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FUMARATE REDUCTASE SYSTEM OF FILARIAL PARASITE SETARIA DIGITATA

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In the cattle filarial parasite Setaria digitata the mitochondria like particles have been shown to possess NADH dependent fumarate reduction coupled with site I electron transport associated phosphorylation. This reduction is catalysed by the fumarate reductase system. The Km for fumarate is 1.47 mM and that for NADH is 0.33 mM. This activity is sensitive to rotenone, antimycin A and o-Hydroxy diphenyl. One ATP is produced for each pair of electrons transferred to fumarate. The fumarate reductase system consisting of NADH-coenzyme Q reductase, cytochrome b like component(s) and succinate dehydrogenase/fumarate reductase is thus very important and hence specific inhibitors of the system may prove useful in the effective control of filariasis.

Parasitic helminth species, studied so far, use oxygen when it is available and show a number of peculiar characteristics (1). According to Barrett (1989) the anaerobic metabolism of parasites differ from that of free-living invertebrates in that, it is continuous, not transient, and persists in the presence of oxygen (2). One or more of the excretory metabolic end-products produced from parasites such as Ascaris lumbricoides, Fasciola hepatica and Trichostrongylus colubriformis show the existence of other pathways of terminal electron acceptors (1,3).

NADH dependent fumarate reduction with ATP production is one such system reported in obligatory anaerobic parasites (4,5,6). In the parasitic nematode A.suum and in cestodes (7,8,9,) this system is well established. Sub-mitochondrial particles from Brugia pahangi and Dipetalonema viteae also possess fumarate reductase activity (10).

The cattle filarial parasite, Setaria digitata shows fumarate reduction associated with ATP generation. The parasite is reported to, be cyanide insensitive, lack characteristic cytochromes, possess two quinones Q_6 and Q_8 and a branched electron transport system (11-14). The existence of phosphoenolpyruvate succinate-glyoxylate pathway has also been reported (15). The fumarate reductase system of S.digitata is studied here.

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Materials and Methods

S.digitata located in the peritoneal cavity of cattle Bos indicus, was collected in Tyrode solution (NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.01%, $NaHCO_3$ 0.015%, Na_2HPO_4 0.05%, glucose 0.5%) from the local abattoir. The worms freed from extraneous materials were used. The weighed live worms were homogenized in 0.25 M sucrose containing 0.1% bovine serum albumin (10 ml/g wet weight) under ice cold conditions and the mitochondria like particles (MLP) were separated by differential centrifugation (11).

Fumarate reductase activity was measured spectrophotometrically at 340 nm by following the disappearance of reduced pyridine nucleotide. The reaction mixture consisted of 25 mM Hepes buffer, pH 7.5, 0.4 mM NADH and MLP fraction in a closed cuvette. The anaerobic condition was expressed by the lack of NADH oxidation (stability of the absorption). The reaction was started by addition of fumarate in the closed cuvette (16,17). The activities of Malic enzyme, Succinate dehydrogenase, Succinate dehydrogenase-coenzyme Q reductase and also ATP generation were assayed by standard methods (18-21). Protein estimation was carried out by Folin's method (22).

Antimycin A, 2-thenoyl trifluoroacetone (TTFA), O-Hydroxydiphenyl (OHD), NADH, NADPH, fumaric acid and Diethylcarbamazine (DEC) were purchased from Sigma Chemicals, U.S.A. Rotenone was a gift from Prof.T.Ramasarma, I.I.Sc. Bangalore.

Results

The different fractions of the subcellular particles were tested for fumarate reductase activity and the MLP fraction had the maximum activity. Table I shows the activities of different enzymes. The effect of different substrates on the activity of fumarate reductase are shown in Table II. The effect of various electron transfer chain inhibitors on ATP generation and fumarate reduction are given in Table III.

Table I . Activities of different enzymes

Enzymes	Coenzyme	Specific activity (n moles/min./mg protein)
Fumarate reductase	NADH	357
" "	NADPH	No activity
Malic enzyme	NAD^+	753
" "	$NADP^+$	910
Succinate dehydrogenase	-	356
Succinate dehydrogenase-coenzyme Q reductase	-	53

Table II. The effect of Malate, Succinate, Citrate, ADP, and Pi on the activity of fumarate reductase

Substrate	Concentration (mM)	% Activity*	% Activation/ Inhibition
ADP	2.0	111	11+
	4.0	168	68+
Pi	2.0	107	7+
	6.0	125	25+
ADP + Pi	2+6	150	50+
	4+6	180	80+
ATP	2.0	55	45-
	4.0	29	71-
	6.0	0	100-
Succinate	2.0	100	Nil
	4.0	40	60-
Citrate	1.5	94	6-
	5.0	62	38-
Malate	0.8	125	25+
	3.0	68	32-

* Expressed as a % of fumarate reductase activity (considered to be 100) using 1 mM fumarate and 0.4 mM NADH.
+ Activation; - Inhibition.

Discussion

Fumarate reductase in S.digitata shows high activity and is a NADH dependent enzyme complex, unlike in the case of cestodes where both NADH and NADPH dependent activities were reported (8,9). The Km for fumarate is 1.47 mM and that for NADH is 0.33 mM.

The addition of ADP, Pi, or (ADP + Pi) activates fumarate reductase while, ATP inhibits the activity. This shows the coupling of ATP generation to the enzyme activity. Succinate and citrate inhibition of enzyme activity may be due to product inhibition (15).

Table III. Effect of inhibitors on ATP generation and fumarate reduction

Inhibitor	Concentration/ mg protein	% Inhibition of fumarate reduction	% Inhibition of ATP formation
Rotenone	31.25 p moles	100	100
Antimycin A	0.5 µg	100	100
OHD	70 µ moles	75	75
TTFA	0.15 mM	75	ND
DEC	16 mM	100	ND

ND - Not done
Using 1 mM Fumarate and 0.4 mM NADH,
Fumarate reduced = 122 n moles/min/mg protein
ATP formed = 134 n moles/min/mg protein.

The fumarate reductase system is the key to malate dismutation (23). As in the case of Spirometra mansonoides (8) a highly active NAD^+ and $NADP^+$ dependent malic enzyme is present in S.digitata. The activity of fumarate reductase was found to decrease with increasing concentrations of malate. Malic enzyme activity produces NADH which drives the reductive step, malate to succinate (15).

The sensitivity towards rotenone shows its dependence on complex I. Anaerobic phosphorylation coupled to fumarate reduction in A.lumbricoides, F.hepatica, S.mansonoides, and Hymenolepis diminuta (1,24) also shows sensitivity to rotenone.

The sensitivity towards antimycin A by fumarate reductase may be due to the presence of b type cytochromes in the complex (13). The fumarate reductase system in A.suum has been shown to be a flavoprotein cytochrome b_{558} complex (23,25). The antimycin A inhibitory effect on fumarate reduction involving fumarate reductase and cytochrome 552, 556 has been reported in Moniezia expansa (26).

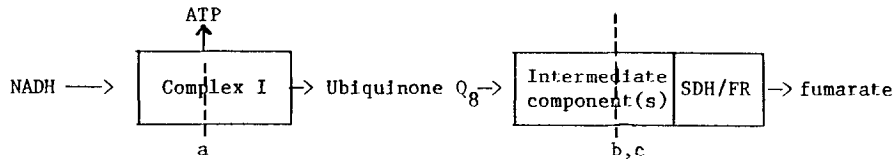
The inhibitory action of TTFA on fumarate reductase shows the presence of non-heme iron containing sites in the enzyme complex. The oxidation of NADH by fumarate is more sensitive to TTFA than the oxidation of succinate (13,27).

OHD, the specific inhibitor of cytochrome o (b type cytochrome) inhibits the enzyme activity. In Ascaris, the cyanide insensitive pathway is linked to cytochrome o, which is sensitive to OHD. 12 μ mole of OHD/mg protein completely inhibited electron transfer in Ascaris (28) while in Setaria it caused only 75% inhibition even at higher concentrations showing a basic difference between the two systems. DEC, the specific drug for filariasis, also inhibits enzyme activity.

The ratio of succinate dehydrogenase to fumarate reductase in S.digitata is 1, indicating an important physiological role for fumarate reductase. This ratio is 0.28 in aerobic Trypanosoma cruzi (29) and 2.0 in the facultative parasitic nematode A.lumbricoides (30). The ratio of succinate dehydrogenase-coenzyme Q reductase to fumarate reductase in S.digitata is 0.15, while it is 1.05 in A.suum egg mitochondria (25).

The branched nature of the electron transport system with different terminal acceptors in parasites has been proposed by Bryant and Behm (23). A similar system has been proposed for S.digitata also (13,14). The findings in the present study are summarised in scheme-1.

The amount of NADH consumed is almost equal to the ATP generated as is clear from Table III. The NADH linked fumarate reduction results in a



SDH - Succinate dehydrogenase
FR - Fumarate reductase
----Inhibition

a - Rotenone
b - Antimycin A
c - o-Hydroxy diphenyl

Scheme 1

site I electron transport associated phosphorylation of ADP which is sensitive to rotenone. The NADH coenzyme Q reductase (Sivan and Kaleysa Raj - unpublished data) shows the same sensitivity to rotenone. ATP generation in S.digitata is sensitive to rotenone, antimycin A and OHD. ATP generation coupled to fumarate reduction is of great biological importance. Under anaerobic conditions, fumarate reductase plays a key role in the electron transport system in transferring electrons to fumarate.

The results show that the fumarate reductase system is a complex composed of NADH-coenzyme Q reductase, succinate dehydrogenase/fumarate reductase and another component similar to cytochrome b termed intermediate component as in the case of Ascaris and Tubifex species (25,31). Therefore, it is postulated that the electrons flow from NADH via NADH coenzyme Q reductase and intermediate component to succinate dehydrogenase/fumarate reductase resulting in the reduction of fumarate to succinate. In many endoparasitic worms (5,17,25,26,32) the component has been identified as a cytochrome related to the b type.

Although the complete elucidation of the proposed mechanism of the electron transport chain in S.digitata, under anaerobic conditions is yet to be completed, even with the available information, it can be stated that it is different from that of mammalian systems. Hence specific inhibitors of the system may prove useful in the effective control of the filarial parasite and thereby the disease, filariasis itself.

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