Changes of Free Amino Acid Pool in the Eggs of the Emma Field Cricket, *Teleogryllus emma* (Orthoptera: Gryllidae)¹

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The amount of any free amino acid in the eggs of the emma field cricket, *Teleogryllus emma*, was less than 1 μmol per 1,000 eggs maximum during the first 2 days after oviposition, but subsequently increased rapidly through the pre-diapause period and initial stage of diapause. Alanine concentration in eggs 6- and 10-days old was 7.8 and 30.1 μmol per 1,000 eggs, respectively. Chilling to induce diapause development resulted in a notable 3.6-fold increase in glutamine, which reached 10.3 μmol per 1,000 eggs during a 40 day period after 20 days of chilling. In addition, asparagine, AMP and glutamine partially broke diapause when 5-day-old eggs were treated with solutions of these amino acids.

INTRODUCTION

The emma field cricket, *Teleogryllus emma*, shows an obligated type of embryonic diapause: it passes one generation per year in a temperate latitude. Mannan-like polysaccharide and glycerol are abundant in newly-laid eggs. These contents do not change during the diapause period, but decrease in parallel with embryonic development in the post-diapause period (IRIE et al., 1979). The contents of ATP, GTP, UTP and CTP increase during pre-diapause but decrease at the initial stage of diapause concurrent with the decrease of oxygen consumption, the decrease of UTP and CTP being particularly conspicuous. The contents of UTP and CTP increase again at the early stages of diapause and embryonic development (IZUMIYAMA and SUZUKI, 1986).

The metabolism of carbohydrates and nucleotides is thought to be associated with that of amino acids. In the eggs of the cricket *Teleogryllus commodus*, glutamine shows the most prominent change among the free amino acids at diapause termination (MACFARLANE and HOGAN, 1966). Egg diapause of this insect is terminated by exogenous ammonia (HOGAN, 1964).

The present study was conducted to analyze the free amino acid pool and the effects of amino acids and nucleotides in relation to diapause breakdown in the eggs of the emma field cricket.

¹ This study is the 5th in the series of "Study on Comparative Physiology of Eggs in Diapause Insects."

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MATERIALS AND METHODS

Insect. The eggs of the emma field cricket absorb water during the 5 to 10 days after oviposition and thereafter immediately enter diapause. When diapausing eggs are chilled at 5°C for a few months and subsequently incubated at 25°C, they renew embryonic development and achieve an eye-spot formation stage about 12 days after being placed in incubation. The period from eye-spot formation to hatching stage is 7 or 8 days. Eggs used in the experiment were obtained from crickets collected in fields in autumn and kept under laboratory conditions. The method of egg preparation for the experimental analyses was described previously (IRIE et al., 1979; IZUMIYAMA and SUZUKI, 1986).

Analysis of free amino acids. The analysis of free amino acids was based upon a modification of the method employed for eggs of the silkworm, *Bombyx mori* (SUZUKI et al., 1984). Fifty eggs in a definite stage were homogenized in 0.5 ml of cold 2.73% sulphosalicylic acid by a glass homogenizer. Homogenate was centrifuged at 10,000 *g* for 15 min and the supernatant was adjusted to near pH 2.2 by 1 *N* LiOH. The preparation was centrifuged at 10,000 *g* for 10 min and 100 μ l of the clear supernatant was subjected to analysis by a Dionex D-500 amino acid analyzer (Dionex Corp., California, U. S. A.).

Application of amino acids and nucleotides. One ml of aqueous solution of various amino acids and nucleotides was poured onto 200 of the 5-day-old eggs on a filter paper in a petri dish. The eggs were kept at 25°C and judged for diapause termination by checking eye-spot formation after 30 and 50 days of incubation.

RESULTS

Changes in the pools of free amino acids during pre-diapause, diapause and embryonic development

From emma field cricket eggs chilled for 100 days after 20 days of oviposition, twenty free amino acids were identified: aspartic acid, threonine, serine, asparagine, glutamic acid, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, β -alanine, lysine, histidine, arginine, and ornithine. Phosphoethanolamine, tryptophan and ethanolamine could not be identified by the present method. Within 24 hr after oviposition, the amount of any free amino acid was less than 1 μ mol per 1,000 eggs at 25°C, but by the 6th day almost all showed a substantial increase. Changes in content of respective amino acids are shown in Fig. 1. Alanine showed the most prominent increase: the concentration was 7.8 μ mol on the 6th day and reached 30.1 μ mol by the 10th day when the eggs entered diapause.

Twenty-day-old eggs were transferred to 5°C and the changes in concentration of the respective amino acids during chilling were compared (Fig. 1 and Table 1). The amounts of all free amino acids decreased during the first 20 days of chilling (data not shown). Most of the amino acids increased in concentration after the 20th day of chilling and attained a plateau level 40 days later. The greatest increase was 3.6-fold in glutamine during 40 days, while that of other amino acids was less than 1.8-fold (1.3 for alanine, 1.7 for glutamic acid, 1.7 for proline and 1.8 for arginine). However, after 60 days of chilling alanine content decreased slightly from what it had been at 40 days.

Fig. 1. Changes of alanine, glutamic acid, glutamine, aspartic acid and asparagine during 20 days of 25°C incubation following oviposition (pre-diapause and diapause), during 100 days of 5°C chilling (diapause development) and then during 25°C incubation (post-diapause) in the eggs of emma field cricket. Δ , alanine (ALA); $\square$, glutamic acid (GLU); $\blacksquare$, glutamine (GLN); $\diamond$, aspartic acid (ASP); $\bullet$, asparagine (ASN). Values are the means ($\pm$ S.D.) of four replications.

Table 1. Changes of some free amino acids in the eggs of the emma field cricket during the period of chilling

Amino acid	Amino acid contents ($\mu\text{mol}/1,000$ eggs)				
	20	40	Days of chilling ^a 60	80	100
Threonine	8.63 ± 1.09^b	11.95 ± 1.83	14.37 ± 1.27	13.93 ± 0.51	14.11 ± 0.33
Serine	17.15 ± 2.36	23.10 ± 3.99	28.46 ± 2.38	24.66 ± 1.29	27.03 ± 1.08
Proline	10.46 ± 1.09	16.05 ± 2.15	18.05 ± 1.76	19.71 ± 0.94	20.18 ± 0.46
Glycine	5.04 ± 0.55	6.90 ± 1.04	8.54 ± 0.53	8.51 ± 0.33	8.62 ± 0.25
Valine	11.27 ± 1.29	15.89 ± 2.38	18.76 ± 1.44	18.81 ± 0.87	19.45 ± 0.59
Methionine	3.49 ± 0.41	4.97 ± 0.72	5.76 ± 0.49	5.21 ± 0.27	5.28 ± 0.20
Isoleucine	8.88 ± 1.12	12.81 ± 1.98	15.18 ± 1.31	14.17 ± 0.63	14.48 ± 0.41
Leucine	15.10 ± 1.92	21.87 ± 3.39	26.28 ± 2.32	24.02 ± 1.11	24.45 ± 0.68
Tyrosine	10.02 ± 1.30	14.81 ± 2.43	18.34 ± 1.78	16.76 ± 0.89	16.42 ± 0.63
Phenylalanine	5.51 ± 0.71	7.93 ± 1.10	8.96 ± 0.94	8.38 ± 0.51	8.71 ± 0.38
β -Alanine	0.51 ± 0.05	0.69 ± 0.14	0.83 ± 0.11	0.76 ± 0.13	0.78 ± 0.09
Lysine	12.95 ± 1.77	17.87 ± 2.70	20.99 ± 2.05	19.60 ± 0.80	19.88 ± 0.62
Histidine	2.88 ± 0.45	3.94 ± 0.73	4.96 ± 0.44	4.68 ± 0.22	4.67 ± 0.17
Arginine	10.61 ± 1.60	15.34 ± 2.54	18.86 ± 1.86	17.88 ± 0.80	17.89 ± 0.76

^a Chilling at 5°C was started 20 days after oviposition.

^b Values are the means ($\pm$ S.D.) of four replications.

Table 2. Effects of amino acids and nucleotides on diapause breakdown in the emma field cricket^{a,b}

Agent	Concentration ($\mu\text{mol/ml}$)	Percentage of diapause-terminated eggs ^{c,d}	
		30-day	50-day
Control (distilled water)		0.0	2.4 $\pm$ 2.4
Glutamine	100	6.5 $\pm$ 6.5	17.4 $\pm$ 12.2
Glutamic acid	100	0.5 $\pm$ 0.6	0.5 $\pm$ 0.6
Asparagine	50	15.5 $\pm$ 0.4	49.2 $\pm$ 9.7
Aspartic acid	25	0.0	2.8 $\pm$ 0.9
Alanine	100	2.0 $\pm$ 2.4	3.8 $\pm$ 4.6
AMP	40	17.2 $\pm$ 13.5	34.2 $\pm$ 20.8
CTP	40	0.0	2.8 $\pm$ 2.3
UTP	40	0.0	4.8 $\pm$ 2.1
Urea	100	1.7 $\pm$ 1.3	6.5 $\pm$ 3.1

^a Two hundred 5-day-old eggs were treated with 1 ml solution of each agent.^b Values are the means ($\pm$ S.D.) of four replications.^c Ratio of the eggs which developed to eye-spot formation stage.^d Dead eggs were excluded from the data.

Table 3. Comparison of amino acid pools in eggs of the emma field cricket and silkworm

Amino acid	Amino acid content ($\mu\text{mol/g}$ eggs)	
	Emma field cricket ^a	Silkworm (<i>Bombyx mori</i>) ^b
Aspartic acid	0.38 $\pm$ 0.04 ^c	2.51 $\pm$ 0.57 ^c
Threonine	0.11 $\pm$ 0.04	0.74 $\pm$ 0.16
Serine	0.51 $\pm$ 0.11	1.40 $\pm$ 0.32
Asparagine	0.56 $\pm$ 0.05	0.35 $\pm$ 0.07
Glutamic acid	1.44 $\pm$ 0.71	7.34 $\pm$ 1.40
Glutamine	0.42 $\pm$ 0.07	0.43 $\pm$ 0.11
Proline	—	0.30 $\pm$ 0.07
Glycine	0.47 $\pm$ 0.13	1.78 $\pm$ 0.40
Alanine	0.56 $\pm$ 0.05	2.34 $\pm$ 0.54
Valine	—	0.51 $\pm$ 0.12
Methionine	0.15 $\pm$ 0.07	0.26 $\pm$ 0.06
Isoleucine	0.07 $\pm$ 0.07	0.27 $\pm$ 0.07
Leucine	0.24 $\pm$ 0.09	0.34 $\pm$ 0.09
Tyrosine	0.22 $\pm$ 0.22	0.79 $\pm$ 0.20
Phenylalanine	—	0.09 $\pm$ 0.03
β -Alanine	0.22 $\pm$ 0.22	—
Lysine	0.25 $\pm$ 0.05	1.96 $\pm$ 0.43
Histidine	0.20 $\pm$ 0.05	2.34 $\pm$ 0.47
Arginine	0.91 $\pm$ 0.11	3.83 $\pm$ 0.80

^a Age of the eggs used was within 24 hr after oviposition.^b Data from SUZUKI et al. (1984). The age of diapause eggs was 6-hr after oviposition.^c Values are the means ($\pm$ S.D.) of four replications.

After chilling for 80 days the eggs were incubated at 25°C to initiate post-diapause development. Under this condition, as shown in Fig. 1, levels of many amino acids continued to decrease during embryonic development. Among them, alanine showed

a dramatic change, decreasing to half during 4 days of incubation, followed by a rapid increase to the initial level during the next 6 days, and then prominently decreasing again during late embryonic development.

Effects of some chemicals on diapause termination of the cricket eggs

When 5-day-old eggs were treated with asparagine, AMP or glutamine, a considerable fraction of them began embryonic development, as judged by eye-spot formation within 50 days of the treatment (Table 2). Dose-dependency of diapause termination for these agents could not be determined because of the higher proportion of egg deaths with the increase of concentration.

Comparison of free amino acid pools in cricket and silkworm eggs

As given in Table 3, the concentrations of several free amino acids greatly differed between the cricket and silkworm eggs when compared at early embryonic stage. The levels of aspartic acid, threonine, serine, glutamic acid, glycine, alanine, lysine, histidine, and arginine in the silkworm eggs were 3 to 12 times greater than those detected in the cricket eggs.

DISCUSSION

Changes in free amino acid pools during diapause and embryonic development were demonstrated earlier in the cricket, *T. commodus* (MACFARLANE and HOGAN, 1966) and the silkworm, *Bombyx mori* (SUZUKI et al., 1984; OSANAI and YONEZAWA, 1986). The increase of free amino acids in *T. commodus* during early embryonic development may reflect the breakdown of chorion cuticle by proteolytic enzyme (MACFARLANE and HOGAN, 1966). An essentially similar pattern of changes in free amino acids was observed in the eggs of *T. emma* in the present study. Most free amino acids increased rapidly during the pre-diapause period and initial stage of diapause and gradually during diapause development, while they decreased during post-diapause (Fig. 1 and Table 1). This suggests that a common metabolic pathway exists in the amino acid pool of the eggs of both crickets, and the prominent increase of most amino acids during early embryo also in *T. emma* may be explained by a possible chorion breakdown. These profiles differ from those of silkworm in which the levels of glutamic acid and arginine remain almost constant and others show various fluctuations during embryonic life.

Alanine is the most abundant amino acid all three species. In silkworm eggs, alanine is partly converted to proline during diapause development, but no such metabolic relationship between these two amino acids was observed in the cricket eggs of *T. commodus* (MACFARLANE and HOGAN, 1966) or *T. emma* (Fig. 1 and Table 1). In the eggs of the emma field cricket, glutamine displayed the most prominent change during diapause development, its content increasing by 3.6-fold during 60 days of chilling (Fig. 1 and Table 1). In the eggs of *T. commodus*, glutamine showed a similar change during diapause development. The content increased 2.2- or 3.5-fold at the maxima compared to the initial level in diapausing eggs (15 $\mu\text{mol/g}$) treated with cold or ammonia (MACFARLANE and HOGAN, 1966). In *Bombyx*, glutamine remained constant at less than 0.5 $\mu\text{mol/g}$ in non-chilled diapausing eggs, but it increased to 3–

4 $\mu\text{mol/g}$ in eggs during diapause development under chilling or in post-diapause development initiated by hydrochloric acid treatment (SUZUKI et al., 1984; OSANAI and YONEZAWA, 1986). Thus, changes in glutamine metabolism seem closely correlated with diapause development.

Exogenous asparagine, AMP and glutamine were somewhat effective in terminating diapause of the emma field cricket (Table 2), while ammonia was found more effective for diapause termination in *T. commodus* eggs (HOGAN, 1964). Hence experiments with more potent diapause-disrupting chemicals may be helpful in understanding the mechanism of embryonic diapause in the emma field cricket.

Interesting information was also gained by the analysis of the free amino acid pool in the emma field cricket and the silkworm, with the amounts of many free amino acids at the early stage of embryonic development in the cricket being far less than those in the silkworm (Table 3). Similarly, the size of the nucleotide pool in the former species was considerably smaller than in the later (IZUMIYAMA and SUZUKI, 1986). Thus, the concentration of low molecular-weight metabolites as maternal stockpiles is quite different between the two species of insects. The differences in the dynamics of free amino acid and nucleotide pools in eggs might reflect the different status of phylogenesis of the two species.

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