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Free Amino Acids in the Gut, Fat Body, Gonads and Haemolymph of *Schizodactylus monstrosus* Drury (Orthoptera: Schizodaclidae) : Possible Role of Intracellular Bacteria

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Analyses of free amino acids in the gut, fat body, gonads and haemolymph of male and female *S. monstrosus* revealed organ-specific variation. In the gut, hind gut exhibited highest amino acid content followed by mid- and fore-gut. Fat body, haemolymph and gonads of female insect showed higher amino acid level than male. Amino acid content in partially aposymbiotic insects revealed total absence of arginine, aspartic acid and valine and decreased level of almost all amino acids except proline and glutamic acid presenting higher concentration.

Key Words: Free amino acids, Intracellular bacteria, *Schizodactylus* **sp.**

Introduction

Considerable information is available on the free amino acids in insects in view of their physiological importance, but attention has been mostly directed to studies on high titres of amino acids in the haemolymph (Barrett 1974, Barrett & Friend 1975, Barrett & Lai-Fook 1976 and Woodring & Blakeney 1980), with only a few references to amino acid concentration in other tissues (Levenbook 1966 and Rakshpal 1973). Blood amino acid level remains in a state of dynamic equilibrium in spite of the rapid turnover due to the influx from feeding and efflux due to cellular growth and peptide-protein secretion (Woodring & Blakeney 1980). This homeostatic limit of blood amino acids is maintained during various physiological activities as a result of dynamic equilibrium between the amino acid levels in haemolymph and various tissues which act as store-house of amino acids (Evans 1972).

Insects harbouring micro-organisms and the roles played by them in maintaining the normal physiological balance of the host is known since last few decades. Their role in the synthesis of essential

and non-essential amino acids has also been reported by many workers (Block & Henry 1961, Henry & Block 1962 and Baker & Mabie 1973). Keeping in view these facts, the present investigation was undertaken to study the amino acid levels in fore-, mid- and hind-gut, fat body, gonads and haemolymph in both sexes of normal and aposymbiotic *Schizodactylus monstrosus*.

Materials and Methods

Gut, fat body and gonads of adult male and female *S. monstrosus* were dissected out. The fore-, mid- and hind portions of gut were separated after clearing the gut contents by ringer solution. Haemolymph was collected through a fine graduated capillary tube, previously rinsed with 1% phenylthiourea to prevent darkening of the blood, into a microbeaker. Sample preparation for amino acid analysis was done following the method of Rakshpal (1973). Amino acid separation was done by two dimensional paper chromatography using phenol:water (4:1) and butanol:acetic acid acid:water (4:1:1) as the two solvents. Quantitative estimation of amino acids was done spectophotometrically by referring the values to the standard curves of each amino acid. Six replicates were prepared to minimize errors.

Role of intracellular bacteria in the synthesis of amino acids was established by rendering the insects aposymbiotic through streptomycin of different doses (0.5%, 1% and 1.5 %) depending upon the body weight. The dose was injected for three successive days and thereafter amino acid analyses from tissues and haemolymph were made. Fluctuation of bacterial number for different days was determined through phase contrast microscope following Noda's (1974) calculations.

Results

Amino acids in different tissues and haemolymph exhibited considerable organ-specific and sex-wise variations. The alimentary canal showed highest concentration of amino acids with significant zonal variation—the hind gut containing highest concentration of amino acids (table 1). Free amino acid variation between the two sexes appeared much pronounced; almost all amino acids except arginine and serine showed higher concentration in the fat body and haemolymph of female insect, ovary showed relatively higher amino acid levels than testis (tables 2 & 3). Examination of the tissue homogenates for the detection of intracellular bacteria revealed that fat body and to some extent gonads contained bacteria, which appeared to be gram-positive. Of the three doses of streptomycin, 1% dose appeared to be much satisfactory in reducing bacterial number/organ (data shown in tables). Partially aposymbiotic insects showed much alterations in the concentration of individual amino acids; arginine, aspartic acid and valine were absent in all the tissues and haemolymph (tables 2 & 3) except gut where their concentration appeared much meagre (table 1). Almost all other amino acids showed considerable lower concentration in aposymbiotic insects except proline and glutamic acid presenting higher concentrations.

Discussion

The results give an idea about the organ-specific variation of free amino acids with sex-wise differences and the role of intracellular bacteria in the metabolism of these amino acids. Higher levels of almost all amino acids in the gut explained temporary accumulation of these amino acids obtained from food. Considerable zonal variation

Table 1 *Free amino acids content (μg/100mg wet tissue wt) in fore-, mid- and hind-gut of* S. monstrosus *(Data are mean±SE of 6 replications)*

Amino acids	Fore		Mid		Hind	
	C	T	C	T	C	T
Alanine	39±1.2	22±1.7	52±1.6	44±1.8	69±1.8	62±2.1
Arginine	21±1.2	—	27±2.1	—	35±2.1	—
Asp. acid	117±1.3	72±2.1	162±1.9	89±1.5	196±1.6	102±2.1
Glut. acid	181±2.7	169±1.9	219±2.1	228±2.3	242±3.1	251±2.7
Glycine	167±1.3	145±2.7	152±1.5	131±2.1	200±3.2	192±2.7
Histidine	129±2.1	119±2.3	107±1.8	93±2.1	129±3.2	119±2.8
Leucine	159±1.9	143±2.7	205±1.3	91±1.8	221±2.6	212±1.7
Lysine	159±1.9	143±2.8	205±1.3	191±2.4	321±1.7	311±1.2
Ph. alanine	19±0.6	8±0.2	42±1.3	18±1.3	59±1.9	41±1.4
Proline	479±2.1	369±1.9	405±2.8	381±2.7	419±2.1	425±1.8
Serine	381±3.8	348±3.1	321±1.8	302±1.9	369±2.1	351±1.6
Threonine	141±1.9	123±2.2	191±1.5	179±3.1	222±1.7	215±1.7
Valine	102±1.4	—	146±2-5	—	199±1.7	—
Methionine	81±2.1	72±3.2	105±2.1	85±2.6	121±1.8	118±1.5

C=control; *T*=treated (1% streptomycin)
'—' indicates absence

Table 2 *Free amino acids content in the fat body (μg/100 mg wet wt) and haemolymph (μg/100ml) and number of bacteria (data × 10/mg tissue wt) in control (C) and 1% streptomycin-treated (T)* S. monstrosus *in both sexes (Data are mean ± SE of 6 replications)*

Amino acids	Fat body				Haemolymph			
	Male		TT Female		Male		Female	
	C	T	C	T	C	T	C	T
Ala.	39±1.4	22±1.8	48±1.9	28±1.9	25±1.7	12±1.5	31±1.5	22±1.3
Arg.	31±1.3	—	19±1.6	—	15±1.4	—	7±0.7	—
Asp.	119±1.8	—	112±1.9	—	74±1.5	—	81±1.9	—
Glu.	185±1.9	205±2.1	191±1.9	209±2.1	85±2.8	105±1.9	99±1.9	72±2.5
Gly.	172±2.6	101±1.9	169±2.0	112±1.9	91±1.9	62±2.1	105±2.9	89±1.7
His.	91±1.9	59±1.2	112±1.7	81±2.1	57±2.5	32±1.3	72±1.8	64±2.1
Leu.	171±1.8	119±2.1	181±2.7	116±2.9	82±1.9	69±1.9	85±1.6	67±1.7
Lys.	277±1.9	242±1.5	281±1.4	239±1.2	189±2.3	141±2.8	182±2.7	165±1.8
P.al.	425±3.2	389±2.9	431±2.8	377+2.9	313±2.9	259±2.8	289±2.8	265±2.9
Pro.	582±2.9	601±3.9	561±3.4	644±3.8	557±2.6	609±2.7	512±2.9	485±2.9
Ser.	92±1.9	89±1.1	81±1.9	72±1.6	72±1.7	92±1.2	41±1.7	29±1.4
Thr.	245±1.8	199±2.3	261±2.7	20±22.9	142±1.9	81±2.1	169±2.9	131±2.6
Val.	121±2.7	—	155±2.6	—	72±2.6	—	95±1.8	—
Met.	92±2.2	65±1.8	115±2.1	71±2.9	65±2.8	25±2.1	89±1.1	32±2.1
No. of bact.	67±1.4	2.3±0.1	68±1.8	2.9±0.7	—	—	—	—

'—' indicates absence

Table 3 *Free amino acids content in testis and ovary (μg/100 mg wet tissue wt) and number of bacteria (data × 10^7/mg wet tissue wt) in control (C) and 1% streptomycin treated (T)* S. monstrosus *(Data are mean ± SE of 6 replications)*

Amino acids	Testis		Ovary	
	C	T	C	T
Alanine	681±.7	32±1.6	75±1.7	39±1.2
Arginine	180±1.8	—	191±1.9	—
Asp. acid	—	—	22±1.1	—
Glut. acid	203±1.8	285±2.7	261±2.8	212±2 7
Glycine	195±2.4	181±1.3	211±2.4	195±2.1
Histidine	123±1.2	111±1.7	139±2.5	109±1.9
Leucine	214±1.7	193±1.6	221±1.7	212±1.8
Lysine	327±3.1	302±1.9	329±2.1	319±2.1
Ph. alanine	365±3.8	341±1.5	371±2.4	359±2.6
Proline	405±3.2	482±3.7	425±2.8	449±3.8
Serine	60±1.2	39±1.2	72±1.9	51±2.1
Threonine	214±2.7	192±2.9	231±2.7	205±1.6
Valine	102±2.3	—	195±2.8	—
Methionine	57±1.7	35±1.2	91±1.9	62±2.1
No. of bact.	12±0.1	1.2±0.3	15±0.4	1.6±0.02

'—' indicates absence

in the levels of respective amino acids indicated regional differences in the permeability of the gut to amino acids and this actually depended upon the internal lining of the gut. Relatively higher level of amino acids in the hind gut might be attributed to the fact that the internal lining of the rectum was sufficiently permeable to many amino acids (Dutta & Majhi 1979). Comparatively higher contents of glycine and leucine in the fore gut in respect to mid gut explained specific absorption of these amino acids to mid gut; corroborating the findings of Dutta and Majhi (1979).

Comparatively high free amino acid levels in the fat body, gonads and haemolymph of female insect might be attributed to the direct and indirect roles of amino acids in reproduction. In comparison to other amino acids, relatively higher levels of methionine and valine in females was supposed to be due to their active roles in the reproduction (Chen 1958, Coles 1965, Davey 1967 and Barrett & Friend 1975).

Predominance of proline in both sexes might be attributed to it's life activities (extremely aggressive) since proline was considered as an active source of energy (Bursell 1963 and Mayer & Canady 1969).

Moderate haemolymph amino acid level was suggested to be regarded as an index to phylogenetic status of the order Orthoptera (Duchateau & Florkin 1958).

Notable decline in the quality and quantity of the free amino acids in partially aposymbiotic insects explained that the intracellular bacteria played some role in the synthesis of essential and

non-essential amino acids (Henry & Block 1962 and Mitlin & Wiygul 1973). For the synthesis of these amino acids in addition to other nutrients, the intracellular bacteria are known to utilize proline and glutamic acid (Bismanis 1976). Slightly increasing levels of these amino acids in partially aposymbiotic insects support this possibility.

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