Change of Carbohydrate Content during Ovarian and Embryonic Development in Emma Field Cricket, *Teleogryllus emma*

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(Received January 19, 1979)

Mannan-like polysaccharide was found as a main carbohydrate component in the fresh eggs of Emma field crickets; accounting for about 97% of total carbohydrates. This polysaccharide was accumulated in developing ovaries and carried over to the eggs, and it was then utilized markedly during the early and the late embryonic development but not during the diapause periods. Glycerol was the sole polyol and was accumulated during the course of ovarian development, and the changes in content were not associated with diapause, but with embryonic development.

**INTRODUCTION**

The relation between the embryonic diapause and polyol production has been, so far, investigated in eggs of some species of grasshopper; polyols did not accumulate at all during diapause in *Melanoplus differentialis* (Randall and Derr, 1965). In *Aulocara elliotti*, mannitol appeared temporarily during diapause, and glycerol did not associate with diapause phenomenon but appeared in pre-diapause and post-diapause stages (Quickenden, 1970). In the eggs of Emma field cricket, Yaginuma (1975) preliminarily detected only glycerol as polyols throughout egg life, and also found that the content of glycogen and free sugars was at a slight level in pre-diapause and diapause periods, but increased gradually after diapause termination.

As for the metabolism of Orthopteran eggs, the utilization of polysaccharide was reported in the grasshopper, *Locusta migratoria* (Yamaska, 1973). Therefore, in the present study, the authors followed the changes in carbohydrate content in the diapause eggs of Emma field cricket, and discussed the diapause of this insect in comparison with the metabolism of other insect diapause eggs.

**MATERIALS AND METHODS**

*Insects.* Cricket larvae and adults were collected from the field at Morioka,

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Iwate prefecture in October 1975 and 1976, and reared on fresh carrots and solid foods for mice in a plastic container (15×28×18 cm) at 25°C. To obtain ovaries developing synchronously, newly emerged adults were collected every day, and at a given stage ovaries were dissected in cold saline solution (0.75% NaCl). On the other hand, the eggs laid down in moist sand were sieved out and stored on wet filter paper in petri dishes at 25°C. The eggs entered diapause 5 days after oviposition. Then, water absorption began and continued for 8 days more to complete at 25°C (MASAKI, 1960). Some of those eggs were transferred to 5°C to break diapause artificially till the 90th day. After chilling of 66 or 90 days, the eggs were transferred to 25°C to continue embryonic development. The others were kept continuously at 25°C till the 60th day as control.

**Extraction and determination of carbohydrates.** The extraction of carbohydrates was done by the methods of YAMASAKI (1973) and YAMASHITA and HASEGAWA (1974) with some modifications. Four hundred to 800 eggs (around 250–500 mg) or approximately 500 mg ovaries were homogenized with 20 folds volume of cold distilled water, and aliquot of the homogenate was mixed with 5 folds volume of 80% ethanol. The mixture was heated at 75°C for 15 min and centrifuged at 3,000 rpm for 10 min, and the supernatant was decanted. After re-extraction, the combined supernatant was evaporated in vacuo at 40°C until dry. The residues were dissolved in 5 ml of distilled water and the solution was washed twice with equal volume of ether. The solution was deionized by passing them through small columns of Dowex 50W-X1 (H+ form) and Dowex 1-X1 (OH− form). The deionized solution was referred to as sugar and polyol fractions.

To extract glycogen, the ethanol insoluble residue was suspended in 5 ml of cold 5% trichloroacetic acid (TCA) and kept at 5°C for over 3 hr. The suspension was centrifuged and the supernatant fluid was drawn off, the precipitate being re-extracted. The combined supernatant was mixed with 3 folds volume of ethanol and one drop of saturated Na2SO4 and kept overnight at 5°C to precipitate glycogen (VAN HANDEL, 1965). By centrifugation the precipitate was washed with ethanol, ethanol-ether (3:1, v/v), and ether successively, and then dried. This fraction was referred to as glycogen fraction.

The residue of TCA extraction was washed with ethanol, ethanol-ether (3:1, v/v) and ether successively, and then dried. The dried material was completely digested with 2 ml of 30% potassium hydroxide (KOH) at 100°C for 30 min. After being cooled, 5 folds volume of ethanol and one drop of saturated Na2SO4 were added, and kept overnight at 5°C. The precipitate was washed with ethanol, ethanol-ether (3:1, v/v) and ether successively, and dried. After extraction with 10 ml of distilled water, the solution was referred to as polysaccharide fraction.

Total sugar content was determined according to phenol sulphuric acid method (Dubois et al., 1956). The amount of glycerol was determined by the method of BURTON (1957).

**Paper chromatography and paper electrophoresis.** In order to identify the sugar components extracted with hot KOH, aliquots of sample were hydrolyzed with 2 N trifluoroacetic acid (TFA) at 100°C for 2 hr (Lee et al., 1971). The hydrolysate was applied to paper chromatography and paper electrophoresis. Paper chromatographic analysis of sugars was carried out using the following solvent system; butanol, acetic acid, water (4:1:2, v/v) or butanol, ethanol, water (4:1:2, v/v). For electro-
phoresis sugars were separated at 20 volts per cm for 2 hr in borate buffer (pH 8.6) cooling by ice (Consdan and Stanier, 1952). The position of sugar components was detected by spraying ammoniacal silver nitrate, and the mobility was compared with the authentic samples co-developed.

RESULTS

Sugar components of cricket eggs

As a preliminary attempt, carbohydrates were sequentially extracted with 80% ethanol, cold TCA and hot alkalin from the newly laid eggs of crickets (Table 1). As shown in Table 1, total sugar content was approximately 22 mg per 800 eggs (about 500 mg), and the main component was extracted with hot KOH. The percentage of each component corresponded closely to that of Locusta eggs (Yamasaki, 1973).

To determine the components of polysaccharide extracted with hot alkalin in the cricket eggs, the hydrolysate was analyzed by means of paper chromatography and paper electrophoresis (Table 2). Both chromatograms exhibited a single spot irrespective of developing systems used, and Rg value of sample similar to that of mannose. It is conceivable that the sugar component is mannose.

Changes in carbohydrate content during ovarian development

The accumulation profile of polysaccharide and glycerol was followed during ovarian development from emergence to 30-day of adults (Fig. 1).

| Table 1. Total Sugar Content of Different Fractions from the Eggs of Emma Field Cricket |
|-----------------------------------------------|---|---|
| **Fraction**                  | **Total sugar** (mg/800 eggs) | **Percentage** |
| Ethanol                        | 0.5                          | 2.3            |
| Cold TCA                       | 0.1                          | 0.5            |
| Hot KOH                        | 20.9                         | 97.2           |

* Eggs within 24 hr after oviposition were used to prepare the fractions.  
  b Total sugar content was shown as D-glucose equivalents.

| Table 2. Analyses of the Hydrolysate of the Hot KOH Fraction |
|-------------------------------------------------------------|---|---|
| **Fraction or Sugar sample**                               | **Paper chromatography** | **Paper electrophoresis** |
| Hydrolysate of Hot KOH fraction                             | Solvent^a | I  | II  |
| D-Glucose                                                   | 121^b     | 114 | 72  |
| D-Mannose                                                   | 100       | 100 | 100 |
| L-Sorbose                                                   | 117       | 114 | 72  |
|                                                             | 104       | 104 | 94  |

* The solvent systems used were: solvent I, butanol/acetic acid/water (4:1:2, v/v); solvent II, butanol/ethanol/water (4:1:2, v/v).  
  b The values were mobilities relative to D-glucose.
As shown in Fig. 1, polysaccharide content was exceedingly low until the 3rd-day after emergence, and began to rise sharply to reach the maximum level in 10-day old with a slight decrease thereafter. Glycerol content continued to increase steeply by the ovarian maturation. In addition, although not shown in Fig. 1, the content of free sugar and glycogen was found at a low level below 1 mg per 500 mg of ovaries, and remained constant throughout the ovarian development.

Changes in carbohydrate content during embryonic development and diapause

In order to bring the cricket eggs to onset of diapause, they were incubated at 25°C for 2 weeks after oviposition. Then, some of the eggs were exposed to 5°C till the 90th-day to break diapause. The others were kept at 25°C till the 60th-day to maintain diapause. The changes in mannan-like polysaccharide and glycerol contents were measured during embryonic development and diapause. As shown in Fig. 2, the content of polysaccharide and glycerol reduced rapidly during early embryonic development. That is, polysaccharide content per 800 eggs

Fig. 1. Changes in polysaccharide and glycerol content during ovarian development in Emma field cricket. The adult ovaries were dissected from emergence to 30th-day old. The content was expressed in terms of mg per 500 mg ovaries.

Fig. 2. Changes in polysaccharide and glycerol content during early embryonic development and diapause. The eggs were kept at 25°C for the first 14 days, and then one group of those diapause eggs was kept at 25°C to the 60th-day. The other group was exposed to 5°C to the 90th-day. The content was expressed in terms of mg per 800 eggs (The same applies to Fig. 3).
(about 500 mg) decreased from 21 to 9 mg during the first 14 days, and glycerol from 14 to 9 mg after a slight increase in the early stage. Both levels, however, were kept at a constant level with slight fluctuation throughout diapause. Further, there were no appreciable differences between the chilled and diapausing eggs.

After being exposed at 5°C for 66 or 90 days, eggs resumed embryonic development by incubation at 25°C. The eggs required 13 days to head pigmentation and 20 days to hatch at 25°C. But some eggs showed a delayed embryogenesis and reached head pigmentation stage several days later. Thus, the eggs of which head pigmentation occurred in 13 days were collected and used for the determination of polysaccharide and glycerol during the late embryonic development (Fig. 3). As indicated on Fig. 3, the content of polysaccharide and glycerol in the eggs exposed to 5°C for 66 days did not notably change during the first 10 days of resumed embryonic development. Subsequently these contents abruptly dropped after head pigmentation stage. Those in the eggs exposed to 5°C for 90 days reduced gradually throughout embryonic development. This difference between the two groups of eggs might be due to the time for breaking diapause by cold exposure. In other words, polysaccharide and glycerol decreased from 11 or 12 to a few mg and from 7 or 10 mg to a trace per 800 eggs during the late embryonic development, respectively.

On the other hand, free sugar and glycogen were in very slight amounts during early embryonic development and diapause, and increased more or less after head pigmentation stage. Specifically, free sugar below 1 mg in the young eggs reached 2.3 or 3 mg just before hatching. The increased free sugars in 18-day old eggs were composed of trehalose, mannose and unknown substance which were analysed qualitatively by paper chromatography (unpublished data).

DISCUSSION

SALT (1961) has proposed that freezing-tolerance in most insects must be related
to the appearance of glycerol or other polyols. But the inadequate information made him venture that the relationship between diapause and glycerol appears to be a coincidence of concurrent timing in general. For example, high glycerol content in mid-winter corresponds to the low supercooling point in overwintering aphid eggs (Sömme, 1969). In addition, the interconversion between glycogen and polyols (Chino, 1957, 1958) or glycogen and sorbitol (Yaginuma and Yamashita, 1977) appeared to be a coincidence of concurrent timing at initiation and termination of diapause in Bombyx eggs.

In the case of the Emma field cricket, the ovaries have already accumulated glycerol during the course of the development and the newly laid eggs contained a high concentration of glycerol. Further, it is of interest that glycerol is restrictively utilized only when the resumed embryonic development proceeds but remains at unchanged levels throughout diapause period. This phenomenon is unique in this insect because much fluctuation in glycerol concentration is observed in many insects according to diapause phenomena. At present the physiological roles of glycerol remain uncertain in the cricket, but it might contribute in part to embryogenesis rather than cold tolerance.

The presence of mannan-like polysaccharide has so far been reported in eggs of the grasshopper Locusta migratoria (Yamasaki, 1973, 1974) and in ovaries of the cockroach Leucophaea maderae (Dejmál and Brookes, 1972). Mannose not mannan-like polysaccharide has further been reported in eggs of the grasshopper Allocara elliotti (Quickenden, 1970). In the cricket, mannan-like polysaccharide was found in developing ovaries and eggs. It was accumulated during ovarian development and decreased throughout early and late embryonic development. Mannan-like polysaccharide is then, the principal carbohydrate in Orthopteran eggs.

The change in the amount of mannan-like polysaccharide of this insect during ovarian and embryonic development periods are comparable to those of glycogen in Bombyx eggs (Chino, 1958; Yamashita and Hasegawa, 1964; Yamashita, 1965). This fact suggests that mannan-like polysaccharide is a main storage form of carbohydrate and is used for energy or materials for embryogenesis. The metabolic pathway of the polysaccharide in relation to glycerol in the cricket eggs remains to be elucidated.

REFERENCES


