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LACTATE OXIDATION COUPLED TO ENERGY PRODUCTION IN MITOCHONDRIA LIKE PARTICLES FROM *SETARIA DIGITATA*, A FILARIAL PARASITE

V.M. SIVAN AND R. KALEYSA RAJ
Department of Biochemistry, University of Kerala,
Thiruvananthapuram - 695 581, India

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In the filarial parasite, *Setaria digitata*, the mitochondria like particles (MLP) show NAD reduction with sodium lactate. The MLP also reduces dye and ferricyanide with lactate. The ferricyanide reduction by lactate is found to be sensitive to the cytochrome o inhibitor orthohydroxy diphenyl (OHD) and complex I inhibitor rotenone, modulated by ADP (+) and ATP (-) and inhibited by pyruvate and oxaloacetate. MLP shows lactate oxidation sensitive to OHD, rotenone and sodium malonate. Thus, the lactate utilizing complex system, consisting of an NADH generating MLP bound lactate dehydrogenase and a lactate flavocytochrome reductase tightly linked to complex I and cytochrome o, produces ATP in functional association with fumarate reductase complex and other enzyme systems. Hence, this study provides new dimensions to the study of metabolism in filarial parasites.

Most of the parasitic helminths have the ability to produce lactate (1) which is considered to be the main excretory product. The enzymes leading to the formation of lactate have been observed in *Brugia pahangi*, *Dipetalonema viteae*, *Litomosoides carinii* (2), *Dirofilaria immitis*, *Draucunculus uniformis*, *Chandlerella hawkengi* and *Setaria cervi* (3-6). However, the well studied *Ascaris lumbricoides* and *Taenia taeniaeformis* systems possessing active cytoplasmic lactate dehydrogenase demonstrates insignificantly low amount of lactate excretion (7,8) and, succinate rather than lactate is the main excretory product in *Hymenolepis diminuta*, *Fasciola* (9) and in some other medically important parasitic helminths (1,7) thus paving the way to speculate on the probable ways of lactate utilization in parasitic helminths.

The electron transport system of *Setaria digitata*, the filarial parasite of cattle, *Bos indicus*, recommended as a model system for human filarial parasites (10), is branched and possesses rotenone sensitive and insensitive pathways for NADH oxidation and anaerobic and aerobic pathways for substrate utilization (11) and is characterized by, the presence of, two quinones, Q_8 and Q_6, transhydrogenases and fumarate reductase, the absence of typical cytochromes and pyruvate dehydrogenase complex (12-15). The pyruvate formed as a result of glycolysis can be utilized by the highly active cytosolic lactate dehydrogenase (16) to form lactate. The mitochondrial utilization of the lactate so formed and its contribution to the energy need of the parasite are the focus of this study.

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Materials and Methods

The mitochondria like particles (MLP) were isolated from live worms by differential centrifugation, after homogenisation in 0.25 M sucrose (10 ml/gm wet wt) (13), with slight modifications. The homogenate was first centrifuged twice at 4000 g in 0.25 M sucrose and the pellet discarded. The pellet collected from the supernatant at 12000 g was saved, thoroughly washed 4 times in 0.25 M sucrose and finally suspended in sucrose (0.25 M) and used for the studies.

The oxygen uptake studies with MLP in buffer (17) were carried out using Gilson Oxygraph. 0.33 mg protein was used for each assay. The system contained 18 mM lactate/mg protein. The lactate dependent NAD reduction by the MLP was assayed spectrophotometrically at 340 nm using Na^+/K^+ phosphate buffer containing 1 mM EDTA, pH 7 (18). 0.06 mg protein was used for each assay. The system contained 50 mM lactate and 4 mM NAD. Lactate flavocytochrome reductase was assayed at 420 nm as ferricyanide reduction by the standard method (18) using the same buffer system. The 1 ml system used in the assay contained 50 mM lactate and 0.5 mM ferricyanide. The same buffer system is used for all other enzyme assays. Standard methods were adopted to follow succinate/lactate DCIP reduction by the MLP, spectrophotometrically at 600 nm, in presence of PMS (19) or quinone (20). The protein was estimated by Folin's method (19).

DL-sodium lactate was purchased from Loba Chemie Ltd, India and all other biochemicals used including L-lactate were purchased from Sigma Chemical Co., U.S.A.

Results and Discussion

The mitochondria like particles (MLP) isolated from *S. digitata* showed NAD reduction with lactate (Table 1). The activity was not only retained, but also showed a marginal increase on repeated washing thus showing the presence of an MLP bound lactate dehydrogenase (MLP-LDH).

MLP showed oxygen uptake with lactate (Table 1). Thus, the anaerobic glycolytic product lactate can be utilized by the MLP through the aerobic route with oxygen as the terminal acceptor even in the absence of detectable pyruvate dehydrogenase complex in the *Setaria* system (15).

The MLP reduces ferricyanide and dye with lactate (Tables 1, 6). Unlike the ferricyanide reduction by MLP with NADH, which is insensitive to rotenone and antimycin A (11) as in yeast (22,23), the ferricyanide reduction by lactate is found to be sensitive to the

Table 1

Activity tested	Test method	Activity*
Lactate dehydrogenase	NAD reduction	16#
Lactate oxidation	Oxygen uptake	23**
Lactate flavocytochrome reductase	ferricyanide reduction	130@

Buffer system as in materials and methods.
* Average of six experiments.
Activity expressed in
n moles of NAD reduced/min/mg protein.
** n atoms of oxygen consumed/min/mg protein.
@ n moles of ferricyanide reduced/min/mg protein.

Table 2 Effect of inhibitors on lactate flavocytochrome reductase*

Inhibitor	Concentration/mg protein	% Inhibition
Rotenone	0.17 n moles	68
OHD	0.20 mM	60

* % Enzyme activity is considered to be 100.
OHD - Orthohydroxy diphenyl.
The enzyme preincubated with rotenone/OHD for 10 minutes.

complex I inhibitor rotenone and the cytochrome o specific inhibitor orthohydroxy diphenyl (OHD) (Table 2). Oxygen uptake with lactate is also sensitive to rotenone and OHD (Table 3). This indicates the involvement of complex I and cytochrome o (b type cytochrome) like component. Thus all indications are that this may be a lactate flavocytochrome reductase similar to the one reported in yeast (18,24). The sensitivity to rotenone and OHD shows that the lactate flavocytochrome reductase of *S. digitata* MLP transfers electrons from lactate to cytochrome o through flavin, Fe-S centers and ubiquinone (Scheme I) and, there has to be a tight active linkage of the enzyme with ubiquinone and cytochrome o as a single functional unit and thus the transfer of electrons to ferricyanide occurs mainly after the level of cytochrome o. Interestingly, the post MLP supernatant did not show ferricyanide reduction with lactate (Table 6).

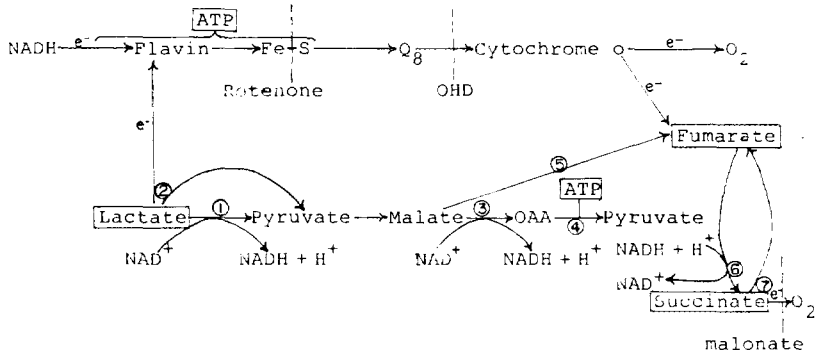
The lactate flavocytochrome reductase activity is stimulated by ADP and inhibited by ATP (Table 4). This gives a clear indication of the involvement of this system in the effective utilization of lactate for the production of ATP.

Unlike lactate and succinate, pyruvate, fumarate and oxaloacetate did not show ferricyanide reduction with MLP (Table 6) but at the same time, ferricyanide reduction by lactate was inhibited by pyruvate and oxaloacetate (Table 5). Thus, the inhibition by pyruvate may be product inhibition and in the absence of pyruvate dehydrogenase complex, the pyruvate produced from lactate can be converted to malate by various routes (25) which can be finally converted to fumarate by fumarase present in MLP (15). Thus the electrons transferred to cytochrome o can be accepted by fumarate and by utilizing the NADH produced by the NAD reducing enzyme

Table 3 Effect of inhibitors on lactate oxidation*

Inhibitor	Concentration per mg protein	% Inhibition
Rotenone	0.08 μ moles	52
OHD	0.16 mM	52
Sodium malonate	12.0 mM	50

* % lactate linked oxygen uptake is considered to be 100.
MLP preincubated with rotenone/OHD for 10 minutes.



1. MLP-Lactate dehydrogenase, 2. Lactate flavocytochrome reductase
3. Malate dehydrogense, 4. Pyruvate carboxylase, 5. Fumarase,
6. Fumarate reductase complex, 7. Succinate dehydrogense
OAA - Oxaloacetate, OHD - Orthohydroxydiphenyl, ----- inhibition

Scheme I

activity of the MLP, fumarate may be reduced to succinate by the highly active NADH fumarate reductase (14) with generation of ATP as hypothesised in Scheme I. The reduction of fumarate to succinate accompanied by Complex I level coupling has been reported in *S. digitata* (14). The inhibition of lactate oxidation by sodium malonate (Table 3) is an indication of succinate formation and its subsequent oxidation. The same concentration of sodium malonate inhibits succinate oxidation and fumarate reductase activity of the MLP (25). L-lactate also gave oxygen uptake, inhibited by sodium malonate (Data not shown).

The MLP showed DCIP reduction with lactate (Table 6). The dye reduction may be accounted for either by, lactate flavocytochrome reductase transferring electrons from lactate to the dye as in yeast (18) or succinate dehydrogenase transferring electrons from lactate derived succinate to the dye or both. Succinate DCIP reduction by MLP, in presence of PMS or quinone,

Table 4 Effect of ADP and ATP on lactate flavocytochrome reductase

Addition	Concentration (mM)	% stimulation/ inhibition
ADP	2.4	50+
ATP	0.8	13-
	3.2	34-
	4.8	45-

Each assay contained 0.06 mg protein.
+ Stimulation, - Inhibition.

Table 5 Effect of pyruvate and oxaloacetate on lactate flavocytochrome reductase#

Addition	Concentration (mM)	% Inhibition
Pyruvate	1.6	28
	2.4	40
Oxaloacetate	1.6	36
	3.2	50

% Enzyme activity is considered to be 100.
Each assay contain 0.06 mg protein.

is also given in Table 6. The purified beef heart LDH does not reduce dye even in presence of NAD (Table 6). Thus, lactate oxidising flavocytochrome system in *Setaria* is extremely unique, totally different from the mammalian host system and is exclusively MLP linked. Hence, study of this complex system and elucidation of its relation with the quinone system is promising and provides new dimensions to the role of lactate in *S. digitata* and gives a new insight into the biochemistry of medically important parasites in general.

Table 6

Assay	Activity* (n moles/min/mg protein)
MLP	
Lactate ferricyanide reduction	130
Succinate ferricyanide reduction	64
Pyruvate ferricyanide reduction	0
Fumarate ferricyanide reduction	0
Oxaloacetate ferricyanide reduction	0
(Lactate + PMS) DCIP reduction	87
(Succinate + PMS) DCIP reduction	100
(Lactate + quinone) DCIP reduction	24
(Succinate + quinone) DCIP reduction	43
PMST	
Lactate ferricyanide reduction	0
Beef heart LDH (Purified)	
(Lactate + PMS) DCIP reduction	0
(Lactate + NAD + PMS) DCIP reduction	0
Lactate NAD reduction	288

* Average of six experiments.
Buffer system (1 ml) as in materials and methods. 50 mM DL-sodium lactate, 0.5 mM ferricyanide, 0.05 ml 2% PMS, 0.5 mM 2,3-Dimethoxy-5-methyl-1,4-benzoquinone,0.04 µmole DCIP and 4 mM each of succinate, fumarate, oxaloacetate and NAD were used.
PMST - Post MLP supernatant, PMS - Phenazine methosulphate,
DCIP - 2,6-dichlorophenolindophenol.

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References

1. Smith, J.D. and Mc Mannus, D.P. (1989) The Physiology and Biochemistry of Cestodes, pp.77-113, Cambridge University Press, Cambridge.
2. Middleton, K.R. and Saz, H.J. (1979) J. Parasitol. 65, 1-7.
3. Hutchinson, W.F. and Turner, A.C. (1979) Comp. Biochem. Physiol. 62B, 71-73.
4. Von Brand, T., Bowman, I.B.R., Weinsten, P.P. and Savior, T.K. (1963) Exptl. Parasitol. 13, 128.
5. Srivastava, V.M.L., Ghatak, S. and Krishnamurthi, C.R. (1968) Exptl. Parasitol. 23, 338.
6. Anwar, N., Ansari, A.A., Ghatak, S. and Krishnamurthi, C.R. (1977) Comp. Biochem. Physiol. 29, 1135-1147.
7. Barrett, J. (1981) Biochemistry of Parasitic Helminths, pp.72-148, Macmillan Publishers Ltd., London.
8. Eugene, C., Weinbach and Von Brand, T. (1970). I, 1, 39-56.
9. Lee, R.M. and Vasey, H.M. (1970) Int. J. Biochem., 1, 274-280.
10. Hawking, F. (1978) Diethyl carbamazine, WHO/ONCHO, 78- 142.
11. Sivan, V.M. and Kaleysa Raj, R. (1992) Biochem. Biophys. Res. Commun., 186, 698-705.
12. Santhamma, K.R. and Kaleysa Raj, R. (1990) Biochem. Biophys. Res. Commun. 167, 568-574.
13. Kaleysa Raj, R., Puranam, R.S., Kurup, C.K.R. and Ramasarma, T. (1988) Biochem. J. 256, 559-564.
14. Unnikrishnan, L.S. and Kaleysa Raj, R. (1992) Biochem. Biophys. Res. Commun. 184, 448-453.
15. Rafi, M.M. and Kaleysa Raj, R. (1991) J. Bio. Sci. 16- 3, 121-126.
16. Hepsibah Thilagavathy (1989) Ph.D. thesis submitted to the University of Kerala.
17. Estabrook, R.W. (1967) in: Methods in Enzymology, Vol.10, pp.41-47.
18. Labeyrie, F., Baudras, A. and Lederer, F. (1978) in: Methods in Enzymology, Vol. 53, pp.238-239.
19. Susheela, L. and Ramasarma, T. (1971). Biochim. Biophy. Acta, 242, 532-540.
20. Ziegler, D. and Rieske, J.S. (1967). In: Methods in Enzymology, Vol.10, pp. 231-235.
21. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
22. Mackler, B., Haynes, B., Person, R. and Palmer, G. (1980). Biochim. Biophys. Acta, 591, 289-297.
23. Davis, K.A., Mackler, B., Haynes, B. Person, R., Bevan, C., Grace, R., Palmer, G. and Carter, K. (1982). Biochem. Int. 5, 49-58.
24. Baudras, A. and Spyridakis, A. (1971). Biochimie 53, 943.
25. Unnikrishnan, L.S. (1993). Ph.D. thesis submitted to the University of Kerala.