Note

Specific Incorporation of L-Glutamine into Volicitin in the Regurgitant of Spodoptera litura

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Volicitin, [N-(17-hydroxylinolenoyl)-L-glutamine], was identified as an elicitor of plant volatiles from a Spodoptera exigua regurgitant. It has been proposed that gut microbes synthesize volicitin from glutamine, a predominant amino acid component in the insect gut. However, we found that glutamine was not a major component in the regurgitant of Spodoptera litura, although L-glutamine was exclusively incorporated into volicitin by S. litura fed on diets enriched with various amino acids. This selectivity of glutamine as a substrate was not due to a dominant occurrence in the insect gut.

Key words: insect-produced elicitor; volicitin; biosynthesis; Spodoptera litura

Volicitin [N-(17-hydroxylinolenoyl)-L-glutamine], which was first identified in the oral secretion of larvae of the beet armyworm, Spodoptera exigua (Fig. 1),1,2) induces excised maize seedlings to release the same blend of volatile terpenoids and indole as that released when they are damaged by caterpillar feeding. Volicitin-related compounds have also been found in the oral secretion of Noctuidae,3–7) Geometridae,4) and Sphingidae.7–9) Interestingly, the proportion of volicitin-related compounds in the secretion of some caterpillars was species-specific.4,7) Although volicitin-related compounds are thought to be key compounds to understand the arena of plant-induced resistance to herbivorous arthropods, details of the biosynthesis of volicitin still remain unknown. A mass spectrometric analysis of volicitin obtained from S. exigua larvae fed on 13C-enriched corn seedlings has clearly demonstrated that the larvae synthesized volicitin from plant-derived linolenic acid by hydroxylation of the fatty acid at C-17 and conjugation with insect-derived L-glutamine.3,5) Additionally, Spiteller et al. have suggested that endosymbiotic bacteria present in the gut segments of herbivores were involved in the production of volicitin.10) The bacterial strains isolated from the gut displayed a very broad substrate tolerance in in vitro experiments, and most of the twenty proteinogenic amino acids were observed to be readily incorporated into N-acylamides upon co-incubation with linolenic, linoleic and oleic acids. In contrast to the low substrate specificity, volicitin-related compounds in the oral secretion of insects comprise only glutamine and glutamic acid.1,4–9) Spiteller et al. have suggested that the observed preference for glutamine was due to the predominant occurrence of glutamine in the insect gut and that the dominant occurrence could result from the selective transport of glutamine from the hemolymph into the gut.10)

In the present study, we report quantitative analyses of the amino acids in an oral secretion of Spodoptera litura larvae fed on artificial diets. The results of feeding experiments with amino acid-enriched artificial diets show that L-[α-15N] glutamine was incorporated into volicitin and N-linolenoyl-L-glutamine. A total of 13 amino acids have been identified as their TBDMS derivatives from the oral secretion of the caterpillar.11,12) The amino acids were identified by comparing the retention times and fragmentation patterns with those of authentic samples; for exam-

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Abbreviations: ESI, electrospray ionization; GABA, γ-aminobutyric acid; MTBSTFA, N-(tert-butyldimethylsilyl)-N-methyl trifluoroacetamide; TBDMS, tert-butyldimethylsilyl
Fig. 2. Amino Acid Composition in the Oral Secretion of *S. litura*.

Each value is shown as the mean ± SEM of three replications. Analytical conditions and sample preparations are described in the Experimental section.

ple, glutamine was identified as tri-TBDMS-glutamine which gave a base ion at *m/z* 431 and characteristic ions at *m/z* 488, 473 and 329. It was confirmed that negligible conversion of glutamine to glutamate and to pyroglutamate occurred during the sample preparation and GC-MS analysis. The mean quantities of the amino acids, in μM, for three batches of *S. litura* oral secretions are shown in Fig. 2. Alanine, aspartic acid, glutamic acid, serine, leucine and valine were the major free amino acids in the regurgitant. This result is consistent with the amino acid composition of the artificial diets. In contrast to previous literature, glutamine was not a dominant amino acid in this case. Although this difference might have been due to a different species of caterpillar used and fed on a different type of artificial diet, it was clearly demonstrated that glutamine was selectively incorporated into volicitin even when glutamine was not a dominant component in the regurgitant. To confirm the observed selectivity of glutamine, appropriate feeding experiments with amino acid-enriched artificial diets were conducted.

After 9 hours of feeding, the regurgitant was collected and analyzed by HPLC-MS. As shown in Fig. 3, the labeled amino acid was incorporated into volicitin and N-linolenoyl-L-glutamine, the percentage incorporation being calculated to be 25 ± 1.0% and 42 ± 2.1%, respectively. On the contrary, a negligible amount of the conjugate with L-leucine was detected. The retention time of N-linolenoyl-L-leucine under the HPLC-MS analytical conditions employed was confirmed by using a synthetic compound. When an alanine- or glutamic acid-enriched diet was fed, the corresponding acylamino acid conjugates were not detected in the secretion.

These results, together with the quantitative analyses of amino acids clearly suggest that the exclusive incorporation of glutamine into volicitin and N-linolenoyl-L-glutamine was not due to the dominant occurrence of this amino acid in the insect gut. Additional studies should be carried out to clarify how volicitin is synthesized within caterpillars.

**Experimental**

**General.** L-[α-15N] glutamine (15N, 98%) and L-[U-13C5] glutamic acid (13C, 20%) were purchased from Cambridge Isotope Laboratories. Caterpillar rearing, sample preparations and analyses of volicitin-related compounds from the oral secretions of *S. litura* were conducted as described. N-Linolenoyl-L-leucine was synthesized as described.

**Analysis of free amino acids.** A 100-μl volume of the sample was separated by an ODS Sep-pak cartridge (Waters), eluting with water. The eluate was further chromatographed in a column of cation-exchange resin [Dowex 50W × 8 (H+), 200–400 mesh, Muromachi Kagaku Kogyo Kaisha, 500-μl volume], which was eluted first with 5 ml of water and then with 5 ml of a 2 N ammonia aqueous. This cation-exchange clean-up procedure has been used for determining the free amino acids in physiological fluids.

The ammonia fraction (50-μl oral secretion equivalent) was concentrated in vacuo. Acetonitrile (150 μl) and MTBSTFA (50 μl) were added to the dried residue, and silylation of the compounds was accomplished as described. After diluting with dichloromethane, aliquots (1.0 μl) were analyzed by GC-MS (HP-5890 Plus series II gas chromatograph with a 30 m × 0.32 mm, 0.33 μm film thickness, HP-5MS capillary column, interfaced to an HP-5989B.
mass spectrometer; Hewlett-Packard). The column temperature was held at 60°C for 2 min after injection and then programmed to 10°C/min to 290°C.

Quantitative analyses of the amino acids used 10 µl of a GABA solution (2.5 mg/ml) added to the ammonia fraction as an internal standard. After silylation, the samples were analyzed by GC (HP-6850 gas chromatograph with a 25 m × 0.2 mm, 0.33 µm film thickness, HP-1 capillary column) under the same analytical conditions as those just described.

Feeding experiments with the amino acid-enriched diets. The last instar larvae of S. litura were fed on an artificial diet enriched with the amino acids (750 µg) L-[α-¹⁵N] glutamine, L-[U-¹³C₅] glutamic acid, L-leucine and L-alanine. The test amino acid was dissolved in an appropriate amount of water, and aliquots were added to a small amount of the diet (ca. 50 mg). The larvae consumed the amino acid-enriched diet within 30 min. The larvae were then fed on the artificial diets (not enriched with amino acid) for 9 hours prior to collecting the oral secretions.

¹⁵N isotopic enrichment measurements. Calculation of the percentage incorporation of the labeled glutamine into volicitin and N-linolenoyl-L-glutamine was accomplished as follows: \( \left( \frac{(m + 1)/m}{m/9} \right) \times 100 \), where \((m + 1)/m\) is the area ratio \( m/z 422 \) to \( m/z 421 \) for volicitin and the area ratio \( m/z 406 \) to \( m/z 405 \) for N-linolenoyl-L-glutamine. “Sample” represents the isotopically enriched conjugates \((m + 1)/m\) and “control” is the ratio \((m + 1)/m\) obtained for the control oral secretion. Each calculated result is presented as the mean ± SEM of three replications.

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References


