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ORIGINAL ARTICLE

LIPIDOMICS OF HOUSEFLY (MUSCA DOMESTICA) LARVAE BY MALDI-TOF-MS.

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Abstract:

Housefly, Musca domestica is distributed worldwide and considered as a pest of both farm and home. These insect species are completely dependent on lipids for their metabolic requirements. About 19-20% of the biochemical constituents are the lipids in housefly. The primary storage organ is fat body. Neutral lipids are stored as triglycerides. Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat soluble vitamins, monoglycerides, diglycerides, phospholipids and other. MALDI-TOF-MS analyses of lipid of housefly larval extract after total lipids were isolated using MTBE. The obtained data or spectrum were analysed based on their mass per charge ratio (m/z). (m/z) range between 500-900. For the analysis of lipids MALDI-TOF-MS technique has been used.

KEYWORDS:

Musca domestica, Larvae, Lipidomics, MALDI- TOF-MS.

INTRODUCTION :

Lipidomics is the study of networking and pathway of cellular lipids in the living systems. (Wenk, 2005; Watson 2006).Lipidomics is involved in identification of various and quantification of various cellular lipids and their correlation with other components like proteins as well as other lipids (www.wikkipedia. org/wiki/lipidomics).

Lipid profiling is very useful because it provides a comprehensive analysis of various lipid species within cell or tissue. Profiling is mainly based on the use of mass spectrometry (MS) which provide the quantitative data (www.lipid maps. Org/data/index/.html). Several workers have studied relationship between lipids and immune functions. Norris and Denis, (2012) studied dietary fish oil omega-3 fatty acids role in eliciting cardio protective and anti inflammatory effects. Bhikshapati et al., (2011) have studied the differential fatty acid expression in native, injured, and infected housefly Musca domestica larva. They have noticed that the fly larva have abundant phosphotidyl ethnoalamine and phosphotidyl choline.

Kihari et al., (2009) studied therapeutic effects on multisclerosis by using mPEGS-1-PGE-2and stated that targeted lipidomic study can be useful in a significant way for the treatment of multiple sclerosis. Ghioni et al.,(1996) studied the total neutral lipids and total polar lipids from fresh water insects namely Stonefly nymph, Beetle larvae, Chirnomidae, water batmen and Mayfly nymph. Joseph.Vitale and Selwymbroytmen ,(1981) studied the role of lipids in relation to the immune function and stated that the dietary lipid play a role in modulating immune functions.Corone and Bridges, (1963) studied

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phospholipids of housefly Musca domestica. They have summarised that it contains phosphotidyl ethnoalamine (65%) phosphotidyl choline (17%) Phosphotidyl serene (3.5%0and phosphotidyl inositide (3%) etc. They have further observed that the housefly contains low plasmatogen content (1.3%) and absence of sphingomylein.

MATERIALAND METHOD:

Lipid extraction from Housefly larvae for lipidomic study:

The reared housefly larvae were homogenated in PBS buffer and semidried using speed vacuum and150µl of methanol was added to a semidried homogenate and vortexed vigorously for 10- 15 minutes and prepared homogenous turbid mixture. The entire mixture was suspended in 500 µl of MTBE and was kept for vigorous vortexing for 1 hr and about 200 µl of water was added to the mixture and centrifuged at 10,000 rpm for 10 minutes. The protein pellet settled down at the tube and MTBE layer at the top of it. Without disturbing the water layer, the top layer of MTBE was collected in another tube and semidried under speed vacum. The total lipids are obtained in MTBE layer. This MTBE mixture is spotted on MALDI plate for lipidomic analysis.

RESULTS AND DISCUSSION-

The total lipids expressed in housefly larvae shows about 18-20 lipids. Among these lipids, glycerophospholipids are the major constituents. About 14 types of glycerophospholipids are expressed. Whereas three types of polyketaids, 1 type of glycerolipid, 1 type of sterol lipid and 1 type fatty acid were observed on the MALDI-TOF- MS analysis

The obtained lipidomic graph and table shown below-

Table 1: MALDI data of total lipids

Observed M/Z	Exact M/z	Systematic Name	Formula	Category	Main class
504.2502	504.25	1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3-phospho-(1'-sn-glycerol)	$C_{24}H_{41}O_9P$	Glycerophospholipids [GP]	Glycerophosphoglycerols [GP04]
522.4979	522.54	pentatriacontanoic acid	$C_{35}H_{70}O_2$	Fatty Acyls [FA]	Fatty Acids and Conjugates [FA01]
549.3889	549.38	1-(10E-octadecenyl)-2-acetyl-sn-glycero-3-phosphocholine	$C_{28}H_{56}NO_7P$	Glycerophospholipids [GP]	Glycerophosphocholines [GP01]
559.4171	559.38	(7E)-(3S,6R)-9,10-seco-5,7,10(19)-cholestatrien-3-ol 6,19-(4-phenyl-1,2,4-triazoline-3,5-dione) adduct	$C_{35}H_{49}N_3O_3$	Sterol Lipids [ST]	Secosteroids [ST03]
575.3949	575.4	1-(13Z,16Z-docosadienoyl)-glycero-3-phosphocholine	$C_{30}H_{58}NO_7P$	Glycerophospholipids [GP]	Glycerophosphocholines [GP01]
577.4273	577.41	1-(11Z-docosenoyl)-glycero-3-phosphocholine	$C_{30}H_{60}NO_7P$	Glycerophospholipids [GP]	Glycerophosphocholines [GP01]
587.4379	586.46	1-dodecanoyl-2-(7Z,10Z,13Z,16Z,19Z-docosapentaenoyl)-sn-glycerol	$C_{37}H_{62}O_5$	Glycerolipids [GL]	Diradylglycerols [GL02]
603.4189	603.10	5-Carboxypyranocyanidin 3-O-(6"-O-malonyl-β-glucopyranoside)	$C_{27}H_{23}O_{16}$	Polyketide [PK]	Flavonoids [PK12]

605.4125	605.41	1-dodecanoyl-2-(9Z-tetradecenoyl)-glycero-3-phosphoethanolamine	$C_{31}H_{60}NO_8P$	Glycerophospholipids [GP]	Glycerophosphoethanolamines [GP02]
615.4767	616.11	Quercetin 7-(6"-galloylglucoside)	$C_{28}H_{24}O_{16}$	Polyketide [PK]	Flavonoids [PK12]
631.4423	631.42	1,2-di-(9Z-tetradecenoyl)-sn-glycero-3-phosphoethanolamine	$C_{33}H_{62}NO_8P$	Glycerophospholipids [GP]	Glycerophosphoethanolamines [GP02]
685.3192	685.47	1-dodecanoyl-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphoethanolamine	$C_{37}H_{68}NO_8P$	Glycerophospholipids [GP]	Glycerophosphoethanolamines [GP02]
701.2883	701.43	1-dodecanoyl-2-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3-phosphoserine	$C_{36}H_{64}NO_{10}P$	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]
704.3148	704.23	Amorphigenin O-vicianoside	$C_{34}H_{40}O_{16}$	Polyketide [PK]	Flavonoids [PK12]
714.3786	714.43	1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-2-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-glycero-3-phosphate	$C_{41}H_{63}O_8P$	Glycerophospholipids [GP]	Glycerophosphates [GP10]
717.2580	717.46	1-tridecanoyl-2-(9Z,12Z-octadecadienoyl)-glycero-3-phosphoserine	$C_{37}H_{68}NO_{10}P$	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]
827.5659	827.57	1-(9Z-heptadecenoyl)-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphoserine	$C_{45}H_{82}NO_{10}P$	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]
854.6014	854.6	1-eicosanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3-phospho-(1'-sn-glycerol)	$C_{48}H_{87}O_{10}P$	Glycerophospholipids [GP]	Glycerophosphoglycerols [GP04]
869.5524	869.61	1-(11Z-eicosenoyl)-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphoserine	$C_{48}H_{88}NO_{10}P$	Glycerophospholipids [GP]	Glycerophosphoserines [GP03]
881.6003	881.59	1-(7Z,10Z,13Z,16Z-docosatetraenoyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl) -glycero-3-phosphocholine	$C_{52}H_{84}NO_8P$	Glycerophospholipids [GP]	Glycerophosphocholines [GP01]

MALDI-TOF-Ms analysis of isolated total lipids from housefly larval extracts was done using MTBE. The lipidomic data was analyzed based on their mass charge ratio (M-Z) and observed (M/Z) the range between 500-900. For the analysis of lipids MALDI-TOF-MS technique has been used and quantitavely described. The lipids amounts in the range of few nanograms are sufficient for MALDI-TOF-MS analysis.

The lipidomic result indicates glycerophospholipids as the key components of lipid bilayer of the cell membrane. They involve in metabolism and signaling pathway, they play important role and ensure the survival of organism by tracing immune system.

Glycerophospholipids are sub divided into distinct classes depending on the nature of the head group such as phosphotidyl choline (PC), phosphotidyl ethyl entanolamine (PE), phosphotedyl serine (PS) are membrane derived secondary messengers. The second major lipid in present data is polyketaids. Polyketaids are produced via biosynthesis of polyketaid synthase (PKS). These PKS are expressed for purpose of defensive mechanism. They are also considered as pheromones. Polyketaids are known to be used by insects for pheromone communications and defense against enemies (Pankewitez, Hilker et al., 2008). They also act as anti microbial and anti-tumor agents. The remaining lipids were expressed at negligible levels. Apart from the proteins, lipids also play a major role to combat the stress and infection to the organism.

The main biological composition of lipids includes as storage of structural components as cell membrane and as important signaling molecules. Lipids may be broadly defined as hydrophobic and ampiphilic small molecules. Biologlical lipids originate entirely or in part from two distinct types of biochemical subunits or building blocks (Fahy et al, 2005). Lipids also have fatty acids and thbewir derivatives as well as other sterol containing metabolites such as cholesterol (Michelle et al, 1993). The discovery of biologically active phospholipids, platelet activative factor has led for further study. Later every individual lipid class has been found to have some unique biological role such as a source of energy as constructional unit of the membrane. The lipids in the membrane function as trafficking in cellular constituents, the regulation of the activities of membrane proteins and signaling were recognized. Some key lipids are analyzed briefly and summarized by MALDI-TOF-MS analysis.

Cellular membranes are semi permeable barriers that enclose and defend the cell and its organelles. They control transport of material including signaling molecules and indeed many reactions occur within membrane, including energy production and biosynthesis of cellular components. The specific lipid composition of membrane provides a barrier to the diffusion of ionic solutes and other molecules in the cellular compartments. Cellular membranes are the first site for react of extracellular signals, the recruit of and activate effector molecules and they are the launch pad for the activated effector molecules throughout the cell.

MALDI-TOF-MS analyses of lipid of housefly larval extract after total lipids were isolated using MTBE. The obtained data or spectrum were analyzed based on their mass per charge ratio (m/z).(m/z) range between 500-900. For the analysis of lipids MALDI-TOF-MS technique has been used. Lipids amounts in the range of few nanometers are sufficient for MS analysis. The initial report of Matrix assisted Laser Desorption Ionization (MALDI) analysis of intact glycerophospholipids and sphingolipids .

Graph 1-Lipdomics of Housefly Musca domestica larva