Changes in the Acid-Soluble Nucleotides during
the Pupal Development of the Rice Stem Borer,
Chilo suppressalis WALKER (Lepidoptera : Pyralidae)

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(Received December 23, 1966)

The acid-soluble nucleotides of pupae of the rice stem borer, *Chilo suppressalis*, were fractionated by anion-exchange chromatography. Adenosine diphosphate, ATP, CTP, GDP, GTP, UDP, UTP, and UDP-sugar(s) were isolated and identified from the pupae at all stages. The ATP and ADP contents are almost constant during the early half of the pupal stage, but rapidly increase from the middle stage to adult emergence. The ratio of ATP to ADP tends to decrease through pupal period. UDP-sugar(s) level becomes maximum on the second day after pupation, then decreases conspicuously to adult emergence. The other nucleotide levels fluctuate with pupal development. These changes of the nucleotide level are discussed in relation to some aspects of metamorphosis.

INTRODUCTION

For a biochemical approach to insect metamorphosis, pupae of Lepidoptera have proved to be highly suitable material. They are essentially a closed system except for respiration, and exhibit specific and profound changes in morphology, physiology and biochemistry during metamorphosis.

Some workers have reported on the acid-soluble nucleotides or phosphates of insect tissues (HESLOP and RAY, 1961; WINTERINGHAM et al., 1955, 1960), but relatively little work has been done on the metabolisms of these compounds during metamorphosis (CAREY and WYATT, 1963; WYATT et al., 1959, 1963). The nucleotides play important roles in many areas of metabolism; they contribute to many phosphorylating systems, energy transferring systems, and electron transporting systems, as well as in the biogenesis of nucleic acids. Studies on nucleotide metabolism may prove important, therefore, for a biochemical analysis of insect metamorphosis. This paper deals with changes in the acid-soluble nucleotides during the pupal development of the rice stem borer, *Chilo suppressalis* WALKER.

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MATERIAL AND METHODS

Extraction of the nucleotides. Diapausing mature larvae of *Chilo suppressalis* were collected in a paddy field in Yamagata Prefecture, Japan in late autumn, and stored at 5°C for 50 days for the termination of their diapause. They were then incubated at 25°C, and the resulting pupae removed daily for the analysis of acid-soluble nucleotides. The mean weight of pupae was 49.5 mg (male) and 59.0 mg (female).

The acid soluble nucleotides were extracted from groups of 20 pupae of either sex taken at 24 hour intervals after pupation. Each group of pupae was homogenized with two volumes of 0.3 N perchloric acid in an ice-cold Potter-Elvehjem glass homogenizer, and the homogenate centrifuged at 3,000 rpm for 20 minutes. The residue was re-extracted three times with two volumes of 0.15 N perchloric acid. The combined supernatants were transferred to a centrifuge tube and neutralized with 5 % aqueous KOH, using phenolphthalein as an indicator and left overnight. All these procedures were carried out below 5 °C. The potassium perchlorate precipitate was then removed by centrifugation, and the supernatant fluid was stored at -20°C until column chromatographic analysis.

Ion-exchange column chromatography. The separation of nucleotides followed the method of HURLBERT et al. (1954), with the use of 1 × 15 cm columns of Dowex 1 × 10 formate resin (200 to 400 mesh). The neutralized extract was poured onto a column, and 5 ml of distilled water was allowed to flow through the column to displace non-adsorbed material. A stepwise gradient elution was employed with the eluting solvent ranging from 1 N formic acid to 4 N formic acid - 0.8 M ammonium formate (shown in Fig. 1). The volume of the mixing bottle was 150 ml. Five milliliter fractions were collected and the optical density of each was read in a Hitachi EPU-II type spectrophotometer at 260 and 280 m μ .

Further separation and identification. The material from each peak was pooled and freeze-dried. The residues were dissolved in small volumes of water and spotted on Toyo Roshi No. 51 paper for descending chromatography. The following solvent systems were employed: (1) iso-propanol-HCl (WYATT, 1951), (2) tert-butanol-HCl (SMITH and MARKHAM, 1950), and (3) iso-butyric acid-ammonium hydroxide (MAGASANIK et al., 1950). After paper chromatography, each spot detected in light of wavelength 253.7 m μ was eluted with 0.01 N HCl and the absorption spectra at pH 2, 7 and 12 were determined.

Aliquots of the eluates from the paper chromatograms were used for determining phosphate, ribose and combined sugars. Phosphate was determined by the method of ALLEN (1940), and ribose by the orcinol reaction (MILITZER, 1946). To detect sugar combined with nucleotides, the nucleotide was hydrolyzed in 0.1 N HCl at 100 °C for 10 minutes, and the hydrolyzate chromatographed in one dimension on paper, with a solvent system of phenol-water (4 : 1). Aniline hydrogen phthalate or ammoniacal silver nitrate solution was sprayed for color development.

Quantitation. Quantitation of the compounds isolated by column chromatography was achieved by integrating the areas (read at 260 m μ) under the peak. In case a peak contained two or more compounds, total absorbancy of the peak at 260 m μ was multiplied by the optical ratio of one compound to the other.

This was estimated by obtaining the absorbancy at $260\text{ m}\mu$ of each compounds eluted from a paper chromatogram. These optical density values were converted to micromoles per gram fresh weight using the following millimolar extinction coefficients at $260\text{ m}\mu$: 14.2, 11.8, 9.9, and 6.2 for adenosine, guanosine, uridine, and cytidine nucleotides, respectively.

Fig. 1. Elution curve of nucleotides extracted from male pupae (8 days after pupation) of *Chilo suppressalis* with perchloric acid. The eluting solvent 1 : 1 N formic acid; 2 : 4 N formic acid; 3 : 0.2 M ammonium formate in 4 N formic acid; 4 : 0.4 M ammonium formate in 4 N formic acid; 5 : 0.8 M ammonium formate in 4 N formic acid. (See text for peaks A, B, C.....)

RESULTS

A typical anion-exchange chromatogram of the acid-soluble nucleotides of the pupae (male, 8 days after pupation) is shown in Fig. 1. The chromatograms of extracts from female pupae closely resembled those from male pupae except for the absence of peak B.

The fractions comprising the various peaks were examined and, where possible, identified as indicated below.

Peak A. This contained many compounds not retained by the ion-exchange resin. No further separation was performed.

Peak B. This peak only appeared in the extract of the male pupae, but it was not decided whether this sexual difference is quantitative or qualitative. Paper chromatography of this peak gave a single spot. The material eluted from the spot had absorption maximum at $299\text{ m}\mu$ and absorption minimum at $262\text{ m}\mu$ (pH 2), but could not be identified.

Peaks C, D and E. Owing to the interference of peak E, precise quantitation of these peaks could not be accomplished. Peak C contained xanthine and

peak D contained uric acid riboside. Peak E consists primarily of uric acid with a small amounts of adenosine monophosphate (AMP) at the end shoulder of the peak.

Peak F. Paper chromatography in solvent system (3) resolved two spots, and the ultraviolet absorption spectra and absorption ratios of those spots were identical with those of AMP and adenosine diphosphate (ADP). It was assumed that the AMP was produced from ADP during chromatography.

Peak G. A guanine compound was indicated by the ultraviolet absorption spectra of the material isolated on the paper chromatogram in solvent system (2). Judging from the effluent position of this peak on the ion-exchange chromatogram, it was determined as guanosine diphosphate (GDP).

Peak H. This peak consisted mainly of uridine diphosphate-sugar complex (UDP-sugar). Paper chromatography in solvent systems (2) and (3) gave two spots, which showed ultraviolet absorption spectra typical for uridine nucleotides. Paper chromatography of the acid hydrolyzate of one spot in phenol-water (4:1) indicated the presence of either N-acetylglucosamine or N-acetylgalactosamine (cf. CAREY and WYATT, 1960). This reacted positively with ammoniacal silver nitrate reagent but not with aniline hydrogen phthalate. The second spot corresponded to uridine diphosphate (UDP) which may result from the decomposition of the labile UDP-sugar during the analysis.

Peak I. This peak contained adenosine triphosphate (ATP) together with cytidine triphosphate (CTP) and UDP. Paper chromatography showed three spots, and they were identified as CTP, UDP and ATP by their ultraviolet absorption date and absorption ratios, and by their effluent position on the ion-exchange chromatogram. The percentage composition of each of the three nucleotides was calculated by the absorbancy at $260\text{ m}\mu$ (pH 2) of each nucleotide eluted from the paper chromatogram.

Peak J. Paper chromatography in solvent systems (1) and (2) revealed two spots. By ultraviolet absorption spectra these two compounds were identified as guanosine triphosphate (GTP) and uridine triphosphate (UTP).

Variation in the levels of the major acid-soluble nucleotides during pupal development of the rice stem borer together with data on newly-emerged adults, are seen in Figs. 2 to 5. No significant sexual difference was noted for any of these nucleotides.

ATP and ADP increased during pupal development, particularly toward the end of the pupal stage (Fig. 2). The GDP and UTP content increased in the middle period of the pupal stage and attained their maximum 7 days after pupation (Figs. 4 and 5). Conversely, the GTP and CTP content declined with pupal age (Figs. 4 and 5). The UDP-sugar(s) also decreased during the pupal stage, whereas no change occurred in the UDP level (Fig. 3). As a result, the percentage of adenosine nucleotides (ADP plus ATP) in the total nucleotides increased with pupal age.

In the newly-emerged adult, the content of ADP and ATP reached the highest level after pupation, whereas the levels of all the other nucleotides were lower than in any stage during pupal development. The percentage of adenosine nucleotides reached a maximum about 70% of the total acid-soluble nucleotides detected at the stage.

(Fig. 2)

(Fig. 3)

Fig. 2. The changes of ATP and ADP content during pupal development of *Chilo suppressalis*, micromoles per gram fresh weight. ○ ATP content in female insects, ● ATP content in male insects, ⊙ ADP content in female insects, ⊙ ADP content in male insects.

Fig. 3. The changes of UDP-sugar(s) and UDP content during pupal development of *C. suppressalis*, micromoles per gram fresh weight. ○ UDP-sugar(s) content in female insects, ● UDP-sugar(s) content in male insects, ⊙ UDP content in female insects, ⊙ UDP content in male insects.

(Fig. 4)

(Fig. 5)

Fig. 4. The changes of UTP and GTP content during pupal development of *C. suppressalis*, micromoles per gram fresh weight. ○ UTP content in female insects, ● UTP content in male insects, ⊙ GTP content in female insects, ⊙ GTP content in male insects.

Fig. 5. The changes of GDP and CTP content during pupal development of *C. suppressalis*, micromoles per gram fresh weight. ○ GDP content in female insects, ● GDP content in male insects, ⊙ CTP content in female insects, ⊙ CTP content in male insects.

DISCUSSION

During the early and middle stages of pupal development of the rice stem borer, the levels of ATP and ADP were fairly constant (0.6 to 0.8 μ moles ATP and 0.05 to 0.1 μ moles ADP per gram fresh pupae), but these rapidly increased from 6 days after pupation to adult emergence. This indicates increased activity of oxidative phosphorylation. Finally the ATP and ADP contents reached 1.4 to 1.7 μ moles and 0.25 to 0.29 μ moles, respectively, at the end of pupal development.

In the early pupal stage of insects, histolysis of larval tissues takes place accompanied by low oxidative metabolism, but during the middle to late pupal stages oxidative metabolism increases as adult development begins (AGRELL, 1953; SHAPPIRIO and WILLIAMS, 1957a, 1957b). WYATT (1959) and CAREY and WYATT (1963) have reported that the ATP content and ^{32}P incorporation into ATP in developing adults of *Hyalophora cecropia* increased two to three times as compared with diapausing pupae. We believe that the middle stage of pupal development of the rice stem borer may correspond to the beginning of adult development of *Hyalophora cecropia*.

Since there is a lower level of ATP and higher ratio of ATP to ADP in the early pupal stage as compared with the late stage, histolysis and histogenesis may occur simultaneously. ATP produced from degradation of larval tissues is rapidly utilized for imaginal differentiation resulting in a rapid turnover of ATP. On the other hand, ATP might increase from the middle stage to adult emergence due to increasing cellular respiration via the electron transport system. The tendency toward a lower ratio of ATP to ADP suggests that histolysis terminates at these stages and histogenesis nears completion.

Remarkable changes occurred in the UDP-sugar content (Fig. 3). The UDP-sugar(s) level reaches a maximum 2 days after pupation and gradually decreases thereafter until it is almost absent from the adult. As UDP-glucose could not be detected in this experiment, the UDP-sugar(s) may be UDP-N-acetylglucosamine or UDP-N-acetylgalactosamine. If this is right, the decreasing level of UDP-sugar(s) during the pupal period may be related to the active formation of adult cuticle. In comparison with the UDP-sugar(s), the UTP content is the mirror image while UDP is approximately constant in the course of the pupal stage (Figs. 3 and 4). It is not clear, however, how these uridine nucleotides are related to one another.

CTP and GTP gradually decrease, but GDP increases with the advancement of pupal stage (Figs. 4 and 5). Changes in these compounds may be a reflection of increases in nucleic acid synthesis needed for the synthesis of adult proteins. In addition, the cytidine nucleotides may be involved in phospholipid synthesis.

ACKNOWLEDGEMENTS

We wish to thank Dr. Takeshi YUSHIMA of the National Institute of Agricultural Sciences, Tokyo, Professor G. R. WYATT of Yale University, New Haven, and Professor L. I. GILBERT of Northwestern University, Evanston, for their kindness of reading the manuscript and contributing valuable comments.

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