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Proteomic analysis of *Aedes aegypti* midgut during post-embryonic development and of the female mosquitoes fed different diets

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ABSTRACT

In this work we analyzed protein expression in the *Aedes aegypti* midgut during the larval (fourth instar, L4), pupal, and adult stages [including newly emerged (NE), sugar-fed (SF) and blood-fed (BF) females]. Two-dimensional electrophoresis showed 13 spots in the midgut of larvae, 95 in the midgut of pupae, 90 in the midgut of NE, and 76 in the midgut of SF or BF females. In the larval midguts, high serpin expression was noted, while in the pupae, protein abundance was lower than in the NE, SF, and BF females. The spots related to proteins linked to energy production, protein metabolism, signaling, and transport were highly expressed in the NE stage, while spots related proteins involved in translation were abundant in SF and BF females. The differential abundance of proteins in the midgut of *A. aegypti* at different developmental stages supports the necessity for midgut development during immature stage followed by the necessity of proteins related to digestion in adults.

1. Introduction

The yellow fever mosquito *Stegomyia aegypti* (=*Aedes aegypti*) is associated with the living spaces of humans, and is the primary vector of the dengue virus, and other viruses such as Chikungunya (CHIK) [1]. In general, *A. aegypti* females require a bloodmeal for ovarian development and oviposition to occur. When female mosquito feeds on blood, it is temporarily stored in the midgut, where digestion, absorption of nutrients, and infection by blood-borne pathogens (including viruses) occurs [2].

The ingestion of blood causes significant morphological and biochemical changes in the midgut of the adult female mosquito, resulting in epithelial flattening and increased protein synthesis of the peritrophic matrix (PM) and digestive enzymes (e.g., trypsin) in the posterior region of the organ [3]. Studies investigating gene expression in the midgut of *A. aegypti* females at a post-transcriptional level have shown that 3 and 7 days after feeding on sugar or blood, females had a similar protein expression profile, except for defensin, which increase in expression after blood-feeding [4]. On the other hand, when infected with CHIK virus or Dengue virus type 2 (DENV-2), modified expressions of proteins associated with regulatory, metabolic and structural pathways in the midgut were noted [5]. Moreover, midgut proteins linked to DENV-2 infection, including enolase, beta-ARK, and cadherin that can act as receptors, and the elongation factor EF-1 alpha/Tu that is important for viral replication [6], have recently been identified.

During its life cycle *A. aegypti* move from an aquatic to a terrestrial environment, and during this passage, the midgut undergoes changes in morphology and gene expression. The midgut remodeling during metamorphosis includes the replacement of digestive cells in the larval epithelium during pupation by the digestive cells of the adult. These changes allow the insect to change its diet and it ceases to feed on microorganisms and decomposing detritus and begins to feed on plant sugars and/or blood [7–9]. In this study, protein profiles of the *A. aegypti* midgut in the larval, pupal, and adult stages (newly emerged and fed with sugar or blood) were studied. In addition, we examined the possible roles of these proteins during the mosquito life cycle and during feeding, which contribute to a better understanding of the working dynamics of the midgut under different feeding conditions.

2. Material and methods

2.1. Mosquitoes and protein extraction

A. aegypti specimens (PPCampos strain, Campos dos Goytacazes, Rio de Janeiro) were obtained from a colony maintained in the insectary of the Department of General Biology at Universidade Federal de Viçosa, Brazil. Insects were raised in plastic trays containing dechlorinated tap water and containing turtle feed as a food source (Reptolife®-Alcon). The insects were maintained under conditions of controlled

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temperature (25 ± 5 °C), relative humidity (60 ± 5%), and a defined photoperiod (12:12 light/dark).

Two-hundred mosquito specimens were used to study each stage: 4th instar larvae (L4), female pupae 24 h after ecdysis (pupa), newly emerged adult females (0 − 12 h) (NE), adult females 3–4 days old fed on sugar solution (10% sucrose) (SF), and females fed sugar and provided with a blood meal (BF) from mouse anesthetized with Dopalen (Ceva). The midguts were dissected in PBS (0.1 M sodium phosphate buffer, pH 7.2). For L4, the midguts were gently pressed with tweezers for the expulsion of the food bolus surrounded by the PM. The midguts of BF were dissected 24 h after the blood meal. In this case, the ingested blood surrounded by the PM was discarded, and only midguts with no residual blood were used in the experiments.

After dissection, the midguts were gently washed in PBS and transferred to 50 μL of PBS at 4 °C containing 1 mM thiourea, 1 mM benzamidine, and 1 mM phenylmethylsulfonyl fluoride (PMSF), sonicated three times on ice, and centrifuged at 14,000 × *g* for 10 min at 4 °C. To the supernatant, 150 μL of cold acetone was added and maintained at − 20 °C for 3 h. After this period, the material was centrifuged at 14,000 × *g* for 10 min at 4 °C. The resulting pellet was resuspended in 50 μL of solubilization solution, consisting of 7 M urea, 2% CHAPS (w/v), and 2 M thiourea, and protein concentration was quantified by the Bradford method using BSA as standard protein.

2.2. Two-dimensional electrophoresis (2-DE) and image analysis

The 2-DE was performed at the Proteomics and Protein Biochemistry Laboratory, Universidade Federal de Viçosa. Midguts were taken from each mosquito developmental stage, and three 2-DE gel (technical replicates) from a pool of 200 mosquitoes was done for each stage or feeding condition. Each Immobiline DryStrip pH 3–10, 7 cm (GE Healthcare) was hydrated for 16 h with 125 μL of a solution containing 85 μg protein added Destreak (GE Healthcare), 40 mM dithiothreitol (DTT), and 2% (v/v) IPG buffer pH 3–10 (GE Healthcare). Isoelectric focusing (IEF) was performed using the IPGphor3™ (GE Healthcare) system in accordance with the manufacturer's instructions.

After IEF, strips were reduced for 15 min in a 5 mL buffer solution containing 75 mM Tris-HCl pH 8.8, 1% DTT, 6 M urea, 30% glycerol (v/v), 2% SDS (w/v), and 0.002% (w/v) bromophenol blue, and then alkylated in the same buffer by substituting DTT with 2.5% iodoacetamide. The second dimension was performed using polyacrylamide gel, 12% T glycine-SDS-PAGE (Sodium dodecyl sulfate

Table 1
Proteins of *A. aegypti* midgut identified by MS/MS. NE — newly emerged adults; SF- sugar fed adults; BF — blood fed adults.

Spot	Code	Protein	Sequence	Ion Score/C.I. %	e-Value	% Sequence coverage	Protein Score/C.I.,%
L481	gi|157116805	Serine proteinase inhibitors (serpins)	FKIEFDLDLKETLEK	115/100	8e-10	4	161/100
L482	gi|157116805	Serine proteinase inhibitors (serpins)	FKIEFDLDLKETLEK	108/100	2e-06	9	166/100
			GFRSEAESVNFQDNTATAK	23/72			
P6	gi|108877579	Trypsin-like serine protease	YNNPQFHNDIALVK	58/100	2e-9	5	91/100
	gi|157113343	Trypsin-like serine protease	IVQHPQFSYSTIDYDYSLLK	62/100	1e-15	7	
NE1	gi|403182713	Glyceraldehyde 3-phosphate dehydrogenase	LISWYDNEFGYSNR	65/100	1e-09	7	119/100
			IQVFQERDPK	21/90			
NE3	gi|94468602	Catalase [*A. aegypti*]	IWPQAEFPLIPVGR	28/97	3e-12	5	77/99
			LFAYTDTHR				
NE34	gi|157113061	Calreticulin family	FFNDEENDKGLQTSQDAR	30/96	8e-13	4	82/100
NE37	gi|157131648	ATP synthase alpha, central domain.	EAYPGDVFYLHSR	55/100	5e-10	5	124/100
			TALAIDTIINQQR	45/100			
NE38	gi|157168005	WD40	FSPNHSNPIIVSAGWDR	61/100	3e-12	5	122/100
NE39	gi|108871874	Conserved domains on Citrate synthases	ALGVLASLVWDR	60/100	2e-07	5	129/100
			SGQVVPGYGHAVLR	32/98			
NE41	gi|157124666	Voltage-dependent anion channel (VDAC)	EFGGLIYQR	35/99	9e-04	3	72/99
NE44	gi|108871874	Citrate synthases	LPVVAATIYR	22/90	7e-05	4	43/100
			ALGLPIERPK	21/90			
NE47	gi|403182713	Glyceraldehyde 3-phosphate dehydrogenase	LISWYDNEFGYSNR	50/100	7e-10	4	50/100
NE58	gi|108884060	Protein disulfide isomerase (PDI)	FVTAQALPLIVDFSHETAQK	68/100	3e-14	4	149/100
NE59	gi|108881686	*F*1 ATP synthase beta	IINVIGEPIDER	21/90	8e-07	2	99/100
NE60	gi|157121051	Enolase	GNPTVEVDLVTDLGLFR	88/100	1e-12	9	191/100
			SGETEDTFIADLVVGLSTGQIK	57/100			
NE64	gi|157120956	Arginine or creatine kinase [*A. aegypti*]	AVQQQLIDDHFLFK	43/100	1e-08	6	125/100
			IPFSHHDR	32/97			
NE65	gi|301641424	Actin partial [*A. aegypti*]	SYELPDGQVITIGNER	68/100	3e-10	7	125/100
			GYSFTTTAER	64/100			
NE67	gi|108880329	Aldo-keto reductases (AKRs)	LVPITSAAGHPYHPFEKEEF	56/100	3e-15	6	56/100
NE68	gi|157114623	Pyridine nucleotide-disulfide oxidoreductase	DGSKQELEFDVLLVSVGR	79/100	8e-12	3	145/100
NE69	gi|108879763	Sugar kinase/HSP70/actin superfamily	SYELPDGQVITIGNER	36/98	1e-10	4	71/100
NE70	gi|108879763	Sugar kinase/HSP70/actin superfamily	SYELPDGQVITIGNER	63/100	4e-10	7	105/100
			IWHHTFYNELR	29/82			
NE71	gi|108872600	14-3-3 epsilon	LGLALNFSVFYYEILNSPDR	32/97	1e-07	12	63/98
			YLAEFATGDDRK	22/62			
NE72	gi|157114501	14-3-3 protein	LGLALNFSVFYYEILNSPDK	125/100	6e-15	8	147/100
NE75	gi|301641424	Actin partial [A. aegypti]	SYELPDGQVITIGNER	60/100	1e-10	7	88/100
NE76	gi|765339426	Glutathione S-transferase (GST)	LVTLNSEVIPFYLEKLDDIAR	83/100	2e-07	17	235/100
			FLLSYGNLPFDDIR	78/100			
BF16	gi|94468818	HSPA5	IEIESFYEGDDFSETLTR	34/99	4e-13	3	34/99
BF18	gi|157119815	V/A-type ATP synthase catalytic subunit A	RVGYYELVGEIIRL	41/99	5e-08	8	123/100
			RALDDFYDKNFQEFVPLRT	27/99			
			KFGYVFAVSGPVVTAERM	22/99			
BF25	gi|357604983	Alpha_tubulin	RNLDIERPTYTNLNRL	39/99	1e-19	9	105/100
			RFDGALNVDLTEFQTNLVPYPRI	25/99			
BF28	gi|301641424	Actin	KSYELPDGQVITIGNERF	27/99	4e-07	14	97/100
			kIWHHTFYNELRV	26/99			
BF30	gi|108882996	Enolase	RGNPTVEVDLKTAKGLFRA	30/99	4e-07	4	95/100
BF33	gi157103617	Translation_Factor_II_like superfamily	R.AGQVGYLVCNMRTV.E	21/99	1e-03	2	92/100