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Changes in Soluble Proteins during the Development of Malaria Vector—*Anopheles stephensi* (Diptera : Insecta)

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Qualitative and quantitative changes in soluble proteins during the development of *An. stephensi* as examined by SDS-PAGE and Spectrophotometry revealed in all 97 polypeptides bands of molecular weight ranging from 10 to 205 KDa (eight polypeptides were common in all the stages examined). The stage-specific proteins have been suggested to have regulatory role during embryonic and pupal development. 71KDa polypeptide was present in the highest concentration in the early pupa seems to be similar like "calliphorin" molecule. The decline in the number of proteins and their quantity with advancing pupation has been attributed to the process of histolysis. The adult emergence was marked with the appearance of nine new polypeptides, particularly when adult tissue histogenesis was at its peak. Four low molecular weight sex-specific polypeptides (10, 14, 15 and 16 KDa) were also identified. The changes in these proteins have been correlated with the morphological and physiological events occurring during development.

Key Words : Mosquito, *Anopheles stephensi*, Insect, Development, Polypeptide

Introduction

The major paradigm of developmental genetics is differential gene expression from the same nuclear repertoire. Different cell types make different sets of proteins which impart specific quality to them. Therefore the studies of proteins is the first step towards the understanding of the mechanism of differentiation programme of development.

The temporal changes in the level of soluble proteins in specific tissues during development (Rank et al. 1982, Shivanna & Ramesh 1995) have provided valuable insight into the control of gene activity and cellular differentiation in these tissues (Chen et al. 1985). However, studies with specific tissues do not address the problem of identifying the major protein fluctuations in the entire organism. The changes in the protein pattern during insect development particularly during metamorphosis in holometabolous insects (Chen et al. 1985, Gakhar & Maleyvar 1985) reflects the radical process of histolysis and histogenesis. Most of the earlier studies (Brzyski & Ruh 1982, Turchetto et al. 1987, Faulde et al. 1990 Banks et al. 1994, Wheeler & Buck 1995) pertain to arbitrary choosen stages of development and do not indicate specific

age and sex, that too are lacking particularly in mosquitoes. The present investigation is an attempt to analyse major protein species and quantitative analysis during the development of *An. stephensi*, an important vector of malaria parasite, *Plasmodium falciparum* on the Indian subcontinent (WHO 1982) where their control has been hampered by insecticidal resistance (Subbarao et al. 1984).

Materials and Methods

Organism

The culture of *An. stephensi* (Delhi strain : stock obtained from Malaria Research Centre, New Delhi) was maintained in our laboratory at 28 ± 2°C and 70-80 % RH as detailed earlier (Gakhar & Vandana 1989). Different developmental stages of known chronological ages were pooled for further analysis.

Preparation of Protein Extract

The tissues were homogenised in 60 µl of phosphate buffer saline (8.0g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.2g KH_2PO_4 and distilled water to 1000 ml, *p*H 7.4) containing Phenylmethyl sulfonyl fluoride.

The supernatants were collected by centrifugation at 10,000 rpm for 15 min. at 4°C. To each supernatant equal volume of 2x sample buffer (100 mM Tris-HCl pH-6.8, 4% SDS, 0.2% bromophenol blue, 20% Glycerol, 2% β-ME) was added and thereafter heated at 100°C for 2-3 min. The samples were stored at 20 °C until used.

Electrophoresis

Electrophoresis was carried out in 10% polyacrylamide gels using *Tris*-glycine buffer system containing 0.1% SDS as described (Laemmli 1970).

After electrophoretic run, the gels were fixed in 10% trichloroacetic acid for 20 min. and stained with 0.15% coomassie brilliant blue in methanol : acetic acid : water (10 : 07 : 83, vol.).

Molecular Weight Determination

The apparent molecular weight of various protein bands was determined using the following molecular weight standards — Cytochrome-c (12 KDa), Carbonic anhydrase (29 KDa), Bovine serum albumin (68 KDa), Phosphorylase-b (97 KDa) and β-galactosidase (116 KDa).

Quantitative Estimation

The quantity of protein was assayed by the method of Lowry et al. (1951).

Test for Glycoproteins

The periodic acid schiff (PAS) test was performed according to the method described by Hames and Rickwood (1986) for glycoproteins.

Results

The qualitative and quantitative variations of soluble proteins of *An. stephensi* are presented in figures 1 to 3. In total 97 polypeptides (mol. wt. 10 to 205 KDa) could be resolved. The total number of polypeptides present in each stage has been depicted on the top of each lane of electropherogram (figure 2). The glycoproteins have been

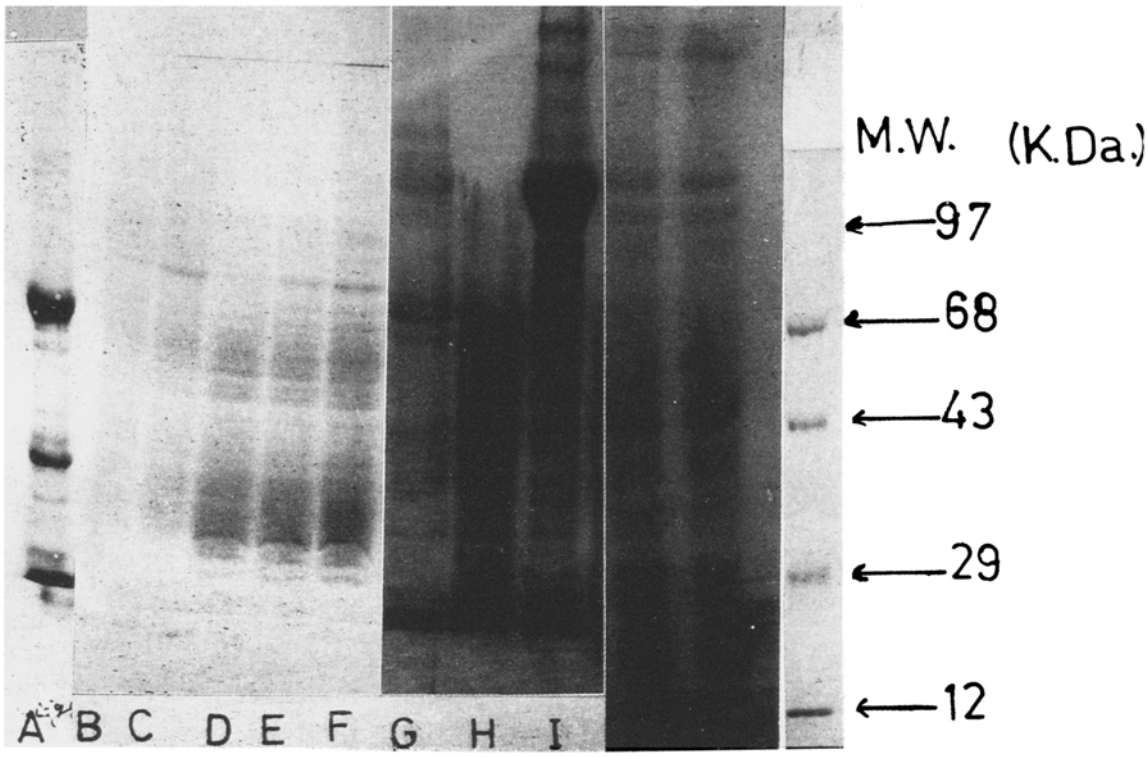

Figure 1 Original gel showing the SDS-PAGE of soluble proteins in different developmental stages of *An. stephensi*.

A. Egg ; *B*. Instar- I larva (Early) ; *C*. Instar-I larva (Late) ; *D*. Instar-II larva ; *E*. Instar-III larva ; *F*. Instar-IV larva ; *G*. Pupa-late ; *H*. Pupa-mid ; *I*. Pupa-early ; *J*. Newly emerged adult-male ; *K*. Newly emerged adult-female

indicated by putting asterisk on their molecular weight in the following text.

Eight low molecular weight polypeptides viz., 11*, 12*, 13*, 19*, 21*, 28*, 31* and 33* KDa were found to be common in all the stages of development examined.

Six polypeptides - 36, 73, 110, 115, 125 and 140 KDa were exclusively found during embryonic stages. The concentration of 36 KDa was very high in the eggs. Similarly, three polypeptides - 16.5, 48 and 59 KDa were found to be present only during all the larval stages.

The protein pattern of pupal stage was observed to be quite different from earlier stages. It is characterized by the appearance of 18 new polypeptides (37, 43, 54, 60, 65, 67, 69, 71, 94*, 105*, 120, 128, 137, 145, 152, 180*, 190* and 205 KDa) and disappearance of 10 polypeptides (16.5, 38, 39.5, 48, 53, 59, 75, 76*, 86 and 91 KDa) in the early pupa. Similarly 4 new proteins (58*, 85*, 87 and 90* KDa) were synthesized along with the

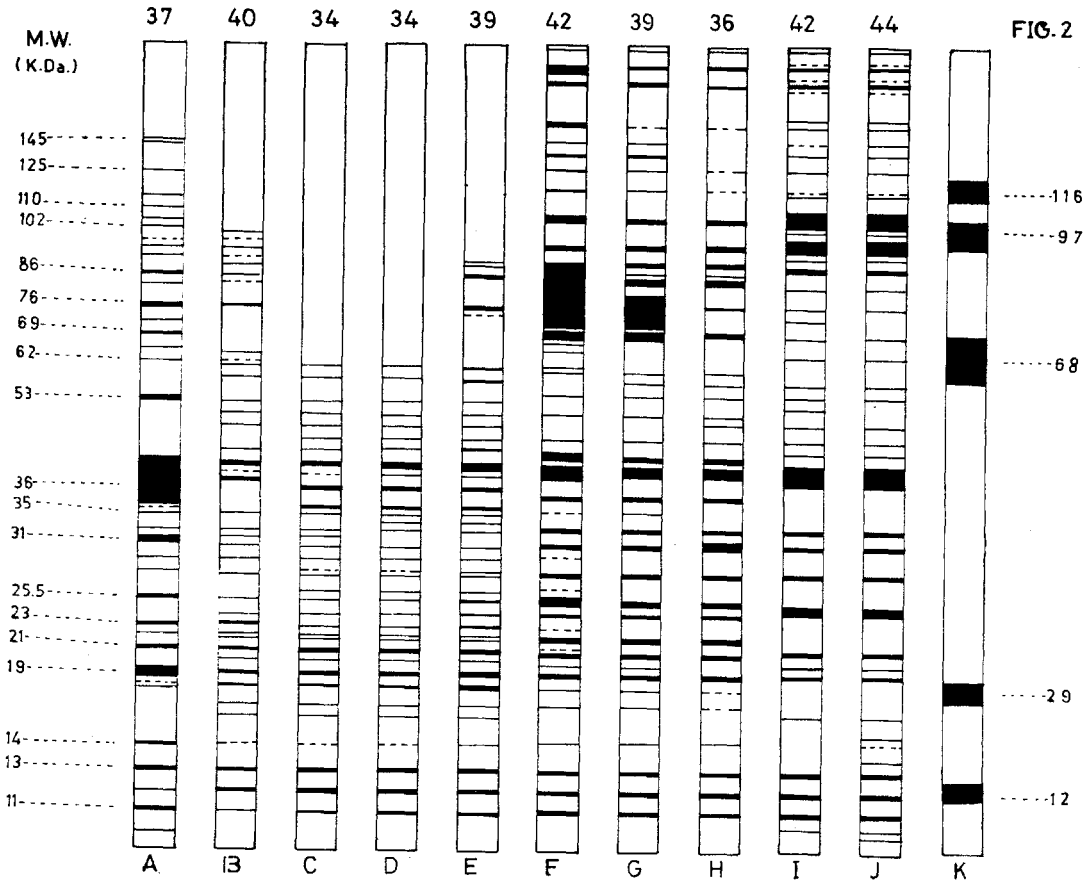

Figure 2 Electropherogram showing the pattern of soluble proteins in different developmental stages of *An. stephensi*.

A. Egg ; *B.* Instar-I larva ; *C.* Instar-II larva ; *D.* Instar-III larva ; *E.* Instar-IV larva ; *F.* Pupa-early ; *G.* Pupa-mid ; *H.* Pupa-late ; *I.* Newly emerged adult-male ; *J.* Newly emerged adult-female ; *K.* The molecular weight standards.

disappearance of 7 pre-existing minor polypeptides (21.5, 23, 27, 30, 61, 65 and 67 KDa) during mid pupal stage. The late pupal stage is characterized by the synthesis of 2 polypeptides (50 and 76* KDa) and disappearance of 4 polypeptides (20, 71, 137 and 145 KDa). The 37, 43, 60 and 71 KDa proteins were present only during the pupal stage.

The concentration of 71 KDa polypeptide was very high in early pupa, started declining with the advancing pupal stages and finally disappeared on adult emergence.

The adult emergence coincided with the synthesis of 9 polypeptides (25*, 64, 93*, 102, 117*, 155, 175, 185 and 195 KDa)

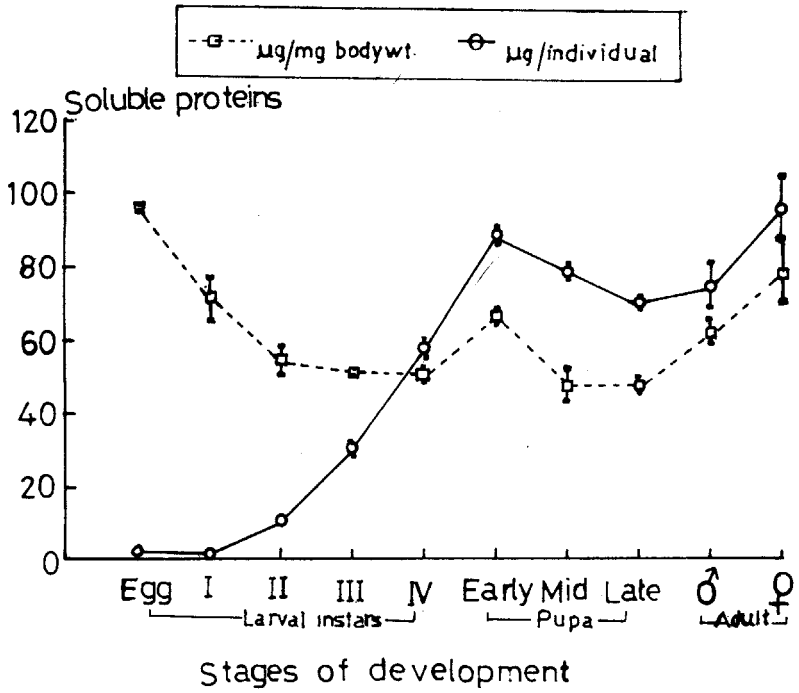

Figure 3 Quantitative variations in soluble proteins during the development of *An. stephensi*.

while 10 pre-existing proteins (17.5, 18.5, 22, 24, 26, 37, 43, 51, 60 and 87 KDa) disapppeared. The concentration of 5 polypeptides (40', 94', 105', 180' and 190' KDa, were high during the adult life. However, of these only 6 bands (25', 117', 155, 175, 185 and 195 KDa) were observed to be adult specific. The remaining proteins were present in some of the other successive stages of development as indicated in the table 1.

Regarding sex-specific differences, 4 female-specific, low molecular weight (10, 14, 15 and 16 KDa) polypeptides could be identified.

Quantitatively, the total soluble proteins declined by about 25.6% ($P<0.05$) on hatching of eggs. During larval stages,there was a decrease by about 28.8% ($P<0.05$) in the instar-IV as compared to instar-I. The soluble proteins declined further during metamorphosis by about 29.44% ($P<0.05$). The protein concentration increased significantly by about 33% ($P<0.05$) in male and 67% ($P<0.05$) in female in freshly emerged adults.

Discussion

The results show that the soluble proteins of *An. stephensi* varied in quantity, quality and their synthesis during different developmental stages.

The present study demonstrated different stage and sex-specific

Table 1 *Pattern of only newly synthesised polypeptides during various stages of development of* ***An. stephensi***

Mol. Wt.	Larval Instars				Pupa			Adult	
(KDa.)	I	II	III	IV	Early	Mid	Late	♂	♀
16.5	+	+	+	+	-	-	-	-	-
17.5	+	+	+	+	+	+	+	-	-
20	+	+	+	+	+	+	-	++	+
22	+	+	+	+	++	++	++	-	-
24	+	+	+	+	++	++	++	-	-
25	-	-	-	-	-	-	-	+++	+++
26	-	+	+	+	+	-	-	-	-
32	+	+	-	-	-	-	-	-	-
34	-	-	+	+	-	-	-	-	-
35.5	-	++	++	++	+	+	-	-	-
37	-	-	-	-	++	++	++	-	-
38	-	++	++	++	-	-	-	-	-
39	++	-	-	-	-	-	-	-	-
39.7	+	+	+	+	-	-	-	-	-
40	++	++	++	++	+++	+++	+++	+++	+++
43	-	-	-	-	+++	++	++	-	-
45	+	+	+	+	-	-	-	+	+
47	-	+	+	+	+	+	+	+	+
48	+	+	+	+	-	-	-	-	-
50	-	-	-	-	-	-	+	+	+
51	+	+	+	+	+	+	+	-	-
54	-	-	-	-	+	+	+	+	+
58	-	-	-	-	-	+	+	+	+
59	+	+	+	++	-	-	-	-	-
60	-	-	-	-	+	+	+	-	-
61	+	+	+	++	+	-	-	-	-
64	+	-	-	-	-	-	-	+	+
67	-	-	-	-	+	+	-	-	-
71	-	-	-	-	+++	+++	-	-	-
90	+	-	-	+	-	++	++	++	++
94	-	-	-	-	++	++	++	+++	+++
117	-	-	-	-	-	-	-	+	+
120	-	-	-	-	+	+	+	+	+
128	-	-	-	-	+	+	+	+	+
137	-	-	-	-	+	+	-	+	+
152	-	-	-	-	++	+	+	+	+
155	-	-	-	-	-	-	-	+	+
175	-	-	-	-	-	-	-	+	+
180	-	-	-	-	++	++	++	++	++
185	-	-	-	-	-	-	-	+	+
190	-	-	-	-	+++	++	++	++	++
195	-	-	-	-	-	-	-	+	+
205	-	-	-	-	+	++	+	+	+

+ = Low concentration ; ++ = Moderate concentration ; +++ = High concentration

polypeptides which may be the product of the events occurring during that particular stage of development. These specific polypeptides are suggested to be associated with specific tissues rather than being general cellular proteins (Rank et al. 1982, Faulde et al. 1990).

Eight polypeptides which were common in all the stages indicate their continuous synthesis, common origin and perhaps similar functions in all the developmental stages.

The eggs had relatively higher protein content. This may be due to insect vitellogenins found in eggs in relatively large amounts (Marinotti and Bianchi 1986). Insect vitellogenins are synthesized by female fat bodies that participate in yolk protein-vitellins production (Izumi et al. 1994). The very high concentration of 36 KDa along with the presence of 5 others proteins (73, 110, 115, 125 and 140 KDa) exclusively in eggs suggest their regulatory function during embryonic development. However, it is premature to speculate.

Similarly, the appearance of 71 KDa polypeptide only during pupation in very high concentration indicates that it may be required for the regulation of pupation (Rank et al. 1982) . The abundance and the pattern of this protein during pupation suggest that it may be similar to the "calliphorin" molecule observed to decrease during pupation in *calliphora* sp. (Levenbook & Bauer 1980) and *Dacus oleae* (Kefaliakou-Bourdopoulou et al.1981). The protein content at the onset of pupation decreases as pupae are characterized by the low metabolic and biosynthetic activity followed by remarkable increase in metabolism at the adult emergence.

During transition from pupa to adult in *An. stephensi*, proteins already present seem to serve as the main constituent for the construction of adult tissue proteins and that this re-distribution may be mediated by proteolytic enzymes. This hypothesis is in accordance with the conclusion drawn by Turchetto et al. (1987).

Four female-specific polypeptides identified may be synthesized in the female fat body and selectively sequestered by developing oocyte (Yadav et al. 1988, Izumi et al. 1994). The conspicuous absence of these female-specific bands in male is supportive of the above statement and further suggests that the male of this species does not synthesize vitellogenin in contrast to few male insects of other species (Yadav & Kumar 1986, Thymianou et al. 1995).

The decline in the protein level during larval growth of *An. stephensi* is comparable with that of other insects viz., *Sitophilus* (Singh & Sinha 1977), *Trabala vishnou* (Gakhar & Maleyvar 1985) and *Ripotortus clavatus* (Chinzei et al. 1991). The decline of soluble proteins during the early phase of metamorphosis is attributed to the process of histolysis of larval tissues. This coincides with the disappearance of 7 polypeptides in the mid-pupal stage. The increase of soluble

proteins before adult emergence may be due to the process of histogenesis of adult tissues. The soluble proteins built up during the pupal stages of *Trabala vishnou* had been attributed to the metabolism of glycogen and chitin (Gakhar & Maleyvar 1985).

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