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THERMAL ACCLIMATIVE RESPONSES EXHIBITED BY SILK GLAND TRANSAMINASES OF ERI-SILKWORM, *PHILOSAMIA RICINI*

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S. P. Singh, M. K. Singh and G. B. Singh (1985) Thermal acclimative responses exhibited by silk gland transaminases of eri-silkworm, *Philosamia ricini*. *Bull. Inst. Zool., Academia Sinica* **24**(1): 111-116. There occurs an enhancement in the activities of glutamate-pyruvate and glutamate-oxaloacetate transaminases of silk gland tissue of eri-silkworm during cold adaptation at all temperatures of measurement. However, a higher degree of compensatory response observed for the GPT than that for the GOT indicates a slightly better capacity of cold acclimation exhibited by the former than the later.

The adaptive responses of a large number of metabolic and non-metabolic enzymes of poikilotherms against thermal stress have been studied (Wilson, 1973; Prosser, 1973; Hazel and Prosser, 1974; Shaklee *et al.*, 1977), which have been reported to be expressed mainly as a result of specific changes in the protein synthesis and gene regulation (Somero and Hochachka, 1976). However, little informations are available on thermal acclimatory responses of the enzymes from insects, although possible augmentation of the enzyme activities during thermal acclimation has been indicated by Marzusch (1952), Thiessen and Mutchmor (1967), Anderson and Mutchmor (1971), Singh and Das (1977), and Das and Das (1982a and b). The present communication reports the compensatory changes for temperature in the activities of silk gland transaminases (glumamate-oxaloacetate transaminase, EC. 2.6.1.1 and glutamate-pyruvate transaminase, EC. 2.6.1.2) of eri-silkworm, *Philosamia ricini*.

MATERIALS AND METHODS

The eri-silkworms were reared in the laboratory at 23±1°C. The minimal relative humidity was maintained at 70-80% by keeping wet cotton pads in the rearing trays (Singh and Singh, 1978). It was particularly important for the insects living at higher temperature of adaptation. The laboratory reared larvae were transferred right on the first day of the 3rd instar in B.O.D. incubators maintained at 15±1° and 30±1°C.

The enzymatic assays were made on the tissues obtained from the late 5th instar larvae of both thermal habitats because of higher levels of transaminase activities during the period. Thus, the period of whole 3rd instar (5 days), 4th instar (5 days) and the early and middle parts (4 days) of the 5th instar were available to the insects for thermal adaptation.

The silk gland of both sides of an insect from each thermal eategory was taken out after dissecting it in an ice-cold water. The middle and posterior portions of the silk gland of both sides were separated and placed in an ice-cold distilled water for half an hour when the liquid silk was extruded out (Shimada and Hayashiya, 1975). The tissue fractions of the

middle and posterior silk gland were pooled together and homogenized in 5 ml of phosphate buffer (pH 7.4). The supernatant, so obtained, was used for the assay of transaminase activities according to the procedure described by Reitman and Frankel (1957) with slight modification in the amount of the sample taken. A part of the supernatant used for the enzyme assay, was analysed for the estimation of total protein content present therein, according to the method of Lowry *et al.* (1951). The enzyme activities were expressed as micromole oxaloacetate or pyruvate formed/hour/mg protein. The measurements of enzyme activities in the silk gland tissues of cold and warm adapted insects were made at 15°, 20°, 25°, 30° and 35°C. The values of thermal coefficient (Q_{10}) for the transaminases in a specific thermal range were calculated according to Van't Hoff's equation:

$$Q_{10} = \frac{V_1^{\frac{10}{(t1-t2)}}}{V_2}$$

Where V_1 is the velocity at higher temperature t_1 and V_2 being the velocity at lower temperature t_2.

RESULTS AND DISCUSSION

The results of the investigation on the activities of the gultamate-oxaloacetate transaminase (GOT) and glutamate-pyruvate transaminase (GPT) of the silk gland of cold and warm adapted eri-silkworms as a function of temperature of assay have been presented in Figs. 1 and 2. The calculated values of 'Q_{10}' for the enzymatic reactions have been tabulated in Table 1. The findings of the present work clearly demonstrate that the activities of GOT and GDT in the silk gland tissue be modulated by the eri-silkworm during thermal acclimation. As evident from Figs. 1 and 2, the cold adapted insects possess a higher level of activity of both the transaminase in their silk gland tissue at all temperatures of measurement compared to the warm adapted insects. The degree of difference between the enzyme activities of the insects from two thermal habitats is consistent throughout the thermal range used for

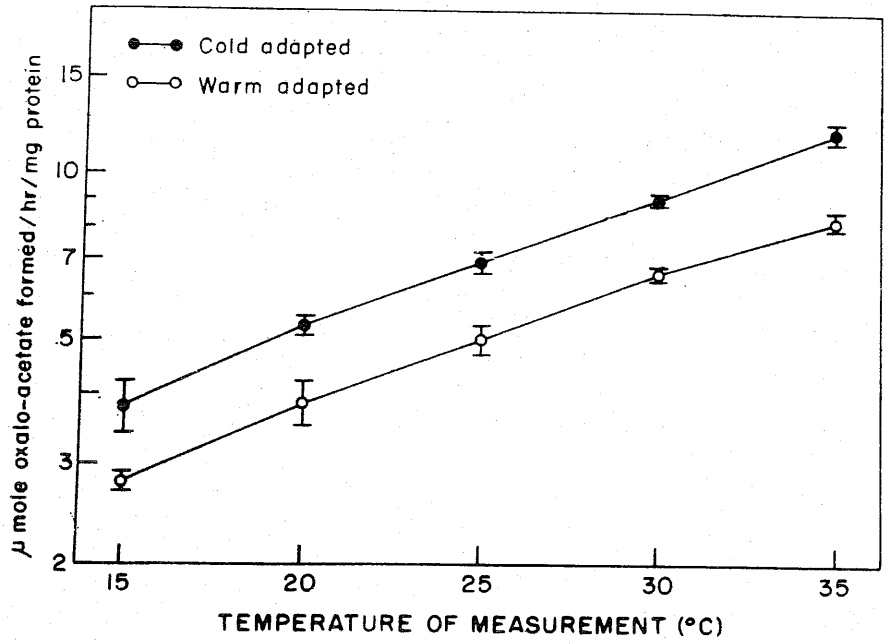

Fig. 1. Effect of temperature on the glutamate-oxaloacetate transaminase activity in the silk gland tissue of cold and warm adapted eri-silkworm (Mean of 5 replicates±S. D.).

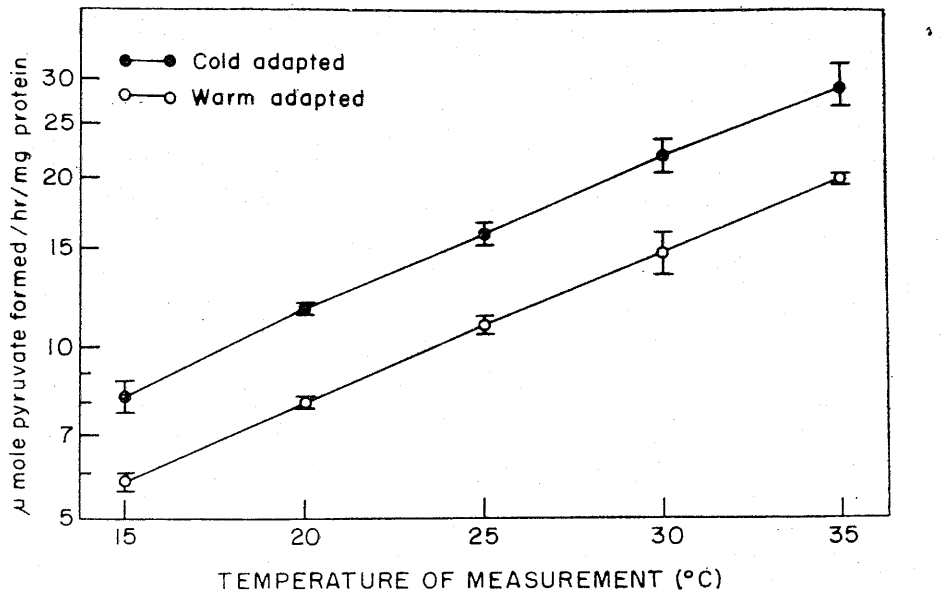

Fig. 2. Effect of temperature on the glutamate-pyruvate transaminase activity in the silk gland tissue of cold and warm adapted eri-silkworm (Mean of 5 replicates±S. D.).

measurement of the enzyme activity. Hence, both the transaminases present an almost translational pattern (Prosser's pattern II-A) of thermal acclimation. However, a higher degree of difference for the GPT (44–48%) than that of the GOT (34–44%) indicates a slightly better capacity of cold acclimation exhibited by the GPT than that by the GOT of the silk gland tissue. Translational pattern of thermal acclimation results in shifting of the R-T curve towards the left, which indicates the probability of a quantitative change in the enzymatic protein concentration and a change in controlling conditions like ionic strength, pH and water activity or a change in relationship among enzymes in series or parallel. A translational quantitative strategy exhibited by the GOT and GPT in the silk gland tissue of eri-silkworm during cold acclimation indicates a plausible explanation to the augmentation of protein concentration (47.1%) in the silk gland tissue of 15°C-adapted late 5th instar larvae in comparison to 30°C-acclimated individuals, as reported by Singh and Singh (1984).

Similar translational compensation against thermal stress has been observed by Singh and Das (1977) for the salivary amylase of nymphal cockroach, *Periplaneta americana*. Burr and Hunter (1970) observed a higher glutamate-aspartate transaminase activity for the

TABLE 1

Approximate 'Q_{10}' values for the transaminases of the silk gland tissue of cold and warm adapted eri-silkworms

Kind of transaminase	Nature of Adaptation	Thermal Range	
		15°–25°C	25°–35°C
Glutamate-oxaloacetate transaminase	Cold adapted	1.80	1.75
	Warm adapted	1.75	1.67
Glutamate-pyruvate transaminase	Cold adapted	1.95	1.77
	Warm adapted	1.90	1.82

15°C-acclimated female *Drosophila melanogaster* and young adult males and females of *Drosophila imigrans* than that for the 25°C-acclimated insects when measured at 20°C. However, these authors failed to observe any compensatory response in the transaminase activity of two steno-thermal species, *D. preudoobscura* and *D. willistoni*. Accordingly, the conclusion was made by the workers that the eurythermal species demonstrate a greater capacity of physiological adaptation than do the stenothermal species. The insects of the present investigation (eri-silkworm) is certainly an eurythermal species exhibiting a marked compensatory response against cold stress with respect to the two transaminases of its silk gland tissue.

The decrease in the value of thermal coefficient at higher temperature is characteristic of many rate processes (Rao and Bullock, 1954). However, this statement does not seem to be true for both transaminases of the silk gland tissue of eri-silkworm, which do not demonstrate any significant alteration in their 'Q_{10}' values due to a change in either nature of thermal adaptation or in thermal range of the enzyme assay (Table 1). This is well anticipated for a thermal acclimatory response exhibiting a complete translational pattern.

It is interesting to point out that the level of GPT activity of the silk gland tissue is always higher (about two times) than that of the GOT from the insects of similar thermal category at any temperature of measurement (Figs. 1 and 2).

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Eri-silkworm, *Philosamia ricini* 其絲腺中氨基移轉酶對溫度適應之反應

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Eri-silkworm 低溫適應實驗中，在所有測試溫度下，絲腺組織中麩胺酸—丙酮酸及麩胺酸—草醋酸氨基移轉酶 (glutamate-pyruvate and glutamate-oxaloacetate transaminases) 兩者的活性都有增加的現象，但是麩胺酸—丙酮酸氨基移轉酶有較麩胺酸—草醋酸氨基移轉酶爲高的補償反應，顯示前者有較後者略佳之低溫適應力。