家蚕休眠卵の炭水化物代謝に関する研究

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Studies on the Carbohydrate Metabolism in Diapause Eggs of the Silkworm, *Bombyx mori*, with Special Reference to Phosphofructokinase Activity

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The onset of embryonal diapauses of the silkworm, *Bombyx mori*, accompanys the conversion of glycogen into glycerol and sorbitol, and the diapause termination is followed by glycogen synthesis from these sugar alcohols (CHINO, 1958). The mechanism of this interesting phenomenon was extensively investigated by CHINO (1960, 1961, 1963) and further by ISHIKAWA (1966), but not elucidated in its regulatory mechanism. Recently, OKADA (1917) in silkworm diapause eggs suggested that the diapause initiation and termination, and, further, glycogen conversion into two sugar alcohols, and *vice versa* would be induced by changes of permeability of oxygen through the chorions of diapause and post-diapause eggs.

KAGEYAMA and OHNISHI (1971) in carbohydrate metabolism in silkworm eggs stated that phosphofructokinase activity could not be demonstrated in the diapausing and young developing eggs although aldolase, glyceraldehydephosphate dehydrogenase, glycerol-3-phosphate dehydrogenase were detected. Then, they postulated that the conversion of glycogen into two polyols occurs predominantly through pentose phosphate pathway instead of glycolytic pathway suggested by CHINO (1958)

During the course of the experiments on carbohydrate metabolism in silkworms (SUZUKI *et al.*, 1973), phosphofructokinase activity, one key enzyme of glycolytic pathway, was observed in silkworm eggs contrary to the result of KAGEYAMA and OHNISHI. The attention was paid by the authors on this enzyme and it was found that phosphofructokinase activity was demonstrable in pre-diapause, young diapause, and developing eggs. The present paper deals with the activity of phosphofructokinase in silkworm eggs together with those of glucose-6-phosphate dehydrogenase and fructose-1, 6-diphosphatase which function as key enzymes of pentose phosphate pathway and gluconeogenesis, respectively.

The following abbreviations were used in this paper: **AMP** adenosine-5′-monophosphate, **ATP** adenosine-5′-triphosphate, **F6P** fructose-6-phosphate, **FDP** fructose-1, 6-diphosphate, **G6P** glucose-6-phosphate, **NADH** nicotinamide-adenine-dinucleotide (reduced form), **NADP**$^+$ nico-

tinamide-adenine-dinucleotide phosphate (oxidized form), **NADPH** nicotinamide-adenine-dinucleotide phosphate (reduced form), **ALDase** aldolase, **FDPase** fructose-1, 6-diphosphatase [D-fructose-1, 6-diphosphate 1-phosphohydrolase (EC 3. 1. 3. 11)], **G3PDHase** glycerol-3-phosphate dehydrogenase, **G6PDHase** glucose-6-phosphate dehydrogenase [D-glucose-6-phosphate: $NADP^+$ oxidoreductase (EC 1. 1. 1. 49)], **PFKase** phosphofructokinase [ATP: D-fructose-6-phosphate 1-phosphotransferase (EC 2. 7. 1. 11)], **PGIase** phosphoglucose isomerase, **TPIase** triosephosphate isomerase.

MATERIAL AND METHODS

Experimental animals: Silkworms used were a hybrid of Shunrei and Shogetsu or Kinshu and Showa conditioned to lay diapause eggs after adult emergence. The newly laid eggs were kept at 25°C and divided into three groups. One group of these eggs was treated by hydrochloric acid 24 hr after oviposition to break the diapause, one group was chilled 11 days after oviposition and kept for 100 days to break diapause artificially and the last one was kept at 25°C until sacrifice.

Chemicals: AMP (disodium salt), ATP (disodium salt), F6P (disodium salt), FDP (trisodium salt), G6P (disodium salt), NADH (disodium salt), $NADP^+$ (disodium salt), NADPH (tetrasodium salt), ALDase, G3PDHase, G6PDHase, PGIase and TPIase were purchased from Boehringer Mannheim GmbH. Bovine serum albumin was prepared by Sanko Junyaku Co.

Preparation and assay of the enzyme: Silkworm eggs (0.5 g) were ground in a mortar with 3 ml of 50 mM Tris-HCl buffer (pH 8.5) and then homogenized thoroughly with a Teflon homogenizer. The homogenates were contrifuged at 15,000 g for 30 min, and the supernatants were used as enzyme preparations (The preparations different from those at 15,000 g for 30 min were denoted in the captions).

PFKase activity was determined by the method of UNDERWOOD and NEWSHOLME (1965), *i. e.* the reaction mixture was as follows; 1.65 ml of 50 mM Tris-HCl buffer (pH 8.5), 5 μl of ALDase (0.45 units), 5 μl of G3PDHase (0.2 units), 5 μl of PGIase (1.75 units), 5 μl of TPIase (25 units), 5 μl of KCN (120 mM), 25 μl of ATP (80 mM), 25 μl of AMP (160 mM), 25 μl of imidazol (1.6 M), 25 μl of $MgCl_2$ (400 mM), 50 μl of NADH (4 mM), 100 μl of KCl (4 M), 25 μl of G6P (160 mM), and 50 μl of enzyme preparation in 2 ml of a final volume. After pre-incubation for 30 min, the decrease in extinction of the reaction mixture at 340 nm was followed at intervals of 2.5 min at room temperature (25°C). The activity was corrected with controls in which G6P was omitted.

G6PDHase assay was carried out by the modified method of CHINO (1960). The reaction mixture comparized 1.85 ml of 50 mM Tris-HCl buffer (pH 8.5), 50 μl of $MgCl_2$ (200 mM), 25 μl of G6P (160 mM), 25 μl of $NADP^+$ (12 mM), and 50 μl of enzyme preparation in 2 ml of a final volume. After pre-incubation for 30 min, the increase in extinction of the reaction mixture at 340 nm was followed at intervals of 2.5 min at room temperature (25°C). The activity was corrected with controls in which $NADP^+$

was omitted.

FDPase assay was carried out according to the method of the previous paper in pupal ovaries of the silkworm (SUZUKI *et al.*, 1973). The reaction mixture comparized 1.25 ml of 50 mM Tris-HCl buffer (pH 8.5), 50 μl of $MgSO_4$ (300 mM), 50 μl of $NADP^+$ (4.5 mM), 25 μl of PGIase (17.5 units), 25 μl of G6PDHase (8.75 units), 50 μl of FDP (3 mM), and 50 μl of enzyme preparation in 1.5 ml of a final volume.

Protein concentration: Protein concentration in each enzyme preparation was estimated by the method of LOWRY *et al.*, (1951).

RESULTS AND DISCUSSION

Demonstration of PFKase activity in silkworm eggs

To see whether PFKase activity was demonstrable in silkworm eggs or not, the method of UNDERWOOD and NEWSHOLME (1965) was adopted in a preliminary experiment using 24-hr old (pre-diapause), 5-day old, and 30-day old diapause eggs kept at 25°C after oviposition. PFKase activities were measured at intervals of 2.5 min and the results were indicated in Fig. 1.

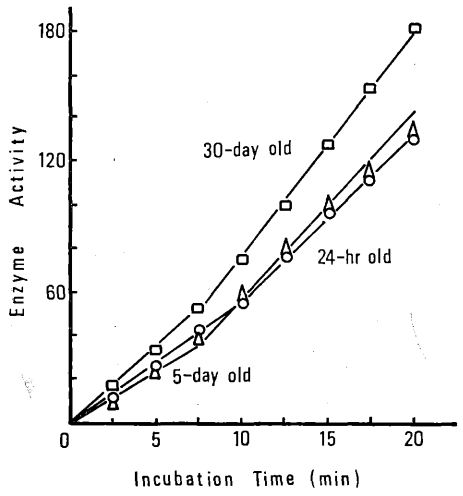

Fig. 1. Time course of PFKase activity in silkworm eggs. The homogenate of 30-day old eggs was centrifuged at 8,000 g for 30 min. Enzyme activity was expressed in terms of n moles of NADH oxidized per mg protein. ○—○, 24-hr old eggs; △—△, 5-day old eggs; □—□, 30-day old eggs, at 25°C after oviposition.

As indicated in Fig. 1, enzyme preparations of 24-hr old (pre-diapause) and 5-day old diapause eggs showed almost identical, PFKase activities, increasing with the lapse of time, although the activities in 30-day old diapause eggs were slightly higher than those in the former two cases. These results suggested that enzyme PFKase was present in silkworm eggs, including pre-diapause and diapausing eggs contrary to KAGEYAMA and OHNISHI (1971). They stated that PFKase activity was not demonstrated in the young developing and diapausing eggs. To find the cause of the discrepancy, a series of experiments was carried out with the assays of PFKase adopted by KAGEYAMA and OHNISHI (1971), and UNDERWOOD and NEWSHOLME (1965). Fig. 2 shows time course of PFKase activity in diapausing and hydrochloric acid-treated eggs. In the method of KAGEYAMA and OHNISHI (1971), silkworm eggs were homogenized in distilled water, but both distilled water and Tris-HCl

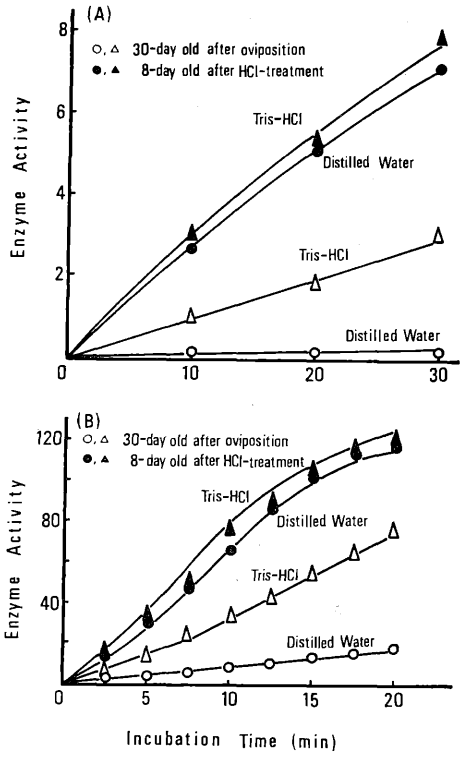

Fig. 2. Time course of PFKase activity assayed by two methods in silkworm eggs. (A)—PFKase activity was determined by the method of KAGEYAMA and OHNISHI (1971) with the exception of 0.56 M hydrazine sulfate. ○—○, 30-day old eggs at 25°C after oviposition, homogenized in distilled water, and enzyme preparation contained 1.8 mg of protein; △—△, 30-day old eggs at 25°C after oviposition, homogenized in 50 mM Tris-HCl buffer (pH 8.5), and enzyme preparation contained 3.96 mg of protein; ●—●, 8-day old eggs at 25°C after HCl-treatment, homogenized in distilled water, and enzyme preparation contained 1.8 mg of protein; ▲—▲, 8-day old eggs at 25°C after HCl-treatment, homogenized in 50 mM Tris-HCl buffer (pH 8.5), and enzyme preparation contained 2.97 mg of protein. Enzyme activity was expressed in terms of (O.D. at 540 nm) × 10 per 0.3 ml of enzyme preparation. (B)—PFKase activity was determined by the method of UNDERWOOD and NEWSHOLME (1965). ○—○, 30-day old eggs at 25°C after oviposition, homogenized in distilled water, and enzyme preparation contained 0.195 mg of protein; △—△, 30-day old eggs at 25°C after oviposition, homogenized in 50 mM Tris-HCl buffer (pH 8.5), and enzyme preparation contained 0.42 mg of protein; ●—●, 8-day old eggs at 25°C after HCl-treatment, homogenized in distilled water, and enzyme preparation contained 0.175 mg of protein; ▲—▲, 8-day old eggs at 25°C after HCl-treatment, homogenized in 50 mM Tris-HCl buffer (pH 8.5), and enzyme preparation contained 0.345 mg of protein. Enzyme activity was expressed in terms of n moles of NADH oxidized per 50 μl of enzyme preparation.

buffer were used in this experiment.

As indicated in Fig. 2, PFKase activities increased with time, in the 8-day old eggs after HCl-treatment which were homogenized in either distilled water or Tris-HCl buffer. However, in 30-day old diapause eggs, when the eggs were homogenized in distilled water, PFKase activities were lower than those in Tris-HCl buffer. In particular, PFKase activity in 30-day old diapause eggs assayed by the method of KAGEYAMA and OHNISHI (1971) could not be demonstrated till 30 min after the initiation of reaction. These results suggested that absence of PFKase activity in the diapausing and young developing eggs stated by KAGEYAMA and OHNISHI (1971) would be brought about by homogenizing the eggs in distilled water. It seemed that PFKase activity in silkworm eggs did not become more labile by homogenizing in distilled water, but aggregated in the precipitated fraction (unpublished data). This interesting phenomenon will be investigated in future.

During the course of this experiment, the authors encountered sometimes very low PFKase activity in the eggs stored in a deep freezer. To confirm whether or not PFKase activity would be denatured in storage, following experiments were undertaken. Diapausing and developing eggs were frozen at about −30°C for 6 days, and PFKase activities were assayed together with G6PDHase and FDPase activities. The results are tabulated in Table 1.

Table 1. Effect of freezing on PFKase, G6PDHase, and FDPase activities in silkworm eggs

Egg used	Enzyme preparation made from	PFKase**	G6PDHase***	FDPase***
Diapause egg				
8-day old after oviposition	Non-treated eggs	10.1	15.2	—
	Frozen egg*	0.5	17.1	—
25-day old after oviposition	Non-treated eggs	12.6	15.8	—
	Frozen eggs*	0.0	16.3	—
Developing egg				
6-day old after 100-day chilling	Non-treated eggs	13.0	17.2	4.6
	Frozen eggs*	10.4	15.6	4.8

Silkworm eggs used: diapause eggs 8- and 25-day old at 25°C after oviposition and developing eggs 6-day exposed at 25°C after 100-day chilling; *: Eggs were exposed to about −30°C for 6 days; **: PFKase activity was expressed in terms of n moles of NADH oxidized per min per mg protein from 10 to 15 min after the initiation of reaction; ***: Activities of G6PDHase and FDPase were expressed in terms of n moles of $NADP^+$ reduced per min per mg protein during 5 min after the initiation of reaction (The same applied to Figs. 5, 6, 7 and 8). The homogenates in diapause eggs 8-day and 25-day old at 25°C after oviposition were centrifuged at 8,000 g for 30 min.

As indicated in Table 1, G6PDHase activity was little changeable by freezing, but PFKase activity was almost completely lost except in developing eggs. Thus, freezing of silkworm eggs had remarkable effect on PFKase activity in diapause eggs but not on that in developing eggs. These results suggested that PFKase activity in diapausing eggs was more labile to freezing than in developing eggs, indicating no availability of frozen eggs for PFKase assay. When enzyme preparations were kept in the ice chest for 24 hr after centrifugation, PFKase activity was almost lost even prepared from developing eggs although those of G6PDHase and FDPase were little affected. Thus, fresh enzyme preparation of not frozen eggs should be used for PFKase assay as soon as possible after preparation.

One attempt was carried out to stabilize the enzyme *in vitro* by the aid of some chemicals, such as 2-mercaptoethanol (20 mM) and dithiothreitol (5 mM), and ethylenediaminetetraacetic acid (EDTA, 5 mM) and diethylenetriaminepentaacetic acid (DTPA, 5 mM).

As indicated in Fig. 3, when the eggs were homogenized in only Tris-HCl buffer

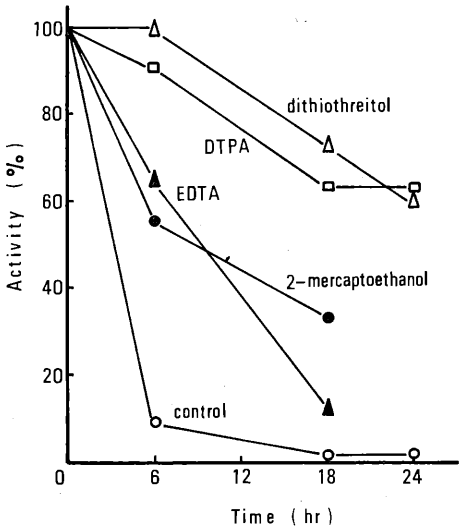

Fig. 3. Effect of 2-mercaptoethanol, dithiothreitol, EDTA, and DTPA on the stabilization of PFKase activity in diapausing eggs. Sampled eggs were homogenized in 50 mM Tris-HCl buffer (pH 8.5), control (○—○), or 50 mM Tris-HCl buffer (pH 8.5) containing 20 mM 2-mercaptoethanol (●—●), 5 mM dithiothreitol (△—△), 5 mM EDTA (▲—▲) and 5 mM DTPA (□—□). Enzyme preparations were kept in the ice chest from the time assayed immediately after prepared (0 hr) to 24 hr. Each enzyme activity was corrected for the maximal reaction rate assayed immediately after prepared.

and enzyme preparations were kept in the ice chest for 6 hr, PFKase activity in enzyme preparations was lost to below 10 per cent compared with that assayed immediately after prepared. If the eggs had been homogenized in Tris-HCl buffer containing 2-mercaptoethanol, dithiothreitol, EDTA, and DTPA, the loss of PFKase activity was comparatively slight. However, the longer the preservation of enzyme preparations in the ice chest, the less PFKase activity. Dithiothreitol and DTPA were more potent than 2-mercaptoethanol and EDTA for the stablization of PFKase activity. These results implied that the enzyme was stabilized in part in a reducing condition.

Characteristics of PFKase in silkworm eggs

As PFKase was clearly demonstrated in silkworm eggs, kinetic properties were studied using diapausing and developing eggs. First, the effect of enzyme concentration on PFKase activity was analysed using diapausing 8-day old eggs (Fig. 4).

As shown in Fig. 4, PFKase activity increased proportionally from 0.24 to 0.96 mg of protein concentration per 50 μl of enzyme preparation. Thus, the following experiments were undertaken with the material of less than about 1 mg of protein concentration in enzyme preparation.

Next, changes in PFKase activity of silkworm eggs were observed in relation to pH, using 50 mM Collidine-HCl (pH 6.5-7.5), 50 mM Tris-HCl (pH 7.5-9.0), and Glycocol-NaOH (pH 9.0-10.5) buffers. Although the data were not shown, the pH optimum seemed to be present between 8.0 and 9.5. Tris-HCl buffer was somewhat inhibitory comparing to Collidine-HCl and Glycocol-NaOH buffers.

Effect of ATP concentration on PFKase activity was surveyed (Fig. 5). As indicated in Fig. 5, PFKase activity increased according to the concentration of ATP

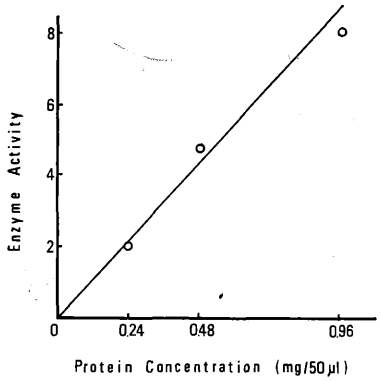

Fig. 4. Effect of enzyme concentration on PFKase activity in diapausing eggs. The homogenate of the eggs was centrifuged at 8,000 g for 30 min. Enzyme activity was expressed in terms of n moles NADH oxidized per min from 10 to 15 min after the initiation of reaction.

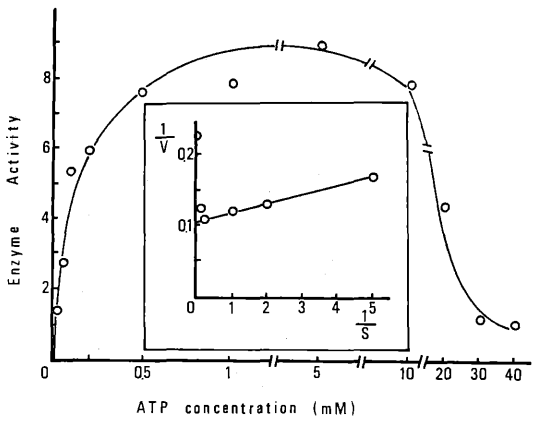

Fig. 5. Effect of ATP concentration on PFKase activity in developing eggs. The inset in this figure showed Lineweaver-Burk plot.

added and the Km value for ATP was 0.12 mM. At higher concentrations of ATP, the activity decreased and half activity was found at 20 mM of ATP. Inhibition profile by higher ATP concentrations was similar to those of rat liver (UNDERWOOD and NEWSHOLME, 1965) and coxal muscles from cockroach (GRASSO and NATALIZI, 1968), but the inhibition was lower excessively than those.

Changes in activities of enzymes during diapause and embryonic development

As described above, PFKase activity was clearly demonstrated in silkworm eggs. Then, the PFKase activity was followed in diapause eggs at various stages together with G6PDHase and FDPase. These activities were measured at 12-hr and at a daily interval from one day after oviposition (Fig. 6).

As indicated in Fig. 6, activities of PFKase and G6PDHase were clearly observed but FDPase activity was hardly observable during this period. G6PDHase activity was comparable to those of CHINO (1960), and KAGEYAMA and OHNISHI (1971), and the result supports that the enzyme provides NADPH necessary for the formation of sugar alcohols in diapause initiation. As for PFKase, the activity remained about half that of G6PDHase during early 10 days after oviposition. This result was different that of KAGEYAMA and OHNISHI (1971), in which no activity was found in diapausing and young developing eggs. From the absence of PFKase activity, they concluded that glycogen is converted mainly into sugar alcohols by way of pentose phosphate pathway in diapause eggs. The result in Fig. 2, however, explained that absence of

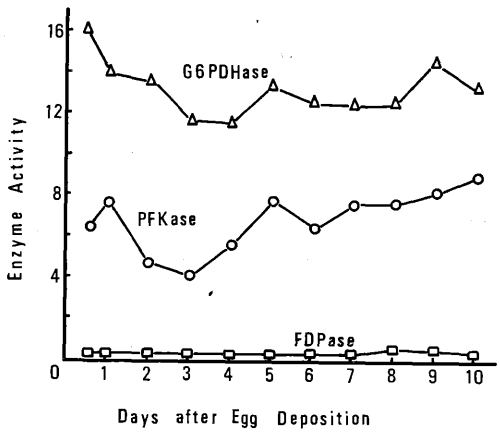

Fig. 6. Changes in activities of PFKase, G6PDHase, and FDPase in diapause eggs. The eggs were exposed to 25°C for 10 days after oviposition. ○—○, PFKase; △—△, G6PDHase; □—□, FDPase.

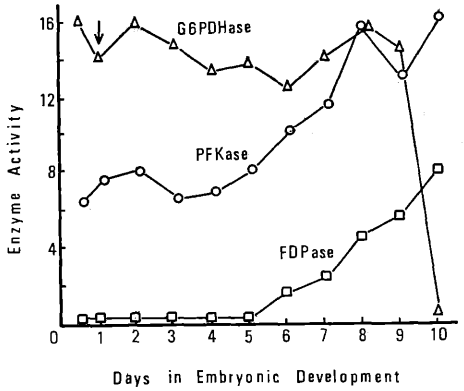

Fig. 7. Changes in activities of PFKase, G6PDHase and FDPase in the eggs treated with HCl 24 hr after oviposition. The arrow indicates the time of HCl-treatment; 10 days in the development; just before hatching; ○—○, PFKase; △—△, G6PDHase; □—□, FDPase.

PFKase in diapause eggs would be brought about by homogenizing the eggs in distilled water. Then as indicated in Fig. 6, it should be inferred that glycolysis as well as pentose phosphate pathway was operating for glycogen conversion to glycerol in diapause initiation. Recently, this was supported by the experiment of incorporation (KAGEYAMA, 1973).

Next the authors tried to follow changes in PFKase, G6PDHase, and FDPase activities during embryonic development. The diapause was broken to initiate embryonic development by HCl-treatment (Fig. 7) or chilling at 5°C for 100 days (Fig. 8).

G6PDHase activity in HCl-treated eggs was maintained at higher level throughout embryonic development and dropped suddenly just before hatching. On the other hand, in the chilled eggs the changes in the enzyme activity showed somewhat like U-shaped curve during the first two-third period of development, and dropped markedly thereafter. In both types of eggs, however, the activities were almost the same level and were higher than those of another two enzymes with the exception of period in a few days before hatching.

FDPase activity in HCl-treated eggs was hardly observable by 5 days of embryonic development, but thereafter rose abruptly until hatching. Same profile of the enzyme activity was shown in chilled eggs. FDPase is generally known as a key enzyme in a part of gluconeogenesis (SCRUTTON and UTTER, 1968). If gluconeogenesis rather than pentose phosphate pathway was involved in glycogen resynthesis from glycerol, it would be inferred that FDPase activity was responsible for the increase in glycogen content. FDPase activity, however, began to rise abruptly from the middle of em-

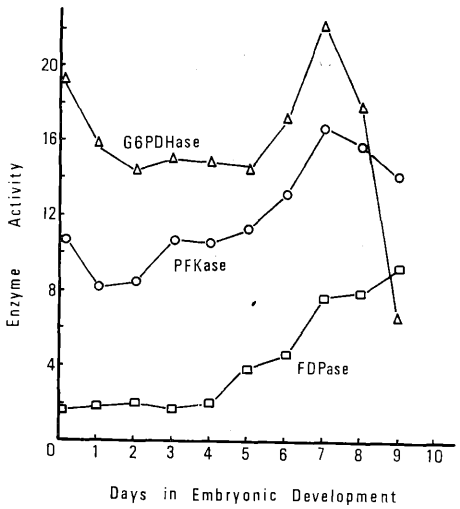

Fig. 8. Changes in activities of PFKase, G6PDHase, and FDPase in the eggs exposed to 25°C after 100-day chilling; 9 days in the development, one day before hatching; ○—○, PFKase; △—△, G6PDHase; □—□, FDPase.

bryonic development. These results suggested that gluconeogenesis involved in FDPase operated intensively from the middle of embryonic development.

As for PFKase, the activity was found clearly on the first day of development, and increased gradually and reached at maximum levels on the late stage of the development, but changes in the activity was somewhat different in HCl-treated eggs and chilled ones. KAGEYAMA and OHNISHI (1971) stated that PFKase activity in HCl-treated eggs first appeared on the 3th day. This difference between two results would be brought about in part by homogenizing the eggs in distilled water in contrast to the authors' case where the eggs were homogenized in Tris-HCl buffer. As stated above, PFKase activity in young diapause eggs would be operating for glycogen conversion to glycerol in diapause initiation. The enzyme activity in developing eggs, however, would be operating for glycogen degradation involved in ATP production, since changes in the enzyme activity were running parallel with tricarboxylic acid cycle-dehydrogenases (KAGEYAMA and OHNISHI, 1971) and typical respiratory enzymes (CHINO, 1963).

Acknowledgements—The authors are indebted to Professor K. HASEGAWA and Dr. O. YAMASHITA of Nagoya University for critical reading of the manucsript.

SUMMARY

Kinetic properties of phosphofructokinase (PFKase) were investigated in the diapausing and developing eggs of the silkworm, *Bombyx mori*. PFKase activity was somewhat stabilized by dithiothreitol and diethylenetriaminepentaacetic acid. The enzyme activity increased according to the concentration of ATP added and the Km value for ATP was 0.12 mM. At higher concentrations of ATP, the activity decreased and half activity was found at 20 mM of ATP.

High activity of PFKase which had not been detected was clearly demonstrated both in diapausing and developing eggs. Glucose-6-phosphate dehydrogenase (G6PDHase) activity was maintained at higher level throughout diapause and embryonic development, and dropped suddenly just before hatching. Fructose-1, 6-diphosphatase (FDPase) activity began to rise abruptly from the middle of the embryonic develop-

ment. The functions of PFKase, G6PDHase, and FDPase were discussed with respect to the carbohydrate metabolism in diapause eggs.

REFERENCES

CHINO, H. (1958): J. Insect Physiol., **2**, 1-12.
CHINO, H. (1960): J. Insect Physiol., **5**, 1-15.
CHINO, H. (1961): J. Insect Physiol., **6**, 231-240.
CHINO, H. (1963): Arch. Biochem. Biophys., **102**, 400-415.
GRASSO, A and G.M. NATALIZI (1968): Com. Biochem. Physiol., **26**, 979-984.
ISHIKAWA, H. (1966): J. Fac. Sci., Tokyo Univ., IV, **11**, 97-108.
KAGEYAMA, T. (1973): Ann. M. Jap. Soc. Developm. Biol., 18.
KAGEYAMA, T. and E. OHNISHI (1971): Development, Growth and Differentiation, **13**, 97-106.
LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR and R.J. RANDALL (1951): J. Biol. Chem., **193**, 265-275.
OKADA, M. (1971): Experientia, **27**, 658-660.
SCRUTTON, M.C. and M.F. UTTER (1968): Ann. Rev. Biochem., **37**, 249-302.
SUZUKI, K., O. YAMASHITA and K. HASEGAWA (1973): J. Sericult. Sci. Japan, **42**, 357-362.
UNDERWOOD, A.H. and E.A. NEWSHOLME (1965): Biochem. J. **95**, 868-875.

摘　要

家蚕休眠卵の炭水化物代謝に関する研究
特にホスホフラクトキナーゼ活性について

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1. 家蚕休眠卵と発育卵を使用し，ホスホフラクトキナーゼの酵素化学的性質を検討した。その結果，酵素活性の安定化に，SH基剤としてはジチオスライトール，キレート剤としてはジエチレントリアミン5酢酸が効果的であった。ATPに対するKm値は0.12 mM であり，高濃度のATPによって基質阻害を受けた。

2. これまで休眠卵と初期発育卵においてホスホフラクトキナーゼ活性の存在が否定されていたが，本研究は両ステージに高い酵素活性を確認した。

3. グルコース-6-ホスフェートデヒドロゲナーゼは休眠および発育中高い酵素活性を保っているが，孵化直前に急激に減少した。フラクトース-1,6-ジホスファターゼ活性は発育中期から増加し始め，孵化直前には最大に達した。

4. 上記より，休眠卵および発育卵の炭水化物代謝におけるそれぞれの酵素の役割を考察した。