

On the Free Amino Acids of Different Stages and Sexes of *Dictyoploca japonica*

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밤나무산누에나방의 發生段階와 性別에 따른 遊離아미노酸의 定性的 分析

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摘 要

밤나무산누에나방(*Dictyoploca japonica*)의 遊離아미노酸을 paper chromatography 法을 利用하여 蛹에서 卵까지 定性的으로 分析하였다.

1. 分析 結果 19 種의 遊離아미노酸이 檢出 되었으며 各 stage 는 遊離아미노酸의 定性的 pattern 의 變動을 보였다.

2. Serine 은 全 stage 에서 높은 농도로 檢出 되었다.

3. 遊離아미노酸의 種類數는 나방의 發生과 더불어 增加하고 一般的으로 male 보다 female 에 있어서 그 數가 많았다.

4. Tryptophane 은 各 stage 에서 全然 檢出되지 않았다.

The fact that the concentration of free amino acids in insect is in general much greater than that of the blood in vertebrate was demonstrated by many workers (Levenbook, 1950; Wyatt et al. 1956; Benassi et al., 1961; Kim & Yoo, 1962; Ganti & Shanmugasundaram, 1963; Duffy, 1964; Ludwig & Jones, 1964). It is well known that amino acid plays an important role in development, growth and metamorphosis of insects.

Duffy(1964) reported that the females of *Curex pipiens*, *Molestus forskal* and *Aedes aegypti* have more free amino acid, nitrogen than the males.

Changes in hemolymph free amino acid during developmental stages of holometabolous insects in relation to the metabolic process had also been studied by Florkin (1959).

Chatterji (1962) reported that *Trogoderma granarium* contains 16 free amino acids and Benassi, Colombo & Allegrì (1961) detected 17 free amino acids in the hemolymph of a locust. The authors have already identified 17 amino acids and an unidentified ninhydrin positive spot in pine moth, at different stages of development.

The present work has been undertaken to investigate the variations of free amino acid in males and females during developmental stages of *Dictyoploca japonica*.

MATERIALS and METHODS

The larvae were collected at the Woo-I-Dong near Seoul City and reared at about 26°C. The analyses were made on the early pupa (two days old), late pupa (4 days old), adult (4 days old) and egg (3 days old) stages.

At the desired age the samples were homogenized with 17% ethyl alcohol and centrifuged. The supernatant was taken and evaporated at room temperature and, 10% trichloroacetic acid added to the supernatant, to aid in the precipitation of proteins.

The free amino acids were separated by two dimensional chromatography using the ascending method adopted by

Table 1. Free amino acids of *Dictyoploca japonica* at different stages.

Amino acids	Early pupa		Late pupa		Adult		Egg
	♂	♀	♂	♀	♂	♀	
Aspartic acid		++	+	+	+	++	+
Glutamic acid	++	+++	++	++	+	++	+
Cystine	+++	+++	+++	+++	+++	+++	++
Serine	++++	++++	++++	++++	++++	++++	++++
Glycine	+++	++	++	+	+	++	++
Threonine	++	+++	+++	++	+++	+++	+++
Alanine	+	++		+		+	+
Histidine	+++	++	++	++	++	++	++
Arginine	+++	+++		++	++	++	++
Lysine	+++		+++	+	+++	+	
Glutamine		+++	+++	+++	+	+	
Proline		+++	+	+++	++	++	+
β -alanine	+++	+	+++	+	++	+	+
Tyrosine	+++	+++	+++	+++	+++	+++	++
Citrulline		+	++	++	+	++	+
Valine	+++	+++	+++	+++	+++	+++	+++
Methionine	+		+		+		+
Phenylalanine	+	+++	++	+	++	+	+
Leucine	+++	+++	+++	+++	+++	+++	+++
Total	15	17	17	19	18	19	17

+ : very small, ++ : small, +++ : large, ++++ : very large.

Wyatt (1959) with n-butanol: acetic acid: water are 4 : 1 : 5 and phenol: water are 4 : 1 as the solvent.

Toyoroshi No. 50 filter paper was used for chromatography. Spots were developed with 0.2% ninhydrin solution. On two dimensional chromatograms amino acids were identified by comparing chromatograms of the extracts with those of standard amino acids. The experiments for each stages were repeated over three times to make accuracy.

RESULTS

The free amino acids identified at each stages are given in Table 1.

As shown in Table 1, the numbers of free amino acid at each stages vary widely and some are not detectable in certain stages. In this experiment, approximately, 19 individual free amino acids have been demonstrated.

1. Cystine, one of the sulphur amino acids unidentified in a free state in the pupae of *Corcyra* was identified in the homogenates of *Dictyoploca japonica*.

2. Tryptophane was not appeared in this experiment as well as in pine moth.

3. Aspartic acid was not present on the chromatogram of male extract of early pupal stage.

4. Serine was present in high concentration in all the stages, whereas, cystine, threonine, tyrosine, valine and leucine were present in relatively large amounts.

5. Aspartic acid, glutamine, proline and citrulline were not detected in male homogenates of early pupal stage.

DISCUSSION

The number of amino acids identified in *Dictyoploca japonica* is the highest, comparing with those of other insects, while 11 amino acids were identified in *Schistocerca gregaria* (TREHERNE, 1959), 17 in *Dendrolimus spectabilis* (KIM & YOO, 1962), 13 in *Corcyra cephalonica* (GANTI, 1963) and 16 in *Trigoderma granarium* (CHATTERGI, 1962) respectively. But in *Dictyoploca japonica* 19 free amino acids were identified as the body constituent. Of course, variations of free amino acids depend on species differences of insect and simultaneously, their distributional patterns also vary with the organs and the tissues in the same body.

Benassi et al. (1961) reported that the differences of amino acid might be due to different analytical methods and to the small concentration of some amino acids, however, it may also depend on species differences and on variations

connected with the physiological and environmental conditions of insects.

The presence of aspartic acid, glutamic acid and alanine seems to show the glutamic-alanine and glutamic-aspartic transaminase activity.

Price (1961) had ever demonstrated such transaminase activities in the housefly.

Tryptophane that is used in the development of dark or yellow ommochrome pigments deposited in the eyes of various species of insects was not identified. Its absence is questionable. This problem requires more study.

Proline that is identified commonly in insects was not detected in pupae of pine moth. But on the contrary, it was appeared in those of the present insect. The presence or absence of this amino acid, presumably, is to be related to the species of insect. It, however, is difficult to give any specific interpretations without further study.

Price (1961) have shown that glutamic acid is concentrated in fly brain cells, and it is thought that glutamic acid plays a highly specific and important role in the transport of potassium ions across cell membranes. It may be that it is playing a similar role also in *Dictyoploca japonica*.

The absence of aspartic acid in male of early pupal stage seems to be due to the smallest concentration and the variation in the presence or absence of amino acids; alanine, arginine, lysine, glutamine, proline, citrulline and methionine also seems to be owing to differences in the metabolic conditions of this insect at various stages of development.

SUMMARY

Free amino acids of *Dictyoploca japonica* were separated paper chromatographically in a series of developmental stages ranging from the pupa to the egg.

1. Free amino acids were identified and each stages showed varying degrees of fluctuation in the qualitative pattern of free amino acids.
2. Serine was present in high concentration in all the stages.
3. The numbers of free amino acid were increased with the development of insect and generally higher in females than in males.
4. Tryptophane was not detected at all in each stages.

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