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7. Lipidic metabolism in parasitic platyhelminthes

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Abstract. This review is an account of research into the biochemistry of lipids of platyhelminth parasites. Taking as high benchmark major metabolic pathways of vertebrates we summarize catabolic and synthesis routes now thought to be characteristic of this group. We emphasize the litlle recent information that exists on intermediary metabolism in these parasites. However, new data may be challenging old paradigms that have rule lipid metabolic studies for decades. Of particular relevance is the finding that fatty acid oxidation is possible under certain conditions, and metabolites related to sterols synthesis have been identified. Recent work on lipids turnover and remodeling of host taken phospholipids is also reviewed. Relevant contributions concerning eicosanoid metabolism are included in this chapter. The review concludes with a brief look at some lipid binding proteins related to the acquisition and transport of lipids from the host and distribution inside the cell.

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Abbreviations

FA:	fatty acids
PL:	phospholipids
TAG:	triacilglyceride
PC:	phosphatidylcoline
PE:	phosphoethanolamine
HMG-CoA:	3-hydroxy-3-methylglutaryl coenzyme A
AA:	araquidonic acid
FABP:	fatty acid binding protein
HLBPs:	hydrophobic lipid binding proteins

Introduction

Lipids are a highly diverse and heterogeneous group of compounds, with crucial roles in cellular structure and metabolism. They are major components of cell membranes and important energy reserves. Lipids also play important roles in enzyme regulation, cell surface recognition, cell interaction, surface antigenic determinants expression and regulation of gene expression.

In contrast to carbohydrate pathways, lipid metabolism has been little studied in parasitic Platyhelminths. In the past, most effort has been done to determine lipid composition and lipids involvement in energy metabolism [1-8]. Two paradigms have risen from these studies and have ruled lipid metabolic studies for decades: the inability of *de novo* synthesis of fatty acids and sterols [9-10], and the lack of fatty acid oxidation [6-7].

Particular interest has been posed on those lipidic molecules involved in host recognition [11], immune response modulation and evasion [12-13], communication [14] and development [15]. Teguments and worm membranes are the first line of attack of immune response, and much work has been done towards the understanding of phospholipids (PL) biosynthesis and function. Prostaglandins have also been identified in these parasites in relation with host response modulation, but with an uncertain function as well [16-17]. Many lipid binding proteins has also been described with special emphasis in those involved in host immune relationship [18]. Members of this family of proteins are highly antigenic and constitute major antigens of cestodes hydatid cyst fluid and vaccine candidates.

It has been demonstrated that these parasites can elongate fatty acids [4] and synthesize complex lipids with preference to phospholipids [19], suggesting a role in membrane synthesis.

Despite the variety of roles played by lipids, the knowledge about lipid metabolism in parasitic forms of Platyhelminthes is scarse. On 1970 Ward

and Fairbarn stated that little was known about helminth lipid metabolism, and almost the same can be said in 2010. The emergence of lipidomics has been renewed interest in the study of lipid components of organisms and we hope that some light could be done in the coming years concerning helminth's lipid metabolism.

Fatty acid oxidation

The energetic metabolism and the presence or absence of fatty acid (FA) catabolism in cestodes remains unclear. Although larvae and adult forms of cestodes are likely to have at least some oxygen supply, in many species the oxygen tension in the central region may be zero. In addition to living in environments with limited oxygen supply, these organisms lack both a circulatory system and respiratory pigments [20]. In this scenario triacilglycerides (TAG) should be the energy source for anaerobic helminth parasites stages. Curiously, despite they contain a large amount of TAG, these compounds did not seem to be the fuel source [6-7]. These conclusions came from *in vivo* experiments with labeled fatty acids. When exposed to ^{14}C-U-palmitate, labeled palmitate was readily taken up by adult parasites and incorporated into the neutral and phospholipid fractions, but no significant labeled CO_2 production was detected. β-oxidation pathway was studied in *Fasciola hepatica* and *Himenolepis diminuta*. Curiosly, *F. hepatica* express all β-oxidation enzymes but with variable activity levels. Acyl-CoA synthetase and 3-hydroxyacyl-CoA dehydrogenase showed low activities in *F. hepatica* [7] and in *H. diminut*a also [6]. In addition acyl-CoA dehydrogenase and enoyl-CoA dehydrogenase indicated low activities in *H. diminuta*. Surprisingly, acetyl-CoA acyltransferase have relatively high activity levels in both organisms.

A number of putative functions were proposed to explain the presence of these enzymes: metabolism of volatile acids [6], involvement in the malonyl-CoA independent elongation of long chain fatty acids [21], a reversal β-oxidation [22-23], or preparation for the free-living stage [7]. Finally, the absence of the oxidative pathway was explained by Barret and Körting by the lack of the enzymes of the trycarboxylic acid cycle to which fatty acid oxidation is tightly coupled.

These subjects have not been reported in other species besides those already mentioned, and the scientific community has assumed from these studies, that parasite Platyhelminths do not perform β-oxidation as the usual energy source.

Recently, Vinaud and co-workers have shown the ability of *Taenia crassiceps* of producing energy from lipids as an alternative energy source [22-23]. When glucose concentration is low, an alteration of the habitat

occurs, or in the presence of antihelmintics, the ability of producing energy from lipids is activated. These experiments demonstrated the secretion of β-hydroxybutirate and propionate, and a higher production of β-hydroxybutirate when the *cysticerci* were exposed to sublethal doses of praziquantel. The doses used did not affect the secretion of the organic acid propionate, therefore not affecting the citric acid cycle and confirming aerobiosis as the preferential energy production pathway. Probably, the drugs prevents glucose uptake from the environment, favouring the use of fatty acids as an alternative energy source [23].

Synthesis of complex lipids

Although parasitic Platyhelminths do not usually obtain energy from lipids degradation, they need them for membrane biosynthesis and renewal due to their continuous growth and asexual reproduction. They can absorb precursors (fatty acids, glycerol, choline, phosphocholine, ethanolamine, phosphoethanolamine, inositol and phosphoinositol) and incorporate them, along with host-derived FAs, into PL, TAGs and sterols [24]. Early works in this sense were done in the cestodes *Spirometra mansonoides* [9] and *H. diminuta* [10]; no recent work has been done in this respect.

On 1997 Brouwers and co-workers published their studies about the incorporation and turnover of fatty acids in adult *Schistosoma mansoni* [25]. They found that FA are easily incorporated by larvae forms of *S.mansoni* and rapidily metabolized and incorporated with preference to phospholipids, mainly phosphatidyl-coline (PC). Schistosomes incorporated 0.6-0.7% of their total fatty acid contents per hour; the incorporated palmitic and oleic acids showed an estimated half life of 24 hs. Only surplus FA was then employed to TAGs synthesis, probably as a store. The function of this store in *S. mansoni* is unclear since these organisms cannot use TAGs for the generation of ATP [26]. This research group proposed that TAG synthesis in schistosomes prevents high intracellular free fatty acid concentrations. PC had a high turnover, in contrast to FA esterified to TAGs, which persisted for extended periods in this lipid class. Once incorporated, fatty-acyl chains remain subjected to deacylation/reacylation and thereby to transfer to another lipid class. When deacylated, oleic acid can be elongated to eicosanoic acid before re-incorporation into either PL or TAGs [25].

Composition of membranes and tegument has been determined and compared with that of the host. Phosphatidylcholine and phosphoethanolamine (PE) are the major components of schistosomes phospholipids fractions [27]. Generally, the PC species, and those of the outer tegumental membranes in

particular, are more saturated than the species from the blood of the host on which they feed [8]. It was found that membrane integral phospholipids of *S. mansoni* display a species composition, and quantities of lipidic species that are very distinctive from that of the host blood [28]. These differences were detected not only in FA composition but in the position of these molecules at the glycerol backbone also. Fatty acid chain on the sn-1 position of glycerol backbone is linked by an ether linkage instead of an ester one indicating that the former is synthesized by this trematode [28]. PC and PE composition are examples of the differences mentioned. Of particular interest was the finding of the plasmalogen specie, (16:0-20:1)PlastEth, a molecule of the PE family which is absent in the host blood. This compound could be a source of eicosanoic acid (20:1 (n-9)), considered a putative second messenger [29]. Its presence on the tegument suggests a role in host response modulation [30]. The high plasmalogen content could provide an optimal environment for schistosomal proteins and prevent an optimal functioning of enzymes involved the immune response of the host [28]. Other unusual and abundant fatty acid specie found in *S. mansoni* tegument was the octadec-5-enoic acid 18:1(5Z) [28]. Interestingly, these fatty acids are specific for *S. mansoni* and confirm the parasite capacity to modify adquired fatty acids.

Fatty acids were also found as excretion products. It was observed that tegumental lipids have a shorter half-life than those in the worm body [31-32]. Two lyso-phospholipids were detected as excretion products of *S. mansoni* schistosomula: monopalmitoyl-PC and lysophosphatidylserine [30].

Sterols metabolism

Platyhelminthes seems to be unable to synthesize steroids *de novo* although they can readily incorporate exogenous steroids and fatty acids into their steroid esters [33]. Interestingly, the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a central enzyme in mevalonate pathway, has been identified in *S. mansoni* [34]. Mevalonate, is a precursor for a variety of sterols, including cholesterol and/or polyisoprenoid, compounds of vital importance to the cells. Entry into this pathway depends on the key enzyme, HMG-CoA reductase, which catalyzes the conversion of HMG-CoA into mevalonate. Despite conserved *S. mansoni* HMG-CoA reductase present different size, membrane topology and regulatory mechanisms [35] when compared with the human homologue. *S. mansoni* enzyme is a protein of 66 kDa [36]. Its deduced primary sequence predicts a hydrophobic amino terminus consisting of seven potential transmembrane domains. In this parasite, HMG-CoA reductase produces 8.8 pmole of

mevalonate/min/mg cell protein [36] and its activity is down regulated by dolichols but not by cholesterol, in contrast to the mammalian enzyme [37].

The biosynthesis of intermediaries of the mevalonate pathway like dolichols, prenols, farnesylpyrophosphate, farnesol, and geranylgeranylpyrophosphate has been demonstrated in several platyhelminth parasites such as *Taenia taeniformis* [38], *S.mansoni* [39-40], and *H. diminuta* [40-41].

Isoprenoid electron transporters, ubiquinone and rodoquinone have been indentified in several species of cestodes [41-44]. Biosynthesis of the former compound has been demonstrated in *S. mansoni* [45], and the latter in *H. diminuta* [41] and *F. hepatica* [46]. The cestode *Penetrocephalus ganapatii* also contains menaquinone (vitamin K) in addition to ubiquinone [44].

Several studies have shown that estrogens were found in many invertebrates (47). However, there is a paucity of information about estrogen production by parasites. In these sense, it was shown that *S. mansoni* have the ability to convert estrone to estradiol [48]. Recently, Valdéz et al., demonstrated the ability of *Taenia solium* to synthetize [^{3}H]testosterone and small quantities of [^{3}H]17β-estradiol from [^{3}H]androstenedione [49].

Among the sterols that have been detected in schistosomes are the ecdysteroids. These were characterized originally as the moulting hormones of insects, but have been identified in a number of non-arthropod organisms [50-51]. The biosynthesis of ecdysteroids by schistosomes has not been detected [52-53], but polar conjugates of ecdysteroid have been found in *S. mansoni* [52].

These findings make us wonder previous hypothesis about the inability of sterols biosynthesis in platyhelmiths parasites [33].

Uptake of cholesterol from host lipoproteins has been demonstrated in *S. mansoni* [54]. Interestingly, low-density lipoproteins (LDL), the major cholesterol-carrying lipoproteins in human plasma, bind to the surface of larval and adult schistosomes [55]. Bound LDL might provide phospholipids and sterols to the worms. Receptors or binding proteins of 60, 35, and 14 kDa involved in interaction with LDL and/or very low-density lipoproteins (VLDL) from schistosomules and adults of *S. mansoni* have been described [56-57]. The receptor search in *S. japonicum* resulted in an unique protein termed Sj43, localized at the parasite's tegument and gut lining with apo-A, apo-B and apo-C binding properties [58]. A molecular model of *S. japonicum* very low-density lipoprotein-binding protein (SVLBP) was depicted [59]. The authors deduced that a single domain might facilitate binding activity of the molecule through an unusual, negatively charged surface, which was maintained by two of the three predicted disulfide bonds. LDL or VLDL bound to the surface of the parasite may inhibit the binding of human anti-schistosomal antibodies and aid in parasite evasion of antibody-dependent

cytotoxic reactions by masking parasite antigens. SVLBP may be released from the membrane and be involved in the turnover of membrane components. This protein may be associated with a GPI-anchor through its COOH-terminal transmembrane domain, thereby also facilitating its release into the plasma. The authors also have suggested the sub tegument as the putative site of synthesis [59].

Eicosanoids metabolism

Although invertebrates in general lack *de novo* synthesis of polyunsaturated fatty acid and eicosanoids, nematode, trematode, and cestode parasites have been shown to generate eicosanoids prostaglandins (PGs), leukotrienes [60-61], and tromboxanes [62]. Since PG production of helminths might modulate the host immune system and the establishment of infections [63] a great effort was achieved to characterize these molecules as well as PG-catabolizing enzymes and receptors.

In vertebrates, araquidonic acid (AA) is derived from linoleic acid desaturation and is the precursor of eicosanoids. It is stored in plasmatic membranes esterifying C-2 position of phosopholipid's glycerol backbone and it is released by the action of phospholipases. Since parasitic helminths lack the abiltiy of *de novo* synthesis of FA and desaturase mechanisms [4] araquidonic acid shoud be taken from their hosts. There is evidence indicating that the synthesis of prostaglandins and thromboxanes from exogenous arachidonic acid or linoleic acid is possible in *T. taeniformis*, *Spirometra erinacei, S. mansoni,* and *Trichobilharzia ocellata* [64-69]. Prostaglandins PGA_2, PGD_2, $PGE_{1,}$ PGE_2, PGF_2, and 6-keto-$PGF_{1\alpha}$ were identified in *Schistosoma spp* [16-17, 67, 70-71]. In addition to prostaglandins, *S. mansoni* synthesizes leukotrienes (LTB4, LTC4) and hydroxyeicosatetraenoic acid (HETE), an intermediate in the pathway of leukotriene synthesis [52].

Parasite enzymes metabolizing eicosanoids are poorly characterized. The search for central enzymes like cyclooxygenases (COX) has not been fruitful, but downstream enzymes involved in the generation of specif prostaglandins have been identified.

Phosopholipase A2, (PLA2) is an enzyme involved in the AA cascade and prostaglandin production, as previously mentioned. The enzyme catalyzes the specific hydrolysis of ester bonds linking the acyl chain to the C-2 position of phosopholipid's glycerol backbone [72]. The enzyme has been detected in *S. japonicum* as an integral membrane protein by western blot using and heterologous antisera. A positive reaction was also obtained with *S. mansoni*

membrane extracts indicating the presence of the membrane protein of both parasites. Immuno-histochemical studies showed a clear signal associated with the parasite gut lining and that no fluorescence was observed in or on the tegument. The authors also demonstrated that the membrane integral protein fraction from both schistosome species contained PLA2 activity.

Sigma class Glutathione S-transferases (GST) have been demonstrated to express prostaglandin synthase activity in trematodes. Sm28GST from *S. mansoni*, excreted in large quantities by the parasite while living within the skin, has PGD_2 synthase activity [73]. Structural studies of the homologous Sh28GST from *S. haembtobium* revealed a possible role of Glutathione as a tightly bound cofactor involved in the catalytic mechanism for prostaglandin D_2 synthase activity [74].

Based on the incapacity of inhibition of PG synthesis using known COX inhibitors, theb absence of annotated sequences with significant similarity to mammalian enzyme and the differences of those characterized synthases in parasitic protozoans with human counterparts led to suggest that a classical COX might not be involved in eicosanoid synthesis in parasites [17]. Although most of the available studies indicate an important role for eicosanoids in parasites and parasitic infections, our current knowledge is still fragmentary and more data are urgently needed.

Lipid binding proteins

The high levels of hydrophobic binding proteins in parasitic helminths, together with their restricted lipid metabolism suggest that these proteins play an important role in metabolism and are putative targets for chemotherapy and vaccine development. A variety of functions have been proposed for these molecules including the uptake, transfer and storage of hydrophobic ligands, targeting ligands to specific organelles or pathways, sequestration of toxic compounds and regulation of gene expression. However, their exact function remains unclear. Proteins of this type are located in the biological membranes as well as the cytosol probably assuming different roles in each compartment.

Unlike what was believed for a long time, plasma membrane offers a barrier to fatty acids translocation. Now there is evidence in support that a protein-mediated FA uptake system is the dominant means by wich fatty acids are taken up [75-77]. In this process, three kind of proteins are involved in a tissue specific way: fatty acid translocase (FAT), fatty acid transport protein (FATP) and fatty acid binding protein from plasmatic membrane (FABPpm). These proteins have not been reported in playhelminths but it is

worth mentioning that there is a sequence of *S. japonicum* annotated in GenBank, with high similarities with CD36/FAT family. With this result in mind we have searched through *Echinococcus multilocularis* Blast server (http://www.sanger.ac.uk/cgi-bin/blast/submitblast/Echinococcus) for membrane proteins involved in uptake according evidence from vertebrates. We found similar sequences to all three reported proteins, CD36/FAT, FATP and FABPpm.

Intracellular lipid traffic is mediated both by membrane vesicles and by a number of non-vesicular pathways facilitated by cytoplasmic lipid binding proteins. The major role of these proteins is to move hydrophobic lipids across the aqueous environment of the cytoplasm independent of vesicular membrane traffic [78-79]. The intracellular lipid binding proteins in mammals are widely distributed and include phospholipid and glycolipid transport proteins, retinol and retinoic acid binding protein, acyl-CoA binding protein and several fatty acid binding proteins.

Since parasitic helmiths lack the ability of *de novo* synthesis of FA, it is, therefore, essential that these parasites have an efficient binding system for the uptake and transport of key hydrophobic molecules. In this direction, two groups of lipid binding proteins have focused attention of scientists: fatty acid binding proteins (FABPs) and hydrophobic lipid binding proteins (HLBPS).

Huge work was devoted to fatty acid binding proteins since FABPs are considered as vaccine candidates. Because of their highly immunogenic character, FABPs are a promising candidate antigen for vaccines against diseases caused by platyhelmiths parasites [80-84]. The FABPs host protective mechanism of action has not been clarified, but current thinking is that it is related to an inability of the worms to obtain nutrients by specifically blocking the binding and transport of fatty acids. Minor work was done to dilucidate their specific function inside the parasite.

FABPs are not exclusive of invertebrates and they belong to a family of small proteins, which bind non-covalently hydrophobic ligands [85]. They are believed to be implicated in fatty acid intracellular uptake and transport, regulation of lipid metabolism and gene expression, and protection from the deleterious action exerted by free long-fatty acids. The precise function of each FABP type remains poorly understood, but sub-specialization of functions is suggested by the tissue specific and temporal expression, in addition to ligand preferences [86-89]. Proteins of this family have been described in *S. mansoni*, Sm14 [90], *Schisotosoma japonicum*, SjFABPc [91], *Schistosoma bovis*, SbFABP [92], *Fasciola gigantica*, FgFABP [80], *Fasciola hepatica* (Fh12/Fh15) [93], *Echinococcus granulosus* (EgFABP1 and EgFABP2) [94-95] and *Mesocestoides vogae* (MvFABPa/b) [96]. Studies using many of these FABPs have been extensive and have involved different

vaccination strategies, particulaly with *S. mansoni* FABP and *F. hepatica* protein [reviewed in 97].

Recent unpublished results of our group showed that EgFABP1 could be targeted to the nucleus suggeting roles in gene expression regulation. Furthermore, displacement assays indicate a preference for arachidonic acid and oleic acid over other fatty acids, phospholipids and other hidrophobic ligands tested [98]. AA could be a relevant ligand since the evidence mentioned in section 5 of this chapter indicates that synthesis of prostaglandins from this FA could be possible in Platyhelminthes.

The second group of lipid binding proteins studied in platyhelminth parasites are the HLBPs. Two classes of lipid or hydrophobic ligand carrier protein have been found in nematodes and cestodes, the group of nematode polyprotein allergens (NPAs) and the group of hydrophobic lipid binding proteins (HLBPs). Both are lipid binding proteins with extremely hydrophobic binding sites. The group of hydrophobic ligand binding proteins, specific to Platyhelminthes is represented by the hydrophobic ligand binding proteins from *M. expansa* [99-100], *H. diminuta* [101-102], *T. solium* [103-104], *Echinococcus granulosus* [18] and a putative immunodiagnosis antigen of *T. crassiceps* [105]. These proteins are oligomeric and have the capability of bind hidrophobic ligands and anthelmintics. Similar sequences from *Taenia hidatigena* and *Taenia multiceps* have been deposited at GenBank.

T. solium HLBP was found to be a hetero-oligomeric complex consisting of multiple subunits of 7, 10, and 15 kDa. The 15 kDa unit represents a glycosylated form of the 10 kDa. The protein is excreted to the medium, has the capability of bind hydrophobic ligands and also co-localizes with lipid droplets and lipase activity at host granuloma wall. The auhtors have suggested that after binding FA the protein-lipid complex could return to the parasite across the syncitial membrane [104].

Recently, a novel *T. solium* lipid-binding protein that may play an important role in membrane trafficking was reported [106]. The protein with homology with the SEC14 domain, interacts with signaling lipid regulators of membrane trafficking. Localization on Golgi membranes of transfected cells supports a role in vesicle flow both in the budding reaction from the trans-Golgy network and in the fusion reaction with the plasma membrane.

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