Lipid and fatty acid compositions of a trematode, *Isoparorchis hypselobagri* Billet, 1898 (Digenea: Isoparorchidae) infecting swim bladder of *Wallago attu* in the district North 24-Parganas of West Bengal

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Abstract Lipid classes and fatty acid compositions of a digenetic trematode, *Isoparorchis hypselobagri* inhabiting swim bladder of *Wallago attu* were analyzed by TLC and GLC. The total lipid (TL) and percent content of neutral lipid (NL, 61.59), glycolipid (GL, 19.78) and phospholipid (PL, 18.62) were recorded. The total lipid is 3.81 % of the wet weight of the body tissue. The total percent of saturates, monoenes, dienes and polyenes in TL, NL, GL, and PL of the parasite were 52.0, 49.7, 57.9, 61.5; 31.7, 31.8, 33.1, 26.3; 5.7, 5.7, 4.7, 4.2; as well as 10.33, 12.6, 4.3, 7.4 respectively. Diunsaturated fatty acids were present in low amount. Palmitic 16:0 (among all saturates) and oleic 18:1 ω 9 (among all unsaturates) acids were the predominant fatty acids in the parasite recorded.

Keywords Digenetic trematode · Fatty acids · *Isoparorchis hypselobagri* · Lipid classes · *Wallago attu*

Introduction

Several species of digenetic trematodes cause economic losses to society through infections of fish, birds, domestic animals, and others are medically important parasites of humans. On the other hand, natural resistance and other factors such as incompatible metabolism and environmental stress are undoubtedly responsible for the failure of certain trematodes to infect certain hosts (Cheng 1986). Adult stages of digenetic trematodes live in an anaerobic condition in a vertebrate host. But the present investigating species *Isoparorchis hypselobagri* Billet, 1898, a digenetic trematode

live in the swim bladder of the Indian catfish *Wallago attu*, which is a gaseous environment with a relatively high oxygen content. Like other macromolecules synthesis, lipid synthesis is an important process in most organisms and the same is true for helminthes. Lipid and fatty acid requirement are different in different animals and are probably related to the need of the animal concerned and its environment. Since helminthes like nematodes, trematodes, and cestodes are unable to synthesize most essential lipids, they have to acquire exogenous lipids from tissues, fluids or cell membrane of the host (Smyth 1994). The adult worms take large amount of lipids to obtain sufficient amount of specific fatty acids and fat-soluble vitamins and the excess lipid is stored and eventually excreted (Cheng 1986). A very common digenetic trematode, *Fasciola hepatica* excretes about 2 % of its net weight per day as polar and neutral lipids (including cholesterol and its esters), and excretion is mainly by way of its excretory system (Roberts and Janovy 2000). The major fatty acids in helminthes are usually C16 and C18. Helminthes have higher percentages of unsaturated C18 acids (Barrett 1981). In mammals unsaturated C18 acids are usually 30–40 % of the total acids whilst in helminthes it is 40–70 %. Usually helminthes contain a small proportion of odd numbered C15 and C17 (Barrett 1981). The principal fatty acid at all evolutionary and trophic levels is mainly C16:0 (Ackman 2000).

The present work is, thus, designed to investigate the major lipid classes and their fatty acid composition in a digenetic trematode, *I. hypselobagri* Billet, 1898 (Digenea: Hemiuridae) inhabiting swim bladder of a cat fish, *W. attu* in North 24-Parganas district of West Bengal, India. The outcome of this work is to be compared with established facts of lipids and fatty acids of those trematodes living in the hepato-biliary tracts and digestive tracts of their final host body.

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Materials and methods

Material

Isoparorchis hypselobagri Billet, 1898 (Digenea: Isoparorchidae), a large flat worm, were collected from swim bladder of *W. attu* from different market places of Gobardanga area of North 24-Parganas district of West Bengal, India. After collection the parasites were pooled and thoroughly washed in 0.85 % Normal saline. Living trematodes were carefully soaked in tissue paper and kept in -4°C . The sample size was 35 and wet weight of the pooled parasite sample taken for the present analysis was 6.5 g.

Methods

Extraction of lipids

The total lipids was extracted from the parasite sample following the method of Bligh and Dyer (1959) using methanol–chloroform (2:1 v/v), methanol–chloroform–water (2:1:0.8 v/v) and then again with the first solvent system. Sample was ground with the solvent, filtered and residue was extracted with the next solvent system. The process was repeated. Finally, the three extracts were pooled, diluted with water and layer is allowed to separate in a separatory funnel. The chloroform layer at the bottom was withdrawn and dried over anhydrous sodium sulphate. The chloroform solution of lipid was evaporated under vacuum, redissolved in distilled *n*-hexane and kept at -20°C for future use. Butylated hydroxy toluene (BHT) was added at a level of 100 mg/l to the solvent as antioxidant.

Thin layer chromatography for lipid class separation

A portion of the total sample was subjected to TLC using silica gel G (SLR) (Rouser et al. 1967). The glycolipids were eluted by the chloroform and acetone, respectively by successive developments. The glycolipid band was marked, scrapped off the plate and extracted by chloroform. The residual band at the bottom of the plate consisting of phospholipids was also extracted by methanol. The solvent was evaporated and fractions were kept in redistilled hexane at -20°C . Each class of lipid was estimated by weighing in a microbalance.

Preparation of methyl esters of fatty acids

A portion of total lipid (TL), glycolipid (GL) and phospholipid (PL) of parasite samples were dissolved in anhydrous methanol containing concentrated sulfuric acid (1 %, v/v) and the mixture was refluxed for 2 h. Methanol was evaporated to a small volume and cooled. Distilled water was added to the cooled mixture and the methyl

esters of fatty acids were extracted three times with aliquots of diethyl ether. The ethereal extracts were dried over anhydrous sodium sulphate, filtered, vacuum dried, redissolved in *n*-hexane and kept for future use.

Purification of fatty acid methyl esters by thin layer chromatography

Fatty acid methyl esters (FAME) were purified in thin layer chromatography (TLC) using *n*-hexane–diethyl ether (90:10, v/v) as a solvent. A standard methyl ester was also run on the same plate in a separate lane. The location of methyl ester bands were done by placing the TLC plate in iodine vapour chamber. The methyl ester bands corresponding to the standard marked and then scrapped off the plate. Methyl esters were recovered by extracting the bands in a mini column with chloroform, the latter was evaporated and the methyl esters were kept in *n*-hexane till analyzed by gas liquid chromatography (GLC). FAME of total lipid (TL), neutral lipid (NL), phospholipid (PL), and glycolipid (GL) were prepared by transmethylation (Christie 2003).

Gas liquid chromatography (GLC)

GLC of fatty acid methyl esters were done on a GLC (Chemito 1000), with flame ionization detector (FID). Quantitation was done by computer using specific software (Clarity Lite).

Analysis of FAME GLC of analysis of FAME was done on a BPX—70 megabore capillary column of 30 m length and 0.53 mm i.d. obtained from SGE, Australia. Oven temperature was programmed from 150 to 240 and 300°C , respectively. Injection port detector temperatures were 250 and 300°C , respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.44 ml/min. Identification of fatty acids was done by comparing their retention times with those of standards, chromatographed under identical operational conditions of GLC. Also a secondary standard of cod liver oil fatty acid methyl ester was used for identification of FAMEs, according to Ackman and Burgher (1965).

The whole experiment was repeated for three times and the results furnished below were the cumulative accumulation of the three experimental data sets.

Results

The TL of *I. hypselobagri* is 3.81 % of the wet weight of the body tissue. Percentage of NL, PL, and GL are 61.59, 18.62, and 19.78 respectively (Table 1). Total 27 fatty acids are identified from the TL and three major lipid classes (NL, PL and GL) of this trematode parasite and their wet weight

percentages are recorded (Table 2). The percent w/w distribution of TL, NL, PL, and GL is represented by pie diagram in Fig. 1. Comparative analysis of fatty acids of TL and their fractions viz. NL, PL, and GL of *I. hypselobagri* is presented in a histogram in Fig. 2. The saturated fatty acid (SFA) is dominated over other groups of fatty acids like monoenes (MUFA), dienes (DUFA), and polyenes (PUFA) in this trematode. This follows the sequences: DUFA (20.3) < PUFA (34.63) < MUFA (122.9) < SFA (221.1) (Table 3). Palmitic (16:0) and stearic (18:0) acids dominate the SFA while Oleic acid (18:1 ω 9) present as highest percent among monoenes (MUFA). Dienes (DUFA) are present in low amount in the lipid classes and linoleic acid (18:2 ω 6) is recorded as highest. In PUFA there is no one fatty acid recorded, which shows highest percentage in all the lipid classes.

Discussion

Digenetic trematodes are primarily facultative anaerobes, i.e., they can be maintained under anaerobic conditions but are capable of utilizing oxygen if available (Cheng 1986). The present parasitic species under study *I. hypselobagri* is such a species lived in a high oxygen content atmosphere of the host organ (swim bladder). The end products of anaerobic respiration are fatty acids and neutral lipids. Parasitic worms display selective mechanisms for absorption of fatty acids from their environment and incorporate these absorbed fatty acids into various lipid fractions (Barrett 1981).

Total lipid content of trematodes varies widely amongst different species. Total lipid content (% wet weight) of *Carmyerius minutus*, *Ceylonocotyle dicranocoelium*, *Cotylophoron cotylophorum*, and *Paramphistomum microbothrium* are 2.72, 3.23, 4.42 and 3.49 % respectively (Awharitoma et al. 1988), whereas it is 3.81 % in present species *I. hypselobagri*. Total lipid of adult *Azygia lucii* is 11.0 % of the dry mass however, it is 39.0 % of the dry mass of the *Echinostoma malayanum* and 29.5 % dry mass lipid in *I. hypselobagri* inhabiting swim bladder of cat fish (Von Brand 1973; Smyth and Halton 1983). In the present study, the abundance of recorded lipid classes in the whole

parasite is NL (61.59 % w/w) > GL (19.78 % w/w) > PL (18.62 % w/w). It can be opined that the parasite selectively absorb much NL from the host. In trematodes, phospholipids accounts for 25 % of total lipids (Goil 1964; Smith and co-authors 1969; Oldenborg et al. 1975). In the present parasite, PL present in moderate amount (18.62 %). Both PL and GL are known to function as an integral part of membrane component and NL probably functions as storage form of fatty acids (Ghosh et al. 2005). Tunholi-Alves et al. (2011) suggested that NL participates in the energy metabolism and structural construction in larval stages of digenetic trematode. Bennett and Caulfield (1991) believed that lipids taken from the host play an important role in immunoprotection in helminthes.

Carmyerius minutus, *Ceylonocotyle dicranocoelium*, *Cotylophoron cotylophorum*, and *Paramphistomum microbothrium* showed similar fatty acid profiles, with chain length varying from 12 carbon atoms to 20 carbon atoms (Awharitoma et al. 1988). In this study we found that fatty acid profiles with chain length varying from 14 carbon atoms to 24 carbon atoms in *I. hypselobagri*. Out of 27 fatty acids recorded from *I. hypselobagri* TL, NL, GL and PL contain 27, 26, 27 and 25 fatty acids. It is interesting to note that SFA is much more in PL and MUFA are more in GL fractions but DUFA and PUFA are much more in NL fractions in *I. hypselobagri*. The level of unsaturation in the fatty acids of the four parasites (*Carmyerius minutus*, *Ceylonocotyle dicranocoelium*, *Cotylophoron cotylophorum* and *Paramphistomum microbothrium*) was over 50 % (Awharitoma et al. 1988). Whereas, in case of the *I. hypselobagri* the level of unsaturation in the fatty acids is <50 % of TL. However, among lipid classes of TL, the level of unsaturation is >50 % only in NL fractions but in GL and PL fractions this level is <50%. In *Fasciola gigantica* the high content of SFAs, which amounted to more than 54 % of the total fatty acids, conferred a high level of saturation on these parasites (Ezigbo and Onyeneke 1987). This supports our present observation in that the level of saturation is also high in *I. hypselobagri* as it is about 55.2 % of total SFA in TL and lipid classes analyzed. This was brought about by a general increase in the values of palmitic acid (C16:0).

Adult *N.girellae*, *S. dumerili* skin and mucus had a relatively high weight-percentage of C16:0, C18:1 ω 9, C18:0 and C22:6 ω 3 fatty acids. Among the SFA of the lipid fractions, C16:0 and C18:0 acids are the chief components recorded in *I. hypselobagri*. The major fatty acid among the UFA in *I. hypselobagri* is oleic acid (C18:1 ω 9) followed by linoleic (C20:1 ω 9) acid. Docosahexaenoic acid, DHA (C22:6 ω 3) shows higher percentage in TL and NL in this trematode. It is probable that the parasite may be synthesized this PUFA in its own body by chain elongation. According to Ghosh et al. (2005) oleic acid is the main C18 fatty acid in parasitic helminths. Similar finding

Table 1 Percentages of various classes of lipids obtained from samples of trematode parasite of the cat fish *W. attu*

Items	w/w (%)
Total lipids (% w/w of wet tissue)	3.81
Neutral lipids (% w/w of total lipids)	61.59
Glycolipids (% w/w of total lipids)	19.78
Phospholipids (% w/w of total lipids)	18.62

Table 2 Fatty acid compositions of total lipids (TL), neutral lipids (NL), glycolipids (GL) and phospholipids (PL) of trematode parasite *I. hypselobagri* of the cat fish *W. attu* as determined by GLC of methylesters. (% w/w of each component in total fatty acids)

Components ^a	<i>I. hypselobagri</i>			
	TL	NL	GL	PL
14:0	1.2	0.9	1.9	1.6
15:0	0.8	0.6	1.1	1.2
16:0	27.5	26.9	34.7	29.2
17:0	0.5	0.5	0.6	0.2
18:0	13.3	11.0	15.6	23.0
20:0	0.6	0.4	0.7	1.9
22:0	6.9	7.9	2.6	3.8
24:0	1.2	1.5	0.7	0.6
∑ Saturated	52.0	49.7	57.9	61.5
14:1	0.2	0.1	0.4	0.2
15:1	0.2	0.2	0.3	
16:1	5.1	5.1	6.2	3.5
18: 1ω9	17.5	17.8	18.1	14.0
20:1 ω9	6.8	6.7	6.4	8.1
22:1 ω11	0.9	0.6	1.5	0.2
24:1	1.0	1.3	0.2	0.3
∑ Monoenes	31.7	31.8	33.1	26.3
16:2	1.0	0.9	1.4	1.5
18:2ω6	4.7	4.8	3.3	2.7
∑ Dienes	5.7	5.7	4.7	4.2
18:3 ω6	0.4	0.3	0.3	0.4
18:3 ω3	0.8	1.0	0.3	0.2
20:3 ω6	1.7	1.8	1.1	1.4
20:4 ω6	0.7	0.8	0.5	0.4
20:4 ω3	0.03	–	0.1	–
20:5 ω3	0.6	0.9	0.2	3.5
21:5 ω3	0.2	0.2	0.2	0.3
22:5 ω6	0.2	0.2	0.1	0.1
22:5 ω3	1.0	1.3	0.4	0.3
22:6 ω3	4.7	6.1	1.1	0.8
∑ Polyenes	10.33	12.6	4.3	7.4

^a First and second figures represent carbon chain length: number of double bonds and the ω values represent the methyl end chain from the center of double bond furthest removed from the carboxyl end

is made in this parasite. Comparison of UFA, C18 total and C18 UFA of TL in *I. hypselobagri* shows that the amount of UFA is higher. In *I. hypselobagri* the total percent of the C18 acids of TL is 36.7. Helminthes possess 40–70 % unsaturated C18 acids (Barrett 1981) which is quite less (23.4 %) in *I. hypselobagri*. Occurrence of stearic acid (C18:0) in the present parasite was 13.3 %, which is not as good in amount as reported by Barrett (1981). It is very difficult to assume the reason behind such variation in fatty acid concentrations among the parasites inhabiting different microhabitat.

Earlier, it was assumed that the chain elongation mechanism of fatty acids in platyhelminthes (cestodes and trematodes) might be similar to that of mammalian mitochondrial system (Barrett 1981). Furlong (1991) reported that trematodes could modify fatty acids by chain elongation. Such mechanism has been demonstrated in *Spirometra*

mansonoides for C16, C18, C18:1, C18:2, C18:3 to C20 and C22 fatty acids (Meyer et al. 1966). The major product of fatty acid synthase is C16:0. Evidence suggests that in most organisms the fatty acid synthase cycle stops at palmitate C16:0 and it acts as precursors for both saturated and unsaturated long chain fatty acids (Barrett 1981). Ackman (2000) commented that palmitic acid (C16:0) is the principal fatty acids at all evolutionary and trophic levels. Therefore, it is also quite probable that palmitate serves as a precursor for both saturates and unsaturates in *I. hypselobagri*. It may be presumed that the trematode follows the strategies of diffusion and mediated transport for uptake of fatty acids those present in lower amount. This assumption was supported by Barrett (1981).

Thus stearic acid (C18:0) in parasitic helminthes is unique in their presence as precursor of other fatty acids required for chain elongation. It was reported that parasitic

Fig. 1 % w/w Distribution of total lipid, neutral lipid, glycolipid, and phospholipid in *Isoparorchis hypselobagri* infecting swim bladder of *Wallago attu*

flatworms especially *F. hepatica* can form C20:1 and C20:2 fatty acids from C18:1 and C18:2 acids (Barrett 1981; Tielens 1997). It is probable that *I. hypselobagri* is also able to modify C18:1 acid to C20:1 fatty acid.

The present piece of work effectively explains the needs of total lipid and different lipid classes for the trematode

parasite. Presence of higher amount of fatty acids such as C16:0, C18:0, and C18:1 in the trematode *I. hypselobagri* parasitizing swim bladder of *W. attu* signify that these fatty acids play an important role in this parasite for higher carbon chain fatty acids modifications. Higher levels of these acids in *I. hypselobagri* prove that these fatty acids might be propagating through trophic levels. So, it can be concluded that this trematode utilizes mostly SFA, MUFA, and PUFA concentrating on C18 acids.

In general, adult trematodes lost their oxygen dependent pathways for synthesis of those sterols and unsaturated fatty acids which occur in their free living states, lost the ability to long chain fatty acids de novo, and unable to synthesize most essential lipids. So, they able to modify fatty acid chain length and have to acquire exogenous lipids from the fluids, body tissues, or cell membranes of their respective host.

The outcome of this work would be a help to helminthologist to use this profile of lipid and fatty acids of the trematode parasite *I. hypselobagri* Billet, 1898 and compare it with its host (both intermediate and final) in the light of host-parasite interrelationship emphasizing the budgetary requirement of lipids and fatty acids but this present piece of finding has no direct practical utility on the pisciculture. However, a line may be presumed that the

Fig. 2 Comparison of occurrence of fatty acids in lipid classes as recorded from *Isoparorchis hypselobagri* infecting swim bladder of *Wallago attu*

Table 3 % w/w Distribution of SFA, MUFA, DUFA, and PUFA in TL, NL, GL, and PL in *I. hypselobagri* of the cat fish *W. attu*. (% w/w of each component in total fatty acids)

Class	TL	NL	GL	PL	Total
SFA	52	49.7	57.9	61.5	221.1
MUFA	31.7	31.8	33.1	26.3	122.9
DUFA	5.7	5.7	4.7	4.2	20.3
PUFA	10.33	12.6	4.3	7.4	34.63

content of dietary lipid in the flesh of cat fish, *W. attu* infected with *I. hypselobagri* would be less in comparing with healthy non-infected cat fish.

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