Indian Journal of Science and Technology, Vol 9(38), DOI: 10.17485/ijst/2016/v9i38/73884, October 2016
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

A Mini Review of Functional Proteins in Silkworm *Bombyx mori L* Haemolymph

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Abstract

This review focuses on the state of the art on haemolymph proteins in lepidopteron insect silkworm *Bombyx mori* L. Insects are most successful animals in the world. Their success is mostly attributed to their physiological well being and adaptability to most of the environmental conditions is an important reason to be considered evaluating their success. Proteins in insect especially the ones circulating in the blood are seen to be the major contributors for the insects successful survive, owing to their open type circulatory system the haemolymph in insects soaks all the major organs systems in the body and thus making it an important body fluid for regulation of homeostasis. This calls for an detailed study on proteomics of the haemolymph of Bombyx mori which along with being an economical insect, it is being considered as model organism for most frontier sciences like biomaterials, biomedical applications and pharmacokinetic studies, Research on lepidopteron insects has been instrumental in the discovery and the biochemical understanding of many haemolymph plasma proteins. In the present review we analyzed the haemolymph proteins based on their function and molecular characterization. We also could deduce the role of these proteins in insect physiology by comparative analysis with earlier reported proteins.

Keywords: Heamolymph Proteins, Hypothetical Proteins, Immune Proteins, Silkworm

1. Introduction

Insects have been particularly successful animals in evolution. Current estimates are that 90% of all known species within the animal kingdom belong to this class. Apart from being an economic avocation the silkworm constitutes an important biological tool for research. The genetics consider it as an important material for studying heredity. The cytologists find it as an excellent tool for studying all biological experiments and its recent exploration for biomedical research is also very promising[1,2]. Haemolymph is the body fluid of insect; most of the insect's functional proteins are present in the haemolymph. Proteins are a major component of the haemolymph plasma. The most abundant proteins in larval heamolymph belong to a class known as storage proteins or hexamerins[3]. The storage proteins are synthesized by the fat body and reach extremely high concentrations in the last instar[4]. Insect haemolymph proteins are extremely difficult to analyze due to the wide spectrum of their physicochemical characteristics as well as the naturally wide range of protein abundances. Proteomics can capture the changes on the protein level (e.g. post-translational processing) that are unapproachable by genomics or transcriptomics[5]. Nevertheless, the promises of proteomic research for the future are indisputable.

Insect plasma proteins, including those involved in immune responses, are synthesized primarily by the fat body, with some contribution from hemocytes and other tissues[4]. Insect haemolymph differs substantially from vertebrate blood, with the absence of erythrocytes and a high concentration of free amino acids being two of

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the common distinguishing features[6]. Haemolymph is composed of water, inorganic salts (mostly Na^+, Cl^-, K^+, Mg^{2+}, and Ca^{2+}), and organic compounds (mostly carbohydrates, proteins and lipids). Haemolymph of many insects contains lysozyme, an enzyme that degrades bacterial cell walls. In addition, low-molecular-weight antimicrobial peptides are synthesized in response to bacterial or fungal infection[7].

Silkworm proteins synthesis mechanism is very straight forward in converting the leaf proteins into the silk protein. While the analysis of haemolymph proteins reveals complex metabolic proteins. In the silkworm haemolymph is in an open system that circulates among all organs, and functions in nutrient and hormone transport, injury, and immunity. To understand the intricate developmental mechanisms of metamorphosis, In addition, haemolymph has a key role in innate immunity response that is triggered when bacteria or fungi enter the silkworm body[8]. All types of antibacterial and antifungal and antiviral proteins are identified in to the silkworm haemolymph.

Since the 1970 s and earlier, the proteins of silkworm haemolymph have been studied to elucidate their role in silkworm development. In 1991, Telfer identified vitellogenin, in a *Hyalophora cecropia*, it is a female specific protein in the haemolymph, it is the first report to found vitellogenin in insects[2]. In 1980 s, two major proteins, SP1 (storage protein 1) and SP2 (storage protein 2), were discovered in silkworm larval haemolymph[9,10]. Hence we tried to elucidate the types of proteins generally present in silkworm heamolymph to observe the proteomics profile. The details of elucidate proteins, their functions and reference are presented in Figure1.

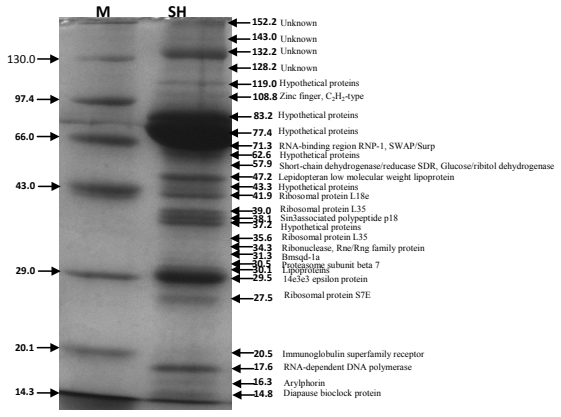

Figure 1. Silkworm heamolymph proteins profile by using 15% SDS-PAGE, M: Marker, SH: silkworm haemolymph

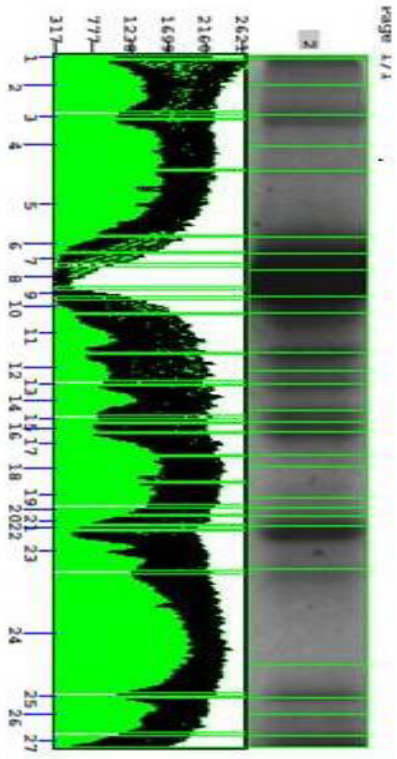

Figure 2. Silkworm heamolymph proteins band intensity.

2. Haemolymph Proteins

2.1 RNA Binding Region RNP-1

The RNA binding region RNP-1 (MW 71.3) is a protein which plays more roles in post transcriptional control of RNAs, such as splicing play adenylation, mRNA stabilization, localization and translation[11]. The abundance of this protein might be attributed to the role of these proteins which is evident that these proteins are required in larger quantities.

2.2 Hypothetical Proteins

Hypothetical proteins are also reported to have wider phyletic distribution and in certain cases are referred as conserved hypothetical proteins[12]. Hypothetical proteins are found in organisms from several phylogenetic lineages but not functionally characterized these functional proteins are a major hindrances in biological studies as it is hard to predict their function. Hypothetical proteins are predicted only from nucleic acid sequences and protein sequences with unknown functions; such hypothetical proteins were also reported in *Danaus pleexippus* with an unknown function. Hypothetical proteins are

Table 1. Silkworm haemolymph proteins MW and their functions

SI. No	Protein name	MW	Function	Reference
1.	Hypothetical proteins	119, 83.2, 77.4, 62.6, 43.3, 37.2,	Half percent of proteins are belongs to this group	10, 11
2.	Zinc finger, C_2H_2-type	108.8	DNA recognition, RNA packaging, transcriptional activation	40
3.	RNA-binding region RNP-1, SWAP/Surp	71.3	post- transcriptional control of RNAs	9
4.	Short-chain dehydrogenase/ reducase SDR, Glucose/ribitol dehydrogenase	57.9	A broad spectrum of metabolic functions	19, 20
5.	Lepidopteran low molecular weight lipoprotein	47.2	Unknown function	12, 13
6.	Ribosomal protein L18	41.9	Structural proteins	37
7.	Ribosomal protein L35	39.0, 35.6	pre-rRNA processing and ribosomal protein assembly function	38, 39
8.	Sin 3 associated polypeptide p18	38.1	responsible for the repression of transcription via the modification of histone polypeptides	14
9.	Ribonuclease, Rne/Rng family protein	34.3	the maturation of all RNA molecules, both messenger RNAs that carry genetic material for making proteins, and non-coding RNAs that function in varied cellular processes	15
10.	Bmsqd-1a	31.3	interacting selectively and non covalently with any nucleic acids	16
11.	Proteasome subunit beta 7	30.5	Multicatalytic proteinase	34
12.	Lipoproteins	30.1	Many enzymes, transporters, structural proteins, antigens, adhesins, and toxins are lipoproteins	22, 23. 24. 25, 26, 27, 28
13.	14e3e3 epsilon protein	29.5	expression of Ras-mediated signaling	35, 36
14.	Ribosomal protein S7E	27.5	Processing of pseudogenes	37
15.	Immunoglobulin superfamily receptor	20.5	Suggesting a distinct biological function	17, 18
16.	RNA-dependent DNA polymerase	17.6	RNA molecule as a template for the synthesis of complementary DNA stands.	31, 32
17.	Arylphorin	16.3	Defense activity	29, 30
18	Diapause bio-block protein	14.8	Metabolism in silkworm diapauses eggs, its transient ATPase activity,	21

Table 2. Haemolymph Proteins their concentration, area and molecular weight in 15% SDS-PAGE gel by using E-box software (Vilber Lourmat)

Lane Number	Volume	Height	Area	Molecular Weight
Band 1	502087	2371	323	152.471
Band 2	5501228	2676	3402	142.326
Band 3	334508	2265	243	131.215
Band 4	5677804	2321	3240	119.138
Band 5	6785208	2290	4131	109.477
Band 6	786479	1869	972	83.757
Band 7	349007	1465	729	77.409
Band 8	549301	921	1377	71.320
Band 9	238510	1152	567	62.584
Band 10	679888	1720	972	57.730
Band 11	2952934	2150	2511	47.198
Band 12	2271944	2283	1782	43.256
Band 13	104259	2114	81	41.783
Band 14	2811427	2375	1944	39.245
Band 15	400987	2226	324	39.092
Band 16	681038	2168	567	37.229
Band 17	2304878	2365	1458	35.156
Band 18	3106109	2409	1620	34.151
Band 19	2660139	2365	1458	31.495
Band 20	795183	2264	486	30.695
Band 21	728040	2220	567	30.521
Band 22	228613	2061	243	29.272
Band 23	17067503	2840	6966	27.545
Band 24	166023	2495	81	20.591
Band 25	232729	2253	162	17.652
Band 26	3768364	2215	2268	16.393
Band 27	886019	1974	809	14.886

expected to reveal new crucial aspects which could to better functional predictions of proteins. Some of hypothetical proteins, their molecular weight 37, 43, 62, 77, 83, 119 kDa were expressed. The hypothetical proteins function can be predicted by domain homology with various confidence levels. These proteins are characterized by low identity to known, annotated proteins[12,13].

2.3 Lepidopteron Low Molecular Weight Lipoproteins

Lepidopteron low molecular weight lipoproteins are extracellular protein of unknown function, its biosynthesis is reported to be stage dependent fashion in the fat body of the insect, and these are of the abundant proteins in the heamolymph of silkworm in fifth instar larvae[14]. These proteins are synthesized in peripheral fat body tissue and translocated into the visceral for utilization during adult development as storage proteins[15]. Their MW is 47 kDa and shows unknown function.

These consist of family of eukaryotic sequences and are also generally referred as SAP18; this complex of proteins is responsible for transcription by the modification of histone polypeptides and is presented to be involved in the regulation of splicing during execution of cell death. Hence in the present context the relevance of these proteins in silkworm heamolymph is to regulate the cellular metabolism of various cells. Their MW is 38 kDa; it is responsible for the repression of transcription via the modification of histone polypeptides[16].

2.4 Ribonuclease

Ribonuclease family proteins are reported from *Wolbachia* pipientis which are generally presented in silkworm as endosymbiont[17]. Ribonuclease, Rne/Rng family proteins are same molecular weight 34.3 kDa. Hence the presence of these proteins in silkworms is relevant.

2.5 Bmsqd-1

These Bmsqd-1a protein is reported in silkworm *Bombyx mori*, they are nucleic acid binds proteins with a molecular functions of interacting selectively and non covalently with any nucleic acids. MW is 31.3 kDa. These proteins are reported from posterior silk glands during the developmental stages[18].

2.6 Ig Super Family

Several types of immunoglobulin related proteins are expressed in insects and as in most cases they are inducible and heat based. These groups of proteins belong to the Ig super family. Large group of cell surface and soluble protein Ig super family proteins are identified with MW 20 kDa. The molecular complexity of innate immune system of insects can be privileged through this family of proteins[19,20].

2.7 Short-chain Dehydrogenase/reducase SDR

The Short-chain dehydrogenase / reducase SDR, Glucose/ribitol dehydrogenase MW is 57.9, its shows a broad

spectrum of metabolic function in specially insects[21]. Some structural proteins like ribosomal protein L18 were identified; their molecular weight is 41.9 kDa. The short chain dehydrogenase/reductases family (SDR) is a large family of enzymes also reported in drosophila[22].

2.8 Diapauses Bio-clock

The protein in the range of 14 kDa is assigned as diapause bio-clock proteins is a countdown clock which depends on esterase A4. Regulation of diapause is a very important step in insect metamorphosis; hence the role of these proteins is very crucial. During metamorphosis the storage proteins are broken down into free amino acids, which are used for synthesis of other proteins required in the adult stage[23].

2.9 Lipoprotein

The most abundant transport protein in haemolymph is lipoprotein, which transports lipids between tissue[24]. Many enzymes structural proteins, antigens, adhesions and toxins belong to lipoproteins[25]. In silkworm heamolymph 30 kDa lipoprotein is identified.

2.9.1 Arylophorin

Arylophorin is a larval storage protein (LPS) which might serve as storage protein used primary scarce of aromatic amino acids for protein synthesis during metamorphosis. It is also reported as a constitute of sclerotization of cuticle and as a carrie of ecdysteroid hormon, works as nutrient reservoir in molecular function[26]. Their molecular weight is 16 kDa, this is a major haemolymph protein in the last larval stage of insects and hexameric glycoprotein[27] and also shows immunological function.

2.9.2 RNA Dependent DNA Polymarase

RNA dependent DNA polymarase proteins have been reported to regulate and control viral infections in insects[28]. Their MW is 17.6 kDa. This is synthesized by complementary DNA stands[29].

2.9.3 Enzyme Like Proteins

Various enzymes like ribonuclease (MW 34 kDa), proteasome subunit beta 7 (MW 30 kDa), RNA-dependent DNA polymerase (17 kDa) were identified. These are involve in the maturation of all RNA molecules, both messenger RNAs that carry genetic material for making proteins and non coding RNAs that function in varied cellular processes (Natalie and Roy, 2010). Proteasome subunit beta 7 is a multicatalytic proteinase[30].

2.9.4 14-3-3 Epsilon

14-3-3 epsilon proteins have been reported in insects and acts as suppression of phenotype characters with expression of Ras-mediated signaling, their MW is 29.5 kDa. This protein belongs to a highly conserved family of molecules that regulate intracellular signal transduction and the cell cycle and prevent apoptosis[31].

2.9.5 Ribosomal Proteins

A few ribosomal proteins like ribosomal protein L18, Ribosomal protein L35, Ribosomal protein S7E were identified depending on their molecular weight, 27, 35, 39, 41 kDa. Ribosomal protein L18 is structural protein[32]. A ribosomal protein is any of the proteins that in conjunction with rRNA make up the ribosomal subunits involved in the cellular process of translation[33], and has several roles in metabolism in insects[34].

2.9.6 Zinc Finger Protein

Zinc finger protein with high molecular weight 109 kDa were identified. Zinc finger proteins are among the most abundant proteins in eukaryotic genomes, their functions are extraordinarily diverse and include DNA recognition, RNA packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly, and lipid binding[35]. Zinc finger, C2H2 type: zinc finger proteins with proteases to contact target metals most of the time metals, have been in silkworm heamolymph is reported to contain several metals in heamolymph. Which might see the interaction of these proteins in silkworm heamolymph. The C2H2, Zn and Fe most common DNA binding metals found in eukaryotic transcriptional factors[35].

2.9.7 Silkworm as a Model for Biomedical Application

Haemolymph contains hemocyanin. Hemocyanin is large copper-containing protein that transports oxygen in the haemolymph of many arthropod and mollusk species[36] they are haemolymph plasma proteins which functions in defense against microbial infection. Phenoloxidase an enzyme stored in hemocytes, and their function is melanization process of after body injury for stop the blood loss. Some times hemocytes are also playing an important role of immune function to protect.

Silkworms have already been established as a model organism various fields of study like bacterial infection study[37], baculovirus expression study, insect immunity, pharmacokinetics study[38], toxicology and metabolism[39]. Most of the research outcome through these studies has proven the correlation and similarity between mammals and silkworms. Recently silkworm is used for assessing the therapeutic effects of chemicals, drug discovery, screening for immunistimulatory agents, detecting test of poisons for drugs and quantitatively evaluating therapeutic effects. Pyriproxyfen residue is changes the level of sugar, urea, uric acid, cholesterol, total protein, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase in heamolymph[40]. The changes of haemolymph protein metabolism after treatment of organophosphorus insecticides[41]. Hormones and neurohormons are identified in haemolymph. The neurohormons are secreted from the central nervous system by the neurosecretory cells of the pars intercerebralis of the brain into the haemolymph to regulate various aspects in a diverse group of insects. In[42] Niimi S, Sakurai S. were synthesized *in vitro* juvenile hormone in corpora allata of the silkworm, *Bombyx mori* for development changes in juvenile hormone and juvenile hormone acid titers in the haemolymph[42].

Some enzymes are isolated from silkworm haemolymph. Daizo K *et al* were purified two isozymes of chitinase from *B. mori* haemolymph and investigated their physicochemical properties, kinetic behavior, and partial amino acid and nucleotide sequences.

The findings of silkworm heamolymph profiling reveals the complex mechanisms involved as evident from the insect physiological mechanisms. It is very relevant to analyze and understand the complex proteins in silkworm *Bombyx mori*. Though till recently it was only looked at as a silk producing organism, with the advent and of natural materials and bioresourceable materials for biomedical applications. The present need of considering the use of silkworm as a model animal for genomic as well as pharmaceuitical applications industrially will be more efficacious considering the ethical and functional aspects. Furthermore there are more advantages of using invertebrates, for arious practical considerations of less space and small equipments for handle of large amount of animals and many biological processes and conserved between mammals and invertebrates. Understanding the hosts protein mechanism will definitely help in realizing more potential application of this economic insect. The function of same as mammalian insulin, *Bombyx mori* bombyxin stimulates the phosphorylation of InR and Akt[43]. Similar to insulin in mammals, bombyxin also regulates sugar and lipid metabolism in *Bombyx mori*. Ueno Y et al were analyzed genetic of basic chymotrypsin inhibitors in the haemolymph of the silkworm[44]. 30K protein of silkworm haemolymph have been reported to exhibit anti-apoptotic activity in various mammalian and insect cell systems and could have therapeutic potential in diseases related to apoptosis[45]. They isolated and characterized the apoptosis inhibiting compounds from silkworm haemolymph[46].

3. Acknowledgement

Dr. Shyam kumar would like to acknowledge to UGC for funding of silkworm immunity research work.

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