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Effects of Nutrition on Storage Protein Concentrations in the Larval Hemolymph of the Silkworm, *Bombyx mori*

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The effects of dietary protein and sugar contents on concentrations of the hemolymph storage proteins were examined in the larvae of *Bombyx mori*. Fourth instar larvae were fed 5 kinds of artificial diets: a high protein, a low protein, a high sugar, a low sugar content, and a standard diet. The hemolymph storage proteins (SP-1: female-specific storage protein, SP-2: arylphorin) and total proteins increased with the rise of the dietary protein concentrations; the two storage protein levels varied much more than those of the other proteins. Although the dietary sugar content did not affect the hemolymph storage protein concentrations of larvae on day 2 of the 4th instar, the newly-ecdysed 5th instar larvae fed on the sugar-rich diet had high concentrations of storage proteins. Extremely low concentrations of hemolymph storage proteins occurred when the larvae were reared from the 1st through the 4th instars on the protein-poor diet. These results showed that the storage protein concentrations in the larval hemolymph were influenced by nutrition, particularly the protein levels in the diet.

Storage proteins are major hemolymph proteins in the larvae of holometabolous insects, playing an important role as reservoirs for amino acids that are utilized for adult development in metamophosis (Levenbook, 1985). There are two kinds of storage proteins in *Bombyx mori*: SP-1 and SP-2 (Tojo *et al.*, 1980). SP-1, even though it is female specific at the 5th instar and during subsequent metamorphosis, is present equally in both sexes from the 1st to 4th larval instars (Mine *et al.*, 1983; Kawaguchi, *et al.*, 1983). SP-2, an arylphorin, is the most abundant protein in the hemolymph of 4th instar larvae. The hemolymph storage proteins have cyclic changes in quantity during larval molts (Nagata and Kobayashi, 1990).

Synthesis of storage proteins in the larval fat body is related with feeding activity (Riddiford and Hice, 1985) and the increase in amount of the storage proteins is evident during the mid-feeding period of an instar. The quantity of storage proteins does not increase during the larval molt or during starvation (Nagata and Kobayashi, 1990). These observations suggest that the accumulation of storage proteins is influenced by nutritional conditions. We report herein our analysis of the effects of dietary

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protein- and sugar-levels on the storage protein concentrations in the hemolymbh of *Bombyx* larvae.

Materials and Methods

Insects and diets: Hybrid races of the silkworm, N140×C140 and Kinshu×Showa, were reared on artificial diets. Protein in the diet was in the form of soybean meal. A standard diet (S) contained 27.52% soybean meal and 7.34% sucrose as dry matter and other ingredients were based on the diet of Horie *et al.* (1973): mulberry leaf powder, 22.94%; potato starch, 5.50%; soybean oil, 1.38%; β-sitosterol, 0.18%; salt mixture, 2.75%; agar, 6.88%; ascorbic acid, 0.92%; citric acid, 3.67%; sorbic acid, 0.28%; and cellulose powder, 20.64%. A vitamin B mixture, propionic acid and chloramphenicol were added to the diet. The water content in the diet was 71.2%. The various protein and sugar contents were based on the amounts of soybean meal and sucrose. In the diets named HP and LP the protein contents were 45.87% and 9.17%, respectively, and in the diets HS and LS the sugar contents were 16.51% and 1.83%, respectively. The total amount of dry matter was adjusted to the same level as that of the standard diet by adding or subtracting the quantity of cellulose powder.

Analyses of hemolymph proteins: Hemolymph from 10 larvae was collected in a tube, to which a small amount of phenylthiourea was added and then centrifuged to remove hemocytes. The supernatant preparation was kept at −60°C until anaiyzed.

Protein components in the hemolymph were analyzed by slab-type polyacrylamide gel electrophoresis (PAGE) with Davis' buffer system (Davis, 1964). A separation gel (0.2×13.8×11 cm) was made in touch with a stacking gel (1 cm height); the concentration of the former was 6, 6.5 or 7.5% and that of the latter was 3.125% of acrylamide. An aliquot of hemolymph (4 μl collected on day 0 of the 4th instar and 2 μl at other stages) was mixed with 20% sucrose and 0.01% bromophenol blue, poured into a sample well (2×5 mm) of the stacking gel and electrophoresed at 20 mA for 2 hr. The gels were stained with 1% fast green-7% acetic acid (Gorovsky, *et al.*, 1970) and destained with 7% acetic acid. Densities of protein bands were measured at 630 nm with a chromatoscanner (Shimadzu CS-910, Kyoto, Japan) connected with an integrator (Hitachi D-2000, Tokyo, Japan). SP-1 and SP-2 were identified tentatively by their positions and quantified by measuring areas of the peaks.

Results

Silkworm larvae were reared on a standard artificial diet until the 3rd ecdysis and then separated into 5 groups. Each larval group

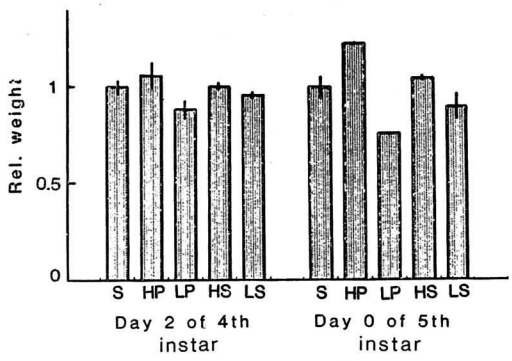

Fig. 1. Body weights of silkworm larvae reared at the 4th instar on different artificial diets after the 3rd ecdysis. The weights were measured on day 2 of the 4th instar and on day 0 of the 5th instar. S: standard diet (27.5% soybean meal and 7.3% sucrose in dry matter), HP: high protein diet (45.9% soybean mean), LP: low protein diet (9.2% soybean meal), HS: high sugar diet (16.5% sugar), LS: low sugar diet (1.8% sugar). Values are expressed by relative weights with the value in larvae reared on the standard diet set at 1.0. Vertical bar in the box indicates standard deviation.

was fed one of the 5 diets containing different protein and sugar contents. The larval weight of each group is shown in Fig. 1. Larvae fed on a high protein diet (HP) weighed the most and grew the fastest. Larvae fed on a high sugar diet (HS) grew at almost the same rate as those on the standard diet (S). The weights of larvae fed on a low sugar diet (LS) were somewhat lower than those on the standard diet. A low protein diet (LP) strikingly affected growth, with the larvae having the smallest gain in weight and the longest duration in the 4th instar: 7-8 days for the LP larvae and 4-5 days for the others.

PAGE showed that SP-2 (arylphorin) was the most abundant protein in the hemolymph. SP-2 made up about 50% of the total hemolymph proteins on day 2 of the 4th instar larvae fed the standard diet (Fig. 2). SP-1 was also a major protein (7-9%), but other proteins were minimal. Both SP-1 and SP-2 were strongly influenced by the protein content in the diet. Fig. 3 showed that differences among 2-day-old, 4th instar larvae fed on the standard, HP and

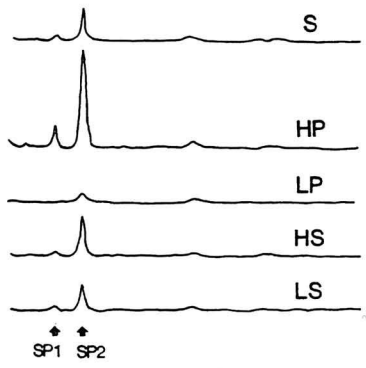

Fig. 2. Densitometric patterns after PAGE of hemolymph proteins on day 2 of the 4th instar. The larvae were reared on different artificial diets after the 3rd ecdysis. Gel concentration was 6%. Arrows indicate storage proteins. Abbreviations are the same as in Fig. 1.

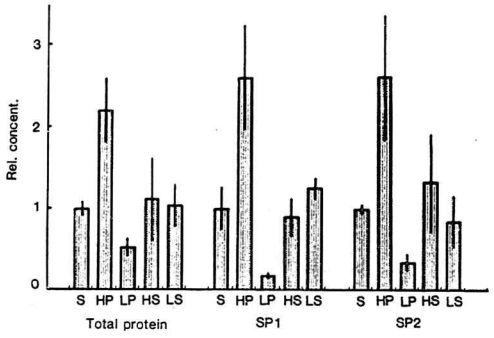

Fig. 3. Densitometric analysis of total and storage protein concentrations in the hemolymph of 2-day-old, 4th instar. The larvae were reared on different artificial diets after the 3rd ecdysis. Values are expressed by relative concentrations with the value in larvae reared on the standard diet set at 1.0. Vertical bar indicates standard deviation. Abbreviations are the same as in Fig. 1.

LP diets were greater in the storage proteins than in the total hemolymph proteins. The SP-1 and SP-2 concentrations in the HP larvae were 2.6 times higher than those in the standard larvae. The LP larvae had 1/5 as much SP-1 and 1/3 as much SP-2 as in the standard larvae. Other proteins, aside from SP-1 and SP-2, also varied with the protein contents in the diet, but their variations were smaller compared to the storage proteins. The diet sugar content had no obvious effect.

The diets clearly affected storage proteins in the hemolymph of larvae just after the 4th ecdysis (Fig. 4). The differences were more striking than those found at the mid-fourth instar. Storage proteins in the larvae fed the LP diet were far below those on the standard diet, whereas the HP larvae had the largest amount of hemolymph storage proteins. The effect of dietary sugar content on the storage proteins was evident in the hemolymph of newly ecdysed larvae, in which high sugar induced high levels of storage proteins and low

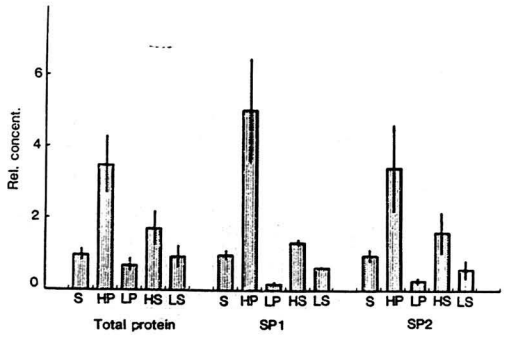

Fig. 4. Densitometric analysis of total and storage protein concentrations in the hemolymphs of newly ecdysed 5th instar larvae. The larvae were reared on different artificial diets after the 3rd ecdysis. Values are expressed by relative concentrations with the value in larvae reared on the standard diet set at 1.0. Vertical bar indicates standard deviation. Abbreviations are the same as in Fig. 1.

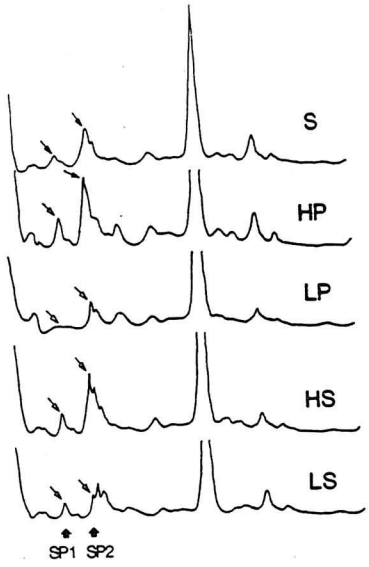

Fig. 5. Densitometric patterns of hemolymph proteins of the newly ecdysed 4th instar larvae reared on different diets from the 1st instar. Gel concentration was 6.5%. Arrows indicate storage proteins. Abbreviations are the same as in Fig. 1.

sugar caused low protein levels.

When larvae were reared on the above diets from the 1st through the 4th instars, the low protein diet seriously affected larval growth and the LP larvae took 16 days to develop to the 4th instar, while larvae on other diets took 11-12 days. Differences in the body weights among the 5 groups followed a pattern similar to that described previously; i.e., the weights of LP larvae were the lowest, those of HP larvae were the highest and those of other groups were between these two.

PAGE patterns of the hemolymph proteins just after the 3rd ecdysis are shown in Fig. 5. Densitometric analysis revealed that the relative concentrations of SP-1 were 1 ± 0.14 in standard, 1.38 ± 0.11 in HP, 0.29 ± 0.13 in LP, 1.43 ± 0.41 in HS and 0.84 ± 0.16 in LS. SP-2 concentrations were 1 ± 0.04 in standard, 2.38 ± 0.78 in HP, 0.04 ± 0.0 in LP, 2.05 ± 1.04 in HS and 0.31 ± 0.07 in LS. The heterogeneous peaks close to the SP-2 area were not recognized as SP-2. Low protein diet resulted in extremely low concentrations of storage proteins. The sugar content in the diets also affected storage proteins; the storage protein levels in the hemolymph increased with the amount of sugar in the diet.

The most abundant protein in the hemolymphs of newly ecdysed 4th instar larvae was not SP-2, but a protein with a relative mobility at 0.67 (dye front set at 1.0). This protein was interesting in that its concentration in larvae did not differ greatly among the 5 groups: 1 ± 0.03 in S, 1.35 ± 0.02 in HP, 1.40 ± 0.26 in LP, 1.27 ± 0.32 in HS and 1.46 ± 0.47 in LS. Thus, the level of this protein in the hemolymph was not influenced by nutrition under the present conditions.

Larval hemolymph on day 2 of the 4th instar was analyzed (Fig. 6). The storage protein concentrations increased with the protein con-

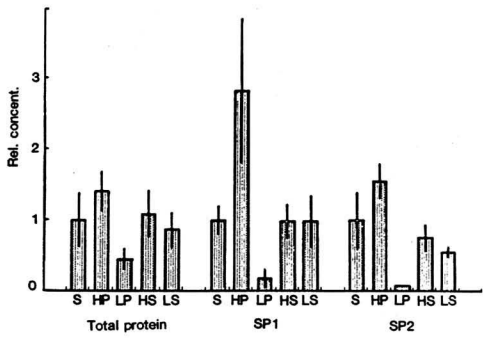

Fig. 6. Total and storage protein concentrations in the hemolymphs of 2-day-old, 4th instar larvae reared on different diets from the 1st instar. Values are expressed by relative concentrations with the value in larvae reared on standard diet set at 1.0. Vertical bar indicates standard deviation. Abbreviations are the same as in Fig. 1.

tent in the diet and their levels varied much more than the total protein levels in the hemolymph. The concentration of SP-1 was more sensitive to the dietary protein as compared to that of SP-2. The effect of sugar was again not obvious in the hemolymphs of larvae on day 2 of the feeding period.

Discussion

Dietary protein influences the hemolymph protein concentration in the silkworm. Horie *et al.* (1971) reported that the total content of hemolymph proteins increased with the amount of protein in the artificial diet. Watanabe and Horie (1980) observed that the dietary levels of essential amino acids were closely associated with the hemolymph protein levels. Our results agreed with their observations, and in addition, we found that the storage proteins were highly sensitive to the dietary protein content. The accumulation of storage proteins in the hemolymph during the feeding period was consistently affected by the levels of protein in the diet. After feeding on high protein diet for 2 days, larvae contained 2.5 times more storage proteins than those fed on the standard diet. On the other hand, the hemolymph of larvae fed the low protein diet had only 18-35% as much storage proteins as compared to that of larvae reared on the standard diet. These results showed that the synthesis of storage proteins were strongly associated with the supply of amino acids in the diet.

The storage protein concentrations decreased after larval ecdysis due to the degradation of the proteins during the molting period (Nagata and Kobayashi, 1990). The dietary sugar content affected SP's levels in the newly ecdysed larvae but not in the feeding larvae, suggesting that the carbohydrates accumulated in the larvae were involved in the storage protein degradation. We speculated in a previous paper that the storage proteins were degraded to amino acids and utilized for tissue formation at the larval molt (Nagata and Kobayashi, 1990). The amino acids may be used mainly for protein synthesis, but there is the possibility that some amino acids are catabolized to other compounds for energy metablism, and in the presence of excess amounts of carbohydrates in the larvae reared on a high sugar diet the storage proteins may be spared at molt.

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永田昌男・小林　淳：家蚕幼虫の血液貯蔵タンパク質濃度に及ぼす飼料の影響

人工飼料中のタンパク質と糖の含量が家蚕血液中の貯蔵タンパク質 SP-1（幼虫型雌特異タンパク質）と SP-2（アリルフォリン）濃度に与える影響を4齢期において調査した。

4齢起蚕よりタンパク質，糖含量の異なる飼料を与え，2日後に調べると，高タンパク質飼料の血液では高い貯蔵タンパク質濃度を示し，低タンパク質飼料では低い濃度となった。一方，糖含量の影響はみられなかった。5齢起蚕時においては，飼料タンパク質量の影響はより強く現れ，さらに糖量の増減も貯蔵タンパク質濃度の高低をもたらした。1齢期より異なる飼料で飼育し，4齢2日で調べると，飼料タンパク質含量の影響は強く，とくに低タンパク質飼料で飼育した場合には，著しく低い貯蔵蛋白質濃度となった。以上のことから，血液貯蔵タンパク質濃度には栄養条件が強く影響することが明らかになった。