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TWO DISTINCT SUCCINATE THIOKINASES IN BOTH BLOODSTREAM AND PROCYCLIC FORMS OF TRYPANOSOMA BRUCEI

TIMOTHY M. JENKINS, ROBERT EISENTHAL and P.DAVID J. WEITZMAN

Department of Biochemistry,
University of Bath,
Bath BA2 7AY, U.K.

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Two succinate thiokinase activities specific for either adenine or guanine nucleotides have been found in Trypanosoma brucei. Key glycolytic and citric acid cycle enzymes were measured to show repression of glycolysis and derepression of the citric acid cycle in the procyclic form, relative to the bloodstream form. A marked rise in adenine-linked succinate thiokinase activity accompanied a rise in activity of citric acid cycle enzymes. However, guanine-linked succinate thiokinase was found to increase only slightly in activity. These results implicate the adenine-linked enzyme as an essential component of the citric acid cycle, whereas the guanine-linked enzyme appears to be under separate control. This communication also reports for the first time the occurrence of citrate synthase activity in the bloodstream (long slender) form of T.brucei.

Succinate thiokinase (succinyl-CoA synthetase) (STK) catalyses the following reversible reaction:

$$NDP + P_i + \text{succinyl-CoA} \xrightleftharpoons{Mg^{2+}} NTP + \text{succinate} + CoA\text{-}SH$$

where NDP and NTP represent nucleoside diphosphate and triphosphate. Originally, animal tissues were thought to possess only the guanine-linked enzyme (1). However, we have recently reported the occurrence of distinct succinate thiokinases in animal tissues specific for guanine (G-STK) or adenine (A-STK) nucleotides (2).

Subsequent studies (3) have indicated that in vivo A-STK and G-STK may be responsible for the operation of the succinate thiokinase reaction in opposing directions. G-STK in mammalian brain shows a marked elevation in activity with increased ketone body utilization. This supports the proposal that the re-cycling of succinate to succinyl-CoA, required for ketone body activation, is achieved directly by the action of G-STK (3). We therefore

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proposed (3) that the A-STK functions in the energy-conserving role of the citric acid cycle and the present work was undertaken to explore this suggestion further.

During the life cycle of Trypanosoma brucei from mammalian host to insect vector, dramatic changes occur in morphology and metabolism (4). In the mammalian bloodstream T.brucei is exclusively dependent upon glucose for energy, being unable to utilise fatty acids or amino acids due to the absence of a functional citric acid cycle (5). In the insect gut the situation is reversed. Here glucose is scarce and amino acids such as proline constitute the main energy source for the procyclic form (6), proline being oxidized and eventually entering a functional citric acid cycle as oxoglutarate (7).

The bloodstream and procyclic cell forms of T.brucei thus provide an example of a eukaryotic organism in which the enzymes of either glycolysis or the citric acid cycle are repressed or derepressed. We have therefore examined the levels of A-STK and G-STK in both bloodstream and procyclic forms of T. brucei, together with key enzymes of the glycolytic and citric acid cycle pathways. The results reported here clearly identify A-STK with the citric acid cycle whereas a different metabolic role is likely for the G-STK.

METHODS

T.brucei in the bloodstream form were isolated from the blood of 250 g Wistar rats, 71h after injection with 10^7 cells of strain MITat 1.1 (obtained from Dr H.P.Voorheis of Trinity College Dublin) by intraperitoneal injection. The cells were separated from blood components by centrifugation (600 x g) for 10 min at 4°C and further purified on a short column of DEAE-cellulose in isotonic phosphate-buffered saline, pH 8.0, containing 10 mM glucose, as described by Lanham and Godfrey (8). T.brucei in the procyclic cell form (strain EATRO-427, kindly supplied by Dr W.Gibson, Tsetse Fly Research Centre, Langford, Bristol) were cultivated at 26°C on SDM-79 medium (9) supplemented with 10% foetal calf serum. The cells were harvested by centrifugation (600 x g) for 5 min at room temperature and then washed in 100 mM Hepes buffer, pH 7.5, containing 25 mM $NaHCO_3$, 50 mM NaCl, 5 mM L-proline, 5 mM KCl and 5 mM glucose.

Pellets of both bloodstream and procyclic cell forms, after their respective washings, were resuspended in 0.1M Na^+/K^+ phosphate buffer, pH 7.5, containing 20% glycerol and disrupted by ultrasonication (MSE 100W sonicator operated at 40W for 6 x 15 sec with cooling). After centrifugation (5000 x g) for 2 min at room temperature, the supernatant solutions were used without further treatment.

Citrate synthase and succinate thiokinase were assayed polarographically as previously described (10,11). The remaining citric acid cycle and glycolytic enzymes were measured according to Bergmeyer (12). Protein concentrations were determined as in (13).

Table 1. Glycolytic and citric acid cycle enzymes in both bloodstream and procyclic cell forms of T.brucei

Enzyme	Enzyme activities (nmol/min/mg protein)		
	Bloodstream (B)*	Procyclic (P)*	Ratio P/B
Citrate synthase (EC 4.1.3.7)	0.34	2.7	7.9
A-STK (EC 6.2.1.5)	5.4	65	12
G-STK (EC 6.2.1.6)	1.0	1.7	1.7
NADP-Isocitrate dehydrogenase (EC 1.1.1.42)	3.4	28	8.2
Malate dehydrogenase (EC 1.1.1.37)	130	1800	14
Hexokinase (EC 2.7.1.1)	460	14	0.03
Phosphoglucose isomerase (EC 5.3.1.9)	500	74	0.15
Pyruvate kinase (EC 2.7.1.40)	31	9	0.29

*Both freshly harvested or frozen cells, stored at -21°C overnight, yielded similar specific activities for each enzyme. Total cell counts of 1.4×10^{10} and 1.02×10^{10} cells were obtained from bloodstream and procyclic cell forms respectively.

RESULTS AND DISCUSSION

In T.brucei the transformation from mammalian bloodstream to insect gut stage involves dramatic changes in morphology and mitochondrial activity. The long slender bloodstream form appears entirely dependent upon glycolysis for its energy requirements. In contrast, the procyclic form, with active mitochondria, relies mainly on the oxidation of amino acids, citric acid cycle intermediates or fatty acids, resulting in greater versatility and less dependence upon glycolysis. In fact the procyclic cell forms are able to grow even in the absence of free glucose or glucosamine (14).

The typical results, presented in Table 1, demonstrate the enzymic changes associated with the differing metabolic requirements of bloodstream and procyclic forms of T.brucei. The specific activities of hexokinase and phosphoglucose isomerase (both glycosomal) and pyruvate kinase (cytosolic) are repressed in the procyclic cell form. Similar changes have also been reported

by Hart et al. (15). As predicted, the specific activities of citric acid cycle enzymes increase in the procyclic cell form. Interestingly, citrate synthase activity was detected for the first time in the long slender bloodstream form of T.brucei. Succinate dehydrogenase now appears to be the only enzyme of the citric acid cycle absent in the bloodstream form, with the remaining enzymes of the cycle all attenuated (16,17).

The results in Table 1 indicate that the specific activities of the citric acid cycle enzymes increase in concert from bloodstream to procyclic form. Significantly, it is A-STK and not G-STK which displays this marked elevation, thus clearly implicating A-STK as a component of the cycle.

As previously mentioned, studies on A-STK and G-STK from mammalian tissues have shown that one of the roles of G-STK is associated with ketone body activation (3). This, together with predicted high ratios of GTP:GDP in mitochondria (18), implies that G-STK functions in vivo in the direction of succinyl-CoA formation. By contrast, mitochondrial ATP:ADP ratio values are less than or close to 1.0, being as much as 2 orders of magnitude lower than GTP:GDP ratios (18). The A-STK is therefore likely to operate in the direction of succinyl-CoA breakdown coupled to the phosphorylation of ADP to ATP. The results presented in this communication strengthen the suggestion that A-STK functions as an integral part of energy generation by the citric acid cycle.

Our findings concerning STK activities and the first identification of citrate synthase in the long slender bloodstream form of T.brucei were greatly facilitated by our use of the polarographic method of enzyme assay (19). We have previously commented on the superiority of this assay system (10,11) in comparison with other assay methods used for these two enzymes.

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REFERENCES

1 Sanadi, D.R., Gibson, D.M., Ayengar, P. and Jacob, M. (1956) J.Biol.Chem. 218, 505-520.
2 Weitzman, P.D.J., Jenkins, T., Else, A.J. and Holt, R.A. (1986) FEBS Lett. 199, 57-60.
3 Jenkins, T.M. and Weitzman, P.D.J. (1986) FEBS Lett. 205, 215-218.
4 Fairlamb, A. (1982) Trends Biochem. Sci. 7, 249-253.
5 Bowman, I.B.R. and Flyn, I.W. (1976) in: Biology of the Kinetoplastida, (Lumsden, W.H.R. and Evans, D.A. eds.) Vol.1, pp. 435-476, Academic Press New York.

6 Broman, K., Ropars, M. and Deshusses, J. (1982) Experientia 38, 533-534.
7 Ford, W.C.L. and Bowman, I.B.R. (1973) Trans. Roy. Soc. Trop. Med. Hyg. 67, 257.
8 Lanham, S.M. and Godfrey, D.G. (1970) Exptl. Parasitol. 28, 521-524.
9 Brun, R. and Schonenberger, M. (1979) Acta Trop. 36, 289-292.
10 Weitzman, P.D.J. (1969) Methods Enzymol. 13, 365-368.
11 Weitzman, P.D.J. and Jaskowska-Hodges, H. (1982) FEBS Lett. 143, 237-240.
12 Bergmeyer, H.U. (1983) in: Methods of Enzymatic Analysis, 3rd edn.,Vol.2, pp. 222-223, 230-231, 246-247, 279-280, 303-304, Verlag Chemie,Weinheim.
13 Bradford, M. (1976) Analyt. Biochem. 72, 248-254.
14 Broman, K., Knupfer, A.L., Ropars, M. and Deshusses, J. (1983) Mol. Biochem. Parasitol. 8, 79-87.
15 Hart, D.J., Misset, O., Edwards, S.W. and Opperdoes, F.R. (1984) Mol. Biochem. Parasitol. 12, 25-35.
16 Ryley, J.F. (1962) Biochem. J. 85, 211-223.
17 Overath, P., Czichos, J. and Hass, C. (1986) Eur. J. Biochem. 160, 175-182.
18 Ottaway, J.H., McClellan, J.A. and Sanderson, C.L. (1981) Int. J. Biochem. 13, 401-410.
19 Weitzman, P.D.J. (1976) Biochem. Soc. Trans. 4, 724-726.