Sci. Rep. Kanazawa Univ.
Vol. 28 No. 1 pp. 17—26
June 1983

Beta-Alanine in the Puparial Case of *Drosophila melanogaster*

Yoshisige UMEBACHI, Motohiko YASUDA, Takashi YOSHIMIYA, and Masahiro TAKADA

Department of Biology, Faculty of Science,
Kanazawa University, Kanazawa, Japan
(Received April 30, 1983)

Abstract 1. Puparial cases of the mutant strain, *yellow* of *D. melanogaster* were fractionated with 70% ethanol, 4% HCl-methanol, 19 M formic acid, and 1 N NaOH, and β-alanine of each fraction was determined. Nearly half of the β-alanine present in the puparial case was found in the NaOH-soluble fraction.

2. ^{14}C-Labelled β-alanine which was given to the last instar larvae was incorporated in the highest degree into the NaOH-soluble fraction.

3. Some chemical properties of the NaOH-soluble fraction were investigated including the molar ratio of β-alanine, the ratio of free amino-group-containing β-alanine to tatal β-alanine, and the release of β-alanine by acid hydrolysis.

Introduction

It is well known that β-alanine is widely distributed in haemolymph of insects (Agrell, 1949; Pratt, 1950; Clark and Ball, 1952; Auclair and Dubreuil, 1953; Chen, 1962, 1971; Levenbook, 1966). Interestingly, in some insects, pupal or puparial cuticle contains also β-alanine. What it means, however, has remained unsettled.

Dennell (1958) reported for the first time that β-alanine was present in the hydrolysate of the puparial cuticle of *Calliphora vomitoria*. Karlson et al. (1969), Gilby and Mckeller (1970), Hackman and Goldberg (1971), and Srivastava (1971) reported also the presence of β-alanine in hydrolysates of the cuticle of various insects including *Calliphora erythrocephala, Lucilia cuprina,* and *Galleria mellonera*. These reports indicate that sclerotized cuticle of pupal or puparial cases contains β-alanine, whearas soft cuticle does not contain it. Lipke and Geoghegan (1971) and Kimura et al. (1976) showed that a large amount of β-alanine was present in the fraction released from the puparial cuticle of *Sarcophaga bullata* by the treatment with N-bromosuccinimide.

Chen and Hanimann (1965), Levenbook (1966), Levenbook and Dinamarca (1966), Pant and Lal (1970), Ross and Morroe (1972), and Hodgetts (1972) reported on the change of β-alanine content in haemolymph during the larval and puparial stages of *Drosophila*

melanogaster, Phormia regina, Sarcophaga ruficornis, and *Musca domestica.* All these reports show that the amount of free β-alanine in haemolymph increases before puparium formation and decreases after the pupariation.

Levenbook et al. (1969), Bodnaryk and Levenbook (1969), Bodnaryk (1970, 1971a and b), and Dunn et al. (1977) reported on Sarcophagine (β-alanyl-L-tyrosine) of the haemolymph of *Sarcophaga bullata.* This dipeptide increases in the haemolymph of the last instar larva, reaches a maximum at the wandering stage, and after puparium formation, rapidly deceases. At this time, the dipeptide is split into β-alanine and tyrosine, and the β-alanine is incorporated into the puparial cuticle.

Seki (1962) found that pupal or puparial case of some black mutants of *Drosophila vililis, Musca domestica,* and *Bombyx mori* lacks β-alanine, whearas the cuticle of wild type strains contains this amino acid. Jacobs and Brubaker (1963) obtained also the similar results in the mutant *ebony* of *D. melanogaster.* After that, Fukushi and Seki (1965), Fukushi (1967), Nakai (1971), Jacobs (1966, 1968), Hodgetts (1972), and Hodgetts and Konopka (1973) investigated this problem in further detail. These black mutant starins have been presumed either to be unable to synthesize β-alanine or to be unable to incorporate or fix this amino acid into the cuticle.

From the results of the above-mentioned reports, the following two questions will arise: (1) what relationship is there between the incorporation of β-alanine and the sclerotization of cuticle ? and (2) what relationship is there between melanin formation and β-alanine ? In order to answer these questions, it is necessary to know about the site and nature of the bonding of β-alanine in sclerotized cuticle.

The present paper deals with the nature of β-alanine in the puparial case of *D. melanogaster.* In this species, there are two mutant strains *ebony* and *black,* of which the cuticle lacks β-alanine. Moreover, the mutant strain *yellow* contains more β-alanine than does the wild type strain (Fukushi, 1967). In the present paper, some properties of the β-alanine present in puparial cases of the strain *yellow* have been investigated including (1)solubility, (2) incorporation of ^{14}C-β-alanine, (3) molar ratio of β-alanine, (4) the ratio of free amino-group-containing β-alanine to total β-alanine, and (5) the release of β-alanine by acid hydrolysis.

Material and Methods

Material

Empty puparial cases of the mutant strain *yellow* of *D. melanogaster* were used. The flies were raised in the culture medium which consisted of malted rice 125g, sugar 50g, agar 12.5g, KH_2PO_4 0.9g, water 1000ml, and yeast. After emergence of flies, the empty puparial cases were gathered and reserved in 99% methanol in the cold.

Fractionation of cuticle components

Empty puparial cases were first treated with 70% ethanol at 40℃, three times. The combined extract is below called 70% EtOH fraction. The residue was then treated with 4% HCl-methanol at room

temperature, three times. The combined extract is referred to as HCl-MeOH fraction. The residue was furthermore treated with 19 M formic acid at room temperature, three times. The combined extract is named formic acid fraction. Finally the residue was treated with 1 N NaOH at room temperature, four times. The combined extract is called 1 N NaOH-soluble fraction. The final residue is referred to below as cuticle residue.

In some cases, the extraction with 19 M formic acid was omitted.

Partial purification of the NaOH-soluble fraction

The 1 N NaOH-soluble fraction was dialyzed against water, and the dialysate was lyophilyzed. The residue was dissolved in water and applied to the Biogel P-200 colum (2.2×50cm). Proteins were moved down with water. The effluent was monitored at 280nm, and then fractions of 3.5ml were collected. After that, the depth of yellowish brown color of each fraction was determined by measuring absorbance at 450nm. The gel filtration pattern is shown in Fig. 1. The contents of peak 2 were pooled and lyophilyzed. The residue was below called the partially purified HaOH fraction. In the peak 2, the peak of absorbance at 280nm and the peak of brown color coincided with each other. The pratially purified NaOH fraction thus obtained is a brown-colored protein fraction.

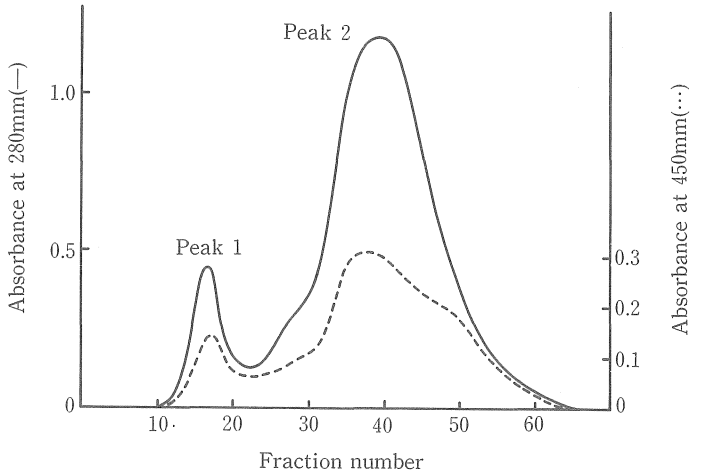

Fig. 1. Gel-filtration of the 1 N NaOH-soluble fraction through Biogel P-200 column.

Hydrolyses

The original empty puparial cases before fractionation and the cuticle residue were hydrolyzed in 1 N HCl for 5 hr without any pre-treatment. The 70% EtOH, HCl-MeOH, and formic acid fractions were evaporated to dryness in a rotary evaporator at 40-60℃ and then hydrolyzed in 1 N HCl at 100℃ for 5 hr. In all the cases, the hydrolysate was filtered through Centriflow CF-25 (Amicon). The filtrate was applied to the Dowex 50W×4 column (1×13cm) and, after being washed with water, amino acids were eluted with 2 or 3 N ammonia water. The amino acid fraction thus obtained was evaporated to dryness in a rotary evaporator at 40-50℃ or lyophilyzed. The residue was dissolved in water and submitted to the determination of β-alanine.

The 1 N NaOH-soluble fraction was hydrolyzed at 100℃ for 5 hr without any pretreatment. The hydrolysate was filtered through Centriflow CF-25 and the filtrate was applied to the Dowex 50W×4 column. The procedure after that was the same as mentioned above.

The hydrolysis of the partially purified NaOH fraction was performed in 6 N HCl at 100℃ for 24 hr. In some experiments, the time of hydrolysis was changed.

Estimation of β-alanine

This was performed in the following three kinds of ways: (1) TNBS-method, (2) capillary tube isotachophoresis, and (3) DNP-method.

The procedure of TNBS-method was a slight modification of Sinoda and Satake's method (Sinoda and Satake, 1961). Sample was submitted to two-dimensional thin-layer chromatography (TLC) with a cellulose sheet (Merck No. 5552, 10×10cm). The developing solvents were 70% methanol for the first direction and a mixture of *n*-butanol, acetic acid, and water (12 : 3 : 5) for the second direction. After development, the chromatogram was sprayed with 0.1% TNBS (2, 4, 6-trinitrobenzene sulfonic acid) in 80% methanol and then with borate buffer 1. After standing overnight in the dark, the area of β-alanine was scraped and put in a test tube. Then, 1ml of 0.1% TNBS in water and 2ml of borate buffer 2 were added and kept at 40℃ in the dark. After 2 hr, 1ml of 2 N HCl was added and centrifuged, and absorbance of the supernatant was measured at 340nm. The above-mentioned borate buffer 1 (pH 8.5) was a mixture of 3.6 vol. of 1/80 M $Na_2B_4O_7$ in 70% methanol and 6.36 vol. of 1/40 M KH_2PO_4 in 70% methanol. The borate buffer 2 (pH 8.0) consisted of 7.25 vol. of a mixture of 1/2.5 M H_3BO_3 and 1/10 M NaCl in water and 7.25 vol. of 1/10 M $Na_2B_4O_7$ in water.

The second method, isotachophoresis, was carried out with the apparatus of capillary tube isotachophoresis, Shimazu IP-1B. Leading electrolyte consisted of 0.01 M HCl, 0.02 M Ammediol (2-amino-2-methyl-1, 3-propandiol) and 0.1% Triton x-100. The pH was 8.9. Terminal electrolyte contained 0.01 M ε-aminocaproic acid and 0.01 M Ammediol. The pH was adjusted to 10.8 with $Ba(OH)_2$. The capillary tube was 26cm in length and 0.57mm in diameter. Migration current was 75 μampere.

The third method was the DNP-method of Sanger and Thompson (1953). After dinitrophenylation of sample, a mixture of DNP-amino acids was submitted to two-dimensional TLC with a pre-coated silica gel sheet (Merck No. 5553, 10×10cm). The solvent for the first direction was the organic layer of toluene, pyridine, chloroethanol, and 0.8 N ammonia water (100 : 30 : 60 : 60) mixture, and for the second direction, a mixture of chloroform, benzylalcohol, and acetic acid (70 : 30 : 3). After development, absorbance of the yellow spot of DNP-β-alanine was measured with a Shimazu TLC scanner 920.

Beta-alanine which has free NH_2-group was determined as follows: The partially purified NaOH fraction was first dinitrophenylated and then hydrolyzed in 6 N HCl at 100℃ for 15 hr. The hydrolysate was diluted to 1 N HCl with water and extracted with ethyl ether four times. The combined ether layer was evaporated to dryness under reduced pressure, dissolved in acetone, and submitted to the above-mentioned TLC. On the other hand, total β-alanine of the partially purified HaOH fraction was determined as follows: the sample was first hydrolyzed in 6 N HCl at 100℃ for 24 hr, and the hydrolysate was evaporated to dryness in a rotary evaporator at 60℃. The residue was dinitrophenylated and submitted to TLC as mentioned above.

Incorporation of ^{14}C-labelled β-alanine

The labelled compound was β-alanine [1-^{14}C] (New England Nuclear), of which the specific activity was 40.51 mCi per mM. About 1.0g of the third instar larvae were raised in the culture medium which consisted of agar 0.25g, sugar 1.0g, water 20ml, ^{14}C-β-alanine 20 μCi, and yeast. The yeast had been killed by being boiled. After emergence of flies, the empty puparial cases were gathered.

The puparial cases thus obtained were fractionated as mentioned above, and the radioactivity of each fraction was determined.

The experiment was carried out two times. In the first experiment, cpm of each fraction was

measured with a Aloka gas flow counter. In the second experiment, dpm was determined with a Beckman LS 9000 liquid scintillation counter. Scintillation cocktail was PCS of Amersham.

Absorption spectrum

The partially purified NaOH fraction was dissolved in water and the absorbance was measured in the range of 230 to 800nm with a Hitachi 240 spectrophotometer.

Amino acid analyses

The puparial cases before fractionation and the 1 N NaOH-soluble fraction were hydrolyzed in 1 N or 6 N HCl at 100℃ for 5 hr or 24 hr. The hydrolysate was filtered through Centriflow CF-25. The filtrate was applied to the Dowex 50W×4 column (1×13cm), and after being washed with water, amino acids were eluted with 2 N ammonia water. The amino acid fraction thus obtained was lyophilyzed and submitted to amino acid analysis. A Hitachi 831-S or KCA-5 amino acid analyzer was used.

In this way, molar ratios of amino acids except arginine could be determined. Most of arginine was lost on the Dowex column, because this amino acid was not completely eluted with ammonia water. Methionine and methionine sulfone could not clearly be determined. Therefore, although Table 3 does not include arginine and methionine, it does not mean that these two amino acids are absent in the cuticle protein.

Results

Beta-alanine content in each fraction from puparial case

Beta-alanine content of each fraction is given in Table 1, which shows that nearly half of the β-alanine present in puparial case is found in the 1 N NaOH-soluble fraction.

Table 1. Beta-alanine in each fraction from puparial case (μg β-alanine per 100 mg dry weight of puparial case)

Fraction	Method	
	TNBS	Isotacho-phoresis
Original puparial case	298.8	345.8
70% EtOH	3.5	6.7
4% HCl-MeOH	37.4	64.0
19 M Formic acid	41.8	68.0
1 N NaOH	127.8	145.3
Cuticle residue	26.8	15.8

Incorporation of ^{14}C-β-alanine into puparial case

Results from the incorporation of the ^{14}C-β-alanine which was given to the last instar larvae are shown in Table 2, which indicates that β-alanine was incorporated in the highest degree into the 1 N NaOH-soluble fraction.

Moreover, the peaks 1 and 2 prepared from the 1 N NaOH-soluble fraction was confirmed to incorporate ^{14}C-β-alanine.

Table 2. Incorporation of ^{14}C-β-alanine into each fraction of puparial case (cpm or dpm per 1 mg dry weight of puparial case)

Fraction	Method	
	Gas flow	Liquid scintillation
	cpm	dpm
Original puparial case	6215	8715
70% EtOH	288	565
4% HCl-MeOH	794	428
19 M Formic acid	1248	1057
1 N NaOH	2279	3842
Cuticle residue	893	780

Amino acid analyses

The results are given in Table 3. Experimental Nos. 2 and 3 show the hydrolysates of the 1 N NaOH-soluble fraction. For the purpose of comparison, the hydrolysate of the original empty puparial case before fractionation is also given in No. 1. Molar ratio of each amino acid is given as the value for 100 of glycine.

Table 3. Amino acid analyses of original puparial case and 1 N NaOH-soluble fraction

Amino acid	Experimental No.		
	1	2	3
	Original puparial case	1 N NaOH-soluble fraction	
	1 N HCl 5 hr*	1 N HCl 5 hr	6 N HCl 24 hr
Asp	126.5**	398.5	304.5
Thr	16.6	29.2	74.6
Ser	45.0	63.9	83.3
Glu	63.3	203.9	392.9
Pro	46.5	102.4	473.3
Gly	100.0	100.0	100.0
Ala	80.2	172.3	325.8
Val	23.5	55.1	422.9
Met	—	—	—
Ile	14.9	30.7	192.5
Leu	11.1	59.9	222.5
Tyr	23.4	26.5	117.9
Phe	6.3	10.5	65.8
β-Ala	68.2	89.2	62.5
Lys	10.6	19.0	89.6
His	5.7	10.2	44.2
Arg	—	—	—

* Hydrolysis: Concentration of HCl and hours of hydrolysis.
**Molar ratios are given as the values for 100 of glycine.

In Experimental No. 2, in which the hydrolysis was performed in 1 N HCl for 5 hr, the molar ratio of β-alanine is higher than in the case of No. 3 (6 N HCl, 24 hr). This fact

shows that β-alanine is more readily released by hydrolysis than most of other ordinary amino acids.

Moreover, the molar ratio of β-alanine in No. 2 is higher than in No. 1. But the difference is not so big as in aspartic acid, glutamic acid, proline, α-alanine, valine isoleucine, and leucine. This may show that even in the original puparial cases, β-alanine is readily released by acid hydrolysis.

Chemical and spectral properties of the partially purified NaOH fraction

Absorption spectrum Absorbance progressively increased from 800 to 230nm, and there was no absorption peak as shown in Fig. 2, though a shoulder was found around 280nm.

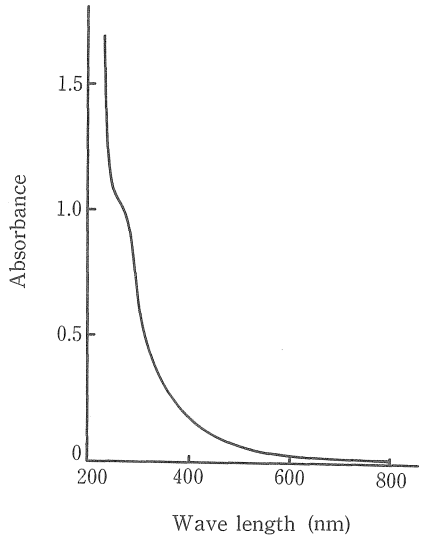

Fig. 2. Absorption spectrum of the partially purified 1 N NaOH-soluble fraction (peak 2). Solvent, distilled water.

Ratio of the free NH_2-group-containing β-alanine to the total β-alanine For the determination of total β-alanine, the partially purified NaOH fraction was first hydrolyzed in 6 N HCl at 100℃ for 24 hr, and β-alanine of the hydrolysate was determined by the DNP-method. The tatal β-alanine content thus determined was 9.7±0.5μg per mg dry weight. For the free NH_2-group -containing β-alanine, the sample was taken from the same partially purified NaOH fraction as used for the determination of total β-alanine. The sample was first dinitrophenylated and then hydrolyzed, and DNP-β-alanine was determined. The experiments were performed three times. The ratio of free NH_2-group-containg β-alanine to total β -alanine was 39.2±1.3% (mean±S. E.).

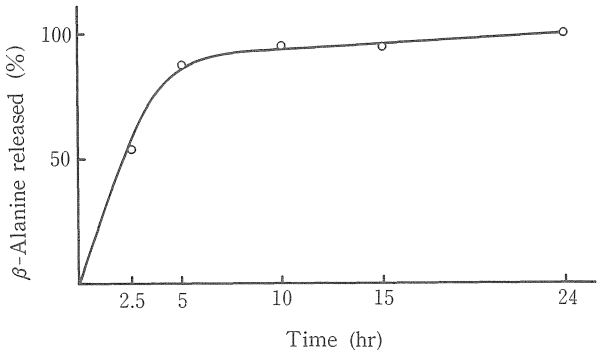

Fig. 3. Release of β-alanine from the partially purified 1 N NaOH-soluble fraction (peak 2) by acid hydrolysis. Hydrolysis, in 6 N HCl at 100℃.

Release of β-alanine by hydrolysis The partially purified NaOH fraction was hydrolyzed in 6 N HCl at 100℃ for 2.5, 5, 10, 15, and 24 hr. Beta-alanine of the hydrolysate was determined by the DNP-method. The result is given in Fig. 3, which shows that β-alanine is rather rapidly released from the cuticle protein, though the complete release of the total β-alanine takes about 24 hr.

Discussion

Jacobs (1978) reported on the possibility that the β-alanine present in the cuticle of *D. melanogaster* may be bound to chitin. But the results of Tables 1 and 2 of the present paper indicate that nearly half of the β-alanine of cuticle is present in the protein fraction. Even if some β-alanine is bound to chitin, therefore, it should be rather a little.

Bodnaryk (1971c) reported in *Sarcophaga bullata* that the β-alanine of cuticle is present as a N-terminal of cuticle protein. It is true that β-alanine is present in protein fraction. But whether or not the β-alanine is present as a N-terminal of protein has remained unsettled. There is also a possibility that β-alanine may be bonded to the side chain of dopamine which is incorporated into cuticle. The possibility that the β-alanine may be present as N-(β-alanyl) dopamine can not be ruled out. Umebachi and Yoshida (1970), Umebachi (1975), Umebachi and Yamashita (1976, 1977), and Rembold et al. (1978) reported that N-(β-alanyl) dopamine derivative is present as a constituent of Papiliochrome. Recently, Hopkins et al. (1982) discussed the meaning of N-(β-alanyl) dopamine in insect cuticle.

In the partially purified NaOH fraction, it is interesting that the β-alanine which has free NH_2-group is only about 40 per cent. This suggests that in the rest (60%) of β-alanine, some substance may be bonded to the NH_2-group of β-alanine. Some amino acid may be bonded to β-alanine through peptide bond. Or there may also be the possibility of β-alanyl -β-alanyl bond.

The results of Experimental Nos. 2 and 3 of Table 3 indicate that the β-alanine of cuticle protein may be more readily released than most of other amino acids. Bodnaryk (1971c) and Lipke and Geoghegan (1971) also reported the same tendency. Does this mean that β-alanine is located in the peripheral region of cuticle protein or that the β-alanine which is on the carbonyl side of peptide bond is more readily hydrolyzed than ordinary α-amino acids in peptide? In this connection, it is interesting that the free NH_2-group -containing β-alanine is only about 40%. Moreover, it is also important that the complete release of β-alanine from cuticle protein needs the hydrolysis in 6 N HCl at 100℃ for 24 hr as shown in Fig. 3. These problems must be the subjects of future research.

Acknowledgements—We wish to express our gratitude to Professor F. Sakiyama of the Institute for Protein Research, Osaka University for analyzing amino acids with the amino acid analyzer. We are also indebted to Professor K. Jozuka of the College of Liberal Arts, Kanazawa University for permitting us to use the apparatus of isotachophoresis.

References

AGRELL I. (1949) Occurrence and metabolism of free amino acids during insect metamorphosis. *Acta Physiol. Scand.* **18,** 247-258.

AUCLAIR J. L. and R. DUBREUIL (1953) Étude sur les acides aminés libres de l'hémolymphe des insectes par la méthode de chromatographie sur papier filtre. *Can. J. Zool.* **31,** 30-41.

BODNARYK R. P. (1970) Effect of dopa-decarboxylase inhibition on the metabolism of β-alanyl-L-tyrosine during puparium formation in the fleshfly *Sarcophaga bullata* Parkar. *Comp. Biochem. Physiol.* **35,** 221-227.

——— (1971a) Studies on the incorporation of β-alanine into the puparium of the fly, *Sarcophaga bullata. J. Insect Physiol.* **17,** 1201-1210.

——— (1971b) Effect of exogenous molting hormone (ecdysterone) on β-alanyl-L-tyrosine metabolism in the larva of the fly *Sarcophaga bullata* Parkar. *Gen. Comp. Endocrinol.* **16,** 363-368.

——— (1971c) N-Terminal β-alanine in the puparium of the fly *Sarcophaga bullata*: Evidence from kinetic studies of its release by partial acid hydrolysis. *Insect Biochem.* **1,** 228-236.

——— and L. LEVENBOOK (1969) The role of β-alanyl-L-tyrosine (Sarcophagine) in puparium formation in the fleshfly *Sarcophaga bullata. Comp. Biochem. Physiol.* **30,** 909-921.

CHEN P. S. (1962) Free amino acids in insects. In *Amino Acid Pools, Distribution, Formation and Function of Free Amino Acids* (ed. by J. T. Holden), pp. 115-135, Elsevier, Amsterdam.

——— (1971) Biochemical Aspects of Insect Development, S. Karger, Basel.

——— and F. HANIMANN (1965) Ionenaustausch-chromatographische Untersuchungen über die freien Aminosäuren und deren Derivate in *Drosophila melanogaster. Z. Naturforsch.* **20b,** 307-312.

CLARK E. W. and G. C. BALL (1952) The free amino acids in the whole bodies of culicid mosquitoes. *Exptl. Parasitol.* **1,** 339-346.

DENNELL R. (1958) The amino acid metabolism of a developing insect cuticle: the larval cuticle and puparium of *Calliphora vomitoria*. I. Changes in amino acid composition during development. *Proc. R. Soc. (B)* **148,** 270-279.

DUNN P. E., R. G. FADER, and F. E. REGNIER (1977) Metabolism of β-alanyl-L-tyrosine in *Sarcophaga bullata. J. Insect Physiol.* **23,** 1021-1029.

FUKUSHI Y. (1967) Genetic and biochemical studies on amino acid compositions and color manifestation in pupal sheaths of insects. *Japan. J. Genetics* **42,** 11-21.

——— and SEKI T. (1965) Differences in amino acid compositions of pupal sheaths between wild and black pupa strains in some species of insects. *Japan. J. Genetics* **40,** 203-208.

GILBY A. R. and J. W. MCKELLER (1970) The composition of the empty puparia of a blowfly. *J. Insect Physiol.* **16,** 1517-1529.

HACKMAN R. H. and M. GOLDBERG (1971) Studies on the hardening and darkning of insect cuticles. *J. Insect Physiol.* **17,** 335-347.

HODGETTS R. B. (1972) Biochemical characterization of mutants affecting the metabolism of β-alanine in *Drosophila. J. Insect Physiol.* 18, 937-947.

——— and R. J. KONOPKA (1973) Tyrosine and catecholamine metabolism in wild-type *Drosophila melanogaster* and a mutant *ebony. J. Insect Physiol.* **19,** 1211-1220.

HOPKINS T. L., T. D. MORGAN, and Y. ASO (1982) N-β-Alanyl-dopamine: Major role in insect cuticle tanning. *Science* **217**, 364-366.

JACOBS M. E. (1966) Deposition of labelled beta-alanine in *ebony* and non-*ebony Drosophila melanogaster* with notes on other amino acids. *Genetics* **53**, 777-784.

——— (1968) β-Alanine use by *ebony* and normal *Drosophila melanogaster* with notes on glucose, uracil, dopa, and dopamine. *Biochem. Genetics* **1**, 267-275.

——— (1978) β-Alanine tanning of *Drosophila* cuticle and chitin. *Insect Biochem.* 8, 37-41.

——— and K. K. BRUBAKER (1963) Beta-alanine utilization of *ebony* and non-*ebony Drosophila melanogaster. Science* **139**, 1282-1283.

KARLSON P., K. E. SEKERI, and V. I. MARMARAS (1969) Die Aminosäurezusammensetzung verschiedener Proteinfraktion aus der Cuticula von *Calliphora erythrocephala* in verschiedenen Entwicklungsstadien. *J. Insect Physiol.* **15**, 319-323.

KIMURA S., H. V. STROUT, and H. LIPKE (1976) Peptodochito-dextrins of *Sarcophaga bullata*: Non-identity of limit glycopeptides from larval and puparial cuticle. *Insect Biochem.* **6**, 65-77.

LEVENBOOK L. (1966) Hemolymph amino acids and peptides during larval growth of the blowfly *Phormia regina. Comp. Biochem. Physiol.* **18**, 341-351.

———, R. P. BODNARYK, and T. F. SAPNDE (1969) β-Alanyl-L-tyrosine. Chemical synthesis, properties, and occurrence in larvae of the fleshfly *Sarcophaga bullata* Parker. *Biochem. J.* **113**, 837-841.

——— and M. L. DINAMARCA (1966) Free amino acids and related compounds during metamorphosis of the blowfly *Phormia regina. J. Insect Physiol.* **12**, 1343-1362.

LIPKE H. and T. GEOGHEGAN (1971) The composition of peptido-chitodextrins from Sarcophagid puparial cases. *Biochem. J.* **125**, 703-716.

NAKAI S. (1971) Genetic and biochemical studies on the β-alanine metabolism in the housefly, *Musca domestica* L.. *Japan. J. Genetics* **46**, 53-60 (in Japanese).

PANT R. and D. M. LAL (1970) Variation of free amino acids in *Sarcophaga ruficornis* during metamorphosis. *Indian J. Biochem.* **7**, 57-59.

PRATT J. J. Jr. (1950) A qualitative analysis of the free amino acids in insect blood. *Ann. Entomol. Soc. Am.* **43**, 573-580.

REMBOLD H., J. RASHER, J. EDER, and Y. UMEBACHI (1978) Partial structure of papiliochrome, the yellow wing pigment of the papilionid butterflies. *Z. Naturforsch.* **33c**, 498-503.

ROSS R. H. Jr. and R. E. MORROE (1972) β-Alanine metabolism in the housefly, *Musca domestica*: Concentration in the larvae, pupae, and adults. *J. Insect Physiol.* **18**, 791-796.

SANGER F. and E. O. P. THOMPSON (1953) The amino-acid sequence in the glycyl chain of insulin. I. The identification of lower peptide from partial hydrolysates. *Biochem. J.* **53**, 353-366.

SEKI T. (1962) Absence of beta-alanine in hydrolyzate of the pupal sheaths of *ebony* mutant of *D. virilis. Drosophila Information Service* **36**, 115.

SRIVASTAVA R. P. (1971) The amino acid composition of cuticular proteins of different developmental stages of *Galleria mellonella. J. Insect Physiol.* **17**, 189-196.

UMEBACHI Y. (1975) Further studies on the dopamine derivative, SN-1 derived from the yellow pigments of *Papilio xuthus. Insect Biochem.* **5**, 73-92.

——— and H. YAMASHITA (1976) Clear evidence for the presence of β-alanine as a consituent of papiliochrome II. *Comp. Biochem. Physiol.* **54B**, 55-62.

——— and H. YAMASHITA (1977) Beta-alanine as a constituent of the dopamine derivative, SN-1 of *Papilio xuthus. Comp. Biochem. Physiol.* **56B**, 5-8.

——— and K. YOSHIDA (1970) Some chemical and physical properties of papiliochrome II in the wings of *Papilio xuthus. J. Insect Physiol.* **16**, 1203-1228.