

The Effect of Indole-3-Acetic Acid (IAA) on the Activity Levels of Dehydrogenases in the Silkgland of Silkworm, *Bombyx Mori L*

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Abstract— The effect of indole-3-acetic acid (IAA) on the glucose-6-phosphate dehydrogenase (G-6-PDH), lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH), iso-citrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) were studied

The stimulation of G-6-PDH activity in the silk gland of experimental larva indicates increased oxidation of glucose resulting in higher levels of NADPH. Increased G-6-PDH activity in the present study suggests this as compensatory mechanism to maintain the structural complexity, functional integrity and metabolic centrality of the cells

The activity of LDH, ICDH, MDH and SDH were increased in the silk gland of IAA treated larvae. The increased activity of the dehydrogenases may be attributed to increased turnover of aminoacids and oxidative metabolism in the silk gland.

The activity level of GDH was increased in silk gland which indicates the increased oxidation of glutamate.

Keywords— *Bombyx mori L.*, indole-3-acetic acid, G-6-PDH, LDH, GDH, ICDH, SDH and MDH.

I. INTRODUCTION

The oxidation of carbohydrates beyond the level of pyruvate is reported by a series of dehydrogenases (Gilmour, 1961). The dehydrogenases involved in both glycolytic and pentose pathway was studied with larval tissues of the silkworm *Bombyx mori L* (Horie, 1967 and Venkatarami Reddy *et al.*, 1992).

GDH is an enzyme active in nitrogen and energy metabolism of tissues. In uricotelic animals such as insects, GDH may act as a key enzyme linking carbohydrates and amino acid metabolism (Male and Storey, 1982 and Prezioso *et al.*, 1985). Succinate dehydrogenase is the oxidative enzyme involved in Krebs's cycle in mitochondria

and this dehydrogenase is considered as an index of aerobic metabolism (Fukuda *et al.*, 1958).

The importance of LDH activity is an index of anaerobic metabolism and that of SDH and ICDH as aerobic metabolism is a well-known fact. The increase in SDH activity is in synchrony with glycolytic pathway. which indicates that TCA cycle is also elevated in tune with pyruvate production during IAA treatment. Where as increased ICDH due to IAA shows increased mitochondrial oxidation (Rajasekhar, 1993).

The increased MDH activity indicates stimulation of intestinal absorption of carbohydrates and glucose utilization by thyroid hormone resulting in enhanced activity levels of TCA cycle enzymes (De *et al.*, 1988). Effect of Throxine on the activities of dehydrogenases in Silkworm, *Bombyx mori L.* (Hemavathi *et al.*, 2002).

Dehydrogenase in carbohydrate metabolism in larvae of the silkworm, *Bombyx mori L.* has been reported (Yasuhiro Horie, 1967). Glutamate dehydrogenase is an enzyme active in nitrogen and energy metabolism of tissues. The role of GDH must be largely dependent on the type of nitrogen metabolism.

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 G-6-PDH (Lohr and Waller, 1965), LDH (Srikanthan and Krishna Murthy, 1955 as modified by Reddanna and Govindappa, 1979), GDH (Lee and Lardy, 1965), ICDH (Korenberg and Pricer, 1951), SDH (Nachlas et al., 1960) and MDH (Nachlas et al., 1960) were assayed in the silk gland of silkworm larvae.

III. RESULTS

The data presented in the table 1 and 2 reveal the changes in the G-6-PDH, LDH, GDH, ICDH, SDH and MDH in the silk gland of silkworm larvae after treatment with IAA.

Glucose-6-phosphate dehydrogenase activity

The per cent increase in the glucose-6-phosphate dehydrogenase activity of silk gland was 45.71 over control.

Lactate dehydrogenase activity

The per cent increase in the lactate dehydrogenase activity of silk gland was 11.67 over control.

Glutamate dehydrogenase activity

The per cent increase in the glutamate dehydrogenase activity of silk gland was 27.07 over control.

Iso-citrate dehydrogenase activity

The per cent increase in the iso-citrate dehydrogenase activity of silk gland was 33.33 over control.

Succinate dehydrogenase activity

The per cent increase in the succinate dehydrogenase activity of silk gland was 31.37 over control.

Malate dehydrogenase activity

The per cent increase in the malate dehydrogenase activity of silk gland was 39.14 over control.

IV. DISCUSSION

Glucose-6-phosphate dehydrogenase is a member of the hexose monophosphate (HMP) shunt or pentose phosphate pathway. In addition to glycolysis, this pathway is also involved in the oxidation of glucose.

The enzymes of HMP shunt including G-6-PDH are present in extra mitochondrial soluble portion of the cell. G-6-PDH catalyses oxidation of glucose-6-phosphate to phosphogluconolactone. G-6-PDH is known to occur in two distinct types-one located in cytosol with NADP specificity (Barkat *et al.*, 1975) and the other located in microsomes which utilizes either NADP or NAD (Shalton *et al.*, 1971).

The stimulation of G-6-PDH activity by IAA in silk gland indicates the increased oxidation of glucose resulting in higher levels of NADPH. As a result of increased energy demand and elevated oxygen consumption, the animals appear to resort to alternative means of energy source may be through HMP shunt (Ghosh *et al.*, 1987 and De *et al.*, 1988).

Higher oxidation of glucose in the HMP shunt as evidenced from increased G-6-PDH activity in the present study suggests this as compensatory mechanism to maintain the structural complexity, functional integrity and metabolic centrality of the cells.

This pathway plays important role in the supply of co-enzyme NADPH for lipogenesis and provision of ribose-5-phosphate for the formation of nucleotides, which are necessary for germ cell differentiation.

Lactate dehydrogenase activity occurs at the terminal stage of glycolysis and oxidation of lactate or reduction of pyruvate depending upon the availability of co-enzyme NAD (Harper *et al.*, 1993).

The increase in NAD dependent LDH activity in the silk gland revealed the possibility of formation of pyruvic acid from lactate. This clearly indicates the magnitude of active mobilization of pyruvate for further processing in the metabolic mill (Rajasekhar, 1993).

The activity levels of NAD-LDH, NADP-ICDH, FAD-SDH were increased in the silk gland. after IAA treatment. The importance of NAD-LDH activity is an index of anaerobic metabolism.

The activity level of glutamate dehydrogenase was increased in silk gland.. GDH is an enzyme of great importance in the intermediary metabolism of amino acids. GDH activity acts as a general marker of amino acid oxidations in the tissues (Harper, 1985) and also acts on the glutamine, glutamic acid, the dicarboxylic acid or its amide glutamate.

The GDH is known to catalyze the interconversion of glutamate and α -ketoglutarate and such as a link between amino acid and carbohydrate metabolism. In other words, glutamate and GDH have a unique role in amino group transfer. It is through, this enzyme that the α -ketoglutarate is made available for the citric acid cycle, at the same time from ATP to release ammonia. The enhanced activity of GDH in silk gland indicates increased oxidation of glutamate (Sailaja, 1999).

Iso citrate dehydrogenase is existing both in mitochondria and cytosol (Lehninger, 1978). It catalyses the conversion

of isocitrate to α -ketoglutarate. The NADP-ICDH activity is increased in silk gland of IAA treated worms indicating increased mitochondrial oxidation. Whereas the α -ketoglutarate form may be utilized for amino transferase functions or goes to the rescue of ammonia detoxification mechanism.

A marked increase in NADP-ICDH activity indicates enhanced synthesis of energy (Singh and Yadav, 1985). The level of ICDH activity indicates the level of oxidation prevailing in Kreb's cycle.

Succinate dehydrogenase is a FAD dependent enzyme, which facilitates the conversion of succinate to fumarate (Fukuda *et al.*, 1958). The importance of NAD-LDH activity is an index of anaerobic metabolism and that of FAD-SDH and NADP-ICDH as aerobic metabolism.

The increase in SDH activity in silk gland is in synchrony with glycolytic pathway which indicates that TCA cycle is also elevated in tune with pyruvate production during IAA treatment perhaps this may be due to increased energy demands (Rajasekhar, 1993).

Malate dehydrogenase is the terminal enzyme in TCA cycle and involved in oxidation of malate to oxaloacetate. MDH is NAD-dependent and exists in both mitochondria and cytosolic fractions of the cell. The NAD dependent MDH activity is increased in silk gland. Similar enhancement in enzyme activities has been reported for metabolic enzymes such as malic enzyme (Murphy and Walker, 1974).

*Table-1: Changes in the levels of Glucose-6-phosphate dehydrogenase ($\mu\text{mol formazan formed/hr/mg protein}$) lactate dehydrogenase ($\mu\text{mol formazan formed/hr/mg protein}$) and glutamate dehydrogenase ($\mu\text{mol formazan formed/hr/mg protein}$) in silk gland of control and experimental (IAA treated) larvae during the 5th instar of silkworm, *Bombyx mori* L. Values are the mean of 6 individual observations. Mean \pm S.D; '+' and '-' indicate per cent increase or decrease respectively over control. 'P' denotes the statistical significance.*

S.No	Component	Control		Experimental
1	Glucose-6-phosphate dehydrogenase activity	0.035 \pm 0.002	+45.71 P<0.001	0.051 \pm 0.004
2	Lactate dehydrogenase	0.197 \pm 0.012	+11.67 P<0.001	0.22 \pm 0.018

	activity			
3	Glutamate dehydrogenase activity	0.181 \pm 0.013	+27.07 P<0.001	0.23 \pm 0.019

*Table-2: Changes in the levels of iso citrate dehydrogenase ($\mu\text{mol formazan formed/hr/mg protein}$), succinate dehydrogenase ($\mu\text{mol formazan formed/hr/mg protein}$) and malate dehydrogenase ($\mu\text{mol formazan formed/hr/mg protein}$) in silk gland of control and experimental (IAA treated) larvae during the 5th instar of silkworm, *Bombyx mori* L. Values are the mean of 6 individual observations. Mean \pm S.D; '+' and '-' indicate per cent increase or decrease respectively over control. 'P' denotes the statistical significance.*

S.No	Component	Control		Experimental
1	Iso citrate dehydrogenase activity	0.27 \pm 0.022	+33.33 P<0.001	0.36 \pm 0.030
2	Succinate dehydrogenase activity	0.153 \pm 0.012	+31.37 P<0.001	0.201 \pm 0.016
3	Malate dehydrogenase activity	0.281 \pm 0.021	+39.14 P<0.001	0.391 \pm 0.032

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