International Journal of Scientific and Innovative Research 2016; 4(1)
P-ISSN 2347-2189, E- ISSN 2347-4971

ASSOCIATION OF LOW TEMPERATURE TREATMENT INDUCED PROTEIN EXPRESSION IN SILKWORM, PHILOSAMIA RICINI (LEPIDOPTERA: SATUNIDAE) TO ACCLIMATION

Anita Singh, *Bechan Sharma

Department of Biochemistry, University of Allahabad, Allahabad, UP, India

*Address for correspondence: Dr. Bechan Sharma, Professor, Department of Biochemistry, University of Allahabad, Allahabad, UP, India
E-mail: sharmabi@yahoo.com

ABSTRACT

The present study was conducted to assess the impact of low temperature (10˚C±1˚C) treatment on the levels of protein and profile of protein expression into three major tissues namely haemolymph, silk gland and fat body of the silkworm, Philosamia ricini, acclimated for different durations (2, 4 and 7 days). The results indicated that the exposure of the insects to low temperature caused significant perturbations in both the levels as well as the expression profiles of proteins in different organs of the insect. The hemolymph showed significant increase (14-69%) whereas the silk gland and fat body tissues exhibited moderate decrease in the protein content up to 12-32% and 10-31%, respectively. The hemolymph showed expression of new proteins of relatively higher molecular weights whereas the silk glands and fat body tissues displayed expression of proteins of lower molecular weights. These results suggested that the low temperature treatment induced alterations in the expression of protein profiles in different body tissues of the insect may be associated to the acclimation of the 5th instar larvae of P. ricini to cold stress.

Keywords: Protein; Proteins Expression; Cold stress; SDS-PAGE; *Philosamia Ricini*

INTRODUCTION

Insects are the most abundant group of species present on earth inhabiting different temperature zones. Insects in their life history may inevitably encounter adverse circumstanced climate, such as scorching hot in summer and terrifying cold in winter. It is absolutely necessary for insects to evolve corresponding strategies to protect themselves against such conditions [1,2]. Many studies have reported that insects display an extraordinary array of adaptations that allow them to sustain metabolic activity during such harsh conditions [3]. A large number of insects usually meet with harsh cold winter. They change physiologically, biochemically or behaviorally for increasing the capacity of cold-hardiness [2,4].

Some studies have been conducted to assess the acclimation of various organisms to low temperature and hypoxic conditions in order to understand the role of a few intermediary metabolites and enzymes under these circumstances. The enzymes concerned with energy metabolism can be divided into three groups, those related to anaerobic metabolism, aerobic metabolism and the pentose phosphate pathway [5]. However, studies are required to be conducted in the direction to understand the role of proteins in cold acclimation of insects including the silkworms exposed to low temperature [6].

An extensive survey of literature suggests that there are no reports available to explain the roles of cold stress induced proteins in different tissues of the insects in general and the silkworm species in particular under low temperature exposure. Therefore, the present study was designed to investigate the impact of low temperature (10 ± 1^0C) treatment for different time periods on the expression of the proteins in different organs of silkworm species, *P. ricini*, which is an economically important silk producing insect in India. The present work illustrates the perturbations in the total protein content and expression of some new induced proteins into different tissues of *P. ricini* exposed to low temperature using the sodium dodesyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique [7-12].

MATERIALS AND METHODS

Silkworm

The Eri silkworm, *P. ricini* is a continuously breeding lepidopteran, which mainly feeds on castor leaves. The life cycle passes through five instar stages and at the culmination of fifth instar stage it spins silk cocoon and changes into pupa [13].

Exposure to cold stress

The newly hatched larvae of *P. ricini* were reared on tender fresh leaves of castor plants (*Ricinus communis*). The 5th instar larvae of the insect were divided into 4 groups; each containing an equal number (50) of larvae in plastic trays. One of the groups served as a control reared at 25±2^{0}C and the other 3 were acclimated at 10±1^{0}C for varying treatment durations (2, 4, and 7 days).

Preparation of cell-free extract

The larvae were dissected in ice-cold Bodenstein's Ringer solution and their silk glands and fat body were excised out [14,15]. The 20% (w/v) homogenates of the tissues dissected were prepared in 50mM Tris-HCl buffer (pH 7.0) in a Potter-Elvehjam homogenizer using Teflon coated pestle under cold condition.

Determination of protein content

The protein content in different extracts of tissues was determined using the colorimetric method of Lowery et al. (1951) [16] using bovine serum albumin (BSA) as a standard protein.

Sodium dodesylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The staining of the proteins from different tissues from *P. ricini* exposed to low temperature were analyzed by reducing polyacrylamide gel electrophoresis (SDS-PAGE) [11,17]. The variations in the protein profiles of control and experimental sets were investigated by loading equal amount of proteins of different samples on to 12% polyacrylamide gel and resolved in cold for 2h [18]. Electrophoresis runs were conducted at constant voltage 150V in Tris-Borate buffer (500 mM Tris, 650 mM Boric Acid, 16 mM EDTA, pH 8.6). The protein bands in the gel were stained using commassie brilliant blue and analyzed with the help of the corresponding bands of marker proteins.

Statistical analysis

Each assay was replicated three to four times. Values were expressed as mean±S.E. of replicates and Student's t-test was applied to locate significant ($p<0.05$) differences between treated and control groups.

RESULTS

The impact of low temperature (10±1^{0}C) treatment on the 5th instar larvae of silkworm, *P. ricini,* was observed. The exposure of the insect larvae to low temperature was expected to lead some changes in its biochemical constituents, so that it can cope up with this harsh situation. Here, the main emphasis is given on evaluation of the impact of low temperature on the levels of proteins and their expression profiles in different tissues of the insect larvae.

The results of the effect of low temperature exposure on the levels of the protein content in three different organs of *P. ricini* larvae are shown in Table 1. In the control set, maximum protein content was recorded in silk gland (108mg/g wet wt tissue) followed by fat body (92mg/g wet wt tissue) and haemolymph (15mg/g wet wt tissue). After treatment of the insect larvae with low temperature, haemolymph exhibited elevation in the level of protein content (14-69%) over different periods of exposure (2 to 7 days). However, a reverse trend was observed in the silk gland and fat body tissues. The cold stress caused decrease in the protein content in these tissues to almost similar extent; the values being 12-32% and 10-30% in silk gland and fat body tissues, respectively (Table 1). The impact of cold stress on the levels of protein content was more pronounced at higher treatment period (7 days) than that at lower durations (2 or 4 days).

Table 1 : Effect of exposure of low temperature (10±1^{0}C) on protein content in three major tissues of 5th instar larvae of *P. ricini* exposed for different days

Tissues	Control (% protein content)	Experimental (% change in protein content)		
		2 Days	4 Days	7 Days
Haemolymph	100	+14.1*	+35.5**	+69.2**
Silk gland	100	-11.7	-21.1**	-32.2**
Fat body	100	-10.3	-19.7**	-30.9**

P. ricini 5th instar larvae was acclimated for seven days at 10±1^{0}C for the experimental set whereas the control group insects were reared at 25±2ºC. The total protein was estimated using the standard procedures as described in Materials and Methods. The values presented into the parentheses indicate the percent increase (+) or decrease (-) over control. *Significantly different at p≤0.05 (Student's 't' test).

Table 2: Effect of low temperature treatment (10±1^{0}C) on the level of expression of proteins in different body tissues of 5th instar *P. ricini* larvae exposed for 7 days

Molecular Weights (kDa) of protein bands in SDS -PAGE					
Haemolymph		Silk Gland		Fat Body	
Control	Experimental	Control	Experimental	Control	Experimental
101.8	101.8	97.4	88.9	101.8	88.9
98.5	98.5	88.9	68.1	98.0	66.0
54.0	**64.0**	68.1	50.0	88.9	50.0
49.7	49.7	50.0	49.7	66.0	47.5
43.0	**47.5**	49.7	47.0	50.0	40.3
25.1	43.0	47.0	39.6	47.5	39.6
14.1	**30.0**	39.6	**28.4**	40.3	28.4
13.8	25.1	28.4	**25.0**	39.6	27.4
12.9	14.1	25.0	**21.4**	28.4	**21.0**
12.0	13.8	22.8	20.4	27.4	14.1
11.1	12.9	21.4	**13.2**	21.0	12.6
	12.0	20.4	12.6	14.1	12.2
	11.1	13.2	12.0	12.6	11.2
		12.6	11.2	12.2	
		12.0		11.2	
		11.2			

Note: The digits in bold represent the variations in the protein expression profile in the cold treated tissues as compared to control. The control group insects were reared at 25±2ºC.

DISCUSSION

Earlier reports have indicated that some organisms, when subjected to low temperature stress, exhibited marked perturbations in their metabolic activity in terms of alterations in the levels of some biomolecules including the activities of certain key enzymes involved in energy metabolism [3,5,19-23]. In the present study, we have shown that the level of protein content consistently increases in the hemolymph of the 5th instar larvae of *P. ricini* due to cold stress over increasing period of treatment. It is important to note that the level of protein gradually decreases over the increasing exposure period in both the silk gland and the fat body tissues to almost equal extent.

Since, the haemolymph of the insect acts as a reservoir of various metabolites entering into it from different organs of the insects [24], it is quite likely that the decrease in the protein content into silk gland and fat body tissue may be due to the mobilization of the protein from these two organs to haemolymph and/or utilization of protein for generation of energy so as to allowing the insect to survive and mitigate the adverse impact of low temperature treatment. The overwintering insects have to contend with osmotic stress [25]. It has been reported that the rapid cold-hardening is associated with increase in the concentration of some metabolites and osmolality of haemolymph [26]. While studying cold tolerance in *Drosophila melanogaster,* Misener *et al*., also have explained their results in the haemolymph of cold stressed insect in similar manner [27]. However, a detailed study is required to characterize different molecular forms of these isozymes from different tissues of the silkworm larvae before reaching to any definitive conclusion.

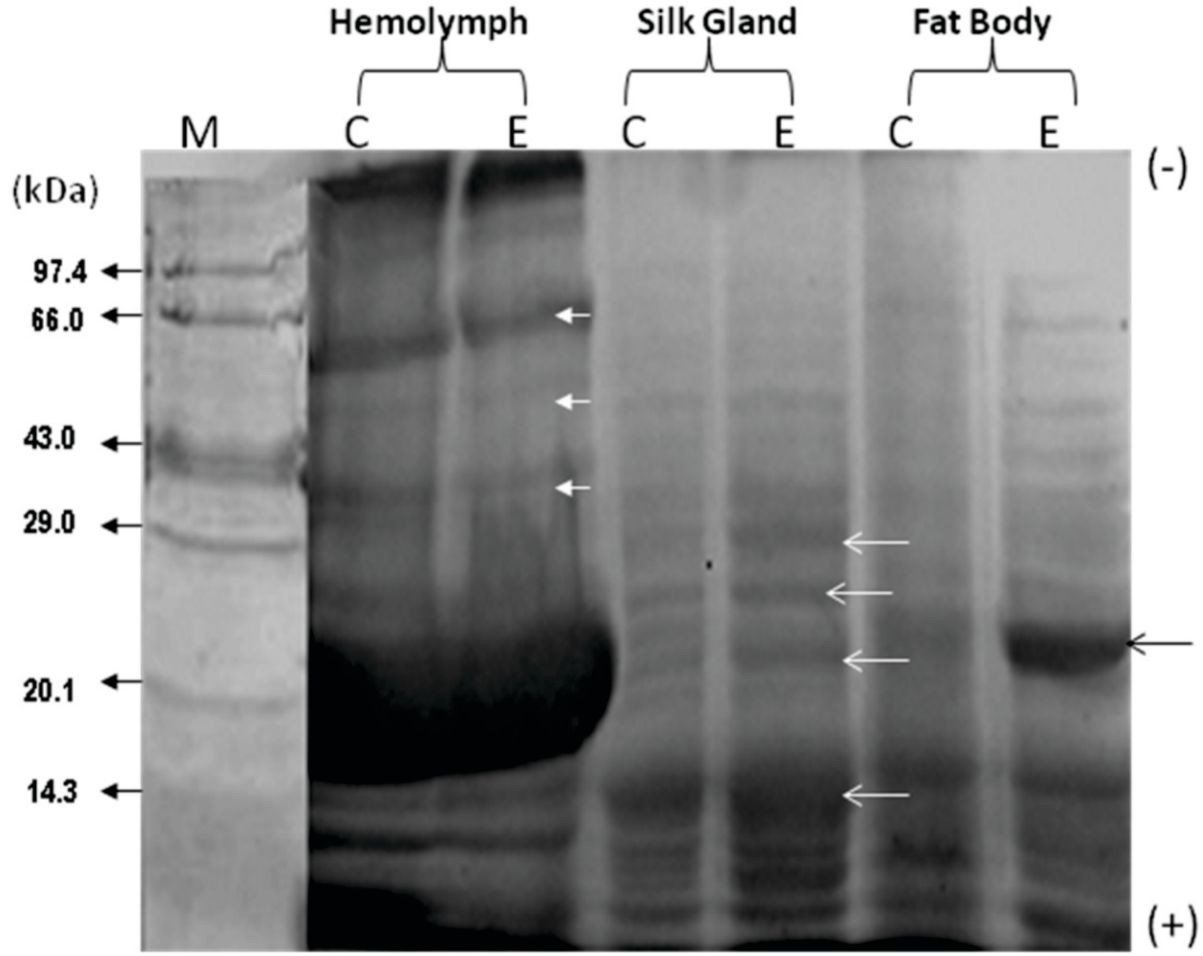

Figure 1

Effect of exposure to low temperature (10±1^{0}C) on the expression of proteins in different organs of the 5th instar larvae of *P. ricini* exposed for 7 days. C= Control, insects reared at temperature (25±2^{0}C), E= Experimental, insects acclimated at temperature (10±1^{0}C) for 7 days. The arrow represents the position of the new protein bands. SDS-PAGE analysis of proteins from Haemolymph, Silk gland and Fat body of 5th instar larvae of *P. ricini*. C=Control, E=Experimental. The protein (50 µg) of each sample was loaded in each well. SDS-PAGE was carried out as described in Materials and Methods following the method described by Laemmli (1970). Lane M represents marker proteins with corresponding molecular weights (kDa) as shown in the figure. The arrows (→) represent the appearance/disappearance of new proteins in the insect due to cold stress.

CONCLUSION

The 5th instar larvae of *P. ricini* displayed accumulation of protein content in the haemolymph and depletion of the same in the silk gland and fat body tissues due to low temperature treatment. The haemolymph displayed emergence of newly induced proteins of higher molecular weights whereas the fat body tissues and lymph glands exhibited expression of low molecular weight proteins. Under this condition, fat body tissues emerged as the main energy supplying organ of the insect larvae supporting its survival. The alterations in the characteristics of the newly expressed proteins may be attributed to the cold acclimation of the 5th instar larvae of *P. ricini.*

ACKNOWLEDGMENTS

The author (AS) is grateful to the University Grants Commission (UGC),New Delhi, India for providing financial support in the form of research scholarship. AS is also grateful to Dr. Shaily Tiwari, Department of Zoology, University of Allahabad for her assistance in isozyme analysis.

REFERENCES

1. Storey KB. Biochemical adaptation for cold hardiness in insects. *Phil. Trans. R. Soc. Lond.* 1990; B326: 635-654.
2. Zhao Z. Progress in the research on mechanism of insect cold-hardiness. *Entomol. Sinica*. 1997; 4(3):265-276.
3. Forcella M, Berra E, Giacchini R, Parenti P. Antioxidant defenses preserve membrane transport activity in *Chironomus riparius* larvae exposed to anoxia. *Archv. Insect Biochem. Physiol*. 2007; 65: 181-194.
4. Duman JD, Wu DW, Xu L, Tursman D, Olsen TM. Adaptations of insects to subzero temperatures. *Quart Rev.Biol*. 1991; 66: 387-410.
5. Su XR, Lv ZM, Li TW, Liu ZM, Paul KC. Analysis of isozymes related to energy metabolism of adult *Tegillarca granosa*. *Chinese J. of Agri. Biotech*. 2007; 4(2):163-166.
6. Panepucci L, Fernandes MN, Sanches JR, Rantin FT. Changes in lactate dehydrogenase and malate dehydrogenase activities during hypoxic and after temperature acclimation in the armored fish, *Rhinelepis strigosa* (Siluriformes, Loricariidae). *Rev. Brasil. Biol.* 2000; 60(2): 353-360.
7. Duncan WP, Marcon JL. Enzymes of energy metabolism in hatchlings of Amazonian freshwater turtles (Testudines, Podocnemididae). *Braz. J. Biol*. 2009; 69 (2): 319-325.
8. Suarez RK, Darveau CA, Welch KC Jr., O'Brien DM, Roubik DW, Hochachka, PW. Energy metabolism in orchid bee flight muscles: carbohydrate fuels all. *J.Exp. Biol*. 2005;208:3573-3579.
9. Zink MW, Shaw DA. Regulation of malic isozymes and malic dehydrogenase in *Neurospora crassa*. *Can .J. Microbiol*.1968;14:907-912.
10. Hopkinson DA, Harris H. Handbook of Enzyme Electrophoresis in Human Genetics. *North Holland, Amsterdam*, 1976.
11. Hu NS, Wan XG. The technology of isozymes and its application. *Human: Science and Technology Press*. 1985; 70-85.
12. Mahamat H, Hassanali A, Morgan H, Mutinga MJ, Mihok S, Massamba NN. Isozymes analysis of Kenyan Phlebotomine sandflies (Diptera: Psychodidae) by Isoelectric Focusing (IEF) on Pharmacia Phast System™ . *Biochem. Systematics & Ecol*. 1992; 20(7): 593-596.

13. Dash R, Mukherjee S, Kundu SC. Isolation, purification and characterization of silk protein sericin from cocoon peduncles of tropical tasar silkworm, *Antheraea mylitta*. *Int J Biol Macromol.* 2006;38:255–258.
14. Ringer S. Concerning the influence exerted by each of the constituents of the blood on the contraction of the ventricle. *J. of Physiol.*1980, 82;3:380-393.
15. Ringer S. Concerning the influence exerted by each of the constituents of the blood on the contraction of the ventricle. *J. of physiol.*1983-84;4:29-42,222-225.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-Phenol reagent. J. Biol. Chem., 1951; 193: 266-275.
17. Horecker BL, Kornberg A. The extinction coefficient of the reduced band of pyridine nucleotides. *J. Biol. Chem*. 1948;175:385-390.
18. Lin J, Somero GN. Temperature-dependent changes in expression of thermostable and thermolabile isozymes of cytosolic malate dehydrogenase in the eurythermal goby fish *Gillichthys mirabilis*. *Physiol.Zool*. 1995; 68(1): 114-128.
19. Gopalkrishanan A, Muneer Abdul PM, Thomas PC, Lal KK, Mohindra V, Basheer VS, Kapoor D, Ponniah AG . Identification of polymorphic allozyme markers for population structure analysis in Horabagrus brachysoma (Gunther, 1864) *Indian J. Fish*.2006; 53(3): 253-261.
20. Ayala FJ. Genetic differentiation during speciation process. *Evolutionary Biology* . 1975;8: 1-78.
21. Alka P, Sant P. Alcohol dehydrogenase isozyme expression in the air breathing fish, *Clarius bactachus* and *Heteropneuster fossilis* , of north eastern India .*Advances in Fish and Wildlife Ecology and Biology*. 1976;9-13. 21.
22. Singh A, Jaiswal SK, Sharma B. Low temperature stress causing biomolecular perturbations in insects with special reference to silkworms. *Journal of Biochemistry Research*.2013a; 1(3): 26-35.
23. Singh A, Jaiswal SK, Sharma B. Effect of low temperature stress on acetylcholinesterase activity and its kinetics in 5th instar larvae of *Philosamia ricini. Journal of Biochemistry Research.*2013a;1(2): 17-25.
24. Singh A, Sharma RK, Sharma B. Low temperature induced alterations in certain biochemical constituents of 5th instar larvae of *Philosamia ricini* (Lepidoptera: Satunidae). *Open Access Insect Physiology*.2010; 2:1–6.
25. Storey KB. Metabolism and bound water in overwintering insects. *Cryobiology*. 1983; 20:365-379.
26. Chen C-P, Denlinger DL, Lee RE Jr. Cold-shock injury and rapid cold hardening in the flesh fly, *Sarcophaga crassipalpis*. *Physiological Zoology*. 1987; 60:297-304.
27. Misener SR, Chen C-P, WalkerVK. Cold tolerance and praline metabolic gene expression in *Drosophila melanogaster*. *J. Insect Physiol*. 2001; 47:393-400.