Nucleotide Pools in the Eggs of Emma Field Cricket, *Teleogryllus emma*, and Two-Spotted Cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae)¹

Shu IZUMIYAMA¹ and Koichi SUZUKI

*Laboratory of Applied Entomology, Faculty of Agriculture,
Iwate University, Morioka 020, Japan*

(Received December 4, 1985)

Oxygen consumption in the eggs of two-spotted cricket, *Gryllus bimaculatus* (non-diapause species) increased gradually with embryonic development, but in the eggs of emma field cricket, *Teleogryllus emma* (diapause species) it decreased after diapause initiation. The contents of ATP, GTP, UTP and CTP in the eggs of two-spotted cricket increased throughout embryonic development. On the other hand, the contents of four triphosphates in the eggs of emma field cricket increased during the period of pre-diapause, but decreased after diapause initiation. In particular, the decrease of UTP and CTP was conspicuous at diapause initiation. The contents of UTP and CTP also increased at early stages of diapause development and embryonic development. UTP and CTP in the eggs of emma field cricket might play an important role in the regulation of early embryonic development, intervening diapause.

INTRODUCTION

The emma field cricket, *Teleogryllus emma* undergoes an embryonic diapause and produces one generation per year in the temperate latitude. Mannan-like polysaccharide and glycerol are contained abundantly in the eggs. These contents do not change throughout the diapause period, but diminish in parallel with embryonic development in the post-diapause period (IRIE et al., 1979). On the other hand in the cricket, *Gryllus bimaculatus* (non-diapause species) found in tropical and sub-tropical latitudes, glycerol is contained in the ovaries and eggs at almost the same level as in the emma field cricket (IZUMIYAMA et al., 1983). Thus, both crickets have the same carbohydrates in the eggs irrespective of diapause.

The authors have aimed to elucidate chemo-physiological events characteristic of egg diapause. The present study determined changes of oxygen consumption and 5'-ribonucleotide pools throughout diapause and embryonic development in the eggs of emma field cricket, and compared them with those in the eggs of two-spotted cricket.

MATERIALS AND METHODS

Insect. The eggs of emma field cricket (*Teleogryllus emma*) absorb water from 5 to

¹ This study is the 4th in the series of "Study on Comparative Physiology of Eggs in Diapause Insects."

² Present address: *Kaken Pharmaceutical Co., Ltd., Bunkyo-ku, Tokyo 113, Japan*

10 days after oviposition and immediately enter an embryonic diapause. When diapausing eggs are chilled at 5°C for a few months and thereafter incubated at 25°C, they restart embryonic development and achieve the head-pigmentation stage about 12 days after incubation. Only the eggs which achieved the head-pigmentation stage were selected to arrange an indefinite embryonic development and they hatched about 20 days after oviposition. The eggs of two-spotted cricket (*Gryllus bimaculatus*) absorb water from 4 to 8 days after oviposition and continue to advance embryonic development. The head-pigmentation and hatching stages are 8- or 9-day-old and 14- or 15-day-old after oviposition, respectively. Eggs of both species were oviposited into moist sand and collected within 24 hr as described in the previous papers (IRIE et al., 1979; IZUMIYAMA et al., 1983). Eggs in each stage were used for the following analyses.

Oxygen consumption. The quantity of oxygen consumption in the cricket eggs were measured by O₂ up tester (Taiyo Scientific Industrial Co., Ltd.). The manometer was composed of capillary tube (1.04 m/m × 28 cm), vessel and holder. One thermometer and five manometers were incubated within water circulating at 25°C. The results were determined directly as ul O₂ of 200 eggs at each stage.

Assay of nucleotide pools. One thousand cricket eggs (about 0.55–1.1 g) were put in the mortar which was chilled with liquid nitrogen. The extraction was in accordance with the methods described in the previous paper (SUZUKI and MIYA, 1983). The identification and measurement of each 5'-ribonucleotide was determined by high pressure liquid chromatography using methods described previously (SUZUKI and MIYA, 1983). All standard nucleotides (AMP, ADP, ATP, GMP, GDP, GTP, UMP, UDP, UTP, CMP, CDP, CTP, IMP) were purchased from Boehringer Mannheim Coop.

RESULTS

Oxygen consumption in the cricket eggs

In the eggs of two-spotted cricket (non-diapause species), oxygen consumption increased gradually with the advancement of embryonic development (Fig. 1). On the other hand, oxygen consumption in the eggs of emma field cricket increased slowly for the first 7 days after oviposition, but thereafter decreased until 10-day-old age. Throughout diapause, oxygen consumption remained at a low level in the eggs of emma field cricket. These results show that a physiological change at diapause initiation is reflected upon oxygen consumption of the eggs of emma field cricket.

Nucleotide pools in the cricket eggs

First of all, free 5'-ribonucleotides in the cricket eggs were identified and their contents were determined. Figure 2 shows changes in ATP, GTP, UTP, and CTP concentrations in the eggs of emma field cricket until 20 days after oviposition. As triphosphate contents were confined to a different order, the indication of changing pattern in ratio of triphosphate contents at each stage for those within 24 hr after oviposition was adopted to simplify the results. As a result, four triphosphates increased until 6 days after oviposition, but thereafter continued to decrease until 20 days. The increase of UTP and CTP contents was about 5 times and that of GTP content was 3.2 times as large as in the first level. For ATP concentration, the maximum increase was 1.7 times as large as in the first level, but this real concentration was 3 times as high as the UTP maximum. These results show that the conversion of increase and decrease

Fig. 1. Changes in oxygen consumption of the eggs of two-spotted cricket (*Gryllus bimaculatus*) and emma field cricket (*Teleogryllus emma*). Eggs of the two species were incubated at 25°C after oviposition. The eggs of two-spotted cricket reached a head-pigmentation stage at 14 or 15 days after oviposition. The eggs of emma field cricket continued to maintain diapause. Values are the means ($\pm$ S.D.) of five replications.

Fig. 2. Changes of ATP, GTP, UTP and CTP throughout the period of diapause initiation in the eggs of emma field cricket. Eggs were incubated at 25°C for 20 days after oviposition. The concentrations of nucleotide triphosphates were indicated as the ratio of each value for it in the eggs within 24 hr after oviposition (ATP, 0.052 ± 0.009 μ moles/1,000 eggs; GTP, 0.009 ± 0.002 μ moles/1,000 eggs; UTP, 0.015 ± 0.002 μ moles/1,000 eggs; CTP, 0.009 ± 0.001 μ moles/1,000 eggs).

Fig. 3. Changes of ATP, GTP, UTP and CTP throughout the period of post-diapause in the eggs of emma field cricket. Eggs were incubated at 25°C after chilling at 5°C for 110 days. The concentrations of nucleotide triphosphates were indicated as the ratio of each value for it in the eggs within 24 hr after oviposition (ATP, 0.424 ± 0.027 μ moles/1,000 eggs; GTP, 0.064 ± 0.010 μ moles/1,000 eggs; UTP, 0.024 ± 0.001 μ moles; CTP, 0.017 ± 0.002 μ moles).

Table 1. Effect of chilling on nucleotide contents in the eggs of emma field cricket

Nucleotide	Nucleotide contents (μ moles/1,000 eggs)		
	20 days at 25°C ^a	10 days at 5°C ^b	80 days at 5°C ^c
IMP	0.341 $\pm$ 0.019 ^d	0.545 $\pm$ 0.040	0.695 $\pm$ 0.025
AMP	0.164 $\pm$ 0.009	0.203 $\pm$ 0.003	0.109 $\pm$ 0.008
ADP	0.124 $\pm$ 0.013	0.140 $\pm$ 0.001	0.119 $\pm$ 0.011
ATP	0.148 $\pm$ 0.006	0.295 $\pm$ 0.022	0.355 $\pm$ 0.014
GMP	0.043 $\pm$ 0.002	0.032 $\pm$ 0.003	0.027 $\pm$ 0.001
GDP	Trace	Trace	Trace
GTP	0.019 $\pm$ 0.001	0.039 $\pm$ 0.001	0.052 $\pm$ 0.004
CMP	Trace	Trace	Trace
CDP	0.006 $\pm$ 0.001	0.008 $\pm$ 0.002	0.007 $\pm$ 0.001
CTP	0.009 $\pm$ 0.001	0.031 $\pm$ 0.003	0.023 $\pm$ 0.003
UMP	Trace	Trace	Trace
UDP	0.009 $\pm$ 0.001	0.008 $\pm$ 0.001	0.006 $\pm$ 0.002
UTP	0.020 $\pm$ 0.001	0.041 $\pm$ 0.001	0.032 $\pm$ 0.001

^a Diapausing eggs incubated for 20 days at 25°C after oviposition.^{b,c} Eggs were incubated for 15 days at 25°C after oviposition, and the following chilling at 5°C was continued for 10 or 80 days.^d Values are the means ($\pm$ S.D.) of four replications.

Table 2. Comparison of nucleotide pools in the eggs of emma field cricket, two-spotted cricket, and silkworm

Nucleotide	Nucleotide contents (μ moles/g eggs) ^a		
	Emma field cricket ^b	Two-spotted cricket ^b	Silkworm (<i>Bombyx mori</i>) ^c
IMP	0.045 $\pm$ 0.003 ^b	0.058 $\pm$ 0.007	0.349 $\pm$ 0.031
AMP	0.034 $\pm$ 0.003	0.855 $\pm$ 0.076	0.076 $\pm$ 0.002
ADP	0.103 $\pm$ 0.002	0.073 $\pm$ 0.018	0.267 $\pm$ 0.032
ATP	0.239 $\pm$ 0.003	0.095 $\pm$ 0.016	2.765 $\pm$ 0.103
GMP	Trace	0.089 $\pm$ 0.009	Trace
GDP	Trace	0.055 $\pm$ 0.011	0.285 $\pm$ 0.006
GTP	0.015 $\pm$ 0.002	0.016 $\pm$ 0.004	2.136 $\pm$ 0.097
CMP	Trace	Trace	Trace
CDP	Trace	Trace	Trace
CTP	0.012 $\pm$ 0.002	0.016 $\pm$ 0.002	0.147 $\pm$ 0.008
UMP	Trace	Trace	Trace
UDP	Trace	0.009 $\pm$ 0.002	0.031 $\pm$ 0.001
UTP	0.026 $\pm$ 0.000	0.027 $\pm$ 0.004	0.359 $\pm$ 0.009

^a The unit of nucleotide contents in the cricket eggs was converted to μ moles per g eggs.^b The age of eggs used was within 24 hr after oviposition.^c Data in non-diapause eggs cited from the results of SUZUKI and MIYA (1983). The age of eggs was 24-hr-old after oviposition.^d Values are the means ($\pm$ S.D.) of four replications.

in four triphosphate contents converges on the stage of water absorption and diapause initiation (MASAKI, 1960; IRIE et al., 1979). In addition, Table 1 shows changes of each nucleotide content in diapausing eggs of emma field cricket during chilling at 5°C. Triphosphate contents changed prominently during chilling at 5°C. Especially, eggs

chilled for 10 days achieved the increase of CTP and UTP faster than ATP and GTP.

Next, changes of four triphosphate contents were investigated throughout embryonic development after chilling for 110 days. This long period was sufficient for diapause breakdown in the eggs of emma field cricket. Thus, Fig. 3 shows changes of four triphosphate contents throughout embryonic development at 25°C. Pyrimidine triphosphates (CTP and UTP) at 3 days after incubation increased 2.2–2.8 times as large as in the first level. These contents increased abruptly after 6 days and attained $0.214 \pm 0.003 \mu$ moles in UTP and $0.107 \pm 0.003 \mu$ moles in CTP per 1,000 eggs at 9-day-old age. Purine triphosphates (ATP and GTP) contents decreased to 50% during incubation, but increased slowly to 12-day-old age (head-pigmentation stage) and did abruptly after this stage (not shown as data). The contents of ATP and GTP at 9-day-old age were $0.491 \pm 0.010 \mu$ moles and $0.082 \pm 0.004 \mu$ moles per 1,000 eggs. These results show that the increase of pyrimidine triphosphate contents during embryonic development is conspicuously faster than purine triphosphates.

Although not shown as figure, changes of triphosphate contents in the eggs of two-spotted cricket were also investigated throughout embryonic development. As a result, four triphosphates increased with the advancement of embryonic development. In 6 days after oviposition, the content of pyrimidine types was over 10 times as large as in the first level. The same phenomenon in purine types occurred 12 days after oviposition.

Comparison of nucleotide pools in the eggs of cricket

Nucleotide pools in the eggs of cricket were compared with the data in silkworm eggs (SUZUKI and MIYA, 1983) (Table 2). As a result, although AMP content in the eggs of two-spotted cricket was exceptional, nucleotide contents at early stage of embryogenesis in cricket eggs were much lower than those in silkworm eggs. In addition, the contents of triphosphate types in silkworm eggs have shown the decreasing pattern (SUZUKI and MIYA, 1983), but those in cricket eggs vice versa showed the increasing pattern.

DISCUSSION

On the study of egg diapause, the previous report elucidated that the emma field cricket contains abundantly glycerol in the ovaries and eggs irrespective of diapausing or developing stage (IRIE et al., 1979). On the other hand, glycerol is contained in adult haemolymph, adult ovaries, and eggs in two-spotted cricket of non-diapause species (IZUMIYAMA et al., 1983). Polyol in these insects might have a role as an energy source rather than as a freezing-protectant or a diapause metabolite. The results of carbohydrates described above do not give us a significant information for the explanation of egg diapause.

Whereas in the silkworm, nucleotide pools of diapause eggs have been compared with those of non-diapause eggs (SUZUKI and MIYA, 1983). At the initiation period of diapause, pyrimidine nucleotide triphosphates decrease prominently. It has not been yet obvious why the decrease of UTP and CTP occurs, but these inhibit phosphorylase kinase activity in silkworm eggs (unpublished data). In addition, the important changes of alanine, glutamine, and proline contents have been found in silkworm eggs throughout each period of initiation, maintenance and breakdown of egg diapause (SUZUKI et al.,

1984). These new information would explain a diapause mechanism in silkworm eggs.

In the emma field cricket, oxygen consumption decreased after diapause initiation and remained at a low level throughout diapause (Fig. 1). This oxygen consumption might be reflected upon the change of ATP content throughout diapause initiation (Fig. 2). In addition, an interesting change of triphosphate type in 5'-ribonucleotides occurred in relation to egg diapause and embryogenesis. That is, triphosphate contents of pyrimidine nucleotides decreased conspicuously throughout the period of diapause initiation (Fig. 2) and increased progressively throughout diapause development (Table 1) and embryonic development (Fig. 3). Whereas in diapausing eggs of silkworm, irrespective of a different insect order, the decrease of triphosphate contents in pyrimidine nucleotides occurs at diapause initiation (SUZUKI and MIYA, 1983). These results suggest that the concentration of UTP and CTP might regulate the advancement of early embryogenesis.

Furthermore in comparing cricket eggs with silkworm eggs, one important phenomenon was found in the increase and decrease of triphosphate contents. Cricket eggs showed the increase of contents paralleling with the advancement of embryogenesis (Table 2, Fig. 3, and unpublished data). However, triphosphate contents in silkworm eggs vice versa continue to decrease throughout embryogenesis (SUZUKI and MIYA, 1983). UTP content in the eggs of dipteran fly also decreases with the advancement of embryogenesis (FAUSTO-STERLING et al., 1974; SCHRANKEL and SCHWALM, 1981). These suggest that the change of triphosphate contents throughout embryogenesis might be an affair related to insect phylogenesis.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. K. B. STOREY of Department of Biology, Carleton University, Canada for advice and critical reading of the manuscript.

REFERENCES

- IRIE, K., K. SUZUKI and K. MIYA (1979) Changes of carbohydrate content during ovarian and embryonic development in emma field cricket, *Teleogryllus emma*. *Appl. Ent. Zool.* **14**: 278-284.
- IZUMIYAMA, S., K. SUZUKI and K. MIYA (1983) Glycerol in the eggs of the two-spotted cricket, *Gryllus bimaculatus* DE GEER. *Appl. Ent. Zool.* **18**: 295-300.
- FAUSTO-STERLING, A., L. M. ZHEUTLIN and P. R. BROWN (1974) Rates of RNA synthesis during early embryogenesis in *Drosophila melanogaster*. *Dev. Biol.* **40**: 78-83.
- MASAKI, S. (1960) Thermal relations of diapause in the eggs of certain crickets (Orthoptera: Gryllidae). *Bull. Fac. Agric. Hirosaki Univ.* **6**: 5-20.
- SCHRANKEL, K. R. and F. E. SCHWALM (1981) Quantification of ribonucleotides and uridine metabolism in ovaries of the kelp fly (*Coelopa frigida*, Diptera) during final stages of oogenesis. *Insect Biochem.* **11**: 117-120.
- SUZUKI, K. and K. MIYA (1983) Nucleotide pools related to embryogenesis and diapause in eggs of the silkworm, *Bombyx mori*. *J. Seric. Sci. Jpn.* **52**: 13-21.
- SUZUKI, K., M. HOSAKA and K. MIYA (1984) The amino acid pool of *Bombyx mori* eggs during diapause. *Insect Biochem.* **14**: 557-561.