Note
Efficient Incorporation of Unsaturated Fatty Acids into Volicitin-Related Compounds in Spodoptera litura (Lepidoptera: Noctuidae)

Takako ABOSHI, Naoko YOSHINAGA, Koji NOGE, RitsuO NISHIDA, and Naoki MORI†

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyoku, Kyoto 606-8502, Japan

Received October 3, 2006; Accepted December 3, 2006; Online Publication, February 7, 2007
[doi:10.1271/bbb.60546]

We introduced efficient incorporation of unsaturated fatty acids into volicitin [N-(17-hydroxylinolenoyl)-L-glutamine] and N-linolenoyl-L-glutamine, insect-derived elicitors of plant volatiles, in the common cutworms Spodoptera litura by the incubation of larval gut tissues with unsaturated (linolenic, linoleic, and oleic acids) or saturated fatty acids (palmitic and stearic acids) sodium salt, and 1-[α-15N]glutamine.

Key words: volicitin; plant defense signal; fatty acids; Spodoptera litura

Plants release a blend of volatiles in response to caterpillar feeding damage, and these volatiles help parasites, natural enemies of caterpillars, to locate their host.1 Volicitin [N-(17-hydroxylinolenoyl)-L-glutamine], a fatty acid amide (FAA), has been identified as an elicitor of plant volatiles from the regurgitant of Spodoptera exigua (Noctuidae).2–4 Volicitin and structurally analogous FAAs, N-(17-hydroxylinolenoyl)-L-glutamine, N-linolenoyl-L-glutamine, and N-linoleoyl-L-glutamine, have been discovered in the regurgitant not only of Noctuidae but also of Sphingidae and Geometridae.3,5–10 However, only volicitin and N-linolenoyl-L-glutamine induce volatile emissions at natural concentrations.5 The absolute configuration of the hydroxylinolenoyl moiety of volicitin has been determined to be all S in high enantiomeric excess.11,12 The fatty acid moiety of volicitin is derived from diet lipids, and conjugation with L-glutamine of insect origin followed by 17-hydroxylation is performed by caterpillars.5,13

It remains a mystery why caterpillars have volicitin at the risk of parasitoid attack. Volicitin is thought to work as a surfactant in caterpillars because of its structure, but Yoshinaga et al. reported the specific incorporation of L-glutamine into volicitin-related compounds in vivo and in vitro in S. litura.8,13–15 Glutamine and linolenic acid are both essential for most lepidoptera larvae. These results indicate that volicitin might also play a role in the metabolism of caterpillars.16,17 Hence, we focused on fatty-acid selectivity as a substrate in FAA biosynthesis in S. litura.

The FAA composition of the last instars of S. litura reared on lettuce was analyzed by LC/MS, as shown in Fig. 1. Each compound was identified by comparing the retention time and molecular ions with those of authentic samples. The ions of volicitin, N-(17-hydroxylinoleoyl)-L-glutamine, N-linolenoyl-L-glutamine, and N-linoleoyl-L-glutamine were detected at m/z 421, 423, 405, and 407 (M − H)− in the negative ion mode. All FAAs are composed of linolenic acid or linoleic acid, and no other fatty acids coupled with glutamine were detected as main compounds.

Four fatty acids were identified as methyl esters by GC/MS from lettuce leaves (Fig. 2). They were identified by comparing the retention times and fragmentation patterns with those of authentic samples. Not only linolenic and linoleic but also palmitic and stearic acids were detected. Interestingly, there was no significant difference in amount between palmitic and linolenic acids, whereas no palmitic acid conjugated with glutamine was found in S. litura larvae. To confirm the selectivity of fatty acids, five fatty acids were incubated separately with gut tissue of larvae to biosynthesize each FAA in vitro, as described.13 The enzyme responsible for coupling fatty acids with glutamine is thought to be in larval gut tissues.

The amounts of FAAs biosynthesized in vitro are shown in Fig. 3. The FAAs were identified as explained above. Linolenic, linoleic, and oleic acids were conjugated with glutamine more than palmitic acid or stearic acid, but there were no significant differences in the amounts of conjugates among the unsaturated fatty acids. These results show that unsaturated fatty acids were more selected as substrates in FAA biosynthesis.

† To whom correspondence should be addressed. Tel: +81-75-753-6307; Fax: +81-75-753-6312; E-mail: mokurin@kais.kyoto-u.ac.jp

Abbreviations: FAA, fatty acid amide; LC/MS, liquid chromatography/mass spectrometry; GC, gas-liquid chromatography; ESI, electron spray ionization; GC/MS, gas-liquid chromatography/mass spectrometry
than saturated acids. Furthermore, the selectivity of linolenic acid in volicitin biosynthesis was not as rigorous as that of glutamine, as reported.15)

It is not well understood how volicitin is produced and metabolized in caterpillars, except for enzymatic decomposition to 17-hydroxylinolenic acid in the fore- and mid-guts.9) As far as we know, this is the first report of efficient incorporation of unsaturated fatty acids in volicitin-related compound biosynthesis.

Experimental

Caterpillar rearing. Larvae of the common cutworm S. littura were reared on an artificial diet, as described previously.13) To investigate the amounts of FAAs in the larvae, they were reared on lettuce leaves from the third to last instars, then dissected to obtain the gut contents.

Fig. 1. Mass Chromatograms of FAAs (Structures Shown) in S. littura Larvae.

1, Volicitin (M-1 = 421, tR = 7.5 min); 2, N-(17-Hydroxylinoleoyl)-L-glutamine (M-1 = 423, tR = 7.8 min); 3, N-Linolenoyl-L-glutamine (M-1 = 405, tR = 10.3 min); 4, N-Linoleoyl-L-glutamine (M-1 = 407, tR = 11.0 min). N-Oleoyl-L-glutamine (M-1 = 409, tR = 11.6 min). N-Palmitoyl-L-glutamine (M-1 = 383, tR = 11.5 min), N-Stearoyl-L-glutamine (M-1 = 411, and tR = 12.9 min) were not detected as main compounds.

Amounts of FAAs in the larvae. FAAs were analyzed using the following procedures: The alimentary canal containing gut contents of a larva was homogenized in 500 µl of 50% acetonitrile in water and centrifuged at 16,000 g for 5 min. The supernatant was filtered through a 0.45 µm hydrophilic PTFE membrane (Millipore, Bedford, MA), and an internal standard (1 µg palmitoleoyl-L-glutamine in 150 µl acetonitrile) was added to 150 µl of the sample. The solution was analyzed by LC/MS (LCMS2010 equipped with a LC-10ADvp pump, Shimadzu, Kyoto, Japan). The CDL temperature was 250 °C, the voltage was 1.5 kV, the nebulizer gas flow was 1.51/min, and the analytical mode was ESI-negative, SCAN. A reversed-phase column (Cosmosil 5C18-AR-o, 50 × 2.0 mm I.D., Nacalai Tesque, Kyoto, Japan) was eluted (0.2 ml/min) with a solvent of 30–90% acetonitrile containing 0.08% acetic acid in water containing 0.05% acetic acid for 15 min. The column temperature was maintained at 40 °C in the oven (CTO-10Avp column oven, Shimadzu).

Amounts of FAAs biosynthesized in vitro. FAAs biosynthesis in vitro was performed as described.13) Five microliters of sodium salt of fatty acid solution (150 µg/µl) and 50 µl of 15N-labeled L-glutamine solution (60 µg/µl) were added to 50 mM imidazole–HCl buffer (100 µl, pH 8.0) containing the whole gut tissue of a larva, and then the mixed solution was incubated at 30 °C for 6 h. After extraction, the amounts of 15N-labeled N-linolenoyl-L-glutamine were determined using a standard curve of known amounts of non-labeled amounts of N-linolenoyl-L-glutamine. The amounts (y ng) of N-linolenoyl-L-glutamine were satisfactory correlated with the [M-1] anion peak-area ratio [x, N-linolenoyl-L-glutamine (m/z 405)/N-palmitoleoyl-L-glutamine (m/z 381, the internal standard)], by the following equations: y (ng) = 0.39 x + 0.80, r2 = 0.99. In the same way, the amounts of N-linoleoyl-L-glutamine (m/z 407), N-oleoyl-L-glutamine (m/z 409), N-palmitoyl-L-glutamine (m/z 383), and N-stearoyl-L-glutamine (m/z 411) were obtained by the following equations: y = 0.26 x + 0.22, r2 = 0.99; y = 0.21 x – 0.03, r2 = 0.99; y = 0.13 x + 0.26, r2 = 1.0 and y = 0.13 x + 0.56, r2 = 0.94 respectively.

Analysis of fatty acids. Lettuce leaves (1 g) were homogenized in 5 ml of chloroform/methanol (2:1, v/v) and extracted overnight. The extracts were hydrolyzed with 20% KOH/methanol–water (4/6, v/v) solution at 85 °C for 1 h. After the solutions were adjusted to pH 1 with 6 N HCl, free fatty acids were extracted with 3 × 3 ml of diethyl ether, and washed with 3 × 3 ml of saturated Na2CO3 solution and distilled water. One portion of the upper layer (500 µl) was concentrated to dryness by nitrogen steam, dissolved in a mixture of methanol/acetic anhydride (200 µl, 1:1, v/v), and heated at 100 °C overnight. The resulting fatty acid methyl esters were dissolved in dichloromethane and analyzed by GC/MS (HP-5890 Plus series II gas chromatograph interfaced to a HP-5989B mass spectrometer; Hewlett-
Packard, Palo Alto, CA) with an HP-5 capillary column (30 m × 0.32 mm, 0.33 μm film thickness, Hewlett-Packard). The column temperature was programmed at 8 °C/min from 50 °C to 190 °C. The injector was kept at 250 °C and the detector at 290 °C.

Quantitative analyses of fatty acids used 100 ng of heptadecanoic acid methyl ester as an internal standard. The samples were analyzed by GC (HP-6850 gas chromatograph with a 25 m × 0.2 mm, 0.33 μm film thickness, HP-5 capillary column) under the same analytical conditions as those just described.

Acknowledgments

This study was partly supported by a Grant-in-Aid for Scientific Research (no. 15580090) and by the 21st century COE program for Innovative Food and Environmental Studies Pioneered by Entomomimetic Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

4) Turling, T. C. J., Alborn, H. T., Loughrin, J. H., and


