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Electrophoretic Studies on Silk Gland Proteins of *Philosamia Ricini*

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Abstract:
Silk is a biological fiber produced by the caterpillars of Philosamia ricini moths at the end of their larval phase. Silk glands of silkworm secrete two major classes of proteins, the fibroin fiber, protein and sericin glue protein. The cocoon of silkworm is mainly composed of these two proteins. The present study focused on the development of new inhibitory protein under the condition of low temperature. The gene, produce these proteins, express in fifth instar stage at 29 ± 2 ^{0} C and humidity 90 % i. e. in normal rearing conditions. It is noteworthy that sericin show distinct bands on polyacrylamide gel electrophoresis, whereas fibroin did not. This suggests that components of sericin, but not fibroin, have definite molecular weights. Electrophoretic analysis of cold stressed silk gland protein in 12.5 % SDS – PAGE showed the development of new high molecular weight proteins in the cold stressed Philosamia ricini larvae when compared to control. Our results fulfills the aim of our study, which describes that when the fifth instar larvae of castor moth were acclimated to 4^0 C for a few days, expression of two major proteins of silk gland was hindered which were expressed in normal rearing conditions. In this case, emergence of new protein takes place comprising the molecular mass of more than 66 KDa.

Keywords : *Hemolymph, eriworm, sericin, fibroin*

1. Introduction

The silkworm has a pair of laterally located silk glands opening by a common orifice or spinneret, on the under lip of the larva. For about 35 days following birth, the larva just keeps consuming castor leaves and becomes 10,000 times heavier than when hatched. At the point it stops feeding and over the next three days ejects from both glands through the spinneret a continuous and reelable thread between 0.7 km and 1.1 km and even 1.6 km, round and round its body to produce a cocoon. The thread from the cocoon is unraveled and converted into the much sought - after yarn. It has been reported that *Philosamia ricini* does not contain higher molecular weight sericin [(1)]. The sericin protein of mulberry silkworm, *Bombyx mori*, was found to mainly consist of three major polypeptides having molecular masses of 400, 250 and 150 KDa estimated by SDS – PAGE [(2)]. SDS – PAGE is the most widely used methods for quantitatively analyzing any protein mixture, monitoring protein purity and to determine their molecular weights. It is based on the separation of proteins according to their size and then locating them by binding to a dye because generally proteins are colorless and hence cannot be visualized directly. That's why suitable dyes are used to stain the samples like coomassie brilliant blue R – 250. SDS or sodium dodecyl sulphate is an anionic detergent that binds strongly to proteins, causing their denaturation. There is a little information about the sericin of non mulberry silkworms. No report on sericin is available for *Antheraea mylitta,* a major contributor of silk among the non – mulberry silkworms. It is possible that the high molecular weight sericin increases the strength of silk, while the lower molecular weight sericin protects the pupa from various environmental stresses. The study was carried out to know that, under the condition of cold shock treatment there is development of new inhibitory cold stressed protein takes place or not.

Figure 1: Picture of Fifth Instar stage Philosamia ricini larvae

2. Material and methods

Larvae of Philosamia ricini were reared in the laboratory and the hemolymph was collected at the fifth instar stage followed by cold shock treatment. Cold stressed larvae were dissected in ice cold Bodenstein's ringer solution and the 20 % (w/v) homogenate of silk gland of both control and treated silkworms was prepared.

Proteins of cold stressed silk glands and hemolymph was isolated by the method of trichloro acetic acid (TCA) precipitation and Lowey et al method [(3)] was applied for quantitation of proteins. After quantitation the required amount of protein samples, dissolved in NaOH (50 mM), was boiled in sample loading or gel loading buffer containing tris buffer (pH 6.8), Sodium dodecyl sulphate (SDS), β- mercaptoethanol which reduces disulphide bonds, glycerol for increasing density and bromophenol blue as a tracking dye and loaded onto wells in the gel. Polyacrylamide gel electrophoresis (SDS – PAGE) was performed as described by Laemmli [(4)].

3. Results and Discussion

Electrophoretic analysis of silk gland protein in 12.5 % SDS – PAGE showed the development of new high molecular weight proteins in the cold stressed Philosamia ricini larvae when compared to control. It is notable that various investigators reported that only mulberry silkworm i. e. Bombyx mori indicates higher molecular weight proteins while non mulberry silkworms show lower molecular mass proteins on polyacrylamide gel electrophoresis. Figure (2) shows that when the larvae were not acclimated to 4 ^{0}C then the silk gland gives one distinct band of approximately 29 KDa. The protein of this band was expressed in the fifth instar larval stage. But under the condition of low temperature, the expression of this protein was hindered and on electrophoresis no band was observed. Instead of this, new protein inculcates which was not observed in non acclimatized worms. The molecular weight of the protein bands was determined by comparing to molecular weight marker run in the same gel. Cold stressed silk gland shows many bands in which one band is very much prominent containing a molecular mass of more than 66 KDa. Other bands showed in the same lane is not much distinguished and having the molecular weights of more than 29 KDa. This data represents that under the condition of low temperature new high molecular weight proteins arises which was not found previously in the non mulberry silkworms. While in the case of haemolymph, there are so many bands were observed whose molecular weight varies between 29 KDa to 66 KDa. Previous investigators declared that silk gland of non mulberry silkworm comprises lower molecular weight protein but not higher molecular mass protein. By satisfying that, our results gives strength to that investigation and also shows generation of new cold stressed proteins.

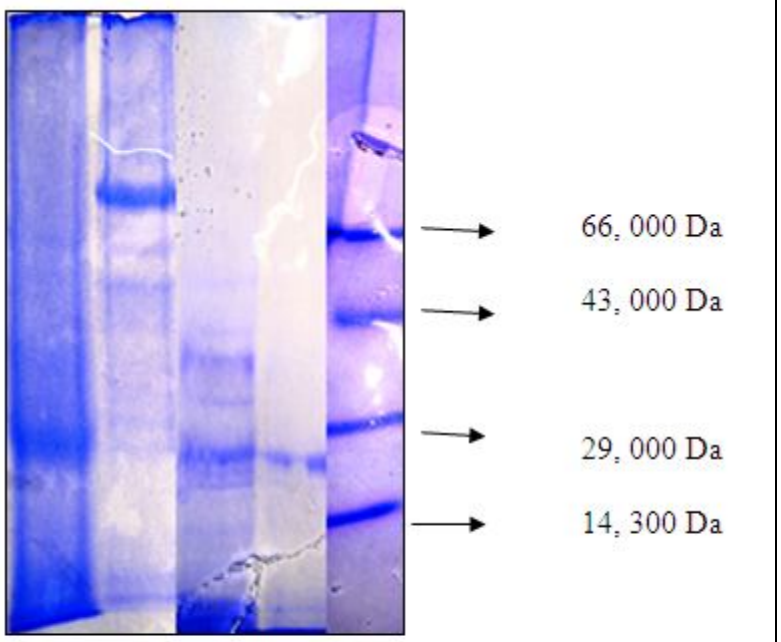

Figure 2: SDS – PAGE of silk gland and hemolymph of cold stressed Philosamia ricini larvae

4. Conclusion

The present study focused on the development of new inhibitory protein under the condition of low temperature. Our results fulfills the aim of our study, which describes that when the fifth instar larvae of castor moth were acclimated to 4 ^{0}C for a few days, expression of two major proteins of silk gland was hindered which were expressed in normal rearing conditions. In this case, the emergence of new protein takes place comprising the molecular mass of more than 66 KDa.

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