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Polyol dehydrogenases in the eggs of the silkworm *Bombyx mori* L.

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Abstract. Activities of some of the dehydrogenases involved in the formation of polyols were examined in diapause, non-diapause and acid treated artificial non-diapause eggs. The studies revealed that NADP-SDH may be important in the production of sorbitol during the onset of diapause and that NAD-GPDH may be playing a more important role than NADP-GDH in the production of glycerol.

Keywords. *Bombyx mori*; dehydrogenases; sorbitol; glycerol.

1. Introduction

A specialised conversion of glycogen to polyhydric alcohols was first demonstrated in the diapause embryo of *Bombyx mori* by Chino (1957a). Two polyols namely sorbitol and glycerol were shown to accumulate in diapause eggs while glycogen content decreased (Chino 1957b, 1958). Many studies have shown that this breakdown of glycogen to form polyols is closely correlated with the onset of diapause in silkworm eggs (Chino 1957a; Yaginuma and Yamashita 1977, 1978).

The enzyme polyol dehydrogenases are involved in the conversion of glycogen to polyols. The presence of these enzymes in the haemolymph of silkworm was demonstrated by Faulkner (1958). Later studies (Chino, 1960; Yaginuma and Yamashita, 1979) demonstrated the presence of the following polyol dehydrogenases in silkworm eggs.

(I) NADP dependent sorbitol dehydrogenase.
(II) NADP dependent glycerol or sorbitol-6-phosphate dehydrogenase.
(III) NAD dependent glycerol phosphate dehydrogenase.
(IV) NAD dependent sorbitol dehydrogenase.

While I and II enzymes were suggested to be playing an important role during the onset of diapause the IV one has been suggested to be playing an important role during the termination of diapause. The significant observation of all the earlier workers is that all these dehydrogenases are present in non-diapause eggs also where polyol formation never occurs. The present studies were undertaken to re-examine the activities of polyol dehydrogenases in diapause eggs following the onset of diapause and during embryonic development in non-diapause as well as artificial non-diapause eggs where diapause has been interrupted using HCl treatment. The activities of polyol dehydrogenases namely NADP dependent sorbitol dehydrogenase (NADP-SDH), NADP dependent glycerol dehydrogenase (NADP-GDH) and NAD dependent glycerol phosphate dehydrogenase (NAD-GPDH) have been studied.

2. Materials and methods

Bivoltine (NB_4D_2) and multivoltine (pure Mysore) races of the silkworm *Bombyx mori* L. were maintained under standard conditions. Eggs laid on polythene sheets were kept at 25 ± 2°C with relative humidity 75%. For breaking diapause, 20 h old eggs were treated with HCl (Sp. gr. 1·075) at 46·1°C for 3–4 min, washed thoroughly with water and kept at 25 ± 2°C.

2.1 *Enzyme preparation*

A 10% (w/v) homogenate of the eggs was prepared using a glass homogeniser fitted with Teflon pestle. The homogenate was filtered through a cotton pad and centrifuged at 5500 *g* for 15 min at 0°C. The supernatant was filtered through Whatman No. 1 filter and the resultant filtrate was used as the enzyme source.

2.2 *Assay of the enzyme activity*

NADP-SDH activity was determined following the method of Takahashi *et al* (1974). The reaction mixture contained 50 mM Tris-HCl buffer of pH 7·5, 3·3 mM fructose, 0·33 mM NADPH and 0·1 ml enzyme extract in a final volume of 1 ml. NADP-GDH activity was determined based on the method of Faulkner (1958). The reaction mixture consisted of 20 mM Tris-HCl buffer of pH 7·5, 4 mM $MgSO_4$, 10 mM dihydroxy acetone, 0·07 mM NADPH and 0·1 ml enzyme solution in a final volume of 1 ml. NAD-GPDH activity was determined based on the method of Baranowski (1949). The reaction mixture consisted of 20 mM Tris-HCl buffer of pH 8·5, 4 mM $MgSO_4$, 10 mM dihydroxyacetone phosphate, 0·07 mM NADH and 0·1 ml of the enzyme extract in a final volume of 1 ml.

The reactions were initiated by the addition of respective substrates. The enzyme activity was determined by measuring the optical density at 340 nm. One unit of the enzyme activity was defined as the amount causing decrease of optical density by 0·01/min. Protein content was determined according to Lowry *et al* (1951) using bovine serum albumin standards.

3. Results

3.1 *NADP-SDH activity*

This activity could be detected in the extracts of both diapause and non-diapause eggs. In non-diapause eggs, though sorbitol is never known to accumulate during embryogenesis, NADP-SDH activity was considerably high at the time of oviposition. The level of activity remained constant throughout the embryonic development (figure 1). In diapause eggs, the activity was higher than in the non-diapause eggs at the time of oviposition. The activity further increased on the first day. But for a small decrease on the second day, the activity remained high upto the 5th day following oviposition which corresponds to the period of rapid glycogen break down in diapause eggs. Following 5th day, the activity decreased as the age increased and it was relatively low by 30 days. In acid-treated artificial non-diapause

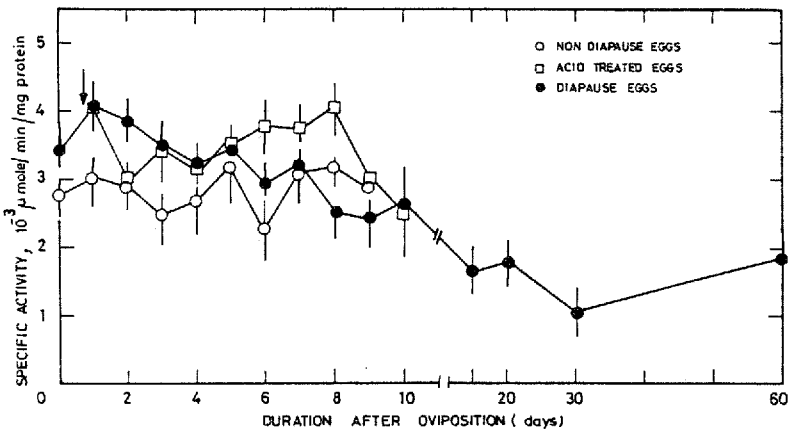


Figure 1. Changes in NADP-SDH activity during diapause and embryogenesis. In the present and all the following figures, Mean values (n = 4) along with SD are plotted and arrow indicates acid treatment.

eggs, the activity of the enzyme decreased significantly following acid-treatment. Though the activity was relatively higher than in the non-diapause eggs, it remained constant as in the case of the latter.

3.2 *NADP-GDH activity*

The activity was quite high immediately after oviposition in non-diapause eggs. The activity further increased on the second day following which it progressively decreased as embryogenesis progressed (figure 3). In diapause eggs, the activity pattern was very much comparable to that observed in non-diapause eggs upto the 10th day. But following this period, the activity increased significantly. In artificial non-diapause eggs, the activity followed a pattern very similar to that observed in the non-diapause eggs.

3.3 *NAD-GPDH activity*

In non-diapause eggs, the activity was quite high immediately after oviposition and it decreased by the second day following which it increased as embryogenesis progressed and decreased just before hatching (figure 4). The activity was found to be higher in diapause eggs at the time of oviposition. It increased subsequently but a significant change was noticed after the 10th day when the activity increased very much. In acid-treated eggs, the activity resembled that seen in non-diapause eggs.

4. Discussion

The studies of Chino (1960) and Takahashi *et al* (1974) showed that NADP-SDH is active throughout the egg life but there was no change in its activity that could be strongly correlated with diapause especially its termination. Secondly, since this

enzyme was found to be equally distributed in both the diapause and non-diapause eggs, it was suggested that this enzyme plays no specific role in sorbitol formation during diapause. NAD dependent sorbitol dehydrogenase has been suggested to be playing an important role during termination of diapause (Yamashita *et al* 1981). Hence, the role of polyol dehydrogenases in the formation of sorbitol during the initiation of diapause is not clear. Present studies showed that in diapause eggs, the activity of NADP-SDH is much higher than in non-diapause eggs. Secondly, immediately after oviposition, a sudden but brief increase in the activity was registered during the second and third days in diapause eggs which actually corresponds to the rapid glycogen break down phase (Chandrashekar and Geethabali 1987). Interestingly, a similar increase in the activity of this enzyme was also recorded (figure 2) by Yaginuma and Yamashita (1979). Such an increase is not observed in non-diapause eggs. Also, the activity somewhat decreased, immediately following acid treatment to reach the level characteristic of a non-diapause egg on the second day. Thus, clearly, there is no increase in the activity of NADP-SDH accompanying the early embryonic development suggesting that the enzyme may be playing an important role atleast during the onset of diapause. While phosphorylase 'a' is said to be the key enzyme involved in providing the initial substrate for polyol formation (Chandrashekar and Geethabali 1987), NADP-SDH may be important in the actual formation of sorbitol.

The increase in the activity of NADP-GDH in diapause eggs 10 days following oviposition indicates that it may be contributing towards the formation of glycerol. However, compared to the level of activity observed at the time of oviposition, this increase is not very significant. Since (i) the enzyme is euqually active in both diapause and non-diapause eggs, (ii) there is no change in its activity correlated with the onset of diapause and (iii) no significant change was seen as a result of acid-treatment, it can be said that though this enzyme may contribute towards the formation of glycerol, it may not be a key enzyme.

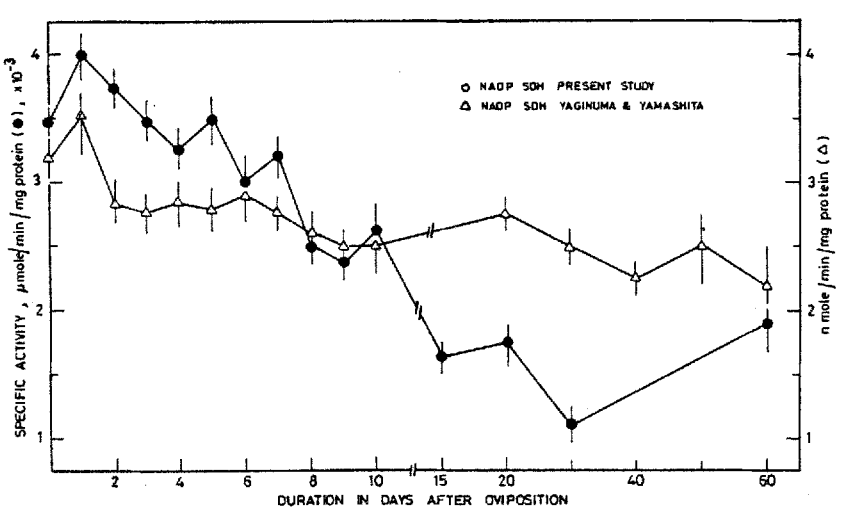
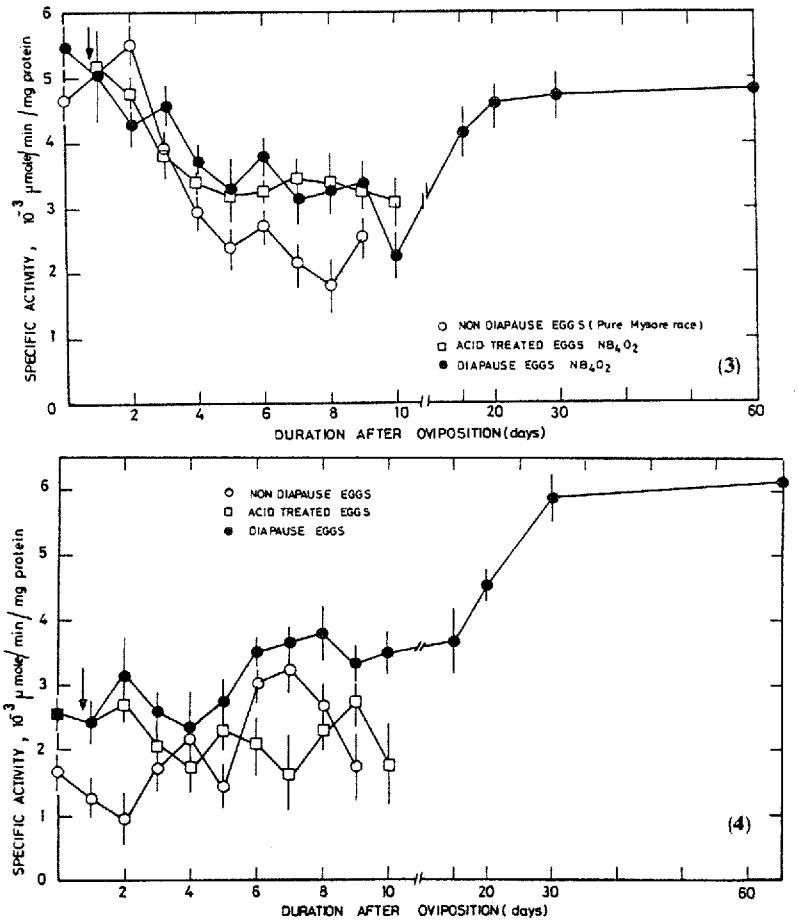


Figure 2. Changes in NADP-SDH activity during diapause. Results from Yaginuma and Yamashita (1979) are presented for comparison. Note the increase in the activity in both the cases on the first day following oviposition.



Figures 3 and 4. Changes in (3) NADP-GDH and (4) NAD-GPDH activity during diapause and embryogenesis.

NAD-GPDH was found to be quite active in both diapause and non-diapause eggs. The activity increased as embryogenesis progressed especially around periods corresponding to blastokinesis and blue egg stages. Hence, it may be important in embryonic development as already suggested by earlier workers (Chino 1960; Horecker, 1968; Yamashita *et al* 1981). In diapause eggs, not only the activity was much higher than that observed in non-diapause eggs, it increased slowly following oviposition up to the 10th day and rapidly thereafter. This closely corresponds to the initial slow increase in the glycerol level followed by its rapid accumulation in diapause eggs (Yaginuma and Yamashita 1978; Geethabali and Chandrashekar 1987). Hence, NAD-GPDH may be playing a more important role than NADP-GDH in the production of glycerol during diapause. However, not much is known about the conversion of glycerophosphate to glycerol in silkworm eggs. This would involve the removal of phosphate either by a specific glycerophosphatase or other general phosphate cleaving enzyme. At this juncture, it can be said that the high activity of NAD-GPDH would lead to the accumulation of glycerophosphate in

silkworm eggs which is a phenomenon common in many insects during diapause or even anaerobiosis (Chefurka 1965). If NAD-GPDH activity is accepted to be contributing mainly to the formation of glycerol, it can be realised that the NADP requirement for glycerol formation is minimised.

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