.01% (v/v) of the detergent was enough to release maximum activity of the transaminases tested. This same concentration of Triton X-100 released maximum activity of glutamate pyruvate transaminase. The similarity in the pattern of release of these

transaminases with that of glutamate pyruvate transaminase indicate that these enzymes are cytosolic in bloodstream forms of T.b. brucei.

Several transaminases are known to be localized within the cytosol. Rat liver enzyme II that catalyses the transamination of leucine, isoleucine and valine is localized in the cytosol. However, liver cell enzymes designated I and III in rats are equally distributed in the cytosol and mitochondria (Ikeda et al., 1976). A cytosolic glutamine transaminase was shown in rat kidney (Cooper and Meister, 1972). Glutamate oxaloacetate transaminase has been reported by Opperdoes and co-workers (1975) to be cytosolic in bloodstream T.b. brucei. Transamination of the aromatic amino acids; phenylalanine, tryptophan and tyrosine in bloodstream Trypanosoma brucei gambiense have been studied (Stibbs and Seed, 1975; Hall et al., 1981). In these studies it was shown that transaminases catalysing these amino acids are found in the cytosol. Presence of transaminases responsible for the transamination of leucine, isoleucine, valine, phenylalanine, tyrosine and tryptophan in the cytosol of bloodstream T.b. brucei is in agreement with the results of other workers who found the cytosol a common location of transaminases as described above. Results in the present study indicate that the tested transaminases are localized in the cytosol and none in the glycosomes of these organisms.

The presence of the transaminases in the cytosol of bloodstream T.b. brucei may have important implications. Studies have shown that alanine is among the most common amino acids found in the proteins of various species of trypanosomes (Williamson et al., 1961). Free alanine has been shown to be in high levels in trypanosomes arising from the transamination between pyruvate and glutamate in the cytosol of trypanosomes (Williamson et al., 1961; Stibbs and Seed, 1975). These workers have suggested that the role of transamination of amino acids in trypanosomes is to form glutamate which is transaminated with pyruvate by glutamate pyruvate transaminase in the cytosol to form alanine. Transaminases present in the cytosol of the bloodstream T.b. brucei play the role of transferring amino groups to various keto acids which become amino acids more vital to protein synthesis. Transamination of glutamate with pyruvate may help to reduce the high levels of pyruvate formed in the cytosol of bloodstream T.b. brucei during aerobic respiration. This role will be discussed further in the next section.

4.2 COMPARISON OF TRYPANOSOMAL TRANSAMINASE ACTIVITIES WITH PYRUVATE AND α-KETOGLUTARATE AS α-AMINO GROUP ACCEPTORS

The specificity of transaminases present in the cytosol of bloodstream forms of Trypanosoma b. brucei

with respect to α-ketoglutarate and pyruvates as the α-amino group acceptors showed the former to be the preferred substrate. Although pyruvate is the end product of glucose oxidation in bloodstream trypomastigotes under aerobic conditions (Ryley, 1956; Fulton and Grant, 1957) it does not seem to be the major α-amino group acceptor.

Several workers have reported on the specificity of transaminases with respect to amino group acceptor in other systems. In Escherichia coli, α-ketoglutarate was shown to be the preferred acceptor of the α-amino group during transamination (Rudman and Meister, 1953). The same ketoacid was the preferred acceptor of the α-amino group for transaminases designated I, II and III present in rat tissues (Ichihara, 1975) and also for rat brain glutamine transaminase (Leuven, 1976). Transamination of tryptophan by bloodstream Trypanosoma b. gambiense required α-ketoglutarate whose substitution resulted in a 80% reduction in the rate of tryptophan metabolism (Stibbs and Seed, 1975). The same ketoacid was found to be a good acceptor of the α-amino group for phenylalanine and tyrosine transaminases in T.b. gambiense (Stibbs and Seed, 1975) where its omission caused a reduction of the enzyme activity to 5%. The apparent major role of α-ketoglutarate as the amino group acceptor in T.b. brucei is consistent with the observation on other organisms (Rudman and Meister,

1953; Ichihara, 1975; Stibbs and Seed, 1975)

On the other hand pyruvate was shown to be a better acceptor of the α-amino group for leucine transaminase present in Acetobacter suboxydans followed by α-ketoglutarate and oxaloacetate (Tachiki et al., 1975). In Leishmania donovani different rates of amino acid transamination were observed when α-ketoglutarate and pyruvate were compared as the amino group acceptors. Among the amino acids tested, the highest rates of transamination of aspartate, methionine, histidine and alanine were observed with α-ketoglutarate as α-amino group acceptor. The highest rates with arginine, ornithine, phenylalanine and tyrosine was observed with pyruvate as the α-amino group acceptor (Chatterjee and Ghosh, 1957). Transaminases in T.b. brucei are different from those in L. donovani in that α-ketoglutarate was the preferred substrate with all the amino acids tested. They are similar to those in rat liver cells, enzymes I, II, and III, E. coli and T.b. gambiense aromatic amino acid transaminases in that α-ketoglutarate is the best α-amino group acceptor.

Glutamate is an end product of α-ketoglutarate transamination. This amino acid may serve a crucial role as one of the major constituents of proteins in T.b. brucei. An analysis of amino acid constituents of proteins in several trypanosomal species showed glutamate to be one of the most common amino acid

(Williamson et al., 1961). It may be transaminated with pyruvate the most abundant ketoacid in bloodstream T.b. brucei, by glutamate pyruvate transaminase to form alanine, which was also shown to be a common protein constituent of trypanosomes (Willaimason, et al, 1961).

In conclusion it is apparent that α-ketoglutarate is the best α-amino group acceptor for the transaminases present in T.b. brucei bloodstream forms which is transaminated to form glutamate. Then glutamate may be transaminated with pyruvate by glutamate pyruvate transaminase to form alanine.

4.3 EFFECT OF pH ON TRYPANOSOMAL TRANSAMINASES AND THEIR STABILITY

Cellular processes are sensitive to pH changes and take place in a medium where the pH is carefully regulated. However, there may be localized intracellular pH variations (Keith and Goulding, 1986). The majority of intracellular processes occur at a pH maintained near neutral pH range where metabolic processes occur at their maximum rate. The control of a constant pH in biological systems is achieved by buffering agents in cellular fluids such as phosphate, bicarbonate, amino acids and proteins (Keith and Goulding, 1986).

Steiger et al., (1974) studied the effect of pH on the activities of alanine aminotransferase and aspartate aminotransferase in soluble crude extracts of bloodstream form of T.b. brucei. In their study maximal activity was obtained at pH 8.0 with Tris buffer for both alanine aminotransferase and aspartate aminotransferase. However, in crude extracts of cultured forms of T.b. brucei maximal activity was obtained at pH 6.0 for both aspartate and alanine aminotransferases (steiger et al, 1974). No further work has been carried out on the effect of pH on other transaminases present in T.b. brucei bloodstream forms.

In the present study effect of pH on the activities of enzymes catalysing the transamination of the branched chain and aromatic amino acids showed that maximum activities occur between pH 7.8-8.5, using phosphate buffer. The pH optima for leucine; α-ketoglutarate, isoleucine: α-ketoglutarate, valine: α-ketoglutarate, phenylalanine: α-ketoglutarate, tyrosine: α-ketoglutarate and tryptophan: α-ketoglutarate transaminases in the present study are similar to those of aspartate transaminase and alanine transaminase in bloodstream form of T.b. brucei (Steiger et al., 1974). Transaminases investigated in this study were found to be cytosolic (Chapter 3). It is also known that alanine transaminase in bloodstream T.b. brucei is localized in the cytosol (Steiger et al, 1980, Visser and Opperdoes,

1980). A cytosolic aspartate aminotransferase in bloodstream T.b. brucei has also been reported by Opperdoes and co-workers (1975). The optimum pH values of between 7.8 - 8.5 for branched chain and aromatic amino acids transaminases observed in this study are also similar to those found in culture promastigotes of Leishmania tarentolae (Fairlamb and Krassner, 1971) even though data in the present study is derived from crude extracts. In L. tarentolae optimum pH range was also observed to be broad as in this study. The maximum activity for ox-heart aspartate amino transferase was observed between pH 7.5 and 8.0 with 50% of the maximum activity being observed at pH 5.5 and 9.2 (Marino et al., 1960). Optimal pH of leucine (methionine) transaminase from rat liver was 8.2 (Ikeda et al., 1976). The pH dependence of glutamine amino transferase from rat liver was also studied and maximum activity observed in the range of pH 8.0-8.7 (Cooper and Meister, 1972). Results by these workers in regard to optimum pH range of transaminases from various sources closely correspond to the results obtained in the present study. However, the effect of pH of leucine: α-ketoglutarate transaminase in this study differ from those of L-leucine: pyruvate transaminase in Acetobacter suboxy-dans. According to Tachiki et al., (1975) L-leucine-pyruvate transaminase from A. suboxydans is most active in the pH range of 5.0 to 5.5 when determined in acetate and

phosphate buffers. They found about 50% of the activity at neutral pH. The transaminase was most stable at pH 8.0. Transamination reaction is dependent on vitamin B_6 as a cofator. Most vitamin B_6 enzymes are known to have a neutral or alkaline pH optimum except decarboxylases (Tachiki et al., 1975). The alkaline optimum pH of transaminases investigated in this study is in agreement with this observation.

Transaminases have been reported to be unstable under various conditions requiring the use of stabilizing agents to preserve their activity (Cammarata and Cohen, 1950; Steiger et al., 1974). Results of leucine: α-ketoglutarate, isoleucine: α-ketoglutarate, valine: α-ketoglutarate, phenylalanine: α-ketoglutarate, tyrosine: α-ketoglutarate and tryptophan: α - ketoglutarate transaminases in the present study (Figures 14 - 19) attempt to show the stability of these enzymes when stored at 25°C and at 4°C. The results show that these enzymes lost their activity at both temperatures but at different rates. There was loss in activity even in crude preparations used in the present study in which protective influences might be expected in accordance to the observation that an increase in purity usually is accompanied with a decrease in stability. This observation correspond to the results obtained on the stability of several transaminases in pig extracts by Cammarata and Cohen (1950).

It was observed that little or no activity remained when the extracts were stored at 25°C for 48 hours. However, between 30 - 50% of the maximum activity remained when the extracts were kept at 4°C for 48 hours clearly showing that these enzymes were more unstable at higher temperatures. The same difference in the rate at which activity is lost with time at different temperatures was observed with glutamine transaminase from rat liver (Cooper and Meister, 1972). In this study whereas no activity was lost when the enzyme was stored at 4°C in phosphate buffer containing 1 mM 2-mercaptoethanol, 20% of the initial activity was lost at 26°C for 3 days in the same buffer.

Results in Table VIII show that the presence of dithiothreitol in the extracts helped to preserve the activity of transaminases at 4°C over 48 hours period. Dithiothreitol was used as a stabilizing agent of alanine aminotransferase and aspartate aminotransferase from bloodstream T.b. brucei extracts (Steiger et al., 1974). Both dithiothreitol and 2-mercaptoethanol have been used in the preservation of transaminase activity from different sources (Cooper and Meister, 1972, Steiger et al., 1974, Tachiki et al, 1975; Ikeda et al., 1976). Both these compounds are reducing agents. Their structural formulae are shown below:

$SH-CH_2-CH_2-CH_2-CH_2-SH$ Dithiothreitol (DTT)

$H-S-CH_2-CH_2-OH$ 2-Mercaptoethanol

They can donate their hydrogen atom from the thiol group to cysteine residues reducing the possibility for their oxidation to form disulfide bridges (Braunstein, 1973). The possibility of the presence of crucial cysteine residues in the transaminases in the present study cannot be ruled out as implied by results with dithiothreitol.

4.4 MULTIENZYME COMPLEX OR INDIVIDUAL ENZYMES

The lack of significant difference in the rate at which the branched chain amino acids were catalysed (Chapter 4) suggests that their transamination is probably by a common enzyme or a multienzyme complex. This possibility is also supported by similarity in which their transaminase activity was lost with time (Chapter 5). Possibility for a common transaminase for the branched chain amino acids has been reported. Ikeda and co-workers (1976) reported the presence in rat liver tissues of leucine aminotransferase which was capable of transaminating leucine, ethionine, nor-leucine, nor-valine in addition to homocysteine. Hog heart enzyme I or leucine aminotransferase was found to transaminate all the three branched chain amino acids; leucine,

valine and isoleucine (Taylor and Jenkins, 1966). Earlier on Rudman and Meister (1953) had reported the presence of a transaminase B in *E. coli* which was found to be specific only for the branched chain amino acids. Similar transaminase activity was found in *Acetobacter suboxydans* (Tachiki and Tochikura, 1975) and in *Pseudomonas aeroginosa* (Norton and Sokatch, 1970). Aki *et al.*, (1968) established that there exist two transaminases for the branched chain amino acids in rat liver. Enzyme II was active for all the three amino acids (leucine, isoleucine and valine) whereas enzyme III was only active with leucine as substrate. Transamination of leucine, isoleucine and valine in the present study resemble those described above in that the same enzyme or a multienzyme complex may be involved in their transamination.

The aromatic amino acids phenylalanine, tyrosine and tryptophan were transaminated at different rates in the presence of α-ketoglutarate (Chapter 4). Their transaminase activity at 25^{o}C and 4^{o}C was also lost at different rates unlike the case observed with the branched chain amino acids (Chapter 5). Although no absolute quantitative significance can be placed on an individual transamination value for a given amino acid it is possible that the different ratios in transaminase activities among the aromatic amino acids are due to a

number of individual enzymes rather than a single one. Presence of individual transaminases for each aromatic amino acid has been reported in T.b. gambiense (Stibbs and Seed, 1975). In rat liver the enzymes responsible for the transamination of phenylalanine, tryptophan and tyrosine have also been reported to be distinct (Lin et al., 1958). Transamination of the aromatic amino acids in T.b. brucei may be similar to that in T.b. gambiense and rat liver. However the specificity of transaminases in bloodstream T.b. brucei needs to be further investigated.

CONCLUSIONS

From the results of this study it is possible to draw the following conclusions on the transaminases in bloodstream form of T.b. brucei:

(i) Enzymes catalysing the transamination of leucine, isoleucine, valine, phenylalanine, tyrosine and tryptophan were shown to be localized within the cytosol with no detectable activity in the glycosomes.

(ii) α-ketoglutarate acid was shown to be the preferred α-amino group acceptor for the transaminases present in T.b. brucei lysates when compared to pyruvate.

(iii) the optimum pH for the activity of the enzymes catalysing both the branched chain and aromatic amino acids was found to be within the range of pH 7.8-8.5.

(iv) Transaminases for both aromatic and branched chain amino acids are unstable when stored at 25°C and 4°C and that thiol compounds such as dithiothreitol can be used to preserve their activity

(v) There is probably a common enzyme or multienzyme complex catalysing the transamination of branched chain amino acids and that the aromatic amino acids are

transaminated by individual enzymes. Electrophoretic studies need to be carried out to confirm these propositons.

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