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Amino acids, aminotransferases and proteins in the metamorphosing silkworm, *Bombyx mori* L.

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Abstract. The total free amino acid levels declined from the early-fourth instar to the mid-fifth instar and were elevated during the late-fifth instar in the metamorphosing silkworm, *Bombyx mori*. The activity levels of aspartate and alanine aminotransferases showed an increase in the silk gland while they decreased in the central nervous system, muscle and hemolymph. The molting period was, however, characterized by low activity levels in all the tissues studied. The total and soluble protein levels increased continuously during metamorphosis. The results are correlated with active protein synthesis and silk production in the developing *Bombyx mori*.

Keywords. *Bombyx mori*; metamorphosis; central nervous system; amino acids; aminotransferases; proteins.

1. Introduction

Species-specific variations in the ontogenic pattern of various biochemical constituents are an essential feature of insect metamorphosis. The study of Chen (1971) provided an innovative report on the role of biochemical constituents during insect metamorphosis. Biochemical studies on the silkworm have been initiated with the aim of understanding the biochemical correlates of silkworm metamorphosis and silk production. The biochemical parameter which attracted considerable attention in this regard is the silk protein fibroin. The metamorphosis of silkworm involves rapid growth of the silk gland and increased protein and fibroin synthesis. Silkworm proteins and their developmental changes have been examined (Robert and Rutt 1982; Sarangi 1985). Pursuits in this direction have led to studies on the amino acids and on the enzymes involved in protein and amino acid metabolism (Bheemeswar and Sreenivasaya 1952; Inokuchi and Yoshitake 1978; Keiji and Daillie 1978; Pant and Jaiswal 1981; Sumio *et al* 1981; Bannikov *et al* 1982; Giordana *et al* 1982; Wanger-Li and Xuting-Sen 1982; Parenti *et al* 1985). These studies were mostly performed on tissues other than the nervous system. Since the nervous system controls the overall activity of the animal it is essential to study the biochemical changes in this system during silkworm metamorphosis, which might eventually give the clue to the role of this facet in silk production. Hence in the present study some aspects of protein metabolism have been examined in the central nervous system (CNS), muscle, silk gland and hemolymph during the development of the silkworm, *Bombyx mori*.

2. Materials and methods

The present investigation was carried out on LR × NB_4D_2 (multivoltine pure Mysore × bivoltine NB_4D_2) hybrid variety of the silkworm, *B. mori*. The silkworms

were reared in large bamboo trays in the laboratory as per Krishnaswami (1986).

The fourth (lasting 5 days) and fifth (lasting 8 days) instar larvae of the silkworm were selected for these studies, as enough tissue could be obtained from them for analysis. For experimental convenience, the duration in both instars was divided into 3 stages, viz. early, middle and late. 1st, 3rd and 5th days of the fourth instar and 1st, 5th and 8th days of the fifth instar were considered as the early, middle and late stages respectively.

The levels of free amino acids, both soluble and total proteins and the activity levels of aspartate (AAT) and alanine aminotransferases (AIAT) were estimated in the CNS (cerebral ganglion + suboesophageal ganglion + ventral nerve cord, VNC) and other tissues such as the muscle, silk gland and hemolymph.

The total free amino acid levels in the tissues were estimated by the method of Moore and Stein (1954), in 1% homogenate of the CNS, 2% homogenates of muscle and silk gland, and 0·05 ml of hemolymph in 10% trichloroacetic acid (TCA). The AAT and AIAT activity was assayed by the method of Reitman and Frankel (1957) in 2, 5 and 10% homogenates of the CNS, muscle and silk gland respectively, and 0·5 ml of hemolymph.

Soluble proteins were estimated by precipitation with equal volume of 10% TCA, which were then separated by centrifugation.

Total protein content was estimated by the method of Lowry *et al* (1951) from the precipitate obtained from tissue homogenates by 10% TCA.

3. Results

The total free amino acid levels declined from the early fourth instar to mid-fifth instar and were elevated significantly during the late fifth instar (table 1). The extent of change was found to vary among the 4 tissues examined. The decrease was 51,

Table 1. Changes in the total free amino acid content during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	36·40	38·28	34·63	33·9	28·40	18·03	46·26
	SD	±0·96	±0·82	±0·96	±1·1	±1·42	±0·56	±0·89
	PC		+5*	−9**	−2*	−16***	−37***	+157***
Muscle	Mean	15·41	16·34	8·38	8·09	8·38	7·27	47·10
	SD	±0·81	±0·32	±0·46	±0·39	±0·21	±0·84	±0·59
	PC		+6·0*	−49***	−3*	+4*	−13*	+548***
Silk gland	Mean	33·47	35·55	45·2	32·4	31·77	18·55	52·57
	SD	±2·03	±0·45	±3·54	±4·7	±3·27	±0·28	±1·65
	PC		+6*	+27***	−28**	−2*	−42***	+183***
Hemolymph	Mean	21·22	24·25	12·45	11·73	9·73	6·47	37·66
	SD	±0·41	±1·1	±0·31	±0·86	±0·86	±0·37	±0·73
	PC		+14***	−49***	−6*	−17**	−34***	+482***

Each value, expressed as μmol of tyrosine/g wet wt. of tissue or 1 ml of hemolymph is a mean ± SD of 4 separate samples, each containing the tissue from 15–20 animals. The per cent changes (PC) (+ or −) following the means and SD for each period were calculated taking the immediately preceding period as the control.

T test: ***$P < 0·001$; **$P < 0·01$; *statistically not significant.

55, 45 and 70% in the CNS, muscle, silk gland and hemolymph, respectively from the early fourth instar to the mid fifth instar. The increase at the late fifth instar was 27, 206, 57 and 78% in the CNS, muscle, silk gland and hemolymph respectively. The decrease was maximum in the silk gland (42%) while the increase was higher in the muscle (548%).

The activity levels of AAT and AlAT showed considerable variations during the metamorphosis of *B. mori* (tables 2, 3). These variations were minor and not significant during the fourth instar, while they were significant during the fifth instar (tables 2, 3). In all the tissues studied this trend of change in these enzyme activities was more or less similar.

During the molting period a general decrease of enzyme activity was recorded in all the tissues studied (table 2). With the exception of a 11% decrease during the late

Table 2. Changes in the activity levels of AAT during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	0·99	0·96	0·85	0·67	1·10	0·87	0·55
	SD	±0·02	±0·01	±0·02	±0·02	±0·04	±0·3	±0·02
	PC		−3*	−11***	−21***	+64***	−21*	+37*
Muscle	Mean	1·23	1·15	1·09	0·94	1·39	1·18	1·03
	SD	±0·04	±0·11	±0·03	±0·04	±0·04	±0·09	±0·01
	PC		−7*	−5*	−14***	+48***	−15**	−13***
Silk gland	Mean	1·20	1·24	1·27	0·69	0·86	1·27	1·34
	SD	±0·23	±0·02	±0·09	±0·08	±0·03	±0·09	±0·26
	PC		+3*	+2*	−46***	+25**	+48***	+6*
Hemolymph	Mean	5·65	5·61	5·58	5·22	6·44	2·73	2·11
	SD	±0·09	±0·03	±0·04	±0·07	±0·10	±0·55	±0·14
	PC		+1*	−1*	−6**	+23***	−58***	−23***

Values are expressed as μmol of pyruvate formed/mg protein/h. The remaining notation is the same as in table 1.

Table 3. Changes in the activity levels of AlAT during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	1·30	1·27	1·26	1·04	2·41	1·07	0·86
	SD	±0·03	±0·02	±0·25	±0·03	±0·06	±0·05	±0·02
	PC		−2*	−1*	−17*	+132***	−56***	−20***
Muscle	Mean	1·62	1·44	1·21	1·08	2·05	1·51	1·30
	SD	±0·06	±0·07	±0·12	±0·06	±0·22	±0·03	±0·07
	PC		−11**	−16**	−11***	+90**	−26***	−14***
Silk gland	Mean	1·79	1·82	1·95	1·39	1·87	2·18	2·20
	SD	±0·18	±0·03	±0·33	±0·04	±0·12	±0·08	±0·19
	PC		+2*	+7*	−29**	+35***	+17**	+4*
Hemolymph	Mean	5·22	4·96	4·20	3·07	1·62	1·39	1·30
	SD	±0·07	±0·16	±0·68	±0·58	±0·16	±0·04	±0·28
	PC		−5*	−15*	−27*	−47***	−14*	−50***

Notations are same as in table 2.

fourth instar, the AAT activity more or less remained constant in the CNS during the fourth instar. The silk gland recorded maximum decrease (46%) followed by the CNS and muscle, while the hemolymph showed a negligible decrease (6%). All the tissues exhibited a significant increase in the AAT activity from the molting period to the early fifth instar. Following this, the tissues recorded a decrease during the middle and late fifth instar. The sole exception to this decrease was the silk gland which showed an increase during this period (table 2).

The changes in AlAT activity were largely similar to those in AAT activity. In CNS, muscle and hemolymph the activity decreased continuously from early fourth instar through the molting period. The activity then showed an increase in these tissues during early fifth instar, but decreased from there during middle and late fifth instar (table 3). Contrary to this pattern the AIAT activity in the silk gland increased from early to the late fourth instar and decreased during the molting period. From then the activity in this organ increased continuously during early to late fifth instar (table 3). The changes in activity in all the tissues from early to late fourth instar were only minor and statistically not significant.

Thus, in general, the AAT and AIAT activities exhibit: (i) little or no change during the fourth instar, (ii) a decrease during the molting period and (iii) an initial increase and a later decrease during the fifth instar. The silk gland provided an exception to this general trend.

Both total and soluble proteins registered an increase from the early fourth instar to the late fifth instar in all the tissues studied (tables 4, 5). However, the level of soluble proteins in the silk gland started declining from the molting period and continued to decrease thereafter. Maximum increase in the protein levels was recorded in the muscle and silk gland.

4. Discussion

It is known that silk-formation is largely controlled by the level of amino acid reserves supplied from the degenerating tissues (Noguchi *et al* 1974). Probably, silk-

Table 4. Changes in the total protein content during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	75·31	77·02	79·35	70·95	81·87	85·65	88·17
	SD	±4·2	±0·44	±7·3	±3·3	±8·8	±6·1	±2·3
	PC		+2*	+3*	−11*	+15*	+5*	+3*
Muscle	Mean	58·65	100·92	137·61	222·75	268·93	323·98	342·88
	SD	±1·92	±6·54	±9·17	±7·76	±11·4	±21·75	±23·7
	PC		+72***	+36***	+62***	+21***	+20**	+6*
Silk gland	Mean	92·68	162·52	203·86	247·48	308·35	371·33	374·13
	SD	±4·27	±22·07	±5·65	±7·51	±11·22	±10·26	±14·82
	PC		+75***	+25**	+21***	+25***	+20***	+1*
Hemolymph	Mean	10·87	11·09	11·57	18·28	28·59	30·47	33·21
	SD	±0·18	±0·27	±0·32	±0·23	±3·57	±1·23	±1·16
	PC		+2*	+4*	+58***	+56***	+7*	+9**

Values are expressed as mg protein/g wt. of tissue or 1 ml of hemolymph. Remaining notation is the same as in table 3.

Table 5. Changes in the soluble protein content during development in *B. mori*.

Tissue		Fourth instar			Molting period	Fifth instar		
		Early	Middle	Late		Early	Middle	Late
CNS	Mean	27·52	32·98	35·45	37·52	42·16	49·33	50·15
	SD	±1·97	±1·05	±0·7	±1·97	±1·07	±1·82	±2·0
	PC		+20***	+8**	+6*	+12**	+17***	+2*
Muscle	Mean	32·17	33·65	34·73	35·46	36·23	37·39	42·96
	SD	±1·35	±0·7	±1·44	±2·4	±2·5	±0·45	±3·07
	PC		+5*	+3*	+2*	+2*	+3*	+15**
Silk gland	Mean	56·97	79·69	108·05	104·96	87·47	60·65	42·92
	SD	±1·24	±1·93	±5·9	±2·43	±5·9	±2·28	±1·72
	PC		+40***	+36***	−3*	−17***	−31***	−29***
Hemolymph	Mean	10·87	11·06	11·57	18·28	28·59	30·47	30·75
	SD	±0·18	±0·8	±0·32	±0·23	±3·57	±1·23	±1·16
	PC		+2*	+5*	+58***	+56***	+7*	+1*

Notations are same as in table 4.

production is also controlled by the hemolymph amino acids, besides their role in osmoregulation and homeostasis (Anderson 1984). The concentration of free amino acids is known to be influenced by various factors such as the changing dietary conditions (Anderson 1984), proteolytic action of the molting fluid on the old cuticle (Wyatt *et al* 1956), age, nutrition and climatic factors (Inokuchi 1970). Presumably, the fluctuating levels of amino acids in *B. mori* reflect general changes in the metabolism during metamorphosis. The increase in amino acid pool at the late fifth instar is reflective of the initiation of proteolytic activity, which is the characteristic feature of the pupal stage.

The aminotransferases (AAT and AlAT) mediate the transfer of amino groups of the amino acids to α-oxo-glutarate, oxaloacetate and pyruvate to form glutamate, aspartate and alanine respectively (Lehninger 1978). The elevation of AlAT and AAT activity levels of the tissues at the beginning of the instar and of the silk gland at the late fifth instar is indicative of increased amino acid turnover and glutamate formation. The decrease in their activity in the CNS, muscle and hemolymph at the late fifth instar is suggestive of their low utilization, thereby increasing the free amino acid concentration in these tissues.

The decrease in the activity levels of AAT and AlAT during the molting period denotes decreased mobilization of amino acid pool required for protein synthesis due to low metabolic activity. It is also likely that the decrease in biochemical constituents during the molting period is due to their loss in body fluids and exuviae (Gakhar and Maleyvar 1985). Simultaneous decrease of transaminase activity in all the tissues except the silk gland, indicates that the focus is on the silk gland during the later half of the fifth instar.

The present study indicates continuous protein synthesis during the metamorphosis of *B. mori*. Several workers have examined the variation in protein content during insect metamorphosis (Mansingh and Smallman 1967; Firling 1977). As supposed by Mansingh and Smallman (1967) the increase in the proteins of the CNS during metamorphosis may be correlated with the increase in cholinergic enzyme activity. Presumably, the increase in soluble proteins in the CNS is

attributable to the synthesis and storage of various enzymes and neurohumoral factors to be made available to the needy tissue during metamorphosis. Similarly, the increase in the level of total proteins probably accounts partially for building the CNS during development.

As reported in other insects (Martin *et al* 1971; Price 1973) the silkworm hemolymph may act as a transitory storage medium for proteins, which might migrate from other tissues such as the fatbody, muscle, CNS, etc. The muscle, being the contractile machinery of the animal, contains high protein levels in order to maintain its structural and functional integrity. The increase in the protein content of the silk gland is evidently due to high rate of fibroin synthesis (Sarangi 1985). The soluble proteins of the silk gland are probably mobilized into the formation of fibroin as evidenced by their decrease during metamorphosis. The fact that the salivary gland of *Calliphora* (Martin *et al* 1971) absorbs the proteins from the hemolymph, suggests a similar mechanism in *B. mori* between the silk gland and hemolymph, since the former is a modification of the salivary gland.

Thus the biochemical constituents studied in the present investigation seem to fluctuate, and undergo transformations that are necessary for an orderly sequence of events during silkworm metamorphosis. Presumably the CNS coordinates all such events during metamorphosis. The observation that certain neurohumoral factors such as the juvenile hormone (Kwangtung College of Agriculture and Forestry 1975) and β-ecdysone (Tai-chu-ying *et al* 1982) could elevate the transaminase activity, provides evidence as to the central nervous control of metabolism. It is probable that the transaminase activity is enhanced by such factors, which in turn increases the amino acid turn-over in the silk gland for fibroin synthesis. It is not known if such a situation exists in *B. mori*, but it presumably does.

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