Vol. 174, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1991 Pages 386-392

QUINONE MEDIATED ELECTRON TRANSPORT SYSTEM IN THE FILARIAL PARASITE SETARIA DIGITATA

SANTHAMMA.K.R. AND KALEYSA RAJ.R.

DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF KERALA,
KARIAVATTOM, TRIVANDRUM 695 581, INDIA

Received November 30, 1990

Setaria digitata, a cattle filarial parasite, is known to have peculiarities such as hydrogen peroxide (H_2O_2) production, cyanide insensitivity, absence of cytochromes and presence of quinones. Estimation of mitochondrial H_2O_2 with different substrates and inhibitors showed that salicylhydroxamic acid (SHAM), the alternative oxidase inhibitor, inhibited the H_2O_2 production maximally. Based on the inhibitory studies with rotenone, antimycin A, o-hydroxydiphenyl, SHAM and 2 thenoyltrifluoroacetone, a mechanism for the electron transport is proposed. Quinone Q_8 seems to have a central role, hence inhibitors at the level of quinones might prove to be effective in designing drugs for filariasis.

Parasitic helminth species so far studied, use oxygen when it is available, at least under in vitro conditions and show a number of peculiar characteristics (1). In Ascaris lumbricoides, Fasciola hepatica and Monieza expansa, the rate of oxygen uptake can be attributed to the functional cytochrome system possessed by them (2,3). The electron transport chain of these parasites differ from mammals in having multiple terminal oxidases (2,3,4). In aerobic respiration of helminths electrons are transported along a chain of electron carriers either to cytochrome oxidase or to alternative oxidases (5). Instead of the common ubiquinone as electron carrier, many helminths possess rhodoquinone capable of anaerobic electron transport mediated respiration (6).

The cattle filarial parasite Setaria digitata possesses rare features, like absence of cytochromes, cyanide insensitivity, H_2O_2 production, presence of two quinones Q_8 and Q_6 etc (7,8). Therefore, we have restricted our studies on mitochondrial H_2O_2 generation in this parasite using different substrates in the presence and absence of inhibitors, which give clues to the nature of electron transport.

Materials and Methods

S.digitata, collected from the local slaughter house, were suspended in modified tyrode solution (NaCl 0.8%, KCl 0.02%; $CaCl_2$ 0.02%; $MgCl_2$ 0.01%;

0006-291X/91 $1.50

$NaHCO_3$ 0.015%; Na_2HPO_4 0.05% and glucose 0.5%) and freed from the host material by repeated washing using the same medium. The worms were maintained in tyrode solution at 37°C until use.

Live worms were blotted dry and homogenized in 0.25 M sucrose containing 0.1% bovine serum albumin (10 ml/gm wet weight). The 'mitochondria like particles' (MLP) were separated by differential centrifugation (8). H_2O_2 assay was done (9) using succinate, α glycerophosphate, fumarate and malate as substrates for MLP fractions. Protein estimation was carried out using Folin's method (10).

Antimycin A, 2 thenoyltrifluoroacetone (TTFA) salicylhydroxamic acid (SHAM) and o-hydroxydiphenyl (OHD) were purchased from Sigma Chemicals, U.S.A. Rotenone was a gift from Prof. T.Ramasarma, I.I.Sc., Bangalore.

Results

Succinate dependent H_2O_2 production in the MLP was found to be maximum. The production of H_2O_2 is continued for a long time with succinate or fumarate while it ceases after some time with α glycerophosphate or malate as the substrate. Table 1 shows the amount of H_2O_2 produced by different substrates and their concentration for maximal activity. Table 2 shows the effect of different inhibitors on mitochondrial H_2O_2 generation. The percentage of inhibition by the different mammalian electron transport inhibitors were at the concentrations reported for complete inhibition. The effect of inhibitors was concentration dependent and the values reported are of the maximum inhibition observed.

Discussion

The succinate dependent H_2O_2 generation through MLP was the maximum and the Km value for succinate was found to be the same for H_2O_2 generation and succinate dehydrogenase (SDH) activity (7) indicating that the same dehydrogenase was participating in both systems.

Rotenone inhibits the NADH dehydrogenase complex (Complex I) of mammalian electron transport chain (11). Rotenone completely inhibited the H_2O_2 production by fumarate in S.digitata which indicates the involvement of

Table 1. H_2O_2 generation in MLP fractions

Substrate	Concn. (m moles)	P mole of H_2O_2 generated/ min/mg protein*
Succinate	6	950 ± 84
Fumarate	4	594 ± 62
Malate	2	235 ± 18
α Glycerophosphate	1	118 ± 12

* Average of eight experiments.

Table 2. Percentage of inhibition by the different inhibitors

Inhibitors	Conc. / mg protein	Percentage of inhibition*			
		Succinate	Fumerate	Malate	Glycero-phosphate
Rotenone	0.03n moles	25	100	50-80	NIL
Antimycin A	0.5 μg	35	100	30	NIL
TTFA	0.15 mM	65	50	25	NIL
SHAM	2.5 μ moles	65	70	80-90	90-100
OHD	15 μ moles	30	30	30	30

* Average of eight experiments.

complex I. NADH dependent fumarate reductase was observed in S.digitata with notable activity (12). Since reducing equivalents from α glycerophosphate are transferred to quinone via FAD, the transfer is not inhibited by rotenone. Fumarate formed by succinate oxidation which undergoes reduction by fumarate reductase explains the continuous production of H_2O_2 by the administration of small quantities of succinate or fumarate. An incomplete inhibition of malate by rotenone pointed to a rotenone insensitive electron transfer in S.digitata. Rotenone insensitive endogenous NADH dehydrogenase which oxidize NAD^+ linked substrates has been reported in yeast and high plant mitochondriae through different pathways (13).

The inhibitory action of antimycin A on succinate, malate and α glycerophosphate showed that an antimycin insensitive electron transfer takes place in S.digitata, or a very low level of a mammalian type of cytochrome b is present. But S.digitata was reported to be devoid of any detectable cytochromes (7). The fumarate mediated H_2O_2 production was completely inhibited by antimycin A providing a chance for the occurrence of b type cytochromes in the parasite in a different position in the electron transfer route from mammals. The fumarate reductase is a flavoprotein cytochrome b_{558} complex (14). The antimycin A inhibitory effect on fumarate reduction involving fumarate reductase and cytochrome 552 556 has been reported in M.expansa (15). The 30% inhibition of malate by antimycin A explains that malate can also be converted to fumarate due to the presence of fumarase. Succinate gets converted to fumarate which in turn undergoes reduction to produce succinate showed partial inhibition. α Glycerophosphate cannot be converted to fumarate and lacks the inhibition by antimycin A.

TTFA, the specific SDH-Coenzyme Q reductase inhibitor (16), cannot completely block the oxidation due to the formation of fumarate which in turn gets reduced to succinate effecting transfer of electrons. Likewise, by using fumarate as substrate the succinate formed by reduction undergoes oxidation through SDH-Coenzyme Q reductase effecting the inhibition by TTFA.

Table 2 showed that SHAM was not able to inhibit succinate, malate and fumarate mediated H_2O_2 production completely. The action of these substrates by fumarate reductase and other respective enzymes pave the way for multiple channel for electron transfer.

OHD, the specific inhibitor of cytochrome O, inhibited all the substrates to a comparable level. In Ascaris, the cyanide insensitive pathway is linked to cytochrome O which is sensitive to OHD and 12 μ moles/mg protein completely inhibited the electron transfer (4). It is assumed that cytochrome O is acting as the terminal oxidase when helminths consume oxygen as cytochrome O is auto-oxidisable and its reaction product with O_2 is H_2O_2(1). S.digitata gave only one third reduction of H_2O_2 production by OHD providing ample evidence for specific alternative system which is more sensitive to SHAM and differs from other helminths which lacks ubiquinone.

Based on all the available informations (7,8) about the peculiar characteristic nature and the H_2O_2 inhibitory studies in S.digitata, a mechanism for the production of H_2O_2 involving the quinones and complexes with multiple terminal electron acceptors is shown in Figure 1.

Rotenone sensitive and insensitive NADH dehydrogenase can transfer its electron to ubiquinone. Q_8 was attributed to NADH oxidase in S.digitata (8). Electrons from succinate pass through the complex II to ubiquinone Q_8 and α glycerophosphate enters the chain at the quinone level. In S.digitata the complexes I, II and fumarate reductase seems to associate with Q_8 with specific binding sites for each and all are linked by the quinone/ hydroquinone system in a modified Q cycle. Two different sites for ubiquinone in NADH-Q reductase and succinate Q reductase have been formulated (17, 18, 19, 20). The electrons from Q_8 can pass on to the alternative oxidase system sensitive to SHAM, or to cytochrome O sensitive to OHD. In S.digitata, electron transfer seems to take place mostly through the SHAM sensitive site. SHAM is reported to inhibit the oxygen uptake and appear to block electron transport between ubiquinone and the oxidase (21). In African trypanosomes oxygen uptake is insensitive to cyanide and sensitive to SHAM and presents evidence for an intermediary ubiquinone like molecule (22). The alternative oxidase isolated from Arum maculatum has been characterized as quinol oxidoreductase sensitive to SHAM and resistant to cyanide and antimycin A (23,24).

The alternative oxidase in S.digitata might be a quinol-oxidoreductase which was sensitive to SHAM and insensitive to cyanide and antimycin A. From this oxidase, the electrons are finally accepted by

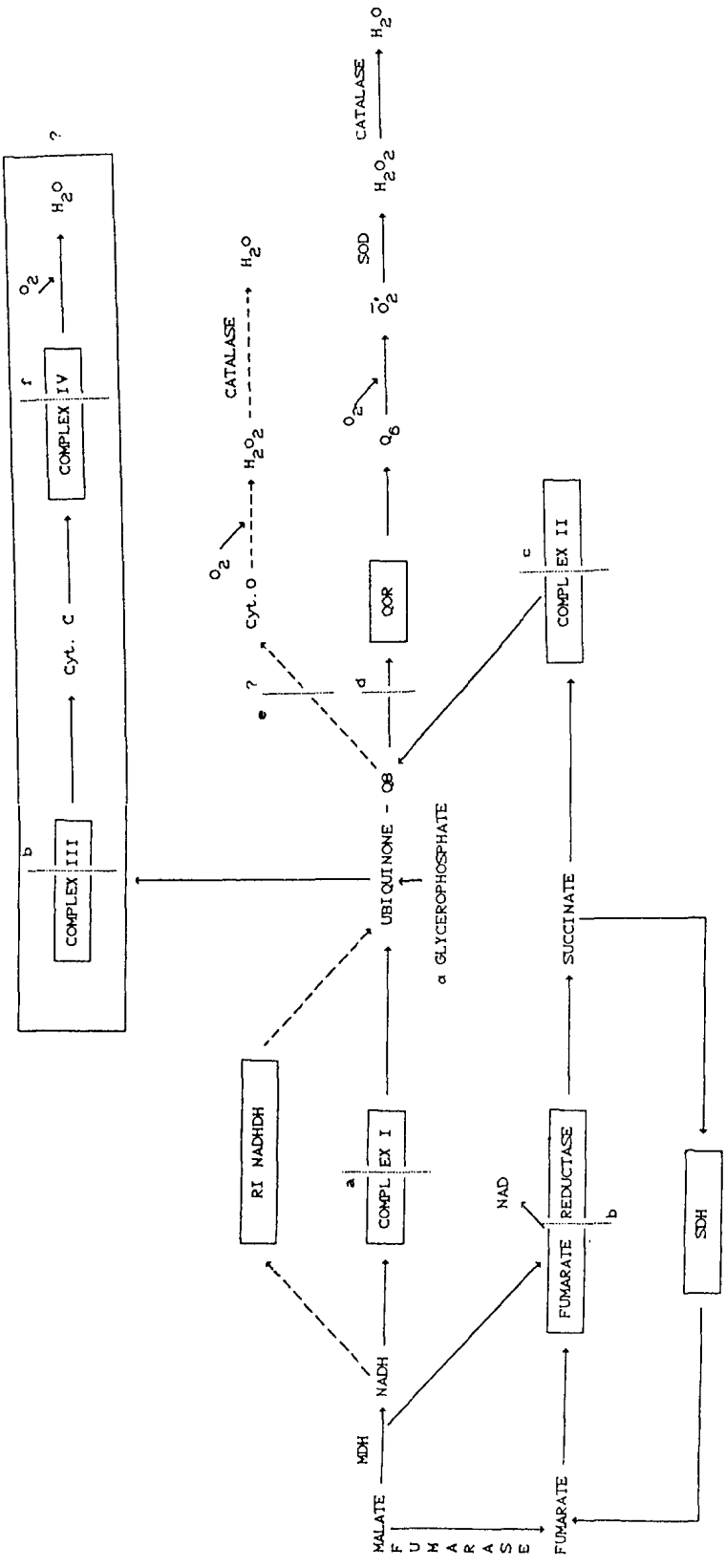

Figure 1. Proposed electron transfer system mediated by quinone. —— major pathway;-----minor pathway;....inhibition. a-rotenone; b-antimycin A; c-TTFA; d-SHAM; e-OHD; f-cyanide; QOR-quinol oxidoreductase; SOD-superoxide dismutase; SDH-succinate dehydrogenase. RINADHDH-rotenone insensitive NADH dehydrogenase.

the auto-oxidizable quinone Q_6 by the production of $\bar{O}_2^{\cdot}$ and H_2O_2. The production of $\bar{O}_2^{\cdot}$ and H_2O_2 during auto-oxidation of quinols in oxygenated buffers have been reported (25). Many of the adult parasitic helminths possesses relatively low activities of aerobic electron transport system which are comparable in function to those of obligate aerobes (14). Catalase present in the parasite (7) ensures detoxification of H_2O_2.

The presence of both aerobic and anaerobic equipment for the parasite seems to be its 'biochemical adaptation' to survive in a fluctuating environment. The inhibitory analyses of H_2O_2 production through mitochondria showed that the quinones have a central role in the transfer of electrons. Hence specific blockers/drugs at the quinone level would prove to be effective in controlling filariasis.

Acknowledgments

The authors wish to thank Prof. Ramasarma, Indian Institute of Science, Bangalore for active interest and helpful criticisms. This work was supported by the Department of Science and Technology, New Delhi and University of Kerala.

References

1. Barret, J. (1981) Biochemistry of Parasitic Helminths, 109-125, Academic Press, Hongkong.
2. Cheah, K.S. (1975) Comp. Biochem. Physiol. 51B, 41-45.
3. Cheah, K.S.(1975) Biochem. Biophys. Acta. 387, 107-114.
4. Cheah, K.S. (1976) Biochemistry of Parasites and Host parasite, relationships (H.Van den Bossche, Ed.) pp. 133-143, Elsevier/North-Holland and Biomedical Press, Amsterdam.
5. Cheah, K.S. (1972) Comparative Biochemistry of Parasites (H.Van den Bossche, Ed.). pp.417-432, Academic Press, London.
6. Kohler, P.(1985) Mol. Biochem. Parsitol. 17, 1-18.
7. Kaleysa Raj.R., Puranam, R.S., Ramakrishna Kurup,C.K., and Ramasarma, T., (1988) Biochem. J. 256, 559-564.
8. Santhamma, K.R., and Kaleysa Raj, R. (1990) Biochem. Biophys. Res. Commun. 167, 568-574.
9. Swaroop, A., and Ramsarma, T. (1981) Biochem. J. 194, 657-665.
10. Lowry, O.H., Rosenbrough, N.J., Farr, A.C., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
11. Slates, E.C. (1967) in Methods in Enzymology (R.W.Estabrook and M.E. Pullman, Ed.) Vol.10, 48-57, Academic Press, New York and London.
12. Rafi, M.M. (1990) Ph.D. Thesis submitted to the University of Kerala, Trivandrum, India.
13. Palmer, J.M.(1976) Ann, Rev.Plant.Physiol. 44, 625-630.
14. Bryant, C., and Behm, C.(1989) Biochemical Adaptation in Parasites pp. 25-245.
15. Cheah, K.S. (1967) Comp. Biochem. Physiol. 23, 277-302.
16. Ziegler, D., and Rieske, J.S. (1967) in Methods in Enzymology (R.W.Estabrook and M.E.Pullman) Vol.10, pp. 231-235, Academic Press, New York and London.

17. Lenaz, G., Daves, D.G., and Folkers, H. (1968) Arch. Biochem. Biophys. 123, 539-550.
18. Lenaz, G., Castelli, A., Littarru, G.P., Bertoli, E., and Folkers, K. (1971) Arch. Biochem. Biophys. 142, 403-416.
19. Lenaz, G., Pasquali, P., Bertoli, E., Parenti-Castelli, G., and Folkers, K. (1975) Arch. Biochem. Biophys. 169, 217-226.
20. Castelli, A., Lenaz.G., and Folkers, K. (1969) Biochem. Biophys. Res. Commun. 34, 200-204.
21. Meshnick, S.R. (1984) in Parasitic Diseases (M.Dekker Ed.) Vol. 2, pp. 165-199, New York.
22. Turrens, J.E., Bickar, D., and Lehninger, A.L. (1986) Mol. Biochem. Parasitol. 19, 259-264.
23. Hua, S., and Palmer, J.M. (1978) FEBS Lett 95, 217-220.
24. Rich,P.R. (1978) FEBS Lett. 96, 252-256.
25. Cadenas, E., Boveris, A., Ragam, C.I. and Stoppani, A.O.M. (1977) Arch. Biochem. Biophys. 180, 248-257.