BIOCHEMISTRY OF THE HAEMOLYMPH OF PHTHORIMAEA OPERCULELLA LARVAE TREATED WITH BACILLUS THURINGIENSIS

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SUMMARY: Quantitative changes in the amino acids of haemolymph of the potato tuber moth Phthorimaea operculella after treatment with Bacillus thuringiensis were detected. The amounts of aspartic acid, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and arginine decreased. Other acids such as glutamic acid, cysteine, histidine and lysine showed a marked increase after treatment with B. thuringiensis. P. operculella larvae treated with B. thuringiensis showed some reduction of calcium and magnesium ions but there was no obvious change in the level of zinc ions. A slight decrease in sodium and obvious increase in potassium and manganese ions occurred. The changes in Na^+/K^+ concentration after treatment throw light on the possibility that they may be among the factors interfering with toxicity caused by B. thuringiensis.

Key Words: Haemolymph, phthorimaea operculella, B. thuringiensis.

INTRODUCTION

The larvae of the potato tuber moth Phthorimaea operculella showed to be susceptible to some varieties of the biocontral agent Bacillus thuringiensis (unpublished). The mode of action of B. thuringiensis on this insect species has to be investigated. Biochemical changes in the haemolymph of insects treated with B. thuringiensis were reported by Cheung *et. al.* (4), Boctor (3) and Salama *et. al.* (15). Changes in the total proteins, amino acids and cations occurred in varying degrees.

MATERIALS AND METHODS

Analysis was performed on the final (fourth) instar larvae of P. operculella, which were still feeding and active. Haemolymph was collected by cutting of one or more prologs. The haemolymph subsequently excluded was collected with a five micro-liter micro-capillary tube and quickly diluted into an appropriate volume of cold water to prevent coagulation. Each larva usually yielded between 0.5 and 1 ul of haemolymph.

Total proteins

Protein reacts with the folin ciocalteau reagent to give a blue colored complex due to the reaction of the alkaline copper with the protein (10). The intensity of the color was measured photometrically by using a bousch and lamb spectrophotometer 710 at 750 nm.

Free amino acids

Free amino acids were determined in pooled haemolymph samples (1-2 ml) using an automatic amino acid analyzer. Separation was achieved with a strong cation exchange column; post-column detection was done with ninhydrin.

Inorganic elements (cations)

The cations potassium, sodium, calcium, magnesium, manganese, copper, iron and zinc present in the haemolymph were measured using an atomic absorption spectrophotometer on haemolymph samples (between 1 and 5 ul diluted into 3 ml of water).

RESULTS

Effect of B. thuringiensis on haemolymph composition

a) Effect of total haemolymph proteins and amino acids

Data obtained show a marked decrease in total proteins in the haemolymph of larvae of P. operculella fed on a diet containing a lethal concentration of B.

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thuringiensis var. galleria HD-234. The total proteins in the control was 2.12±0.095 mg/ml; $ this decreased to 1.61±0.006 mg/ml in the treated larvae. The deproteinized haemolymph of P. operculella larvae was analyzed for the free amino acids and the results are given in Table 1. From the results, it appears that :

1. Seventeen amino acids were detected in haemolymph of the larvae of P. operculella.

2. The pattern of some amino acids in the haemolymph of P. operculella, as affected by treatment with B. thuringiensis var. galleria HD-234 showed an obvious decrease in haemolymph of the treated larvae. These amino acids were aspartic acid, threonine, serine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and arginine. The pattern of other amino acids, such as glutamic acids, histidine, was higher in the haemolymph of treated larvae as compared with that of untreated individuals.

Table 1: Pattern of amino acids (a.a) in haemolymph of P. Operculella as affected by treatment with B. thuringiensis var. galleria HD-234.

No. Amino Acids	Control (untriated) concentrations of a.a. gm/16 gm N	Treated concentrations of a.a. gm/16 gm N
1 Aspartic acid	0.972	0.513
2. Threonine	4.551	1.995
3 Serine	1.0451	0.343
4 Glutamic acid	0.0172	0.128
5 Proline	2.744	0.137
6 Glycine	1.730	0.292
7 Alanine	1.193	0.302
8 Cystine	0.168	0.972
9 Valine	2.526	1.054
10 Methionine	0.258	0.101
11 Isoleucine	0.722	0.149
12 Leucine	1.493	0.140
13 Tyrosine	1.360	0.040
14 Phenylalanine	2.213	0.150
15 Histidine	0.149	0.527
16 Lysine	1.307	0.398
17 Arginine	6.548	2.723

b) Effect of B. thuringiensis on haemolymph cations

The data (Table 2) show a slight decrease in Na^+ concentrations (70%) after treatment with B. thuringiensis. So, in the haemolymph of healthy larvae, the concentration of sodium ions was 439 ppm, this decreased to 312 ppm two days after treatment with B. thuringiensis. On the other hand, the potassium ion concentration in the haemolymph showed a marked increase (143%) after larval treatment. The concentration of K^+ of healthy larvae was 3413 ppm, this increased to 4913 ppm two days after treatment. The Na^+/K^+ was 0.129 in the haemolymph of the healthy larvae as compared to 0.0636 in the larvae treated with B. thuringiensis.

An obvious decrease in the concentration of calcium (51%), magnesium (78%), and copper ions (63%) occurred after treatment with B. thuringiensis. On the other hand, an obvious increase in the concentration of manganese (143%) and zinc (114%) ions was observed in the larval haemolymph after feeding on a diet containing B. thuringiensis. Iron ions, were more or less constant in the haemolymph of both healthy and treated larvae.

DISCUSSION

The present investigations clearly show that the mode of action of B. thuringiensis involves biochemical changes in the haemolymph of P. operculella. The level of proteins in the haemolymph markedly decreased during larval treatment with B. thuringiensis and this agrees with the observation of Salama *et. al.* (15). Analysis revealed a number of free amino acids in the haemolymph of P. operculella, most of which are commonly present in proteins. The most abundant amino acids were arginine, aspartic acid, threonine, serine, glutamic acid, proline, alanine, leucine and isoleucine.

Haemolymph amino acids play an important role in the synthesis of cuticle constituents and in silk production (13,18). Proline and alanine are believed to be involved in cold hardness.

All amino acids found are likely to contribute as a general pool for the synthesis of new proteins and for

the products of protein breakdown and may also be involved in osmoregulation and buffering (13). Quantitative changes in the amino acids of haemolymph after treatment with B. thuringiensis were detected. The amounts of aspartic acid, threonine, serine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine and arginine decreased. Other acids such as glutamic acid, cysteine, histidine and lysine showed a marked increase after treatment with B. thuringiensis. Changes in the free amino acid content of Melolantha larvae infected with Bacillus ribourgensis were reported by Benz (2), with a marked increase in histidine, valine, leucine and alanine contents and with decrease in all peptides. On the other hand, Drilhon *et. al.* (5) observed a reduction in free amino acids content in the haemolymph of larvae of Bombyx mori due to flacherie.

Narayanan *et. al.* (12) reported more amino acids in the larvae of Plutella maculipennis treated with B. thuringiensis than in the healthy ones. This could be correlated to be lysis of cells and tissues which results in the mortality of the caterpillar due to gut paralysis.

Narayanan (11) and Lecadet (9) reported that the presence of various amino acids in diseased caterpillars may be attributed to the presence of bacterial spores and crystals since it has been reported that crystals of B. thuringiensis var. thuringiensis and B. thuringiensis var. sotto contain nearly 8 amino acids. The 6-fold increase in the content of cysteine in diseased larvae may also be attributed to possible dissolution of protein crystals of B. thuringiensis. This could be due to the activity of proteolytic enzymes or the presence of reducing substances in the gut which may lead to the release of high amounts of cysteine.

Salama *et. al.* (15) reported that the level of proteins in the haemolymph of Spodoptera littoralis markedly decreased during larval treatment with B. thuringiensis. Other quantitative changes in the amino acids of haemolymph were also detected. Lepidoptera are know to have haemolymph with a low concentration of sodium and high concentration of potassium, magnesium and organic solutes (6,8,17).

Table 2: Effect of B. thuringiensis var. galleria HD-234 on haemolymph cation concentration of P. operculella.

No	Cations	Concentrations (ppm) of citaons	
		Control	Treated
1	Potassium	3413.00±539.91	4913.33±173.33
2	Sodium	439.85±8.69	312.21±4.12
3	Calcium	213.33±8.82	110.00±15.28
4	Magnesium	453.66±30.36	354.33±17.46
5	Manganese	0.66±0.70	0.93±0.12
6	Copper	4.95±0.11	3.14±0.26
7	Iron	19.65±4.85	19.78±7.41
8	Zinc	18.38±4.09	21.13±0.91

The inorganic constituents such as Na^+, K^+, Ca^{++} are of vital importance in view of their role in the neurophysiology of the insect and their levels inside and outside the nerve membrane and they have to maintained for the propagation of impulses (14). P. operculella larvae treated with B. thuringiensis showed a reduction of calcium (193.9%) and magnesium (71.2%), but no obvious change in the level of zinc ions (114%). There was a slight decrease in sodium (70%) and obvious increase in potassium (143%) and manganese (138%) ions.

The changes in Na^+/K^+ and changes in their concentration after treatment throw light on the possibility that they may be among the factors interfering with the toxicity with B. thuringiensis. In this concern, Tiwari and Methotra (16) reported that the changes observed in pH, sodium and potassium ion concentration and Na^+/K^+ ratio Achaea Janata L. and Spodoptera litura F. treated with B. thuringiensis may not be important as far as the toxicity is concerned. These changes may be a secondary feature and are different for various insect species. Florkin and Jeniaux (6) found that the absolute concentration of various cations in the haemolymph is not as important as the ratio of sodium and potassium (1,7). These authors gave a through account of the general symptoms of intoxication by B. thuringiensis as well as changes in blood pH and midgut histology that follow ingestion of the bacteria by a wide range of lepidopterous pests.

REFERENCES

1. Angus TA and Heimpel AM : Inhibition of feeding and blood pH changes in lepidopterous larvae infected with crystal forming bacteria. Can Ent, 91:352-358, 1959.

2. Benz G : Physiopathology and Histochemistry. In: Insect Pathology. An advanced Treetise Ed by EA Steinhaus, Academic Press, London, NY, pp 299-388, 1963.

3. Boctor IZ : Quantitative differences in the free amino acids between male and female Mediterranean fruit fly Ceratitis capitata Wied. Zool Jb Physiol Bd, 82:349-353, 1978.

4. Cheung PK, Grula EA and Button RI : Haemolymph responses in Heliothes zea to inoculation with Bacillus thuringiensis or Minococcus iysodeikticus. J Invertebr Pathol, 31:148-156, 1978.

5. Drilhon A, Busnel RG and Vaga C : Les acids amines titres et les substances fluorescents due sang et des tubes de Malipighi de la chenille de Bombyx mori L attaints de la maladies a polyedres et de la flacherie. CR Heled Seanc Acad Sci, Paris, 232:360-361, 1951.

6. Florkin M and Jeniaux : Haemolymph composition. In: The physiology of Insecta, Ed by M Rockstein, New York Academic Press, Vol 5, pp 256-302, 1974.

7. Heimpel AM and Angus TA : The site of action of crystalliferous bacteria in lepidoptera larvae. J Insect. Pathol, 1:152-170, 1959.

8. Hoyle G : Potassium ions and insect nerve muscle. J Exp Biol, 30:121-135, 1953.

9. Lecadet MM : La Toxins Figuree de Bacillus thuringiensis. Technique de Separation et Composition en Acides Amines. CR Acad Sci, Paris, Serie D, 261:5693-5696, 1965.

10. Lowry OH, Rosebraugh NJ, Farr AI and Randall RJ : Protein measurement with the folin phenol reagent. J Biol Chem, 193:265-275, 1951.

11. Narayanan K : Pathogenicity of Bacillus thuringiensis Berliner to some lepidopterous pests with particular reference to Plutella maculipennis. M Sci Dis, Univ Madras, 1969.

12. Narayanan K, Jayaraj S and Subromaian T : Sensitivity of Bacillus thuringiensis Berliner to two antibiotics and sulphanilamide. Hind Antibiot Bull, 15:14-15, 1973.

13. Pant R and Argawal J : Free amino acids of the haemolymph of some insects. J Insect Physiol, 10:443-446, 1964.

14. Roeder KD : Insect Physiology. John Wiley and Sons Inc, NY, 1953.

15. Salama HS, Sharaby A and Ragaei M : Chemical changes in the haemolymph Spodoptera littoralis as effected by Bacillus thuringiensis (Berliner). Entomophaga, 28:331-337, 1983.

16. Tiwari LD and Mehrotra KV : Effect of Bacillus thuringiensis on the pH of gut and haemolymph as well as on the sodium potassium concentration in the haemolymph of Achoea janata (L) and Spodoptera litura (P) larvae (Lep, Noctuiade). Z Ang Ent, 92:61-66, 1981.

17. Weevers RDG : A lepidopteron saline: Effects of inorganic cation concentrations on sensory reflex and motor responses in herbivorous insects. J Exp Biol, 44:163-175, 1966.

18. Wyaat GR : The biochemistry of insect haemolymph. Ann Rev Ent, 60:102, 1961.

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