Pattern of changes in the amino acid levels in silk gland of silkworm, *Bombyx mori* L.treated with Indole-3-acetic acid (IAA)

D. Bharathi

Department of Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati-517502, Andhra Pradesh, India

Abstract— *The administration of IAA on the total proteins, protease activity, free amino acids, alanine amino transferase (ALAT) activity and aspartate amino transferase (AAT) activity were studied. The increase of total protein content in silk gland may be either due to increased efflux or decreased proteolysis activity which might lead to accumulation of protein content. The decrease in protease activity level in silk gland may be due to lower rate of histolysis. The decrease in the free amino acid content indicates the faster mobilization of free amino acids into oxidative metabolism in the presence of IAA. The ALAT and AAT activity levels were elevated after treatment indicating the active involvement in the protein synthesis.*

Keywords— Indole-3-acetic acid (IAA), Bombyx mori L., total proteins, protease activity, free amino acids, ALAT and AAT.

I. INTRODUCTION

Proteins play a unique role in determining the pattern of chemical transformations in biological systems. Studies were made to analyse haemolymph proteins in insects such as *Philosamia ricini* (Poonia, 1978), *Bombyx mori* L. (Venkata Reddy, 1984 and Seong *et al*., 1985) and *Calliphora* (Anderson, 1984). Siva Prasad (1987) reported the total and soluble proteins during metamorphosis of the silkworm. Proteases are the most commonly found digestive enzymes in insects (Ann Sorensen *et al*., 1983). Several factors responsible for the secretion of the proteolytic enzymes have been investigated (Briegel and Lea 1975). A strong protease activity has been demonstrated in the digestive fluid of the silkworm *Bombyx mori* L. (Eguchi and Iwamoto, 1976 and Sasaki and Sazuki, 1982).

Amino acids play an important role in the osmotic homeostasis of blood (Beadle and Shaw, 1950). Insects, in addition to sugars and lipids, use amino acids as the readily available source of respiratory fuels (Bursell, 1963Florkin (1949) observed that a very high titre of free amino acids in the haemolymph is characteristic of winged insects. Effect of dietary amino acids in the haemolymph of the larvae was studied (Inokuchi, 1970).

The aminotransferases (ALAT and AAT) mediate the transfer of amino groups of the amino acids to α-oxoglutarate, oxaloacetate and pyruvate to form glutamate, aspartate and alanine respectively (Lehninger, 1978). Amino transferasses have been detected in the tissues and eggs of silkworm (Seshachelam *et al*., 1985).

II. MATERIAL AND METHODS

Polyvoltine pure breed of silkworm *Bombyx mori* L of the race Pure Mysore was used in the present study.

IAA treatment: The larvae were separated into three groups and IAA was given to the silkworm larvae. Each group consists of three replications each of 200 larvae for each treatment.

Fresh mulberry leaves were dipped at least for one hour in Indole-3-acetic acid solution having a concentration of 15µg/lit.

The treated leaves were shade dried and they fed to the silkworm larvae on the first day of the third and fourth instars, and daily during the fifth instar up to 7 days. Optimal conditions were maintained through out the rearing period. The control larvae were fed with mulberry leaves soaked in physiological saline.

The total proteins (Lowry *et al*., 1951), protease activity (Davis and Smith, 1955), free amino acid content was estimated by the method of (Moore and Stein, 1954) as described by (Colowick and Kaplan, 1957). The activity of aspartate (AAT) and alanine amino transferases (ALAT) was determined by the method of (Reitman and Fraenkel 1957) as described by (Bergmeyer, 1965) were assayed in the silk gland of silkworm larva.

III. RESULTS

The data presented in the table reveal the changes in the total proteins, protease activity, free amino acids, ALAT and AAT in the silk gland after treatment with IAA.

Total proteins

Total proteins showed significant increase (P<0.001) after IAA treatment. The increase in protein content in silk gland was 34.62% over control.

Protease activity

Significant decrease was noticed in protease activity (P<0.001). The decrease in protease activity in silk gland was 12.90 % over control.

Free amino acids

Significant decrease was noticed in the amino acid content (P<0.001) after IAA treatment. The decrease in free amino acid content in silk gland was 18.15% over control.

Alanine amino transferase (ALAT) activity

Significant increase was noticed in ALAT activity (P<0.001). The increase in ALAT activity in silk gland was 34.84% over control.

Aspartate amino transferase (AAT) activity

The AAT activity level showed significant increase (P<0.001) in the treated larvae. The increase in AAT activity in silk gland was 37.50% over control.

IV. DISCUSSION

The protein profiles of the cell are indicative of the physiological status of the animal (Harper, 1985) and there exists a dynamic equilibrium between the synthetic and degradative pathways associated with these molecules In the present study the enhancement of proteins was noticed in silk gland of treated larvae which may be supported by the mobilization of more proteins from the haemolymph and fat bodies.

The activity levels of protease showed decrease in silk gland in IAA treated silkworm larvae. Proteases are the enzymes responsible for the hydrolysis of proteins into amino acids. In other words proteases are known to participate in protein digestion in intestine and histolysis in other tissues. The decrease of protease activity level may be due to lower rate of histolysis.

The free amino acid levels showed a decrease in the silk gland which indicates the faster mobilization of free amino acids into oxidative metabolism in the presence of IAA. The increase in protein synthesis and decrease in free amino acid level indicates faster mobilization of free amino acids to meet the energy requirement i.e., TCA cycle through transamination. Amino acids in the silk gland serve as the pool for silk protein synthesis (Siva Rami Reddy *et al*., 1984; Prudhommo *et al*., 1985; Sehnal and Akai, 1990). The amino acids resulting from protein digestion are the most important raw material for the growth of silkworms especially for the growth of silk gland. The decreased free amino acid content may be due to decreased proteolysis (Venkata Rami Reddy *et al*., 1992).

The ALAT and AAT activity levels were elevated significantly after treatment indicating the active involvement in the protein synthesis. The enhanced activity of ALAT and AAT reflected the general index of mobilization of free amino acids into gluconeogenesis and oxidation of amino acids respectively (Venkatarami Reddy *et al*., 1992 and Sinha *et al*., 1996).

Table.1: Changes in the levels of total proteins (mg/gm wet wt), protease activity (µ mol tyrosine equivalents/mg protein/hr), free amino acids (µ mol tyrosine equivalents/gm wet wt), ALAT (µ mol pyruvate formed/mg protein/hr) and AAT (µ mol pyruvate formed/mg protein/hr) in the silk gland of Bombyx mori L. when treated with IAA. Each value represents the mean of 6 individual observations. Mean ± S.D; '+' or '-' indicate the per cent increase or decrease over control respectively. 'P' denotes the statistical significance.

S. No.	Components	Control	Experimental
1	Total proteins	196.56±10.50	264.61±19.72 +34.62 P<0.001
2	Protease activity	1.24±0.11	1.08±0.09 -12.90 P<0.001
3	Free amino acids	23.42±2.17	19.17 ±1.62 -18.15 P<0.001
4.	Alanine amino transferase (ALAT) activity	1.32±0.11	1.78± 0.14 +34.84 P<0.001

5	Aspartate amino transferase (AAT) activity	0.88±0.072	1.21±0.08 +37.50 P<0.001

REFERENCES

[1] Ann Sorensen, A., Rosemary, S.,Kamas and Bradleigh Vinson, S. (1983). The influence of oral sections from Larvae on levels of proteinases in colony members of Solenopsis invicts Buren (Hymenoptera: Formicidae). J.Insect Physiol.Vol.29, pp.163-168.

[2] Beadle, L.C. and Shaw, J. (1950). The retention of salt and the regulation of non-protein nitrogen fraction in the blood of the aquatic larvae Sialis lutaria. J.Exp.Biol.Vol.27, pp.96-109.

[3] Bergmeyer, H.O. and Bruns, E. (1965). In methods of enzymatic analysis. Ed. Bergmeyer, H.A. Academic Press, New York.

[4] Briegel, H. and Lea, A.O. (1975). Relationships between protein and proteolytic activity in the midgut of mosquitoes. J.Insect Physiol. Vol.21, pp.1597-1604.

[5] Bursell, E. (1963). Aspects of the metabolism of amino acids in the Tse tse fly Glossina palpalis (Diptera). J.Insect Physiol. Vol.9, pp.439-452.

[6] Colowick, S.P.and Kaplan, N.O.(1957). Methods in Enzymology. Ed.Sydney P.Colowick and Nathan O.Kaplan, Academic Press Inc.Publications, New York, pp.543.

[7] Davis, N.C. and Smith, E.L. (1955).In: Methods of biochemical analysis. Ed. D. Glick, Interscience Publishers, New York, pp.247.

[8] Eguchi, M. and Iwamoto, A. (1976). Alkaline protease in the midgut tissue and digestive fluid of the silkworm *Bombyx mori* L. Insect Biochem.Vol.6, pp.491-496.

[9] Harper, H.A. (1985). In: Harper's Review of Biochemistry. Eds. D.W. Martin, P.A. Mayes and V.W. Rodwell.18th ed. Lange medical publication, Maruzen-Asia, Singapore.

[10] Inokuchi, T. (1970). Nutritional studies of amino acids in the silkworm *Bombyx mori* L.III Effect of dietary amino acid on the concentration of protein and free amino acids in the haemolymph of larvae. Bull. Expt.Stn.Vol.24, pp.407.

[11] Lehninger, A.L. (1978). Biochemistry, Kalyani Publishers, Ludhiana, New Delhi.

[12] Lowry, O.H., Roserbrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the folin phenol reagent. J.Biol.Chem.Vol.193, pp.265-275.

[13] Moore, S. and Stein, W.A. (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J.Biol.Chem.Vol.211, pp.907-913.

[14] Poonia, F.S. (1978). Studies on food utilization and rate growth during the developmental stages of Eri silkworm Philosamia ricini (Lepidoptera: Saturnidae). Indian J.Seric. Vol.17, pp.48-60.

[15] Reitman, S. and Fraenkel, S. (1957). A colorimetric method for the determination of glutamic oxalo acetic and glutamic pyruvic transaminases. An. N. Cein. Pathol. Vol. 28, pp.56-58.

[16] Sasaki, T. and Suzuki, Y. (1982). Alkaline proteases in digestive juice of silkworm *Bombyx mori* L. Biochem. Biophys. Acta.Vol.703, pp.1-10.

[17] Seong,S.V., Kwang, E., Park, M.N. and Yoshitake, N. (1985). Effect of metamorphosis on the major haemolymph protein of silkworm *Bombyx mori* L. Arch. Insect Biochem. Physic.Vol.2, pp 91-104.

[18] Seshachalam, R.V., Kasturi Bai, A.R. and Krishnamurthy, R.V. (1985). Aminotransferase activities in the developing eggs of two silkworm races (*Bombyx mori* L.) Entomon.Vol.10, pp.13-16.

[19] Siva Prasad, S. (1987). Neurobiological studies on the silkworm *Bombyx mori* L. Ph.D.thesis, S.V.University,Tirupati,India.

[20] Venkata Reddy, S. (1984). Studies of the effects of carbamate insecticide carbaryl on aspects of biology of silkworm *Bombyx mori* L. Ph.D.thesis, S.V.University, Tirupati, India.