

BRIEF COMMUNICATION

FREE AMINO ACIDS IN THE HAEMOLYMPH OF DIFFERENT LIFE STAGES OF THREE ORDERS OF INSECTS

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ABSTRACT

Two dimensional chromatographic studies were conducted on the adults and nymph/larval stages of insects belonging to three different orders. The results obtained indicates that the free amino acids present in the different stages of insects varies. Certain free amino acids are present in the adult but absent in nymph/larval stages and vice versa.

Keywords: Haemolymph, free amino acids, *Periplanata*, *Polytes*, *Aeolopus*.

Insects are the most successful group of animals as evidenced by the large number of species and inhabitation of diverse habitats including those unfavourable for other animals. Insect haemolymph, which fills the haemocoel, consists of fluid plasma with suspended haemocytes, as well as various organic and inorganic constituents. The important organic constituents include proteins, amino acids, end products of nitrogen metabolism, organic acids and lipids. Insect blood is characterised by very high levels of amino acids present in the plasma, constituting 33-65% of the non-protein nitrogen present. About 52-85% are in the form of free amino acids. The concentration of free amino acids in insect blood is very high 0.29-2.43% which is fifty times higher than in human serum (0.05%)². The concentration and types of amino acids present in blood are not identical but vary from species to species, within species including stage of development and feeding¹.

Two dimensional chromatography (ascending) was used to detect the free amino acids in the haemolymph of adult and nymph/larva of insects. Three insects were selected - *Papilio polytes romulus* (order Lepidoptera), *Periplanata americana* (order Dictyoptera), *Aeolopus tamulus* (order Orthoptera).

Standard amino acids were prepared from the amino acid reference collection box for paper and thin layer chromatography, BDH chemicals Ltd., Poole, England. Whatman no. 1 filter paper was used for chromatography. The first solvent used was n-butanol, acetic acid and water (4:1:5) and the second solvent was phenol and water (8:2). Standard reference chromatogram for the twenty amino acids were prepared using both solvents and ninhydrin (triketohydrindene hydrate) was used to develop the chromatogram. Rf values for all the amino acids were calculated.

Table 1 : Free Amino acids in Haemolymph of *Periplanata americana*

	Adult		Nymph	
	Haemolymph Rf	Standard Rf	Haemolymph Rf	Standard Rf
Proline	0.97	0.98	0.97	0.98
Aspartic acid	0.24	0.23	-	-
Histidine	0.59	0.60	-	-
Methionine	0.91	0.92	-	-
Valine	0.85	0.85	-	-
Arginine	0.53	0.52	0.52	0.52
Lysine	0.48	0.48	0.49	0.49
Leucine	-	-	0.96	0.96
Tyrosine	-	-	0.69	0.70
Isoleucine	-	-	0.90	0.91
Ornithine	-	-	0.34	0.34

Table 2 : Free Amino acids in Haemolymph of *Papilio polytes*

	Adult		Nymph	
	Haemolymph Rf	Standard Rf	Haemolymph Rf	Standard Rf
Cystine	0.69	0.70	-	-
Histidine	0.60	0.60	-	-
Lysine	0.48	0.48	-	-
Glutamic acid	0.42	0.41	-	-0.85
Valine	-	-	0.84	0.91
Isoleucine	-	-	0.91	0.34
Ornithine	-	-	0.35	0.34
Aspartic acid	-	-	0.22	0.23
Methionine	-	-	0.93	0.92

Table 3 : Free Amino acids in Haemolymph of *Aeolopus tamulus*

Threonine	0.60	0.61	-	-
Ornithine	0.34	0.34	-	-
Proline	0.99	0.98	-	-
Methionine	0.91	0.92	0.91	0.92
Arginine	0.52	0.52	-	-
Tyrosine	0.69	0.70	-	-
Valine	0.85	0.85	-	-
Aspartic acid	0.22	0.23	-	-

The antenna of adult insect and legs of larva were cut at the base and the haemolymph was extracted with methyl alcohol. Care was taken to prevent squeezing the insect. The extract was kept for a few minutes to allow the alcohol to evaporate. It was then spotted on the filter paper, dried immediately to avoid spreading and subjected to one dimensional chromatography in the first solvent. When the solvent front reached 10 cm level, the paper was removed and dried thoroughly. Then it was rotated at an angle of 90° and placed in the second solvent. The paper was removed when the solvent front reached 10 cm level, dried, sprayed with ninhydrin and placed in the oven at 60°C till coloured spots appeared. The Rf value was calculated and compared with that of the reference amino acids for identification.

Two-dimensional chromatographic studies conducted to detect the free amino acids in different stages of the lifecycle of insects revealed considerable variations. In *Periplanata* adult, about 7 free amino acids were detected. Of these three were near the line of origin because of their failure of migration in the first solvent. The amino acids were identified as proline, aspartic acid, histidine, methionine, valine, arginine and lysine. Seven aminoacids were identified in the nymph of *Periplanata* - leucine, arginine, tyrosine, isoleucine, ornithine, proline and lysine.

In *Papilio* adult, four free amino acids viz. cysteine, histidine, lysine and glutamic acid were seen while in the larva six amino acids, lysine, valine, isoleucine, ornithine, aspartic acid and methionine were identified.

Aeolopus adult had four free amino acids, threonine, ornithine, proline and methionine whereas the nymph had five amino acids arginine, tyrosine, methionine, valine and aspartic acid. Tables 1-3 summarise the free amino acids detected in the three insects.

Differences in the type of amino acids in the adult and nymph/larval stages were observed in the three insects studied. This variation could be attributed to the difference in metabolism of adult and nymph/larval stages. Certain amino acids which were free in the nymph/larval stages were absent in the adult and vice versa. Perhaps this is because the amino acids became bound during certain stages in the lifecycle of the insect.

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