THE NAD-LINKED α-GLYCEROPHOSPHATE DEHYDROGENASE OF TRYPANOSOMES

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SUMMARY. NAD-linked α-glycerophosphate dehydrogenase plays a key role in the α-glycerophosphate cycle of Trypanosoma brucei. The activity in cell lysates was ample for this role. The enzyme was activated by salts (e.g. $MgCl_2$ or NaCl); it had a broad pH-optimum for the reduction of dihydroxyacetone phosphate centred at pH 7.4, with an apparent K_m of 0.5 mM; and it was weakly bound to particulate components of cell lysates. The enzyme from T. vivax was similar to that of T. brucei. These trypanosomal enzymes resemble that of the trypanosomatid Crithidia fasciculata, but are rather different from the enzymes of mammals, birds and insects.

Bloodstream forms of Trypanosoma brucei and similar pathogenic trypanosomes metabolise glucose very actively to pyruvate: the glycolytically-produced NADH is reoxidised non-mitochondrially via a glycerophosphate cycle consisting of NAD-linked α-glycerophosphate dehydrogenase (EC. 1.1.1.8) and a unique, cyanide-insensitive α-glycerophosphate oxidase (1,2). Grant and Sargent (1) and Ryley (3) demonstrated the presence of the dehydrogenase in trypanosomal extracts. Further characterisation of this enzyme from the pathogenic African trypanosomes T. brucei and T. vivax is reported here. The enzyme from the non-pathogenic insect trypanosomatid Crithidia fasciculata (4,5) shows marked similarities to that from these African trypanosomes.

MATERIALS AND METHODS

Trypanosomes. T. brucei was isolated locally from an infected cow and maintained in mice and rats: it was essentially monomorphic (long-slender). T. vivax, strain Y58, was isolated locally from a cow and found to infect mice readily: it was maintained in mice and rats. For both species, rats were infected by syringe-passage to provide the trypanosomes: trypanosomes were isolated from infected blood on DEAE-cellulose by the method of Lanham (6) using phosphate-saline-glucose

buffer, ratio 4:6, pH 8.0. Cells were harvested by centrifugation, and washed in the same buffer. After recentrifuging, the supernatant was removed and distilled water (10 - 20 vol.) added to the packed cells. After dispersion, the lysate was frozen and thawed, and used as the source of enzyme.

Enzyme Assays. Activity was assayed spectrophotometrically at 340 nm and 25 C, assuming a millimolar extinction coefficient for NADH of 6.22 cm^{-1} in a reaction volume of 4 ml. Unless otherwise stated, assay of reduction of dihydroxyacetone phosphate was performed in 20 mM sodium phosphate buffer (pH 7.4) containing NADH (100 μM), dihydroxyacetone phosphate (0.4 mM) and $MgCl_2$ (10 mM). Assay of α-glycerophosphate oxidation was performed in 0.05 M glycine-NaOH buffer (pH 9.3) containing NAD^+ (310 μM), DL-α-glycerophosphate (4.8 mM) and $MgCl_2$ (10 mM). For both types of assay, trypanosomal extract (15 - 150 μg protein) was added last to start the reaction, and controls omitting substrate showed negligible activity.

For determination of pH-optima, buffers (ionic strength 0.05) were as follows: pH 5.9 - 7.4, sodium phosphate; pH 7.7 - 8.3, Tris + NaH_2PO_4; pH 8.8 - 10.1, glycine-NaOH.

Protein was determined by the method of Lowry (7) with bovine serum albumin as standard.

Activity is expressed throughout in μ-moles.min^{-1}.mg $protein^{-1}$, and this is referred to as units/mg.

Reagents. Coenzymes were obtained from British Drug Houses Ltd., Poole, England. Substrates were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A.: dihydroxyacetone phosphate (cyclohexylamine salt, dimethyl ketal) was deionised with Dowex 50(H^+) resin and hydrolysed according to the suppliers' instructions, and assayed enzymatically with NADH and rabbit-muscle α-glycerophosphate dehydrogenase (Boehringer Mannheim GmbH, W. Germany); DL-α-glycerophosphate, Na_2 salt, stated to be 95% pure, was used without further purification. Other chemicals were of reagent grade.

RESULTS

Activity in Trypanosome Lysates. With the assay systems given above, T. brucei lysates reduced dihydroxyacetone phosphate at a rate of 0.59 units/mg; the corresponding figure for T. vivax was 0.46 units/mg. For α-glycerophosphate-oxidation, the figures were 0.15 and 0.19 units/mg respectively. The rates of respiration of intact trypanosomes at 37 C in equivalent units (i.e. μ-gram-atoms oxygen.min^{-1}.mg $protein^{-1}$) are approximately 0.15 for T. brucei (8) and 0.16 for T. vivax, Desowitz strain (9). Assuming a doubling of velocity on raising the temperature from 25 C to 37 C and extrapolating to saturating substrate concentrations, the velocities estimated for reduction of dihydroxyacetone phosphate in vitro at 37 C are of the order of twenty times that neces-

sary to sustain the observed rates of respiration in vivo. Ryley (3) obtained in vitro a rate of triose phosphate reduction of 2.9 units/mg.

Subcellular Location. Ryley (3) reported that the majority of the ∝-glycerophosphate dehydrogenase activity was associated with particulate components in homogenates of T. rhodesiense. This location was confirmed for lysates of T. brucei in the present study: in two experiments, 37% and 42% of the activity was found in the supernatant after a brief (5-minute) centrifugation at 15,000 g. However, when the lysate was brought to an ionic strength of 0.15 (i.e. approximately physiological) with either sodium chloride or sodium phosphate (pH 7.4), 95 - 100% of the activity was found to remain in the supernatant. Therefore the binding between the enzyme and particulate cell components was weak and might involve electrostatic interactions. It is possible that in vivo, when the enzyme is much more concentrated than in the cell lysates employed here, there may be some association with larger components. Bacchi et al. (5) suggested that the enzyme from C. fasciculata may be bound to a carbohydrate moiety in vivo: the partially-purified enzyme was bound by an unsubstituted agarose column and was released by 50 mM NaCl.

Activation by Ions. Sodium chloride or magnesium chloride activate the enzyme from T. brucei in either direction of catalysis (Fig. 1). The effect of sodium is probably comparatively small, because the buffer solutions already contained it. Magnesium chloride gives maximal activation at much lower concentrations than sodium chloride. The basal (unstimulated) activity was unaffected by ethylene diamine tetra-acetate (5 mM). Similar experiments with rabbit-muscle enzyme (Boehringer) gave much smaller activations, probably attributable to the increment in ionic strength alone. Bacchi et al. (5) found that the enzyme from C. fasciculata was activated by Mg^{2+} and Cl^{-}; ethylene diamine tetraacetate did not inhibit the basal activity, and Mg^{2+} could be replaced by polyamines (spermine or spermidine). In the present experiments the basal activity might be affected by endogenous

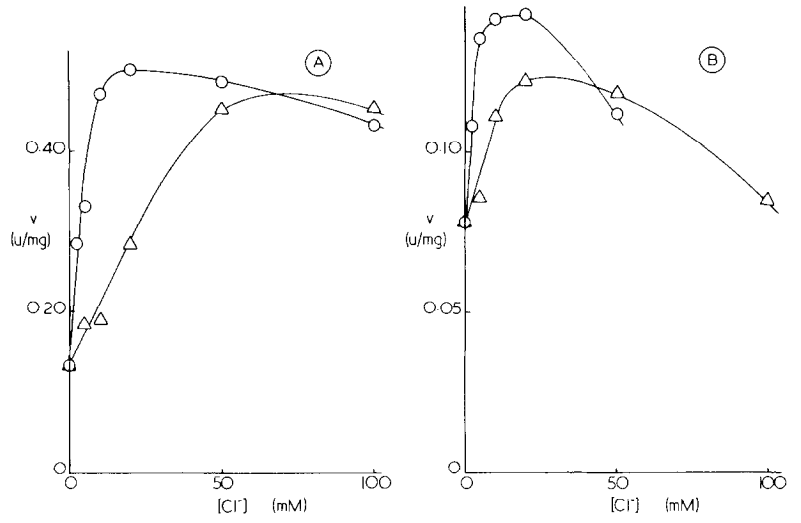

Fig. 1. Effect of Ions on Activity of α-Glycerophosphate Dehydrogenase of T. brucei. A, reduction of dihydroxyacetone phosphate; B, oxidation of DL-α-glycerophosphate. o, chloride added as $MgCl_2$; Δ, chloride added as NaCl. Conditions as in Materials and Methods, except for $MgCl_2$.

activators in cell lysates. The enzyme from T. vivax showed quantitatively similar activations, except that α-glycerophosphate oxidation in the absence of added salts was very low - less than 5% of the activity with 10 mM $MgCl_2$. On storage frozen for two to three weeks, the catalytic activity of cell lysates showed a drop of about 50%, which was accompanied by a decreased degree of activation by salts.

pH-Optima. Reduction of dihydroxyacetone phosphate by the enzyme from T. brucei has a broad pH-optimum, maximum activity being at pH 7.4. Activity was however at least 50% of maximum between pH 6 and pH 9. This broad optimum is similar to that obtained for insects (10) but unlike that for mammalian skeletal muscle (10,11) which gives a sharp optimum near pH 7.6. Activity of α-glycerophosphate-oxidation rose steadily between pH 7.6 and 10.1.

Kinetics. Apparent K_m-values for dihydroxyacetone phosphate were 0.5 mM (T. brucei) and 0.8 mM (T. vivax) (see Fig. 2); only the latter enzyme showed

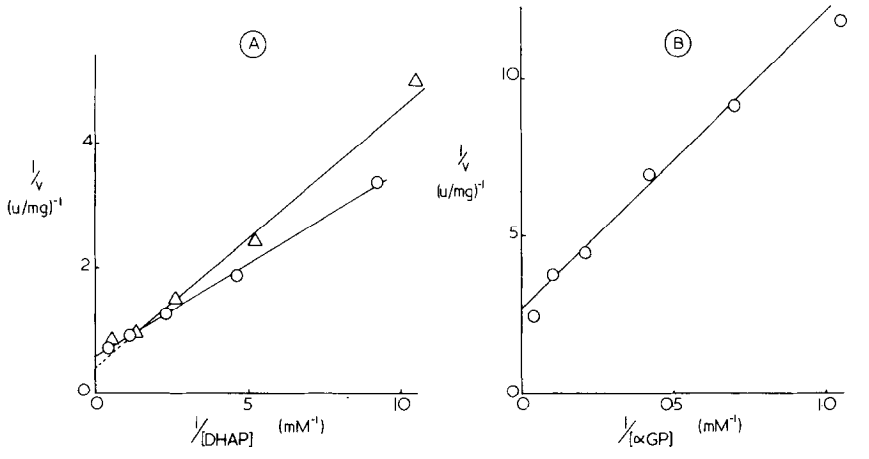

Fig. 2. Double-reciprocal Plots of Variation of Velocity with Substrate Concentration. A, reduction of dihydroxyacetone phosphate (DHAP); B, oxidation of DL-α-glycerophosphate (αGP). o, enzyme from T. brucei; △, enzyme from T. vivax. Conditions as in Materials and Methods, except for substrate concentrations.

some high-substrate inhibition. Some values reported for this enzyme from other sources are 1.2 mM from C. fasciculata (5), 0.33 mM from bee (10), 0.25 mM from rabbit muscle (11), 0.23 mM from chicken muscle (12) and 0.043 mM from chicken liver (12). The enzymes from protozoa thus seem to have rather higher K_m-values than the enzymes from birds, mammals or insects. The apparent K_m-value for DL-α-glycerophosphate-oxidation was 3.7 mM (Fig. 2).

DISCUSSION

The proposed physiological role for NAD-linked α-glycerophosphate dehydrogenase in T. brucei is as part of a glycerophosphate cycle to reoxidise NADH (1). Its role in T. vivax is less clear, but α-glycerophosphate oxidation is comparatively rapid and may also be the chief consumer of oxygen in vivo (2). The results reported here are compatible with a role for the enzyme in a glycerophosphate cycle in each species: the activity is adequate at all likely pH-values in vivo.

The similarities between the enzymes from T. brucei and T. vivax and

the non-pathogenic insect parasite C. fasciculata (4,5) are striking, including the weak binding to cell particles and agarose respectively, and the activation by ions. Crithidia spp. are often used as models for the pathogenic trypanosomes, being comparatively easy to grow in quantity. The results reported here, as well as furthering knowledge of pathogenic trypanosomes themselves, show that the similarities with crithidia extend to individual enzymes; this is not so apparent at the level of overall metabolism, since (for example) Crithidia unlike most bloodstream trypanosomes utilise oxygen via cytochromes (13) and carry out oxidative phosphorylation (14,15).

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