Vol. 205, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1994 Pages 24-29

BIOSYNTHESIS OF ISOPRENOID COMPOUNDS IN CATTLE FILARIAL PARASITE SETARIA DIGITATA

G. AJITHA KUMARI, K.R. SANTHAMMA AND R. KALEYSA RAJ

Department of Biochemistry, University of Kerala
Kariavattom, Trivandrum 695 581, Kerala, India

Received September 28, 1994

The biological significance of isoprenoid compounds such as ubiquinones, prenols and sterols have been well established. The presence and biological function of the two quinones Q_6 and Q_8 in the cattle filarial parasite Setaria digitata have already been reported. Inhibition of the function of quinone was already shown to be an effective means of controlling the filarial parasite. Detailed investigations of the non-saponifiable lipids from S. digitata using column, thin layer, reverse phase and high performance liquid chromatography showed the presence and formation of isoprenoid compounds such as prenols and sterols, in addition to the two quinones. Blocking the biosynthesis of these useful compounds may prove to be an additional means of control of filarial parasites.

Many compounds with isoprene side chain such as ubiquinone, rhodoquinone, farnesol, cholesterol, solanesol etc. are present in nematodes (1). Setaria digitata, a cattle filarial parasite is reported to be similar to human filarial parasite and WHO recommends it as a research model (2,3). The parasite is known to have peculiarities such as H_2O_2 production, cyanide insensitivity, lack of typical cytochromes and presence of two Quinones Q_6 and Q_8 (6,7). In the present investigation experiments were carried out to establish whether it posseses the ability for the de novo synthesis of isoprenoid compounds like prenols and sterols in addition to the two quinones.

Materials and Methods

S. digitata was collected from the local abattoir, freed from host material and maintained in tyrode solution (composition % w/v NaCl 0.8; KCl 0.02, $CaCl_2$ 0.002; $MgCl_2$ 0.01; $NaHCO_3$ 0.015; NaH_2PO_4 0.05 and glucose 0.5) at 37°C.

The non-saponifiable lipids were extracted (4,5) and separated by column chromatography using 4% deactivated

0006-291X/94 $5.00

Brockmann's alumina (neutral) (6,7) into four fractions - petroleum ether (P.E); and 5%, 10% and 20% ether in P.E. The 5%, 10% and 20% fraction were further purified by Thin layer chromatography (TLC) using silica gel (6) with a solvent system of ether and hexane in the ratio 1:4. An aliquot of the concentrate from the 5% ether in P.E. fraction was subjected to reverse phase partition chromatography (RPC) (16-18). The solvent system used was acetone-water 95:5 (v/v).

High-Performance Liquid Chromatography (HPLC) was carried out on Shimadzu (KYOTO, JAPAN) equipment with a UV-spectrophotometric detector (SPD-6A). The normal-phase separation of ubiquinone, dolichol and cholesterol was achieved on a cyanopropyl (CN) column (6 mm x 15 cm) using a solvent system of 0.05% isopropanol in hexane, delivered at 1 ml/min (19). Detection was at 210 nm on a variable wavelength detector set at 0.08 absorbance range. The isotope incorporation study was carried out with [^{14}C] acetate (10 μCi/hr) and [2-^{14}C] mevalonate (2 μCi)/hr).

Cholesterol and Q_6 and Q_8 standards were purchased from Sigma Chemical Company. Dolichol was a gift from Prof. P.S. Sastri (I.I.Sc Bangalore). HPLC grade hexane and isopropanol were purchased from Spectrochem Pvt. Ltd. Bombay.

Results and Discussion

TLC of the 5% fraction of S. digitata showed three iodine positive spots while the 10% and 20% showed only one spot each. The results are shown in Table 1. Both the cholesterol standard and the 20% spot obtained from Setaria had the same R_f value and ran together in chromatography. The dolichol standard and 10% spot obtained from Setaria also had the same R_f value. The standards ubiquinone, dolichol and cholesterol when assayed individually or as a mixture gave retention times 3.8, 5.6 and 11 min respectively in HPLC. The spots scrapped from TLC

Table 1. R_f values of the different spots obtained by doing TLC and RPC with the different column fractions of non-saponifiable lipids of S. digitata

Fractions	Spots	Standards	R_f values	
			TLC	RPC
5%	S_1	Q_6	0.71	0.82
	S_2	Q_8	0.90	0.63
	S_3	-	-	-
10%	S_4	dolichol	0.67	-
20%	S_5	cholesterol	0.35	-

TLC - Thin layer chromatography.
RPC - Reverse phase chromatography.

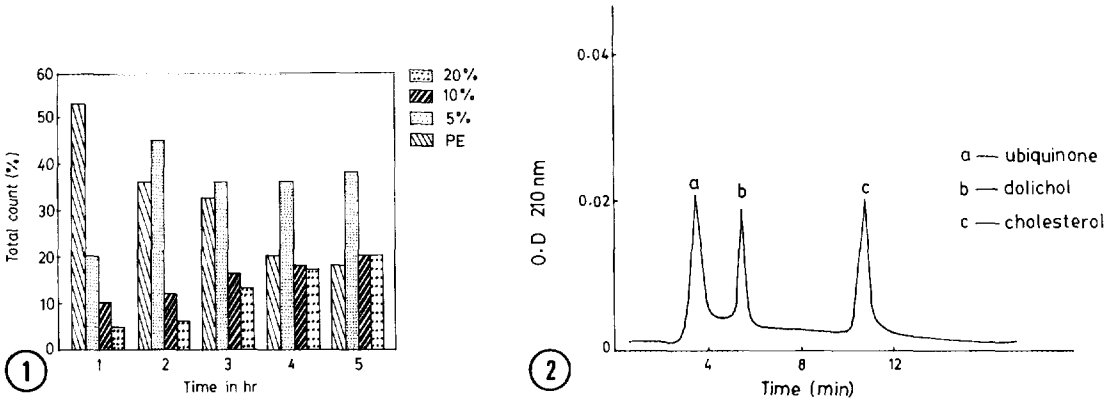

Fig.1. Histogram showing the time dependent label from [^{14}C] acetate (%) in each fraction. PE - Petroleum ether fraction.

Fig.2. HPLC of a mixture of standard ubiquinone, dolichol and cholesterol on a cyanopropyl (CN) column eluted with 0.05% isopropanol in hexane at 1 ml/min.

corresponding to ubiquinone, dolichol and cholesterol obtained from 5%, 10% and 20% fraction of Setaria also gave the same retention times (Figs. 2 and 3), further confirming their presence in the parasite.

The de novo synthesis of isoprenoid compounds in S. digitata was established by the [^{14}C] acetate and [2-^{14}C] mevalonate incorporation studies. Good activity of HMG CoA reductase, the enzyme which catalyses the rate limiting step in the biosynthesis of isoprenoid compounds, was detected in S. digitata (Ajitha and Kaleysa Raj, unpublished data). It is

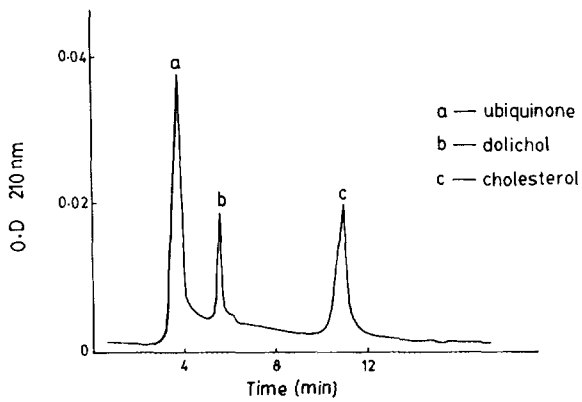

Fig.3. HPLC separation of ubiquinone, dolichol and cholesterol from S. digitata on a cyanopropyl column eluted with 0.05% isopropanol in hexane at 1 ml/min.

Table 2. Level of incorporation of [^{14}C] acetate and [2-^{14}C] mevalonate (cpm/gm wet weight of worm) into different column fractions

Radioactive material used	Time (hr)	Total non-saponifiable lipids	Fractions			
			Petroleum ether	5%	10%	20%
[2-^{14}C] mevalonate	3	83850	24675	32840	7845	10550
[^{14}C] acetate	1	6327±525	3352±20	1284±185	608±70	324±45
	2	15380±1100[a]	5555±345[a]	6980±715[a]	1810±100[a]	950±105[a]
	3	17463±2000[a]	5670±620[a]	6380±690[a]	2794±320[a]	2275±240[a]
	4	18025±2075[a]	3510±400[a]	6430±730[a]	3310±345[a]	3065±350[a]
	5	13806±2505[a]	4220±500[a]	9210±1005[a]	4825±500[a]	4765±500[a]

a - $p < 0.001$, n = 3, cpm in 1 hr incubation is compared to 2 to 5 hr incubation.

evident from tables 2 and 3 that S. digitata is capable of synthesising isoprenoid compounds such as ubiquinones, prenols and sterols. The total count in the non-saponifiable lipids increases with respect to time. The percentage incorporation of [^{14}C] acetate in the petroleum ether fraction decreases with time while time dependent incorporation of label increases in the 5%, 10% and 20% fractions (Fig.1) corresponding to the quinone, prenol and sterol fractions respectively. This is probably due to the conversion of radiolabelled hydrocarbons into other compounds.

Regarding the role of isoprenoid compounds, ubiquinones have a unique role in the electron transport system and the role of ubiquinones in setaria has already been reported (8,9). Dolichols take part in glycoprotein and proteoglycan biosynthesis. Since the microfilaria have a surface coat made up of glycoprotein, the dolichol type prenols detected in Setaria may have a role in the formation of its cuticular components. In the filarial parasite D. immitis a glycosyl transferase in

Table 3. Time dependent incorporation of [^{14}C] acetate and [2-^{14}C] mevalonate (cpm/gm wet weight of worm) into S_1, S_2, S_4 and S_5 spots separated by TLC

Radioactive material used	Time (hr)	Spots			
		S_1	S_2	S_4	S_5
[2-^{14}C] mevalonate	3	15435	4140	2950	7185
[^{14}C] acetate	1	770±60	125±15	380±20	286±15
	2	2115±150[a]	985±80[a]	1158±60[a]	2473±200[a]
	3	6320±500[a]	1088±120[a]	1786±100[a]	3498±300[a]

a - $p < 0.001$, n = 3, cpm in 1 hr incubation is compared to 3 and 5 hr incubation.

which dolichol phosphate promoted the transfer of glycosyl residues from sugar nucleotides to form glycoproteins has already been reported (12).

Cholesterol is a major constituent of the plasma membrane and is the parent molecule of many steroid compounds. Although parasitic helminths have long been considered to lack the de novo synthesis of sterols, there are reports about its synthesis in the sporocysts of Microphallus similis (10) and adult D. immitis (11). Insect moulting and metamorphosis are controlled by ecdysteroid hormones. Nematodes in general undergo several moults during their development. The ecdysteroids have recently been detected in D. immitis and other helminths (13). Hence the sterol found in S. digitata may be used for generating many functional molecules such as moulting hormones in addition to its usual role as a membrane component.

The isoprenoid compounds, isolated from S. digitata are identified as ubiquinones, prenols and sterols. The difference in the isoprenoid metabolism in Setaria compared to the host system offer promise as an effective target of attack for developing drugs. This is more so because S. digitata is very similar to parasites causing human filariasis. Blocking the function of quinones has already been proved to be a target of great significance (16,17). Blocking the pathway of isoprenoid biosynthesis is expected to prove a much more effective target of significance for controlling filariasis, especially because of the difference in the end products formed, for instance Q_6 and Q_8 in setaria in place of Q_{10} in the host.

Acknowledgments

The work was supported by the Department of Science and Technology, New Delhi, and Council of Scientific and Industrial Research, New Delhi.

References

1. Barrett, J. (1981) Biochemsitry of Parasitic Helminths, pp.109-125 Macmillan Publishers Ltd. London Basingstoke.
2. WHO (1987) TDR/FIL-SWG (1987.3)pp.38.
3. Hawking, F. (1978) WHO/ONCHO/78, 142, 1.
4. Kaleysa Raj, R., and Renganathan, S. (1972) Nature, New Biology, 240, 247.

5. Crane, F.L., and Rita Barr (1977) In Methods in Enzymology, 18, 137-165.
6. Kaleysa Raj, R., Puranam, R.S., Kurup, C.K.R. and Ramasarma, T. (1988) Biochem. J. 256, 550-564.
7. Santhamma, K.R., and Kaleysa Raj, R. (1990) Biochem. Biophys. Res. Commun. 167, 568-574.
8. Santhamma, K.R., and Kaleysa Raj, R. (1991) Biochem. Biophys. Res. Commun. 174, 386-392.
9. Santhamma, K.R., and Kaleysa Raj, R. (1991) Biochem. Biophys. Res. Commun. 177,318-323.
10. McManus, D.P., Marshall, I., and James, B.L. (1975) Exp. Parasitology, 37, 157-162.
11. Turner, A.C., and Hutchinson, W.F. (1979) Comp. Biochem. Physiol. 64A, 403-410.
12. Comley, J.C.W., Jafe, J.J., and Chrin, L.R. (1982) Mol. Biochem. Parasitol. 5, 19-31.
13. Mercer, J.G. (1985) Parasitology Today, 1, 96-100.
14. Santhamma, K.R., and Kaleysa Raj, R. (1992) Biochem. Biophys. Res. Commun.
15. Santhamma, K.R., and Kaleysa Raj, R. (1993) Biochem. Biophys. Res. Commun. 190, 201.
16. Threlfall, D.R., and Goodwin, T.W. (1963) Biochim. Biophys. Acta, 78, 532.
17. Goodwin, T.W. (1964) Lab. Pract. April Issue, p.245.
18. Threlfall, D.R., and Goodwin, T.W. (1967) Biochem. J. 103, 573.
19. Palmer, D.N., Maureen, A., Anderson, and Jolly, R.D. (1984) Analytical Biochemistry, 140, 315.