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EFFECT OF AGE & SEX ON THE ACTIVITY OF PROTEASE IN THE MIDGUT AND INTEGUMENT OF FIFTH INSTAR SILKWORM, *BOMBYX MORI* (L) (RACE: PM X CSR_2)

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ABSTRACT
Effect of age and sex on the protease activity in midgut and integument of 5th instar *Bombyx mori* (L) was determined. The results indicated that the midgut tissue protease activity significantly increased during active feeding periods up to the 5th day of larval development. However, in the subsequent periods of 6th and 7th day the enzyme activity in the midgut significantly reduced as a result of non-feeding before they began to spin the cocoons. Sexual dimetabolism in the midgut protease was evident in the female exhibited higher enzyme activity in the integument prior to the spinning process is believed to provide precursors for silk protein synthesis. The feeding activity of the larvae stimulated protease production in the midgut tissue.

KEYWORDS: age, sex, feeding, protease activity, midgut, integument, *Bombyx mori*.

INTRODUCTION

The silkworm *Bombyx mori* (L) is an oligophagous insect that feeds mainly on mulberry leaves (*Morus alba*). The fifth instar larvae show a phenomenal growth rate during the first 5 days of their development. The fat body accumulates large quantity of proteins, lipids and glycogen during the development of fifth instar larval stage. The fifth instar larvae digest and utilize efficiently the nutrient reserves of the mulberry leaves. An active protease in the digestive fluid has been reported in the silkworm (Horie *et al.,* 1963; Eguchi & Yoshitake, 1967; Hamano & Makiyama, 1970; Nishida & Hayashiya, 1974; Eguchi & Iwamoto, 1976). Protease of the digestive fluid has been separated into three components and their enzymatic properties have been reported (Iwamoto & Eguchi, 1978). Most of the studies are confined to the protease of the digestive fluid. It has been suggested that there is a functional differentiation between digestive fluid and midgut tissue, that a molecular proteins are hydrolyzed into peptides in the digestive fluid and into amino acids with peptidases in the midgut tissue (Horie *et al.,* 1963). The present study protease activity of the midgut and integument tissues of 5th in star *Bombyx mori* (L) was examined with reference to age and sex.

MATERIALS AND METHODS

The polyvoltine crossbreed (PM x CSR2) 5th instar silkworm larvae were used as the experimental insects. The rearing technique of larvae was essentially similar to that described by Krishnaswamy *et al*. (1978). Male and female 5th instar larvae were dissected separately in the ice-cold *Bombyx mori* (L) saline (Yamoka *et al*., 1971). The complete alimentary canal was removed from the larva and was flushed with ice cold saline three times so as to remove the leaf debris. The midgut was carefully separated from the rest of the alimentary tract and stored over the crushed ice. After the removal of all the organ systems the integument was scraped and made free from the fat body and trachea and used. The tissue was first cut into smaller fragments and then homogenized in an appropriate ice-cold buffer in a 1 ml capacity glass homogenizer with a Teflon pestle. During the process of homogenization the glass homogenizer was immersed in crushed ice. The homogenate was centrifuged in a high speed refrigerated centrifuge (4^0c) at 8000 g for 15 min. The supernatant was collected and used as crude enzyme source in all the experiments. Protease activity was determined according Birk *et al.* (1962) With a slight modification as outlined by Ishaaya *et al.* (1971) The incubation mixture consisted of 400 micro liters of 1 per cent casein solution (vitamin free); 200 micro liters of 0.2M tris HCl buffer (pH 8.5). The incubation was carried out at 30^0C for 60 min with constant shaking. After 60 min, the enzyme activity was terminated by adding 2.4 ml of 2 per cent trichloroacetic acid. The content of the tube was centrifuged at 8ooog. The absorbance of the supernatant was recorded at 280 nm against a blank in which the enzyme extract was substituted by distilled water. Tyrosine was used as the reference standard. A duplicate sample whose enzyme source was denatured before the addition of the substrate was also run as the control side by side with the original sample. The enzyme activity was expressed in terms of specific activity (microgram tyrosine equivalent liberated/mg protein/min). The soluble protein content of the enzyme source was determined according to the method of Lowry *et al.* (1951). The enzyme activity expressed was the maximum obtainable under the conditions mentioned in this investigation.

RESULTS AND DISCUSSION

The activity of protease of the midgut tissue of 5th instar *Bombyx mori* (L) progressively increased as the larva advanced in age up to the 5th day in both the sexes (Table1). The leaf consumption of 5th instar *Bombyx mori* (L) amounts to more than 75 percent of the total for the

whole larval instars. This high intake of food by the 5th instar larva is to accumulate sufficient energy resources to support its metabolism during non-feeding pupal-adult development. The leaf proteins are first hydrolyzed to peptides by the digestive fluid protease(s) and finally into amino acids by midgut tissue protease. Sexual dimetabolism/ dimorphism in respect of midgut protease was evident in that the female larvae showed significantly higher enzyme level than that of the male. The protein requirement of the female is always higher than male on account of egg production. The protease activity in the midgut tissue was significantly reduced on 6th and 7th day of larval development (Table1).

TABLE 1 : Protease activity in the midgut tissue of 5th instar *Bombyx mori* (L) with reference to age and sex.

	Specific Activity (ug tyrosine liberated/ mg protein/ minute)		
Age (Day)	Female	Male	P. Value
1.	0.028 (± 0.03)	0.027 (± 0.07)	NS
2.	0.33 (± 0.07)	0.027 (± 0.03)	<0.005
3.	0.86 (± 0.03)	0.43 (± 0.04)	<0.001
4.	1.19 (+ 0.03)	1.13 (± 0.12)	<0.5
5.	1.52 (±0.03)	1.50 (± 0.06)	NS
6.	0.39 (± 0.01)	0.64 (± 0.04)	<0.001
7.	0.23 (± 0.08)	0.30 (± 0.02)	< 0.002

- Each figure is the mean of three replications
- The figure in parenthesis with ± sign is the standard deviation

This appeared to be due to the non feeding of the larvae. Normally the *Bombyx mori* (L) larvae stop feeding 4-8 hours before they start spinning the cocoons.The protease activity in the integument with reference to age indicated that the enzyme remained almost at the same level during the first three days of development (Table 2).

TABLE 2 : Protease activity in the integument of the 5th instar *Bombyx mori* (L) with reference to age and sex.

	Specific Activity (ug tyrosine liberated/ mg protein/ minute)		
Age (Day)	Female	Male	P. Value
1.	1.97 (± 0.06)	2.02 (± 0.07)	NS
2.	1.90 (±0.06)	1.86 (± 0.08)	NS
3.	1.80 (±0.09)	1.90 (±0.06)	NS
4.	2.5 (±0.02)	2.15 (±0.02)	<0.001
5.	2.68 (±0.01)	2.45 (±0.01)	<0.001
6.	2.92 (±0.04)	2.80 (±0.03)	<0.05
7.	0.56 (±0.05)	0.40 (±0.85)	<0.05

- Each figure is the mean of three replications
- The figure in parenthesis with ± sign is the standard deviation

TABLE 3: Protease activity in the midgut tissue of five day old 5th instar. *Bombyx mori* (L) in response to feeding activity

Time interval in hour	Specific activity (*u*g tyrosine liberated/mg protein/min.)	P Value
1	1.16 (± 0.01)	
2	2.2 (± 0.13)	<0.025
3	3.6 (± 0.17)	<0.001
4	2.9 (± 0.1)	<0.02
6	1.02 (± 0.08)	NS

- Each figure is the mean of three replications.
- The figure in parenthesis with ± sign is the standard deviation.

The enzyme activity increased thereafter up to 5th day of larval age. It has been proposed that proteins from the disintegrating integument are used for the silk protein synthesis (Koga, 1978). The increased protease activity in the integument before the start of spinning is to hydrolyze integument proteins to release the precursors for the silk protein synthesis by the silk gland. It has been suggested that the midgut enzyme production and secretion varies in insect. In continuous feeding insects the enzyme production and secretion did not vary while in discontinuous feeders enzymes were produced on demand (Applebaum, 1985). In blood sucking insects the production of protease is dependent on the size of the blood meal (Downe *et al*., 1963; Gooding, 1972). While working with protease activity in the larvae of *Spodoptera littoralis* (L), Ishaaya *et al*. (1971) have shown that certain protein factors present in the food can stimulate digestive enzymes probably through a hormonal mechanism. The 5th in star larvae of *Bombyx mori* (L) are constantly fed and may be regarded as continuous feeders. The midgut protease activity significantly increased only 2 hour after the feeding commenced. The enzyme activity

significantly depleted after 4 hours (Table3). The tissue protease may reasonably be regarded as an index of the rate of synthesis of enzyme in secretary cells. In the light of the present observations, it is believed that the feeding stimulates the protease production in the midgut tissue of 5th instar *Bombyx mori* (L). However, further study is required to identify whether or not the factors responsible for the midgut tissue protease production originates from its food plant.

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