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OXIDATIVE PHOSPHORYLATION COUPLED TO ELECTRON TRANSFER IN THE FILARIAL PARASITE SETARIA DIGITATA

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The filarial parasite of Bos indicus, Setaria digitata is reported to have many unique characteristics such as cyanide insensitivity and mitochondrial hydrogen peroxide production. The latter is more sensitive to the alternative oxidase inhibitor salicylhydroxamic acid (SHAM). Studies on the generation of ATP molecules through mitochondriae in the presence of different substrates and inhibitors showed that the oxidative phosphorylation coupled to electron transport occurs mainly at site I and involves the participation of quinone Q8. Based on the data, a scheme for the filarial electron transport system is proposed in which the quinones have a central role. Hence inhibitors at the quinone level might prove to be effective targets for chemotherapy.

All reported parasitic helminths are oxygen conformers. Informations on oxidative phosphorylation in parasitic helminths are nil except Ascaris lumbricoides, Ancylostoma caninum, Monizia expansa, Hymenolepis diminuta, Fasciola hepatica and Schistosoma mansoni (1). These oxygen conformers possess multiple terminal oxidases, produce hydrogen peroxide (H_2O_2) during oxygen consumption and are insensitive to cyanide (2).

Setaria digitata, a filarial parasite of cattle Bos indicus recommended as a model system (3) for human filarial parasites is reported to be devoid of cytochromes, cyanide insensitive, possesses two quinones Q6 and Q8 and several other unique features(4,5). Further analyses with electron transport inhibitors on mitochondrial H_2O_2 production has shown the occurrence of a branched electron transport system (6). To flush out the electron transport system the mitochondrial ATP generation was studied in presence of known electron transport complex inhibitors.

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Material and Methods

S. digitata located in the peritoneal cavity of cattle was collected immediately after opening the abdomen in Tyrode solution from the local abattoir. The worms were freed from the extraneous materials by repeated washing using the same medium and kept in it at 37°C until use.

The weighed live worms were homogenized in 0.25 M sucrose containing 0.1% bovine serum albumin (10 ml/gm wet weight). The mitochondriae like particles (MLP) were separated by differential centrifugation (4). The ATP generated by MLP fraction was estimated (7) using substrates such as malate, succinate, fumarate and α-glycerophosphate. The MLP fractions were provided with ADP and inorganic phosphate. Protein estimation was carried out by Folin's method (8). Antimycin A, 2-thenoyltrifluoroacetone (TTFA), salicylhydroxamic acid (SHAM) and orthohydroxydiphenyl (OHD) were purchased from Sigma Chemicals, U.S.A. Rotenone and 2, 4 dinitrophenol (DNP) were a gift from Prof. Ramasarma. T, I.I.Sc., Bangalore.

Results

The amount of ATP generated by MLP fractions using substrates malate, fumarate and succinate was 150 n moles/min/mg protein irrespective of the concentration of the substrates while α-glycerophosphate produced exactly half the amount. The effect of inhibitors on ATP generation is given in table I. The concentration of the inhibitors used were reported for the complete inhibition of mammalian electron transport system. The effect of inhibitors were concentration dependent and the values given were of the maximum observed inhibition.

Discussion

The same amount of ATP generated by the MLP fractions with succinate, malate and fumarate indicated that the oxidative phosphorylation sites of electron transfer are the same for these substrates.

Table I. Percentage of inhibition by the different inhibitors

Inhibition	Concn/mg protein	% of inhibition			
		Succinate	Fumarate	Malate	Glycerophosphate
Rotenone	0.03 n moles	70	100	100	40
Antimycin A	0.5 µg	35	75	25	35
OHD	15 µmoles	30	75	30	45
SHAM	2.5 µmoles	25	25	25	50
TTFA	0.15 m M	70	35	20	20
DNP	2.5 m M	100	100	100	100

Malate oxidation which is dependent on NADH dehydrogenase complex (complex I) of electron transport chain showed the complete inhibition of ATP generation by rotenone. Partial inhibition shown by malate to antimycin A is due to its conversion to fumarate and succinate by fumarase and fumarate reductase respectively. Antimycin A sensitive fumarate reductase is present in S. digitata (6). Fumarate reduction is dependent on NADH (9) involving the complex I showed the inhibition to rotenone. Partial insensitivity shown by fumarate to antimycin A is due to the conversion to malate by fumarase effecting phosphorylation at site I. The inhibitory effect of rotenone on succinate pointed that the main site of phosphorylation is site I. α -Glycerophosphate enters the electron transport at the quinone level, and its generation of ATP is inhibited by rotenone and SHAM upto 50%. The amount of ATP inhibited by SHAM is the same for all substrates used.

Inhibition shown to OHD (specific inhibitor of cytochrome O) and SHAM (alternative oxidase inhibitor) by all substrates pointed to the branched nature of the electron transport system as detailed elsewhere (6).

Based on the inhibitory studies we have modified the scheme for the electron transport system in S. digitata as shown in figure 1. The electrons from malate and fumarate are transferred to quinone via the complex I, showing complete inhibition to rotenone in ATP generation involving the phosphorylating site I. The complex II inhibitor TTFA could not block fully the succinate mediated ATP production due to the occurrence of fumarate reductase. Glycerophosphate transfers its reducing equivalents to quinone via FAD and thus lacks the inhibition by rotenone in H_2O_2 production (6).

Branching of electron transport occurs at the quinone level. The electrons can either pass through the SHAM sensitive site or OHD sensitive site (figure 1). Since SHAM inhibited the ATP production in equal amount irrespective of the substrate, a fixed proportion of all the substrates might pass through that site and oxidative phosphorylation seems to occur. In S. digitata of the two quinones present Q_8 has been attributed to be associated with complexes I, II and fumarate reductase (6) with specific binding sites for each. The reducing equivalent from Q_8 can pass on to quinol oxidoreductase (6) sensitive to SHAM or to cytochrome O sensitive to OHD. The alternative oxidase isolated

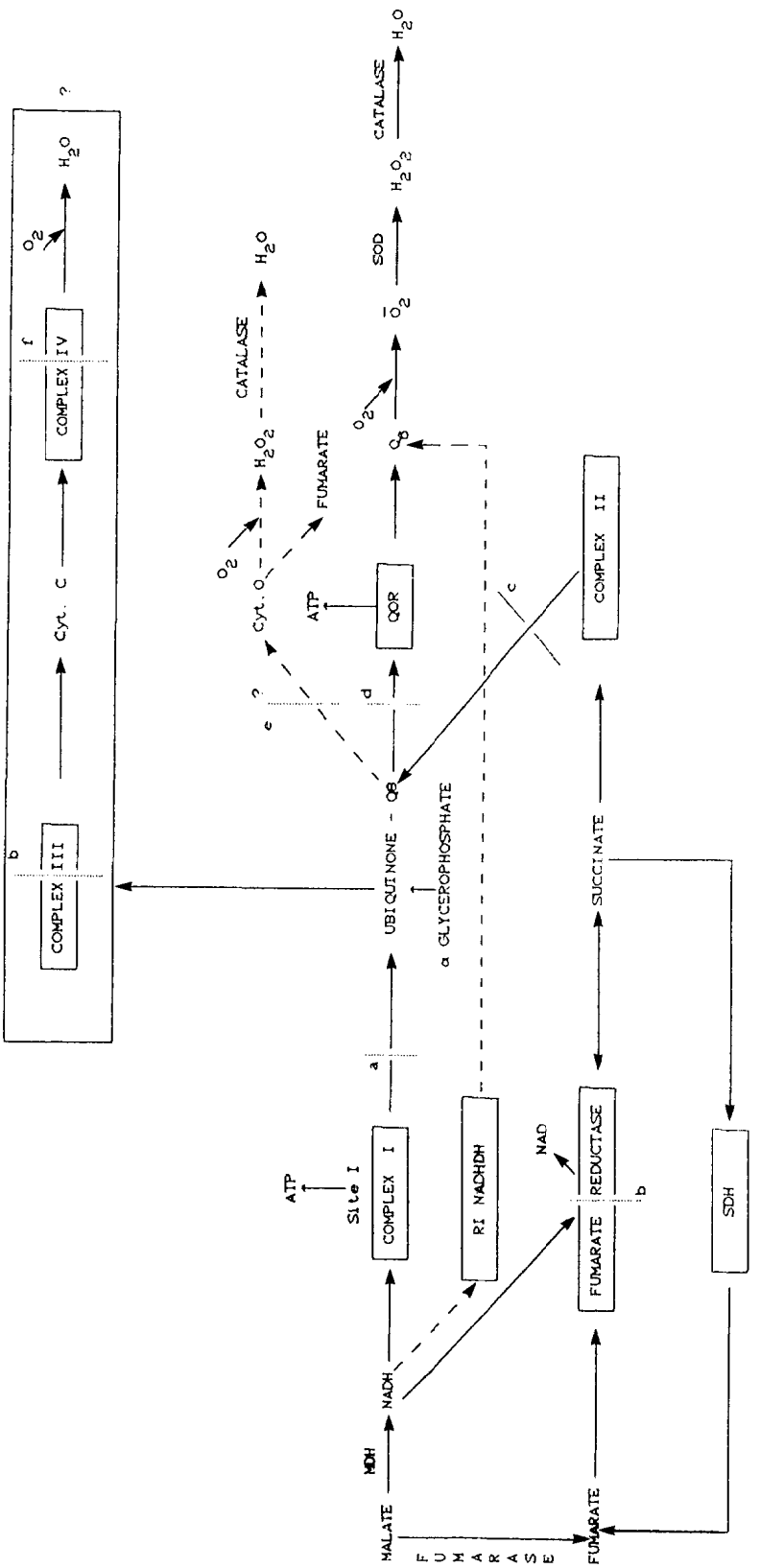

Figure 1. Proposed oxidative electron transport system in S. digitata.

——— major pathway; - - - - minor pathway; ..... inhibition.

a - rotenone; b - antimycin A; c - TTFA; d - SHAM; e - OHD; f - cyanide; QOR - quinol oxidoreductase; SOD - superoxide dismutase; SDH -succinate dehydrogenase; RINADHDH - rotenone insensitive NADH dehydrogenase.

from Arum maculatum has been characterized as quinol oxidoreductase sensitive to SHAM and resistant to cyanide and antimycin A (10, 11). The site between Q_8 and quinol oxidoreductase seems to couple ATP in S. digitata. From quinol oxidoreductase the electrons are finally accepted by auto-oxidizable quinone Q_6 producing H_2O_2 (6).

The electrons accepted by cytochrome O from quinone can pass either to O_2 or to fumarate. Fumarate is reported to act as a terminal electron acceptor, in which no $H_2O_2$is formed (12). Further in helminth systems under anaerobic conditions reduced cytochrome O is reoxidised by the addition of fumarate (1). The acceptance of electrons by fumarate effects reduction due to fumarate reductase involving the complex I which in turn is inhibited by rotenone. Cytochrome O might be an intimate part of the fumarate reductase system functioning as the link between the enzyme and the rest of the chain (1). This explains the rotenone sensitivity of succinate and α-glycerophosphate. When O_2 accepts the electrons from cytochrome O, H_2O_2 is formed.

Since all the substrates could transfer their reducing equivalents as shown in figure 1, showed the inhibition to TTFA and antimycin A.

2,4 dinitrophenol, the uncoupler of oxidative phosphorylation inhibited the ATP production completely for all substrates indicating that the ATP generated is through electron transport.

Q_6 acts as an electron acceptor from rotenone insensitive NADH dehydrogenase (13, 14). In S. digitata oxidative phosphorylation occurs at two sites, one at site I common to all organisms and at SHAM sensitive site which is not reported for any organism.

For every reducing equivalent from succinate, fumarate and malate one molecule of ATP is formed at site I and the same electron can generate another ATP at SHAM sensitive site or can pass through to cytochrome O and again couple ATP at site I. Thus two molecules of ATP are formed for one reducing equivalent. But in the case of α-glycerophosphate, as electrons enter at the quinone level, only one molecule of ATP is formed either at site I (passing through cytochrome O and couple ATP at site I) or at SHAM sensitive site. The studies on electron transport system of S. digitata thus clearly point out very specific targets of attack for effective control of filariasis.

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