Global Journal of Biotechnology & Biochemistry 5 (1): 50-54, 2010
ISSN 2078-466X

Ultrasound Induced Enhancement of Protein Metabolism and Enzyme Activities in the Silk Gland of Fifth Instar Silkworm, *Bombyx mori* L.

[1]P. Naga Jyothi and [2]Y. Suneetha

[1]Department of Fishery Science and Aquaculture, S.V. University, Tirupati Andhra Pradesh, India
[2]Department of Zoology, S.V. University, Tirupati Andhra Pradesh, India

Abstract: The parameters of protein metabolism, such as the levels of total, soluble proteins and free amino acids and also the activity levels of protease, glutamate dehydrogenase, aspartate aminotransferase and alanine aminotransferase were assayed in the silk gland of the fifth instar larva of silkworm, *Bombyx mori*, under the impact of ultrasound. In general ultrasound has an elevatory effect on these parameters. Changes in the levels of these biochemical constituents are correlated with the events of histogenesis and histolysis associated with the silkworm metamorphosis. Under the influence of ultrasound protein metabolism is stimulated to achieve greater turnover of silk proteins, greater spinning activity and consequently greater sericultural output.

Key words: Amino acids · Aminoransferases · *Bombyx mori* · Glutamate dehydrogenase · Protease · Proteins · Silk gland · Ultrasound

INTRODUCTION

Proteins constitute an essential component of all living cells. If carbohydrate metabolism represents the driving force of life, the protein metabolism represents life itself. Essentially, proteins are responsible for the maintenance of structural and functional organization of the cells. They play a vital role in respiration, enzyme catalysis, transport of materials, regulation of metabolism, movement and in body defence. The total protein content of the cell includes both structural and soluble portions, of which the former plays main role in cellular architecture and the latter in cellular metabolism.

Proteins have always been an interesting biochemical tool for insect biochemists because of their prominent role in development, morphogenesis and the intermediary metabolic pathways in insects. Mihaeslu *et al.* [1] observed biochemical difference in the eggs of *Bombyx mori* and *Philosamia ricini* with regard to their total proteins and amino acids. The first ever observation on insect haemolymph proteins was made by Lauffer [2] in the silkworm *Bombyx mori.* Subsequently a series of detailed physico-chemical studies on haemolymph proteins of *Bombyx mori* appeared [3]. Tojo *et al.* [4] made a detailed account of storage proteins while Sasaki *et al.* [5] investigated the intracellular transport and secretion of fibroin in the posterior silkgland of *Bombyx mori.* A novel approach in silkworm research is the manipulation of biochemical machinery through exogenous modulators that could boost the silk production. This obviously, included the administration of certain neuro-humoral factors, vertebrate hormones and various other chemicals like cyclic AMP and prostaglandins, which could have a profound influence on the growth rate, larval life cycle and fecundity [6, 7]. Significantly, the positive impact of vertebrate thyroxine on silkworm biology, especially in improving the pre- and post cocoon parameters is well documented [8]. Another vertebrate hormone, namely prolactin induced improvement in the growth and reproductive potential of silkworms [7]. The dietary administration of vertebrate sex hormones like ethynyl estradiol and norethindrone to the silkworm increased the larval weight, cocoon and shell weights, female pupal and adult weights, but the larval, pupal periods and the egg-hatchability were significantly reduced [9].

These investigations opened up alternative strategies for improving the economic parameters of the sericulture industry by regulating the biochemical machinery. One such option is the ultrasound, whose impact on larval life in Drosophila has been reported [10]. Further, it was reported that ultrasound irradiation does not cause any detectable deterioration in behavioral responses such as mating, oviposition, larval development and pupation in insects [11]. In view of its harmless nature, ultrasound has been used as an exogenous modulator in the present

Corresponding Author: Dr. P. Nagajyothi, Department of Fishery Science and Aquaculture, Sri Venkateswara University, Tirupati - 517 502 Andhra Pradesh, India

investigation for the manipulation of protein metabolism in the silkgland silkworm and to examine the possibility of its utility in sericulture.

MATERIALS AND METHODS

Multivoltine x bivoltine hybrid variety of the silkworm, (Pure Mysore x NB_4D_2) *Bombyx mori* L used in the present investigation were obtained from the Central Seed Farm at Tondavada, a suburb of Tirupati, A.P., India.

Ultrasound Treatment: Silkworm eggs were irradiated with ultrasound waves, 10-12 h after hatching (blue-egg stage) by water bag method. Prior to exposure, the eggs were kept in a sealed, water-filled polythene cover, smeared with gel so as to prevent the diversion of ultrasonic waves. The duration of exposure was standardized by exposing the eggs to varying intensities of ultrasound weaves, at different time intervals, viz. 2, 5, 10, 15, 20, 25 and 30 minutes. Promising results were obtained at 1 MHz, continuous wave of ultrasound at an intensity of $9W/Cm^2$ for 2 minutes. The larvae that emerged from the exposed (experimental) and unexposed (control) eggs were used in the investigation.

Tissue Separation: Silkgland was isolated by dissecting the larvae in ice-cold silkworm Ringer [12] were used for the biochemical assay.

Analysis of Protein Metabolism and Enzyme Assay: Day-to-day changes in biochemical parameters of protein metabolism such as total, soluble proteins and free amino acid, the activity levels of protease, aminotransferases and glutamate dehydrogenase were examined in the fifth instar larvae. The protein content was estimated by the method of Lowry *et al.* [13], the free amino acid content by the method of Moore and Stein [14] as described by Colowick and Kaplan [15] and the protease activity by the method of Davis and Smith [16]. The activities levels of aminotransferases, viz, aspartate aminotransferase (AAT) and alanine aminotransferase (AlAT) were estimated by the method of Reitman and Frankel [17] as described by Bergmeyer and Bruns [18] and the activity of Glutamate dehydrogenase (GDH) was assayed by the method of Lee and Lardy [19].

RESULTS

The levels of total and soluble proteins recorded an increasing trend in the silkgland from day 1 to day 6 during the development of fifth instar larvae. The levels of total proteins increased from 81 mg to 124 mg in silkgland (53% rise). Ultrasound has an elevatory effect on total protein content. The elevation is about 54% to 93% (Table 1). The levels of soluble proteins increased from about 31 mg to 72 mg. Ultrasound in general caused an elevation in the levels of soluble proteins also, with

Table 1: Day-to-day changes in the silk gland protein metabolism during the 5th instar of *Bombyx mori* under the impact of ultrasound ($9W/Cm^2$ for 2 minutes). Each value is the mean ± Standard Deviation (SD) of six separate observations. For each observation, tissue from at least 10 larvae was pooled. The percent changes for all days were calculated taking control as the reference

		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Total Protein							
(mg proteins/g wet weight)	Control	80.89 ± 5.97	82.38 ± 7.0	84.88 ± 9.48	99.37 ± 6.21	113.87 ± 15.62	124.22 ± 12.4
	Treated	124.2 ± 12.4	153.21 ± 18.97	163.56 ± 9.48	171.84 ± 9.48	188.40 ± 9.49	219.46 ± 18.97
	% Change	53.5	85.9	92.7	72.9	65.5	76.7
	t-Test	5.4366**	6.0662**	10.1585**	11.0723**	7.0603**	7.2736**
Soluble Proteins							
(mg proteins/g wet weight)	Control	31.05 ± 6.21	35.19 ± 15.63	43.48 ± 6.21	49.69 ± 5.07	60.04 ± 10.55	72.46 ± 15.63
	Treated	55.9 ± 6.21	64.18 ± 9.48	68.32 ± 6.12	72.46 ± 7.75	86.96 ± 6.21	99.38 ± 6.2
	% Change	80.0	82.4	57.1	45.8	44.8	37.2
	t-Test	4.8976**	2.7452 NS	4.8989**	3.4778*	3.2527*	2.7715 ns
Free amino acids							
(mg of tyrosine equi/g wet weight)	Control	30.48 ± 2.34	33.76 ± 0.93	35.63 ± 0.94	37.05 ± 0.38	37.98 ± 0.38	38.92 ± 0.47
	Treated	34.07 ± 1.17	36.11 ± 0.94	37.70 ± 0.70	38.45 ± 0.77	39.39 ± 0.94	40.80 ± 0.47
	% Change	11.8	6.9	5.8	3.8	3.7	4.8
	t-Test	2.3718 NS	3.0656*	3.0469*	2.3177 NS	2.3237 NS	4.8989**

NS - Not significant at 0.05 level; * Significant at 0.05 level; ** Significant at 0.01 level

Table 2: Day-to-day changes in the silk gland enzyme contents during the 5th instar of *Bombyx mori* under the impact of ultrasound (9W/Cm^2 for 2 minutes). Each value is the mean ± Standard Deviation (SD) of six separate observations. For each observation, tissue from at least 10 larvae was pooled. The percent changes for all days were calculated taking control as the reference

Enzyme activity		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Protease (µmoles of tyrosine							
equivalents/mg protein/h)	Control	0.64 ± 0.01	0.67 ± 0.015	0.67 ± 0.03	0.7 ± 0.02	0.74 ± 0.02	0.8 ± 0.02
	Treated	0.70 ± 0.01	0.72 ± 0.02	0.73 ± 0.02	0.760 ± 0.02	0.8 ± 0.02	0.84 ± 0.02
	% Change	9.8	7.5	8.7	8.6	8.1	5.0
	t-Test	7.3484**	4.5434*	2.8736*	1.8370 NS	1.2247 NS	2.4494 NS
Aspartate aminotransferase							
(µmoles of pyruvate formed/mg protein/h)	Control	0.7 ± 0.02	0.74 ± 0.03	0.77 ± 0.02	0.82 ± 0.02	0.84 ± 0.03	0.88 ± 0.02
	Treated	0.74 ± 0.03	0.77 ± 0.02	0.79 ± 0.02	0.84 ± 0.02	0.87 ± 0.03	0.92 ± 0.02
	% Change	5.7	4.1	2.6	2.4	3.6	4.6
	t-Test	4.8976**	2.7452 NS	4.8989**	3.4778*	3.2527*	2.7715*
Alanine aminotransferase							
(µmoles of pyruvate formed/mg protein/h)	Control	2.33 ± 0.02	2.42 ± 0.02	2.54 ± 0.04	2.62 ± 0.02	2.76 ± 0.02	2.82 ± 0.03
	Treated	2.38 ± 0.02	2.47 ± 0.02	2.57 ± 0.03	2.67 ± 0.03	2.78 ± 0.02	2.95 ± 0.04
	% Change	2.2	2.1	1.2	1.9	0.7	4.6
	t-Test	2.5114 NS	2.3348 NS	1.1470 NS	2.1879 NS	1.6263 NS	1.9604 NS
Glutamate dehydrogenase							
(µmoles of formazon formed/mg protein/h)	Control	1.5 ± 0.02	1.61± 0.04	1.68 ± 0.01	1.74 ± 0.02	1.82 ± 0.02	1.92 ± 0.02
	Treated	1.56 ± 0.02	1.67 ± 0.02	1.74 ± 0.02	1.8 ± 0.02	1.87 ± 0.03	1.62 ± 0.56
	% Change	3.3	3.7	3.6	3.5	28	-15.6
	t-Test	2.3348 NS	2.6261 NS	3.6706*	3.6742*	2.5298 NS	0.9064 NS

NS - Not significant at 0.05 level; * Significant at 0.05 level; ** Significant at 0.01 level

varying intensities. The range of increase is about 37 to 82% was observed (Table 1). The levels of free amino acids showed a similar trend during fifth instar. The level of increase is about 30 to 41 mg (36% rise). The impact of ultrasound on free amino acid levels is positive. The range for increase is about 4 to 12% in silkgland (Table 1).

The activity levels of protease recorded an upward trend through out the fifth instar. The elevation is more pronounced in the silkgland (0.64 to 0.8 µmoles). Ultrasound caused an elevation (2% to 30% increase) in the enzyme activity in the selected tissues (Table 2). The activity levels of aspertate aminotransferase enzyme activity increased in silkgland (0.7 to 0.88 µmoles/mg protein/h). The impact of ultrasound is elevatory, with an overall increase of 2% to 5% in silkgland (Table 2). Alanine aminotransferase activity showed a similar trend during fifth instar development. The enzyme activity increased from 2.33 to 2.82 µmoles/mg protein/h in the silkgland. Ultrasound caused an elevation in the activity notwithstanding its minor fluctuations in controls. The over all elevation is about 1 to 4 % (Table 2). The glutamate dehydrogenase (GDH) activity was increased in the silkgland (1.5 to 1.9 µmoles/mg protein/h) during the fifth instar development. Ultrasound caused an elevation in GDH activity levels. The elevation in silkgland is about3% (Table 2).

DISCUSSION

The impact of ultrasound on protein metabolism is profound as evidenced by upsurge in the levels of all the biochemical parameters examined. Though, increased levels of proteins were observed in silkworm tissue, these parameters were not analyzed with reference to ultrasound. However, some earlier investigations [20] attempted to elucidate the effect of ultrasound on protein synthesis. Obviously, the intensification of these two behaviors is of paramount importance for the fifth instar larvae. In silkgland, the proteins are used for the synthesis of silk proteins, viz, fibroin and sericin [21]. The Increased levels of total, soluble and structural proteins in silkworm tissue indicates the growth-promoting nature of ultrasound when applied in lower dosages and indicate a promising future for the sericultural industry. Apparently, ultrasound seems to enhance the protein synthesis in general, with a bias towards the silk proteins in silkgland. Amino acids are the

building blocks of the proteins. Ultrasound irradiation caused an elevation in the levels of free amino acids. The silkworm and other lepidopteran insects are known to contain unusually large amounts of free amino acids [22]. Insect metamorphosis is a dynamic process involving both histogenesis and histolysis [23]. Obviously, the amino acid pool in silkworm is derived both from proteins through histolysis and from non-protein sources like carbohydrates and lipids through *de novo* synthesis. Continuous increase in the levels of free amino acids following ultrasound-treatment is attributable to the synthesis of amino acids from non-protein sources like glucose and fatty acids [24].

Protease activity levels recorded a continuous increase throughout the fifth instar development as reported earlier in silkworm and other insects [25]. The positive impact of ultrasound on enzyme activity indicates its ability to degrade proteins by activating proteolytic enzymes. Histolysis seems to be more pronounced in silkgland as evidenced by increased turn over of amino acids. This could probably bring about the degeneration of silkgland during pupal stage, leaving the fatbody that forms the bulk of pupal weight. The presence of aspartate (AAT) and alanine (AlAT) aminotransferase activity was detected in silkgland of silkworm as reported in earlier investigations [26]. Ultrasound caused an elevation in the activity levels of both AAT and AlAT in silkworm tissues (Table 2), indicating the increased turnover of amino acids and glutamate formation during metamorphosis in silkworm. The higher levels of free amino acids observed in the present investigation (Table 1) support this assumption. Ultrasound has an elevatory effect on GDH activity in all the tissue of silkworm (Table 2). Some reports are available on GDH activity in silkworm, *Bombyx mori* [26]. The enhanced activity of GDH in silkgland is indicative of increased oxidation of glutamate in this tissue. The α-ketoglutarate generated by this enzyme is probably used-up in ensuring sperm mobility in silkworm [27]. The actual mechanism of ultrasound irradiation on protein metabolism is not clear.

The role of ultrasound in protein metabolism needs special mention in economically viable insects like the silkworm, in view of its profound and positive impact on biochemical parameters. Under its influence entire biochemical machinery in silkworm is geared up to synthesise silk proteins in silkgland during fifth instar development. While the former are used up as the raw materials for the cocoon, the latter are used for generating a muscular mechanism necessary for spinning the cocoon at the end of fifth instar. The increase in the concentration of amino acids with concomitant increase in the levels of total and soluble proteins in silkgland under the impact of ultrasound reflects this fact. Metabolically silkgland seems to occupy a pre-eminent position during metamorphosis in silkworm. It may be concluded that under the impact of ultrasound protein metabolism is stimulated to achieve greater turnover of silk proteins, greater spinning activity and consequently greater sericultural output.

REFERENCES

1. Mihaeslu, A.N., N. Stancioiu and A. Alexandrina, 1983. Variations in some physiological and biochemical parameters of silkworms with and without diapause. An. Univ. Bucur. Biol., 32: 63-66.
2. Lauffer, M.A., 1943. Ultra centrifugation studies on the blood of normal and Jaundice diseased silkworm. Proc. Soc. Exp. Biol. Med., 52: 330-332.
3. Ogawa, K. and S. Tojo, 1981. Quantitative changes of storage proteins and vitellogenin during the pupal adult development in the silkworm *Bombyx mori* (Lepidoptera: Bombycidae) Appl. Ent.zool., 16: 288-296.
4. Tojo, S., M. Nagata and M. Kobayashi, 1980. Storage proteins in the silkworm *Bombyx mori*. Insect Biochem., 10: 289-303.
5. Sasaki, S., E. Nakagima, Yoshiaki Fu-Jii-Kuriyama and Y. Tashiro, 1981. Intracellular transport and secretion of fibroin in the posterior silkgland of the silkworm. J. Cell. Sci., 50: 19-44.
6. Thyagaraja, B.S., E.P. Master, T.J. Kelly and A.B. Borkovec, 1991. Thyroxine induced haemolymph protein and ecdysteroid increases in the silk worm, *Bombyx mori* L. Effect on larval growth and silk production. J. Insect Physiol., 37: 153-160.
7. Bharathi, D., 1993. Effect of PGF 2 on the organic constituents of haemolymph of silk worm larvae, *Bombyx mori* (L). J. Seric., 1: 25-28.
8. Chaudhuri, A. and A.K. Medda, 1992. Thyroxine-induced alterations in glycogen content of fat body of female silkworm *Bombyx mori* (race Nistari) during larval, pupal and adult stages of development. Annals of Entomol., 10: 17-21.
9. Saha, B.N. and A.R. Khan, 1977. Effect of vertebrate Sex-hormones on *Bombyx mori* L. Sericologia, 37(1): 19-25.

10. Child, S.Z., E.L. Carstensen and W.K. Law, 1981. Effects of ultrasound on *Drosophila*: III. Exposure of larvae to low temporal-average-intensity pulsed irradiation. Ultrasound Med. Biol., 7: 167.
11. Koehler, P.G., R.S. Patterson and J.C. Webb, 1986. Efficacy of ultrasound for German cockroach (Orthoptera: Blattellidae) and oriental rat flea (Siphonaphera: Pulicidae) control. J. Econ. Entomol., 79: 1027-1031.
12. Yamaoka, K., M. Hoshino and T. Hirai, 1971. Role of sensory hairs on the anal papillae in oviposition benabiours of *Bombux mori.* J. Insect Physiol., 47: 2327-2336.
13. Lowry, O.H., N.J. Rosenbrough, L. Farra and R.J. Randall, 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem., 1933: 265-275.
14. Moore, S. and W.A. Stein, 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem., 211: 907-913.
15. Colowick, S.P. and N.O. Kaplan, 1957. Methods in Enzymology. Academic Press, New York, 63: 28.
16. Davis, N.C. and E.L. Smith, 1955. Assay of proteolytic enzymes. Meth. Biochem. Anal., 2: 215-257.
17. Reitman, S. and S. Frankel, 1957. A colorimentric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvic transaminases. Am. J. Clin. Pathol., 28: 56.
18. Bergmeyer, H.O. and E. Bruns, 1965. Methods of enzymatic analysis, Ed., H.A. Bergmeyer, Academic Press, New York.
19. Lee, Y.L. and H.A. Lardy, 1965. Influence of thyroid hormones on phosphate dehydrogenase and other dehydrogenases in various organs of the rat. J. Biol. Chem., 240: 1427-1432.
20. Vijaya, Kumari D., 1997. Effect of ultrasound on basic metabolism of liver of mouse, Ph.D. thesis, S.K. University, Ananthapur, A.P., India.
21. Horie, Y., K. Watanabe and E. Shirohara, 1971. Effect of dietary composition on growth, silkglands and components in haemolymph of the silkworm. Acta Seric. Japan, 78: 44-50.
22. Sinha, A.K., U.S.P. Sinha, S.S. Sinha and K. Sengupta, 1991. Studies on free amino acids, proteins, carbohydrates and phosphorous compounds in the tissue extracts of healthy and pebrine infected moths of Tasar silkworm, *Antheraea mylitta* D. Indian J. Seric., 30: 103-106.
23. Anderson, O.D., 1984. Developmental changes in protein content, volume and amino acid pools in the larval fat body and haemolymph of *Calliphora erythrocephala*. Comp. Biochem. Physiol., 77: 161-165.
24. Bose, P.C., S.K. Majumder and K. Sengupta, 1989. Role of amino acids in silkworm, *Bombyx mori* (L). Nutrition and their occurrence in haemolymph, silkgland and silk cocoon. Indian J. Seric, 28: 17-31.
25. Bharathi, D. and Y. Miao, 2003. Changes in the protein and lipid profiles of silkworm, *Bombyx mori* L. infected with NPV. Bull. Ind. Acad. Seri., 7(2): 76-80.
26. Venkata Rami Reddy, K., O.K. Ramadevi, S.B. Magadum, K.V. Benchamin and R.K. Datta, 1992. Uzi parasitisation and gluconeogenic precursor levels and related enzyme activity proteins in silkworm, *Bombyx mori* L. Indian J. Seric., 31: 123-129.
27. Osanai, M., T. Aigaki and H. Kosuga, 1987. Arginine degradation cascade as an energy yielding system for sperm maturation in the spermatophore of silkworm *Bombyx mori*. In: New Horizons in Sperm Cell Research, eds., P.P. Mohr, pp: 185-195.