Essential Amino Acids for the Growth of the Smaller
Brown Planthopper, *Laodelphax striatellus*
FALLÉN (Hemiptera : Delphacidae)

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The smaller brown planthopper, *Laodelphax striatellus*, was reared on holidic diets (MED-1) deficient in single amino acids in order to discern the essential amino acid requirements for the growth of this planthopper. Rearing on a diet lacking cysteine or methionine resulted in death during the middle stage of nymphal development. Dietary deficiency of any one of the remaining 21 amino acids did not prevent the planthoppers from completing nymphal development. The planthoppers, however, died at the first instar when these amino acids were deleted all together. Cysteine was thought to be utilized by being converted to cystine, and its minimum concentration for allowing growth was estimated as 5 mg per 100 ml. When cystine was used instead of cysteine, its minimum effective concentration was 10 mg per 100 ml. The minimum effective concentration of methionine was found to be 40 mg per 100 ml.

INTRODUCTION

Regarding artificial rearing of sap-sucking insects, aphids were the first to be reared for generations on synthetic diet (AUCLAIR and CARTIER, 1963). This facilitated nutritional studies on sap-sucking insects including aphids. In aphids, there have been some reports on the requirements of sugars, amino acids, vitamins and trace metals (AUCLAIR, 1965, 1967; DADD, 1967; DADD et al, 1967; DADD and KRIEGER, 1968; MITTLER et al., 1970; AUCLAIR and SRIVASTAVA, 1972; MASSONIE, 1973; LECKSTEIN and LLEWELLYN, 1973, 1974; SRIVASTAVA and AUCLAIR, 1974), but information is still limited.

It has become possible to rear the smaller brown planthopper, *Laodelphax striatellus* FALLÉN, on a holidic diet for more than 10 generations (MITSUHASHI and KOYAMA, 1971). Moreover, it has been known that some amino acids can be deleted without appreciable effects on nymphal growth (MITSUHASHI and KOYAMA, 1972).

In the present study, essential amino acids for the growth of *L. striatellus* nymphs were determined by examining the effects of feeding on a holidic diet, MED-1, deficient in single amino acids, upon development and the incidence of mortality.

MATERIALS AND METHODS

L. striatellus used in this study was a red eye mutant isolated in this laboratory and maintained on rice seedlings in short glass tubes (15 mm × 100 mm). In order

Table 1. COMPOSITION OF AMINO ACIDS IN MED-1 DIET (MG PER 100 ML)

L-Alanine	100	L-Isoleucine	200
γ -Aminobutyric acid	20	L-Leucine	200
L-Arginine hydrochloride	400	L-Lysine hydrochloride	200
L-Asparagine	300	L-Methionine	100
L-Aspartic acid	100	L-Phenylalanine	100
L-Cysteine	50	L-Proline	100
L-Cystine hydrochloride	5	DL-Serine	100
L-Glutamic acid	200	L-Threonine	200
L-Glutamine	600	L-Tryptophan	100
Glycine	20	L-Tyrosine	20
L-Histidine	200	L-Valine	200
DL-Homoserine	800		

to commence experiments with nymphs immediately after hatching without bringing the planthoppers into contact with rice plants, eggs were collected by MITSUHASHI's method (MITSUHASHI, 1970), whereby nymphs were allowed to hatch on moistened filter paper.

Small glass cylinders (25 mm in inner diameter, 30 mm in length) were adopted as rearing vessels. Test solutions consisted of a MED-1 diet (MITSUHASHI and KOYAMA, 1971) from which an individual amino acid was deleted. The composition of amino acids in MED-1 is shown in Table 1. The same test solution was placed in sachets on both sides of openings of a rearing vessel. *L. striatellus* was reared individually in such vessels. Experimental rearing was conducted at 25°C and under 16 hours illumination per day. For each test solution, 100 nymphs were individually reared until total mortality occurred.

RESULTS

Sequential deletion of single amino acids

When cysteine or methionine was removed from the MED-1 diet, no nymphs grew to the adult stage. Most nymphs died at the first instar, although some reached the 4th instar. Deletion of other amino acids did not severely affect the growth of *L. striatellus*. The nymphal durations, when rearing was on the diets lacking one of these amino acids, were not significantly different from that of the control grown on the complete MED-1 diet (Fig. 1). The mortality during the nymphal period, however, was affected by the deletion of these amino acids. The deletion of γ -aminobutyric acid, aspartic acid, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, phenylalanine, proline, or serine increased the mortality markedly. On the other hand, the deletion of α -alanine, homoserine or tryptophan decreased the mortality considerably (Fig. 2).

Deletion of amino acids as a group

Since all amino acids except cysteine and methionine did not prevent the nymphal growth of *L. striatellus* when omitted singly, an experiment was conducted where amino acids were deleted altogether from the MED-1 diet. As a result, total mortalities were observed at the 1st instar.

Fig. 1. Nymphal durations of *L. striatellus* reared on MED-1 diets lacking single amino acids.

Fig. 2. Mortality of *L. striatellus* nymphs reared on MED-1 diets lacking single amino acids.

Sulfur-containing amino acids and the growth of nymphs

In the preceding experiments, methionine and cysteine were found to be necessary for nymphal growth. Both amino acids are known to contain sulfur. In addition to these, MED-1 diet contained another sulfur-containing amino acid, cystine. When all of the three sulfur-containing amino acids were deleted, all nymphs died within 10 days after hatching. Deletion of cysteine and cystine permitted growth up to the 3rd instar. When fed on diets lacking methionine or cysteine but containing very little cystine, nymphs grew to the 4th instar (Fig. 3).

In the next series of experiments, diets, in which the amount of the 3 above-

Fig. 3. Maximal growth of *L. striatellus* nymphs reared on MED-1 diet lacking certain sulfur-containing amino acids.

Fig. 4. Effects of the concentration of methionine in MED-1 diet on nymphal duration and ratio of adult emergence.

mentioned amino acids was changed without alteration of the other components of the MED-1 diet, were prepared in order to discern the minimum concentration required for nymphal growth. For methionine, the minimum concentration was estimated at 40 mg per 100 ml, while the optimum concentration was between 100 and 160 mg per ml (Fig. 4). The optimum concentration was roughly equivalent to the concentration of methionine in the MED-1 diet. Below the minimum concentration, the nymphs grew only to the 4th instar. Nymphal duration was not affected very much by change in methionine concentration, but its fluctuation increased as the concentration diverged from the optimum one.

It has been known that cysteine is easily oxidized to cystine. In fact it was recognized by the amino acid analysis of diets that 5–10 per cent of cysteine in MED-1 diet was converted to cystine during a 24 hour period at 25°C. In order to evaluate the effect of cystine and cysteine separately, the concentration of cystine in MED-1 diet lacking cysteine was first tested at different levels. From Fig. 5, the minimum concentration for the completion of nymphal growth was estimated at 10 mg per 100 ml. Since MED-1 diet contained only 5 mg per 100 ml cystine, this concentration was not sufficient. The optimum concentration appears to be 10 mg per 100 ml. Above this concentration, the rate of adult emergence decreased with increase in the concentration. The nymphal duration was not seriously affected, but its deviation became larger when the concentration was increased. It became evident, therefore, that cystine could take the place of cysteine.

In order to examine the effect of cysteine, cysteine in a MED-1 diet lacking

Fig. 5. Effects of the concentration of cystine in MED-1 diet lacking cysteine on nymphal duration and ratio of adult emergence.

Fig. 6. Effects of the concentration of cysteine in MED-1 diet lacking cystine, on nymphal duration and ratio of adult emergence.

cystine was tested at various concentrations. From Fig. 6, the minimum concentration was found to be 5 mg per 100 ml. The rate of adult emergence and the nymphal duration were improved with an increase in its concentration.

DISCUSSION

Experiments of individual amino acid deficiency showed that methionine and cysteine were essential for the growth of *L. striatellus* nymphs. However, cystine was found to be a substitute for cysteine. Since conversion of cysteine to cystine is known to occur readily the active form of cysteine might be cystine. In the single deletion experiments, the diet lacking cysteine but containing cystine did not support nymphal growth. This might be due to the fact that cystine contained in the MED-1 diet was less than the minimum concentration required for nymphal growth.

In the conversion of cysteine to cystine, two molecules of the former aggregate to form one molecule of the latter by formation of a disulphide bridge. Even if 100 per cent of cysteine is converted to cystine, the minimum effective concentration of cysteine (5 mg per 100 ml) would be half of that of cystine (10 mg per 100 ml). It is, therefore, likely that cysteine itself is also active, and further it may have synergistic action with cystine. The parallel relationship between the rate of adult emergence and the concentration of cysteine may also support this assumption.

Some non-essential amino acids impaired the rate of adult emergence markedly when they were deleted. The presence of these amino acids should be favourable

for nymphal growth although they are not necessary for its completion. On the other hand, the presence of some other non-essential amino acids seems unfavourable, because deletion was shown to increase the rate of adult emergence.

The deletion of all the non-essential amino acids together caused a drastic decrease in survival time. There should be compensatory action when these amino acids were deleted one at a time. From results of rearing on the MED-4 diet (MITSUHASHI and KOYAMA, 1972), it has already been shown that γ -aminobutyric acid, arginine, cystine, glycine, homoserine, phenylalanine, proline, threonine, tryptophan, tyrosine and valine can be deleted altogether without adverse effect on the growth of *L. striatellus*.

In general, about ten amino acids are known to be essential for insect growth. In hemipterous insects, however, the essential amino acids are limited. They are reported to include methionine, histidine and isoleucine in *Myzus persicae* (DADD and KRIEGER, 1968), and alanine, histidine, methionine, proline, serine, cystine, phenylalanine, and tyrosine in *Aphis fabae* (LECKSTEIN and LLEWELLYN, 1973, 1974). In *L. striatellus*, the essential amino acids were found to be methionine and cystine (cysteine). In aphids, the role of intracellular symbiotes has been considered as a possible reason for limited number of essential amino acids. Since *L. striatellus* also lodges yeast-like and rickettsia-like microorganisms as intracellular symbiotes, these symbiotes may synthesize and supply some amino acids to the host cells. In order to solve nutritional problems of hemipterous insects, studies on their intracellular symbiotes is considered a prerequisite.

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