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Full Length Research Paper

Malate dehydrogenase from blood stream *TRYPANOSOMA VIVAX* exists in isoenzyme forms

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Malate dehydrogenase was isolated and partially purified from blood stream *TRYPANOSOMA VIVAX*. The enzyme exists in isoenzyme forms (MDH_1 and MDH_2). The relative molecular weights of MDH_1 and MDH_2 determined by Sodium dodecyl sulphate polyacrylamide gel electrophoresis were 61 and 63 kdal. respectively. MDH_2 lost activity immediately after purification. It has purification fold of 15 and percentage recovery of 31%. Similarly, MDH_1 was purified 21 fold from the crude with 54% recovery. This isoenzyme was considerably stable. There was no MDH activity when the live parasites were incubated with the assay medium suggesting that the enzyme was not secreted by parasites. However, addition of detergent led to a surge in MDH activity suggesting that the enzyme is membrane bound. Malate dehydrogenase from the infective form of *T. VIVAX* has a stable and non – stable isoforms.

Key words: Malate dehydrogenase, isoenzyme, parasites, *Trypanosoma vivax.*

INTRODUCTION

Typanosoma vivax is one of the protozoan parasites that retard economic growth and agricultural production in Africa. The parasite affects wide range of domestic animals in the region. Available chemotherapeutic agents, which are less efficient, (Gutteridge and Coom, 1977) are targeted at cellular components enzymes or metabolic pathways of the parasite. Parasite mitochondria have therefore, been one of the targets for chemotherapy. Gutteridge and Coom (1977), reported that the enzyme pyruvate kinase of the trypanosome is likely the target of inhibition by the arsenicals. Trypanosome alternative oxidase, which is the terminal oxidase of glycerol-3-phosphate oxidase system does not exist in the host and has been attracting attention as target for trypanocides (Kita et al., 2001). Nok (2002) also reported the specific inhibition of the mitochondrial glycerol-3-phosphate dependent electron transport of *Trypanosoma congolense* by azanthraquinone.

The blood stream forms of the parasite possess non-functional mitochondrion and survive exclusively by substrate-level phosphorylation with glucose readily available in the environment as t he energy source. In contrast, the procyclics living in the tse-tse flies where amino acids are the predominant substrates possess well developed mitochondria with citric acid cycle enzymes and a respiratory chain.

Malate dehydrogenase is an oxido-reductase enzyme that is vital in carbohydrate metabolism. It catalyses the oxidation of L-malate to oxaloacetate in the TCA cycle of cells. Information about this vital enzyme from the blood stream *T. vivax* was not documented. This work was designed to isolate and partially purify malate dehydrogenase from blood stream *T. vivax* and also to determine whether the enzyme is membrane bound or secreted by the parasite.

MATERIALS AND METHODS

Experimental animals

Four adult goats purchased from Zaria market were screened for possible infection by parasites. They were acclimatized for two weeks in the department of Veterinary Parasitology, A.B.U. Zaria. The animals were well fed and water was provided *ad libitum.*

The parasite

T. vivax (Agai strain) was kindly provided by the National Institute of Trypanosomiasis (NITR), Jos, Nigeria.

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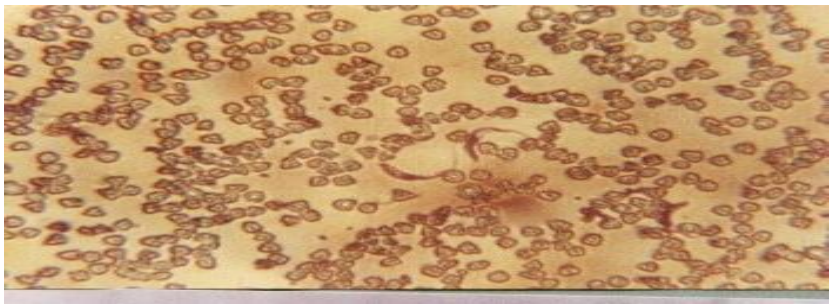

Figure 1. Photograph of *Trypanosoma vivax* in the infected blood.

Reagents

Substrates (oxaloacetic acid, L – Malate), coenzymes (NADH, NAD+), α - ketogluterate, Succinic acid, pyruvic acid, isosuccinic acid, DEAE – cellulose – 52, TEMED and bromophenol blue were products of Sigma chemical company, U.S.A. Acrylamide, N, N, methyl bis acrylamide (Bis), ammonium persulphate, coomaise blue, sodium dodecyl sulphate (SDS), mercaptoetyhanol, glycine, sodium dihydrogen phosphate, disodium hydrogen phosphate and heparin were obtained from BDH chemical, pool, England. All other chemical used were of analytical grade.

Inoculation of goats with the *TRYPANOSOME*

A suspension of *T. vivax* of approximately 10^3 parasites per field was intraperitoneally injected into the apparently healthy goats. The level of parasiteamia was monitored everyday for two weeks.

Parasiteamia determination

Parasiteamia was determined by the wet blood film technique. A smear of the infected blood was made on a slide and covered with a cover slip. The blood film was then examined under the microscope (mg × 40). Motile parasites were counted per field by the method of Herbert and Lumsden (1976).

Isolation of the *TRYPANOSOMES*

Trypanosomes were isolated from the infected goats by the method of Lanham and Godfrey (1970). At peak parasiteamia, blood was drawn through the jugular vein using heparanised needles. The blood was loaded unto previously packed column (1.5 x 30 cm) with DEAE- cellulose and the parasites were eluted using phosphate buffered saline.

Preparation of the crude enzyme

The reconstituted pure parasites were divided into two portions. One of the portions was frozen and thawed three times to solubilize the *Trypanosome*. To the other portion, 1% triton X – 100 was added by intermittent shaking for about 30 min. The two portions were assayed for malate dehydrogenase activity and protein content.

Assay for malate dehydrogenase

Malate dehydrogenase activity was assayed spectrophotometrically in the direction of oxaloacetate (OAA) reduction, by following the disappearance of NADH at 340 nm. The assay medium in the cuvette contained 0.134 mM NADH, 0.2 mM OAA in 25 mM phosphate buffer (pH 7.2) in a total volume of 500 ∝l.

Protein determination

Protein concentrations were determined by the biuret method, using bovine serum albumin BSA as standard.

Ion-exchange chromatography

Charged slurry of DEAE – 52 cellulose was packed into a chromatographic column (1.5 × 30 cm) and equilibrated with 25 mM phosphate buffer pH 7.2. 5 ml of the crude enzyme was dispensed into the column. The enzyme was eluted by linear phosphate gradient of 0.02 – 1 M. Fifty fractions of 5 ml each were collected and assayed for MDH activity and protein content.

SDS polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis of the active eluents from the ion-exchange chromatography was performed as described by Webster and Pringle (1972). Prepared test samples and reconstituted protein standards were separately dispensed on to the gels (12% running gel and 6% stacking gel) and electrophoresis was performed at a current of 5 mA/gel tube for about 1.5 h.

At the end of electrophoresis, the molecular weights of the partially purified enzymes were extrapolated from a calibration curve of $\log_{10}$ of the molecular weights of the standard proteins against the RF values.

MDH activity from the live parasite

Harvested live parasites were incubated with the assay medium and MDH activity determined in the direction of oxaloacetate reduction as described before. Various volumes containing different concentration of the life parasites were used. Drops of triton – x – 100 were added to the harvested parasites in an increasing order as the activity of the enzyme was determined at intervals.

RESULTS

Figure 1 shows the photograph of blood stream *T. vivax* before separation from the blood. After separation, the

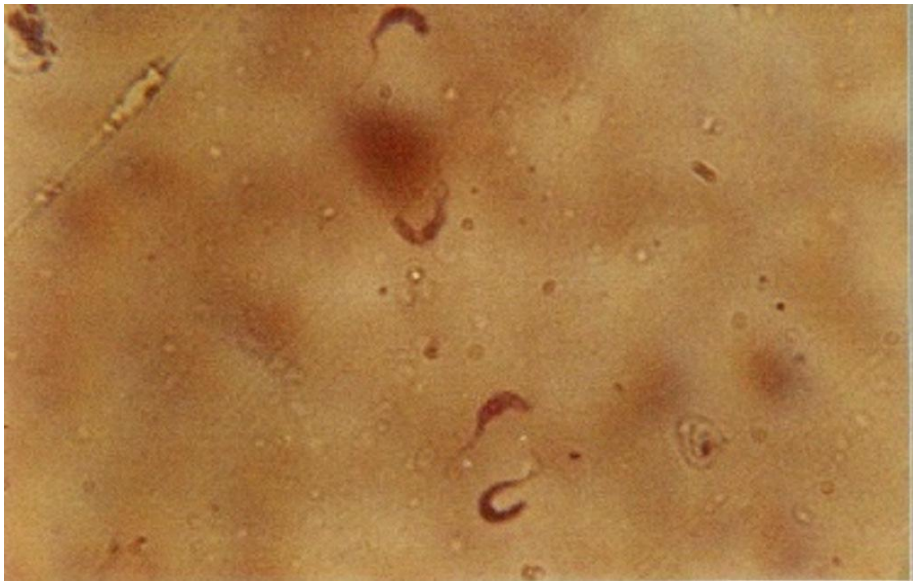

Figure 2. Photograph of *Trypanosoma vivax* after separation from the blood.

Table 1. Purification table.

Step	Volume (cm^3)	Protein (mg/ml)	Total protein (mg)	Activity/ min ml	Total activity (units)	Specific activity (units/mg)	Purification fold	% yield
Crude	12	2.0	24.0	0.0051	0.0612	0.00255	1	100
DEAE cellulose chromatographyPeak1(MDH_1)	5	0.1	0.5	0.0069	0.0345	0.069	27.1	56.4
DEAE – Cellulose chromatographyPeak2 (MDH_2)	5	0.1	0.5	0.0016	0.0080	0.016	6.3	13

parasites were motile and retained their morphology as shown in Figure 2.MDH was released into the solution after three cycles of freezing and thawing.

Total and specific activity of the enzyme is presented in Table 1. Elution of the enzyme by linear phosphate gradient on DEAE – cellulose resulted into two distinct peaks Figure 3. The active peak gave purification fold of 27.1 and percentage recovery of 56.4% (Table 1).

When the two distinct peaks were separately subjected to electrophoresis single bands were observed corresponding to the two bands out of the several bands observed from the crude enzyme.

The molecular weights of the partially purified MDH1 and MDH_2 were determined by the sodium dedocyl sulphate polyacrylamine gel electrophoresis. The logarithms of the molecular weight of the standard protein were plotted against the relative mobility expressed as percentage of bromophenol blue (Figure 4). The relative molecular weight of the MDH_1 and MDH_2 were found to be 61 and 63 kDal respectively.

Figure 5 show the activity of MDH when various concentrations of the life parasites were incubated with the substrates NADH and OAA in the reaction medium. There was no activity observed initially, however, when different volumes of triton X-100 were added, the activity of MDH was observed and it increases with increase in parasite concentration.

DISCUSSION

Various isoenzymes of malate dehydrogenase have been

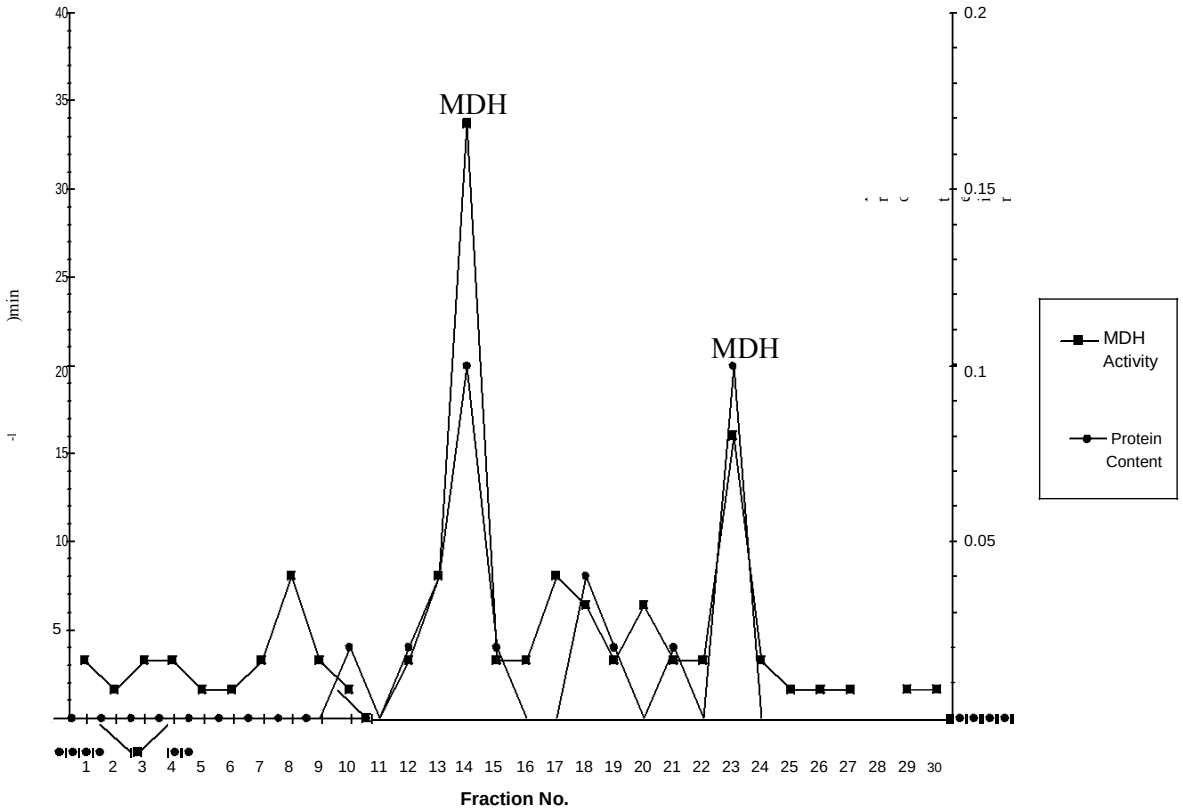

Figure 3. Elution profile of MDH by linear phosphate gradient from DEAE 52 Cellulose.

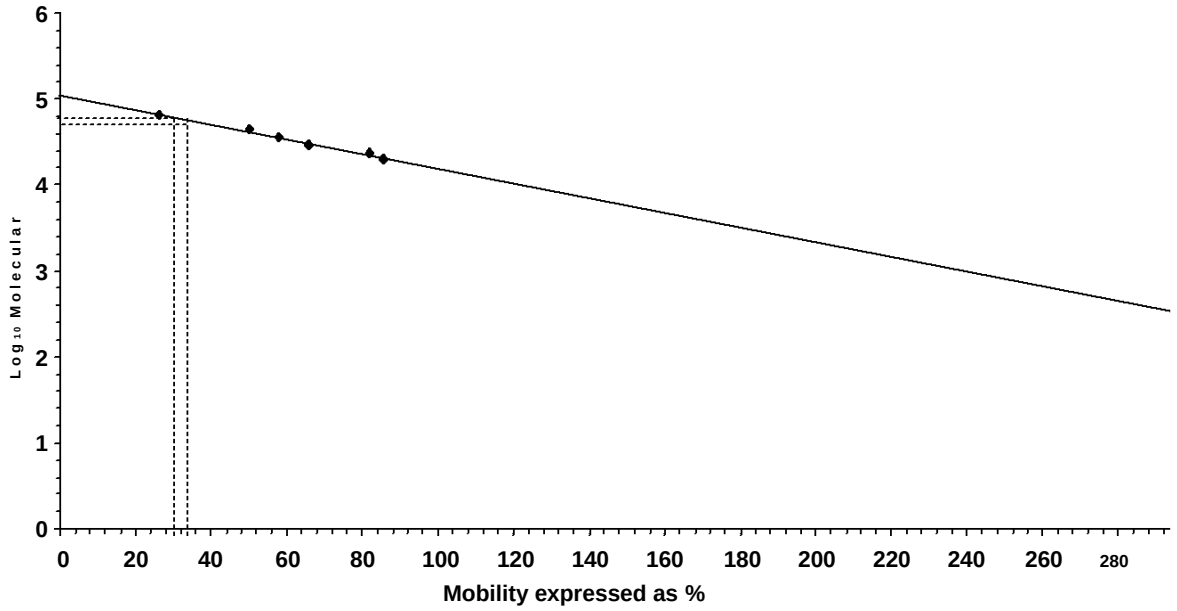

Figure 4. SDS – PAGE Calibration curve for determination of molecular weight of MDH.

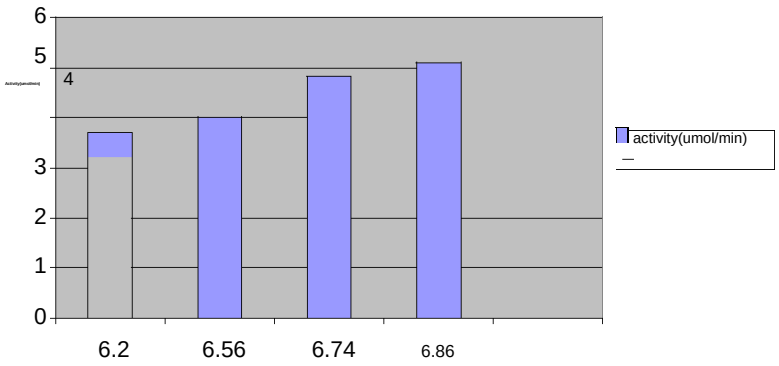

Figure 5. MDH Activity from the live parasites.

purified from different sources (Genda et al 2006; Bokato et al., 1995; Yelena et al., 2008; Lang-Unnasch, 1992). Studies have shown low degree of primary structural similarity among isoenzymes of malate dehydrogenases, however, positions for nucleotide binding, catalysis and sub unit interface were found to be conserved (Van Hellemond et al., 2005; Embley et al., 2003). This may account for the identical properties observed in isoenzymes of malate dehydrogenases. The differences observed between MDH from organelles and those from the cytosol is that a common ancestral MDH - gene may have been duplicated before inversion of premodial eukaryotes by bacteria to produce mitochondria according to the probable endosyonbiotic origin of these organelles (Hrdy et al., 2004).

MDH_1 was stable while MDH_2 lost its activity immediately and could not be characterized. It might be that MDH_2 is the mitochondrial enzyme that lost its activity due to thermal inactivation or post translational modification. MDH_2 could have been glycosylated after translation because of the environmental conditions. This could account for the higher molecular weight of the MDH_2 observed. Under blood steam condition substrate availability could be limited and the mitochondria are not fully functioning. It is also important to note that the parasite life cycle alternates between the vector and the host and it must adjust to the different environmental conditions. Therefore, it might be possible during the developmental stage of the parasite in the vector, where the mitochondria is fully functioning and substrates for both catabolism and anabolism are available, MDH_2 may not be glycosylated, may be active and its molecular weight may not be high as observed.

When MDH activity was assayed on the live parasites, no activity was observed indicating that the parasites do not secrete the enzyme. However, addition of detergent led to a surge in MDH activity. Increased activity was also observed as the concentration of the parasite was increased in the reaction medium. Both observations strongly suggest that MDH from *T. vivax* is membrane linked. Variant surface glycoprotein which protects the blood stream parasite from attack by the host immune system (Vickerman, 1985) and procyclic acidic repetitive protein are anchored to the plasma membrane by glycosyl phosphatidyl inositol (GPI) (Ferguson et al., 1985). In the present work it is obvious that MDH from the blood stream *T. vivax* is membrane bound but whether the linkage is via GPI remains to be elucidated.

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