Proc. Indian natn. Sci. Acad. **B57** Nos. 3&4 pp. 239-244 (1991)

Analysis of Cast-off Skins of *Trabala vishnou* Lef (Lepidoptera: Insecta) of Various Stages of Development

S K GAKHAR

Department of Bio-Sciences, Maharshi Dayanand University, Rohtak 124 001, *Haryana*

(Received 19 September 1990; after revision 12 February 1991; Accepted on 18 April 1991)

Qualitative and quantitative analysis of free amino acids have been carried out of all the skins casted off during the development of *Trabala vishnou* Lef. Reducing carbohydrates, lipids and proteins have also been analysed in the cast-off cuticles. Higher concentrations of these constituents were noticed in the exuviae and cocoons of female larvae. Egg-shell and pupal-shell showed a similar FAA composition except for methionine, proline and valine. γ-aminobutyric acid was significantly higher in the cast-skins of female stages. The loss of all these constituents generally increased in the exuviae of successive larval instars. The loss of these FAA in the cast skins has been proposed as the energy saving device.

Key Words: Cast-off skins, *Trabala vishnou*, Free amino acids

Introduction

The present investigations complement the detailed qualitative and quantitative ontogeny of free amino acids (FAA) at very close intervals of *Trabala vishnou* (Gakhar & Maleyvar 1984 abcd). There seems not to be even a single entry about the information of FAA in the cast-off cuticles of the successive stages of insect development, as compared to the bulk of information available about this insect body itself (Chen 1971). However, cuticular bound amino acids have been studied by some workers (Hackman & Goldberg 1958, Karson et al. 1969, Kawasaki et al. 1971 and Pant & Sharma 1972, 1974).

The study was undertaken with a view to examine the qualitative and quantitative patterns of FAA in all the different skins of two sexes separately which were casted-off during development. In addition, the loss of reducing carbohydrates, soluble proteins and lipids have also been analysed in order to assess the total loss of these constituents in the exuviae.

Materials and Methods

The larvae of *T. vishnou* collected from the Kurukshetra University Campus were reared in the laboratory on the leaves of castor, *Ricinus communis* L. at 28 ± 2°C and 70% RH in an incubator as described earlier (Gakhar 1983). Egg-shells left over after the emergence of larvae were collected, freed from unhatched eggs and contaminants. This insect passes through five instars. The cast-off cuticles were collected carefully at each instar termination. The fifth instar/pre-pupal cuticle was collected after the pupa formation. Pupal-shells were collected after the adult emergence from the cocoons by splitting them with the sharp blade and also avoiding the contamination from each other. All the cast-off cuticles were washed separately with distilled water and were homogenised in 80% ethanol. FAA after extraction (Ganti & Shanmugasundram 1963) were analysed qualitatively and quantitatively by spectrophotometrically as described earlier in detailed by Maleyvar and Gakhar (1982).

Total reducing sugars were estimated from ethanolic extract with the help of Dreywood's anthrone reagent (Morris 1948). For quantitative determination of lipids, known dry weights of various cast-off skins were extracted with chloroform; methanol (2:1 v/v) for 24 hrs (Folch et al. 1957). The proteins were determined by the method of Lowry et al. 1951. The larvae could be sexed during the final phase of instar V on the basis of difference in their live weight (Maleyvar & Gakhar 1984).

All the data were subjected to students 't' test to determine statistical significance.

Results

The FAA composition varied in cast-off skins of various developmental stages and is represented in tables 1 & 2.

The egg-shell had the maximum number of FAA (18) as compared to all other cast-off skins including 8 essential amino acids viz., arginine, histidine, leucine/phenylalanine, lysine, methionine, threonine, tryptophan and valine. Quantitatively, GABA, leucine/phenylalanine and proline were in high titres (ranged between 12 to 15% of the total FAA). However, asparagine, glutamine, glutamic acid and tyrosine were only in trace amounts.

Qualitatively, the exuviae had only 6 FAA in all, of which 3-FAA asparagine, aspartic acid and serine/glycine were common to exuviae of all the five instars (table 2). Statistically the total FAA content did not change significantly in the exuviae of first four instars. However, it was interesting to note that this level rose by about 14-folds in the exuviae of last instar-V ($p < 0.01$).

Silken-cocoon had only four non-essential FAA (table 1) which were also common to those present in the exuviae. Asparagine and aspartic acid were the main titres (ranged from 23 to 27% of the total FAA).

Table 1 *Concentration of various FAA (mg/g dry weight ± SD) of the cast-off cuticles of successive larval instars of* ***T. vishnou***

Name of FAA	I Instar	II Instar	III Instar	IV Instar	V Instar pre-pupa-exuviae	
					Male	Female
Alanine	Trace	—	—	—	—	—
Asparagine	0.0091 (±0.01)	0.0080 (±0.00)	Trace	0.0316*** (±0.01)	0.2000*** (±0.02)	0.2101 (±0.003)
Aspartic acid	0.0210 (±0.00)	0.0120 (±0.00)	Trace	0.0267*** (±0.01)	0.1011 (±0.001)	Trace
γ-Aminobutyric acid	—	—	—	—	0.0527 (±0.00)	0.1482*** (±0.01)
Glutamine	0.0200 (±0.01)	0.0149 (±0.00)	0.0395** (±0.01)	—	—	—
Leucines/Phenyl alanine	—	—	—	—	0.1573 (±0.01)	0,1483 (±0.01)
Serine/glycine	0.0200 (±0.01)	0.0311 (±0.00)	0.0369*** (±0.00)	0.0062*** (±0.00)	0.3001*** (±0.01)	0.3102 (±0.01)
Total FAA	0.0701 (±0.01)	0.0668 (±0.01)	0.0764 (±0.01)	0.0645 (±0.01)	0.8112*** (±0.12)	0.8168 (±0.11)

(e) = Essential amino acid
(*) = Significant at P = 0.1
(**) = Significant at P = 0.05
(***) = Significant at P = 0.01

Compared with the preceeding value

Table 2 *Concentration of various FAA (mg/g dry weight ± SD) of the cast-skins i.e. egg-shell, cocoon and pupa-shell of different sexes of* ***T. vishnou***

Name of FAA	Egg-shell	Silken cocoon		Pupa shell	
		Male	Female	Male	Female
Alanine	0.2602 (±0.01)	— —	— —	0.4562 (±0.01)	0.4673 (±0.01)
Arginine (e)	0.3253 (±0.01)	—	—	1.2185 (±0.12)	1.4398 (±0.15)
Asparagine	Trace	0.1210 (±0.01)	0.1221 (±0.01)	0.2167 (±0.02)	0.1736 (±0.03)
Aspartic acid	0.4649 (±0.01)	0.1311 (±0.01)	0.1201 (±0.01)	0.2101 (±0.02)	0.1295*** (±0.02)
γ-Amino butyric acid	1.0700 (±0.10)	—	—	0.1088 (±0.02)	0.0410** (±0.01)
Glutamic acid	Trace	0.0625 (±0.00)	0.1257** (±0.02)	0.5100 (±0.08)	0.6314 (±0.09)
Glutamine	Trace	—	—	0.5294 (±0.07)	0.7704** (±0.09)
Histidine (e)	0.6579 (±0.02)	—	—	0.9112 (±9.09)	0.9500 (±0.08)
Leucines/phenyl alanine (e)	0.8170 (±0.03)	—	—	0.3124 (±0.02)	0.3031 (±0.03)
Lysine (e)	0.2602 (±0.01)	—	—	0.3919 (±0.02)	0.2210*** (±0.02)
Methionine (e)	0.1590 (±0.01)	—	—	—	—
Ornithine	0.4338 (±0.01)	—	—	0.1421 (±0.01)	0.1421 (±0.02)
Proline	1.0700 (±0.03)	—	—	—	—
Serine/glycine	0.0939 (±0.01)	0.0343 (±0.01)	0.0295 (±0.00)	0.6180 (±0.02)	0.6094 (±0.03)
Threonine (e)	0.5061 (±0.01)	—	—	0.3101 (±0.01)	0.3220 (±0.01)
Tryptophan (e)	0.1590 (±0.001)	—	—	0.5110 (±0.08)	0.6978** (±0.02)
Tyrosine	Trace	—	—	0.6014 (±0.02)	0.6314 (±0.03)
Valine (e)	0.4699 (±0.00)	—	—	—	—
UN-1	—	0.1300 (±0.01)	0.1101 (±0.01)	0.2200 (±0.01)	Trace

(e) = Essential amino acid
UN = Unidentified spot
* = Significant at P = 0.1
** = Significant at P = 0.05 Compared with the preceeding value
*** = Significant at P = 0.01

Pupal-shell had the same composition of FAA which was present in the egg-shell except that three FAA methionine, proline and valine were absent. The total FAA content was highest in the pupal-shell as compared to other casted-off cuticles during development. Two essential FAA, histidine and arginine were in high titres (ranged from 12 to 19% of the total FAA). Regarding the sex-specific differences, GABA and aspartic acid were noticed in high titres in the exuviae of female larvae ($p < 0.05$), in addition to glutamine, lysine and tryptophan into the female pupal-shell, and only glutamic acid in cocoons ($p < 0.05$).

Soluble proteins were maximum in the egg-shell, 76.4% dry weight. Its content was very low during the first four instars exuviae (ranged between 12-13%). Its level became more than double in the exuviae of instar-V ($p < 0.05$). Reducing carbohydrates were very low in the cast-off cuticles. They ranged from 0.5% in exuviae to 5% in the pupal-shell. However, lipid concentration ranged between 25% in the pupal-shell and 96% in the egg-shell in all the cast-skins except for cocoons where the concentration was very low, i.e. only 3-4% of dry weight. All these bio-chemical constituents were generally in higher concentration in the cast-skins of females than the males of their counterparts ($p < 0.01$, $p < 0.05$) (table 3).

Discussion

From the present study it may be concluded that the four non-essential amino acids asparagine, aspartic acid and serine/glycine appear to have some special significance as these FAA were present in all the cast-off cuticles and in the body in high titres during *T. vishnou* development (Maleyvar & Gakhar 1982, 1984, Gakhar & Maleyvar 1984 a, b). The FAA compositon of *T. vishnou* larvae was also roughly similar to that of *R. communis* leaves (Gakhar 1983). No other worker appears to have examined the FAA in the cast-off skins. However, some workers (Karlson et al. 1969, Hackman 1988, Kawasaki et al. 1971 and Pant & Sharma 1972, 1974) studied the cuticular proteins bound amino acids. The deposition of FAA in the cast-off skins may be taken as one of the alternative methods of nitrogen elimination and which may also be the energy saving device whereby the synthesis of uric acid can be avoided atleast partially.

The similar composition of FAA in the egg-shell and the pupal-shell is suggestive of the fact that some fluid is, however, secreted before the process of hatching of larva and adult, which may contain so many FAA which are deposited in an unknown manner in the egg-shell and pupal-shell respective-

Table 3 *Concentration of soluble proteins, reducing carbohydrates and lipids in the cast-skins of* ***T. vishnou*** *during development (mg/g dry wt). Standard deviations have been mentioned in parenthesis* (±)

Sr. No.	Stage of development	Proteins	Reducing carbohydrates	Lipids
1.	Egg shell	763.75 (±15.19)	2.1755 (±0.49)	76.00 (±2.14)
2.	Exuvia I instar	125.50*** (±6.21)	0.5012 (±0.13)	29.30 (±0.98)
3.	II Instar	122.00 (±4.16)	0.5230 (±0.06)	34.70 (±1.12)
4.	III Instar	130.00 (±4.83)	0:6499 (±0.08)	35.00** (±1.11)
5.	IV Instar	125.00** (±2.05)	6.7271** (±0.22)	45.10*** (±1.21)
6.	Pre-pupa or V instar			
	Male	270.00 (±6.75)	8.4014 (±0.84)	50.00 (±1.24)
	Female	326.25*** (±8.36)	13.1678*** (±0.81)	55.00** (±1.27)
7.	Pupa shell			
	Male	326.25 (±8.33)	54.2144 (±3.25)	25.00 (±1.78)
	Female	305.00 (±8.33)	50.9269 (±2.97)	27.00 (±1.99)
8.	Silken cocoon			
	Male	599.25 (±8.71)	9.0554 (±0.75)	3.25 (±0.21)
	Female	627.50*** (±8.11)	10.9416* (±0.61)	4.17 (±0.75)

*Significant at P = 0.1
**Significant at P = 0.05
***Significant at P = 0.01

ly. The egg-shell contains all the essential FAA. Presence of methionine may be important as this sulphur containing amino acid has been shown to help in providing covalent links by −SH and S−S bonds in side chains of proteins of egg-shell as to impart specific secondary configuration (Kawasaki et al. 1971). The egg-shell contains about 31% of the total FAA present in the fertilized egg (Maleyvar & Gakhar 1982) before hatching. Similarly about 40% and 50% of FAA were present in the male and female pupal-shell, respectively, when compared with the total FAA of pupa on last day of metamorphosis (Gakhar & Maleyvar 1984c).

The presence of only six FAA in the exuviae inspite of the continuous supply through feeding larval stage, might be due to their incorporation into cuticular/larval proteins or due to their utilization in various metabolic activities. Tyrosine and alanine are mainly involved in the hardening of insect cuticle (Hackman & Goldberg 1971). Proline acts as a precursor of cuticular proteins during sclerotization (Hackman 1959). The absence of sulphur containing amino acids has also been reported in the cuticular proteins of exuviae of *Philosamia ricini* (Pant & Sharma 1974) and *Agrianoma spinicollis* (Hackman & Goldberg 1958). Though the seventeen amino acids have also been identified in moulting fluid of fourth instar and pre-pupa of Bombyx (Zielinsker & Laskowska 1958).

Silken cocoon is secreted by salivary glands which has about 0.9% FAA in male and 1.7% in female when compared to total FAA present in the larval body during last instar (Maleyvar & Gakhar 1984). Silk protein fibroin is rich in glycine and serine which together contribute 45-90% of the total amino nitrogen (Corrigan 1970). In the present study, the free state of these amino acids constitutes about 5-7% of total FAA pool present in the cocoon.

It was surprising that GABA and tryptophan were present in higher concentration in the cast-off skins of female but they were absent in the body of larva and pupa of both the sexes before moulting (Maleyvar & Gakhar 1982 and Gakhar & Maleyvar 1984c). GABA participates in nitrogen assimilation and is not incorporated into proteins (Chen 1971).

The concentrations of other bio-chemical constituents compare favourably with that of *Philosomia ricini* (Pant & Sharma 1974).

References

Chen P S 1971 Monographs in development biology; *S. Karger*, London **3** 230

Corrigan J J 1970 Nitrogen metabolism in insects; in *Comparative Biochemistry of Nitrogen Metabolism: The Invertebrates* 387-487 ed. by Campbell (London: T.W.C. Academic Press)

Folch T, Leos M and Sloane-Stanley G H 1957 A simple method for isolation and purification of total lipids from animal tissue; *J. Biol. Chem.* **226** 497-506

Ganti and Shanmugasundram E R B 1963 A study on the free amino acids during growth and metamorphosis of *Corcvra cephalonica St; J. Exp. Zool.* **152** 1-4

Gakhar S K 1983 Studies on the development of *Trabala vishnou* Lep. (Lepidoptera: Insecta); Ph.D. Thesis, Kurukshetra University, Kurukshetra, 271 pp

——and Maleyvar R P 1984a Qualitative sex-specific changes in free amino acids during metamorphosis of *Trabala vishnou; Comn. Physiol. Ecol.* **9** 185-188

——and—— 1984b Analysis of the excreta during the various larval instars of *Trabala vishnou* Lep. (Lepidoptera: Insecta), *Monitore Zool. Ital.* **18** 199-305

——and—— 1984c Age and sex related free amino acids during metamorphosis of *Trabala vishnou* (Lepidoptera: Insecta) and sex-specific differences; *Proc. Indian natn. Sci. Acad.* **B50** 277-285

——and——1984d Age and sex related free amino acids changes in *Trabala vishnou* (Lepidoptera: Insecta); *Proc. Indian Natn. Sci. Acad.* **B50** 362-367

Hackman R H 1959 In *Biochemistry of Insects* Vol. 12, 48 ed. by L Lavenbook (New York: Pergamon Press)

——and Goldberg M 1958 Proteins of the larval cuticles of *Abrianoma soinicollis* (Coleoptera); *J. Insect Physiol.* **2** 221-231

——and—— 1971 Studies on hardening and darkening of insect cuticles; *J. Insect Physiol.* **17** 335-347

Karlson P, Sekeri K E and Marmaras V I 1969 Die aminosaurezusammensetzung verschredener protein fraction on aus der cuti cula von *Calliphora erythroceohale* in ve.schiednen entwicklungsstadien; *J. Insect Physiol.* **15** 319-324

Kawasaki H, Sato H and Suzumi M 1971 Structural proteins in the silkworm egg-shells; *Insect Biochem.* **8** 130-148

Lowry O H, Rosebrough M J, Farr A L and Randal R J 1951 Protein measurement with the folin-phenol reagent; *J. Biol. Chem.* **193** 265-275

Maleyvar R P and Gakhar S K 1982 Changes in free amino acids during the embryonic development of *Trabala vishnou* (Lasiocampidae: Lepidoptera); *Proc. Indian Natn. Sci. Acad.* **B48** 199-206

——and——1984 Sex-specific differences and changes in free amino acids during the larval growth of *Trabala vishnou* Lep. (Lepidoptera: Insecta); *Biologia* **30** 277-282

Morris D L 1948 Quantitative determination of carbohydrates with Dreywood's anthrone reagent; *Science* **107** 254-255

Pant R and Aggarwal H C 1963 Chemical composition of egg-shells of *Philosamia ricini; Naturwissenchaften* **50** 647

——and Sharma K K 1972 Amino acid composition of the egg-shell of *Antherea mylilla; Indian J. Exp. Biol.* **10** 390-391

——and——1974 Amino acids composition of the cuticle of *Philosamia ricini* during various stages of larval development; *Indian J. Exp. Biol.* **12** 192-194

Zielinsker Z M and Laskowska T 1958 Amino acids and amino sugars in the moulting fluid of the silkworm (*Bombyx mori*); *Acta Biol. Exp.* **18** 209-219