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Phosphoenolpyruvate-succinate-glyoxylate pathway in the filarial parasite *Setaria digitata*

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Abstract. *Setaria digitata,* a filarial parasite of cattle possesses certain unique characteristics like cyanide insensitivity, and lack of cytochromes. In the present study, we have shown that the parasite has an incomplete tricarboxylic acid cycle with the absence of activities of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and succinyl-CoA synthase. However the parasite showed the existence of glyoxylate cycle and phosphoenolpyruvate-succinate pathway. The widely used antifilarial drug diethylcarbamazine caused general inhibition of all enzymes of phosphoenolpyruvate-succinate pathway and glyoxylate cycle except that of fumarase and isocitrate lyase. The results may pave the way for new targets for chemotherapy in the control of filarial parasites.

Keywords. Filariae; *Setaria digitata;* mitochondriae; tricarboxylate cycle; phosphoenolpyruvate-succinate pathway; glyoxylate cycle.

1. Introduction

All helminths examined are capable of utilizing glucose and consume oxygen under appropriate conditions (Saz 1972). Filarial parasites are reported to be homolactate fermenters (Barrett 1981; Saz 1981). However there is no evidence that any parasitic helminth exhibits homolactate fermentation in the strict sense of the term (Bryant 1989). There is considerable evidence now suggesting aerobic respiration in filarial parasites (Bryant and Behm 1989). Studies using mitochondrial isolates from *Brugia pahangi* and *Dipetalonema viteae* have shown the oxygen uptake to be completely inhibited by cyanide, while rotenone and antimycin A suppress it by about 80% (Barrett 1983).

Setaria digitata is a filarial parasite of cattle recommended as a model system by the WHO, due to its similarity to the human filarial parasites *Wuchereria bancrofti* and *Brugia malayi* (Hawking 1978). *S. digitata* possesses rare characteristics such as cyanide insensitivity, hydrogen peroxide production, absence of cytochrome, lipid peroxidation and substrate-dependent oxygen uptake (Kaleysa Raj *et al* 1988). A distinct difference was observed between *S. digitata* and those of *B. pahangi* and *D. viteae* in their sensitivity to cyanide (Barrett 1983; Kaleysa Raj *et al* 1988). The occurrence and formation of quinones Q_6 and Q_8 was reported recently by Santhamma and Kaleysa Raj (1990). They also showed generation of H_2O_2 (Santhamma and Kaleysa Raj 1991a) in *S. digitata.* These prompted a detailed study of the activities of enzymes connected with the tricarboxylic acid (TCA) cycle and PEP-succinate pathway in the parasite.

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2. Materials and methods

Adult *S. digitata* were collected from the peritoneal cavity of freshly-slaughtered cattle. The worms were washed well to remove host material and incubated for 2 h at 37° C in Tyrode solution (composition: w/v sodium chloride 0·8%, potassium chloride 0·02%, calcium chloride 0·02%, magnesium chloride 0·01%, sodium bicarbonate 0·015%, disodium hydrogen phosphate 0·05% and glucose 0·5%). The incubated worms were minced and homogenized in ice-cold 0·25 M sucrose solution and the homogenate was subjected to differential centrifugation. The pellet collected between 800 and 12000 g was used for the studies. The experimental fraction was termed mitochondria-like particulate fraction (MLP) as described by Kaleysa Raj *et al* (1988) and used for the assay of mitochondrial enzymes. The supernatant collected after removing MLP was used for the study of the cytosolic enzymes (PMS).

Enzyme assays were carried out by the following methods: pyruvate dehydrogenase (EC 1·2·4·1, Sumegi and Alkomyi 1983), citrate synthase (EC 4·1·3·7, Shephard and Garland 1966), aconitase (EC 4·2·1·3, Racker 1950), isocitrate dehydrogenase (ICDH) (EC 1·1·1·41, Chen and Plaut 1963), α-ketoglutarate dehydrogenase (KDH) (EC 1·2·4·2, Sanadi 1969), succinyl-CoA synthase (EC 6·2·1·4, Ramaley *et al* 1967), succinate dehydrogenase (SDH) (EC 1·3·99·1, Susheela and Ramasarma 1971), fumarase (EC 4·2·1·2, Kanarek and Hill 1964), malate dehydrogenase (MDH) (EC 1·1·1·37, Englard and Siegel 1969), malic enzyme (EC 1·1·1·40, Ochoa 1955), fumarate reductase (EC 1·3·1·6, Holwerda and Zwaan 1959), malate synthase (EC 4·1·3·2, Dixon and Kornberg 1959), pyruvate carboxylase (EC 6·4·1·1, Seubert and Weicker 1969), pyruvate kinase (EC 2·7·1·40, Bucher and Pfleiderer 1955), phosphoenolpyruvate carboxykinase (EC 4·1·1·32, Ward *et al* 1969), glucose-6-phosphatase (EC 3·1·3·9, Shull *et al* 1956), fructose-1, 6-diphosphatase (EC 3·1·3·11, Shull *et al* 1956), citrate lyase (EC 4·1·3·8, Srere 1959), isocitrate lyase (EC 4·1·3·1, Dixon and Kornberg 1959), and lactate dehydrogenase (EC 1·1·1·27, Kornberg 1955). The protein content of the preparations was determined after trichloroacetic acid precipitation by the method of Lowry *et al* (1951). Assays were carried out in a Shimadzu UV-240 spectrophotometer.

The effect of diethylcarbamazine (DEC) on the activities of selected enzymes was studied by preincubating the enzyme preparation with neutralized aqueous solution of DEC under different concentrations.

3. Results and discussion

The activities of ICDH, KDH and succinyl-CoA synthase were not detected in MLP under the experimental conditions. The activities of citrate synthase, aconitase, SDH, fumarase, malate dehydrogenase (MDH), isocitrate lyase, malic enzyme, malate synthase and fumarate reductase are given in table 1.

Thus *S. digitata* apparently has an incomplete TCA cycle. This is not in agreement with the information available in the case of certain other filarial parasites such as *B. pahangi* and *D. viteae* (Bryant and Behm 1989). These parasites are reported to have low levels of TCA cycle enzymes, in agreement with the cyanide sensitivity shown by them (Barrett 1983). Another filarial parasite *Litomosoides carinii* also behaved like *B. pahangi* (Ramp *et al* 1985). *S. digitata* is different from them in its cyanide insensitivity (Kaleysa Raj *et al* 1988).

Table 1. Specific activities of TCA cycle and glyoxylate cycle enzymes in MLP of *S. digitata.*

Enzymes	Specific activity* (nmol/min/mg protein)
Pyruvate dehydrogenase	ND
Citrate synthase	19 ± 1
Aconitase	5 ± 0·3
Isocitrate dehydrogenase	ND
α-ketoglutarate dehydrogenase	ND
Succinyl-CoA synthase	ND
Succinate dehydrogenase	366 ± 12
Fumarase	757 ± 18
Malate dehydrogenase	820 ± 18
Isocitrate lyase	85 ± 3
Malate synthase	81 ± 6
Fumarate reductase	58 ± 4
Malic enzyme	135 ± 8

ND, Not detected.
*Average of 6 independent experiments.

In the context of the observed incomplete TCA cycle, the presence of glyoxylate cycle enzymes complete the cycle of events from acetate to oxaloacetate. In addition, the absence of ICDH and KDH indicates the absence of carbon loss as CO_2. Thus there is a possibility of complete conservation of the carbons of acetyl CoA entering the TCA cycle.

The findings that the activity of aconitase was very low suggest that it may be the rate-limiting step. It is also of significance because oxygen deficiency greatly reduced the activity of aconitase (Hirsch 1952). The reduction of fumarate to succinate catalysed by NADH-dependent fumarate reductase was also detected in mitochondrial isolates from *S. digitata.* The ratio of SDH to fumarate reductase was 6·3. This ratio is in agreement with the values reported for facultative anaerobes (Prichard and Schofield 1968).

Malate formed by fumarase action can be decarboxylated to pyruvate by malic enzyme. Malate may be converted by mitochondrial MDH to oxaloacetate which condenses with acetyl-CoA to form citrate by citrate synthase. Since the activity of pyruvate dehydrogenase complex is absent, the source of acetyl-CoA is from oxidation of fatty acids and catabolism of amino acids. Isocitrate lyase cleaves the isocitrate formed from citrate into succinate and glyoxylate. Glyoxylate condenses with acetyl-CoA to form malate and the net result is the condensation of two acetyl-CoA to yield a molecule of succinate. Recent studies from this laboratory showed continuous production of H_2O_2 by isolated mitochondria of *S. digitata* with either succinate or fumarate (Santhamma and Kaleysa Raj 1991a) with generation of ATP (Santhamma and Kaleysa Raj 1991b), indicating a cyclic reaction taking place involving SDH, fumarate reductase and fumarase system.

The activities of certain cytosolic enzymes (table 2) clearly suggest the presence of PEP-succinate pathway in *S. digitata.* Based on the observations, a scheme for substrate and product shuttling between the cytosol and mitochondria involving the glyoxylate cycle and PEP-succinate pathway is shown in figure 1. Such a pathway is reported in many parasites such as *Ascaris lumbricoides, Onchocerca voluvlus,*

Table 2. Specific activities of some enzymes in cytosolic fraction of *S. digitata.*

Enzymes	Specific activity* (nmol/min/mg protein)
Pyruvate kinase	103±6
PEP-carboxykinase	94±4
Pyruvate carboxylase	57±8
ATP-citrate lyase	66±4
Lactate dehydrogenase	89±3
Glucose-6-phosphatase	ND
Fructose-1, 6-diphosphatase	57±2

ND, Not detected
* Average of 6 independent experiments.

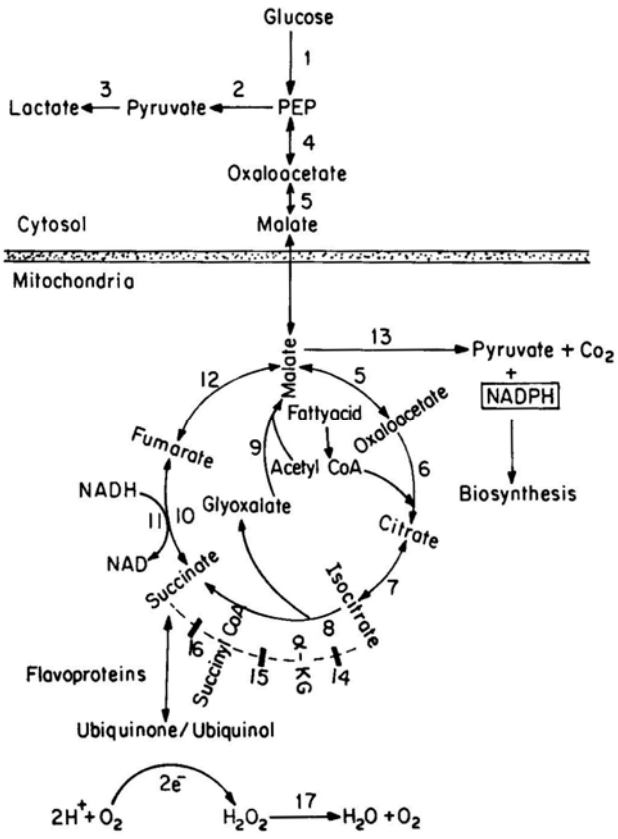

Figure 1. Proposed PEP-succinate-glyoxylate pathway in *S. digitata.*

1. Glycolytic enzymes; 2, pyruvate kinase; 3, lactate dehydrogenase; 4, PEP-carboxy kinase; 5, malate dehydrogenase; 6, citrate synthase; 7, aconitase; 8, isocitrate lyase; 9, malate synthase; 10, succinate dehydrogenase; 11, fumarate reductase; 12, fumarase; 13, malic enzyme; 14, isocitrate dehydrogenase; 15, α-ketoglutarate dehydrogenase; 16, succinyl-CoA synthase; 17, catalase.

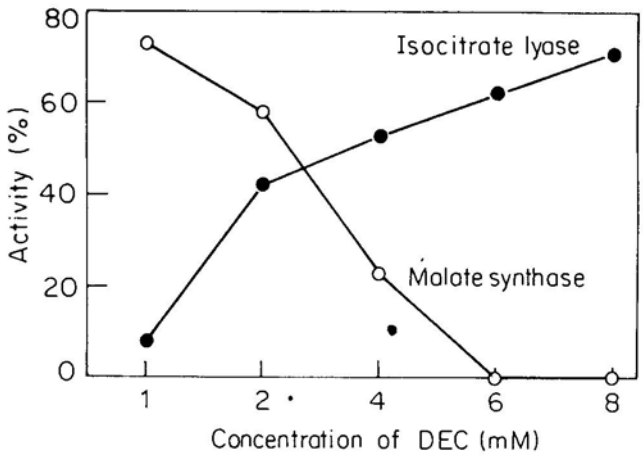

Figure 2. Effect of DEC on glyoxylate cycle enzymes.

Schistosoma mansoni, Hymenolepis diminuta and *Fasciola hepatica* (Behm and Bryant 1976) and also in *S. cervi* (Hussain *et al* 1990). However in none of these parasites, is glyoxylate cycle reported and many of them are entirely or partially cyanide sensitive.

The effect of DEC, a widely used antifilarial drug, when tested against the *Setaria* system, showed a generalized inhibition of the activities of enzymes of the PEP-succinate pathway, except that of fumarase (unpublished results). DEC showed a differential effect on glyoxylate cycle enzymes. It stimulates the activity of isocitrate lyase and inhibits the activity of malate synthase. This result is presented in figure 2. Because of this, lesser amounts of malate will be available for generating oxaloacetate and fumarate for the continuation of the cycle and a resultant decrease in the generation of ATP will lead to paralysis of the parasite.

Since *S. digitata* possesses electron transport system associated with two quinines Q_6 and Q_8, a unique feature of this parasite (Santhamma and Kaleysa Raj 1990), cyanide insensitivity (Kaleysa Raj *et al* 1988), incomplete TCA cycle, glyoxylate cycle and PEP-succinate pathway (present study), inhibitors specific to at least one of these systems may prove very effective targets of attack for controlling filariasis.

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