Structural Analysis of New Syringopeptins by Tandem Mass Spectrometry

Akira ISOGAI,1,2 Haruhisa IGUCHI,2 Jiro NAKAYAMA,2 Akihiko KUSAI,3 Jon Y. TAKEMOTO,4 and Akinori SUZUKI2

1 Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma-shi, Nara 630-01, Japan
2 Department of Applied Biological Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
3 JEOL Company Ltd., Akishima-shi, Tokyo 196, Japan
4 Department of Biology, Utah State University, Logan, Utah 84322, U.S.A.

Received February 22, 1995

New syringopeptins SP(SC)-1 and -2 were isolated from culture filtrates of phytopathogenic bacterium strain SC1 of Pseudomonas syringae pv. syringae. These syringopeptins were composed of a β-hydroxy fatty acid, a long sequence of aliphatic amino acids, and a lactone moiety of eight amino acids. The amino acid sequences were deduced from a comparison of their tandem mass spectra with those of known syringopeptins SP-22a and SP-25a. SP(SC)-1 and SP(SC)-2 resembled SP-22a, but differed from the latter by 3 amino acids.

Pseudomonas syringae pv. syringae is a phytopathogenic bacterium that causes various diseases in many agronomically important plants. Many strains of this species are known to produce phytoxic substances as virulence determinants.1,2 Until now, three phytoxins have been known: syringomycin, syringotoxin, and syringostatin. These toxins are cyclic lipodepsipeptides with a lactone ring composed of nine amino acids and a β-hydroxy fatty acid.3 Similar lipodepsipeptides have recently been isolated from another strain of P. syringae.3

Another group of more virulent phytoxins are produced by P. syringae pv. syringae and are termed syringopeptins. These are also cyclic lipodepsipeptides, but with higher molecular weights and longer peptide chains. Two types of syringopeptins, SP-22 and SP-25, are known.4,5 The syringopeptins are more phytotoxic than the three shorter phytoxins and may act synergistically with the latter three.3

From our research of phytoxins produced by a strain of P. syringae pv. syringae isolated from sugar cane (SC1), we have reported that this bacterial isolate produced syringomycin.5,6 We further analyzed the culture filtrate of the bacterial isolate and found two substances similar to syringopeptins. However, they were structurally different from SP-22 and SP-25, and were termed SP(SC)-1 and SP(SC)-2. We report here tandem mass spectroscopic analyses of SP(SC)-1 and SP(SC)-2, and propose the deduced structures of these new phytoxins.

Bacterial strain SC1 of P. syringae pv. syringae was grown in a potato-dextrose medium.6,7 The semi-purified fraction containing syringomycin was applied to an ODS column for HPLC. Two peaks were observed with longer retention times than that of syringomycin E, and these peaks roughly corresponded to SP-22 and SP-25.41 The peak materials were isolated, and a FAB mass analysis indicated (M + H)+ ions at m/z 2157 and 2185, respectively. This indicated that the substances were different from the known syringopeptins, probably not in the length of the fatty acids, but in the species of the amino acids. These putative novel substances were termed SP(SC)-1 and SP(SC)-2, respectively.

Tandem mass spectrometry is useful for sequence analyses of peptidyl compounds,8 although the limiting molecular weight applicable to this analysis is normally considered to be around 2000 daltons. Thus, for high-molecular-weight compounds, a doubly charged ion generated in a four-sector mass spectrometer is sometimes selected for tandem mass analysis.

Two known syringopeptins, SP-22a (MW 2142) and SP-25a (MW 2397) in Fig. were used to test this strategy, and to explore the potential of tandem mass spectrometry for structural elucidation of the new syringopeptins. Initially, we selected a doubly charged ion from each of these specimens for further analysis, because rather strong peaks were observed at the double-charged positions. However, neither gave fine fragment ions for comparison and for use in the structural analysis. We then tried to degrade the (M + H)+ ions and succeeded in obtaining clear data indicating the sequence of the peptides as shown in Table. In the upper parts of the spectra (m/z 750), sequential Y-type fragments accompanying smaller X- and Z-type ions were detected. The smallest Y-type ion was observed at m/z 776 and 793 in SP-22a and SP-25a, respectively. These would correspond to the ions composed of the lactone moieties, and sequence information within these moieties was reflected in the spectra. The difference between the two lactone parts was a single amino acid substitution. The lower parts of the spectra were composed mainly of N-terminal B-type ions. Furthermore, similar B-type fragments starting from the prolyl residue were observed. These were probably generated after initial cleavage of the peptide bond between the 2-aminoxybutyroyl and prolyl residues and are tentatively termed B-type ions (Table). Thus, most of the observed ions in the tandem mass spectra could be assigned.

SP(SC)-1 and SP(SC)-2 indicated (M + H)+ ions at m/z 2157 and 2185, respectively. The difference in the molecular weights of 28 mass units would be derived from the difference in fatty acid residues, since the fatty acids are elongated by 2-carbon units and this relationship is found with other syringopeptins. Furthermore, the molecular weight difference between SP-22a and SP(SC)-1 of 14 mass units was not due to a difference in the fatty acid residues, but rather to a difference in the peptide parts. When SP(SC)-2 was applied to tandem mass spectrometry, it gave a similar spectrum to that of SP-22a, but with slight differences. The fragment ions were compared with those in the spectra of the specimens and are summarized in Table.

The hydrophobic properties of the linear parts in syringopeptins SP-22 and SP-25 were taken into consideration in deducing the sequences of SP(SC)-1 and SP(SC)-2. In the spectrum, the Y-type fragments were dominant in the higher part, and sequential B-type ions were observed in the lower part. The C-terminal Y-type ions from m/z 777 to 1172 are common in the spectra of SP-22a and SP(SC)-2. This suggests the structural identity of the C-terminal half (after the 10th amino acid), including the lactone ring between the two peptides. However, information on the sequence within the lactone parts could not be obtained for SP(SC)-2. The Y14 fragment in the spectrum of SP-22a at m/z 1255 corresponds to an m/z 1241 fragment for SP(SC)-2, indicating that 2-
Structures of New Syringopeptines

| SP-22a | HDE. Dhb-Pro-Val-Ala-Ala-Val-Ala-Dhb-Ala-Thr-Ser-Ala-Dhb-Ala-Dab-Dab-Tyr |
| SP-25a | HDE. Dhb-Pro-Val-Ala-Ala-Leu-Ala-Dhb-Dalh-Ala-Ala-Dhb-Thr-Ser-Ala-Ala-Dab-Dab-Tyr |
| SP(SC)-2 | HDE. Dhb-Pro-Leu-Ala-Ala-Leu-Val-Dhb-Ala-Ala-Ala-Dhb-Thr-Ser-Ala-Ala-Dab-Dab-Tyr |
| SP(SC)-1 | HDE. Dhb-Pro-Leu-Ala-Ala-Leu-Val-Dhb-Ala-Ala-Ala-Dhb-Thr-Ser-Ala-Ala-Dab-Dab-Tyr |

Fig. Structures of SP-22a, SP-25a, SP(SC)-1, and SP(SC)-2.

Abbreviations: HDE, 3-hydroxydodecanoic acid; HDD, 3-hydroxydodecanoic acid; Dhb, 2-aminomethylenebutyric acid (dehydrothreonine); Dhp, 2-aminomethylenepropionic acid (dehydroalanine).

Table Fragment Ions in the Tandem Mass Spectra of SP-22a, SP-25a, and SP(SC)-2

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SP-22a</td>
<td>777</td>
<td>860</td>
<td>931</td>
<td>1002</td>
<td>1103</td>
<td>1172</td>
<td>1255</td>
<td>1354</td>
<td>1453</td>
<td>1524</td>
<td>1595</td>
<td>1694</td>
<td>1793</td>
<td>1890</td>
<td>1973</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP-25a</td>
<td>793</td>
<td>876</td>
<td>947</td>
<td>1018</td>
<td>1119</td>
<td>1188</td>
<td>1271</td>
<td>1370</td>
<td>1453</td>
<td>1524</td>
<td>1595</td>
<td>1708</td>
<td>1878</td>
<td>1949</td>
<td>2048</td>
<td>2145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP(SC)-2</td>
<td>777</td>
<td>860</td>
<td>931</td>
<td>1002</td>
<td>1103</td>
<td>1172</td>
<td>1255</td>
<td>1354</td>
<td>1453</td>
<td>1524</td>
<td>1595</td>
<td>1708</td>
<td>1878</td>
<td>1949</td>
<td>2048</td>
<td>2145</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aminodehydrobutyric acid (dehydrothreonine) at the 9th position in SP-22a was replaced by a homologous amino acid, 2-aminomethylenepropionic acid (dehydroalanine) in SP(SC)-2. Two valines at the 4th and 7th positions in SP-22a were also replaced with leucine in SP(SC)-2. Since leucine and isoleucine could not be differentiated in the mass spectra, we tentatively represent both leucine and isoleucine by leucine. Thus, only three amino acids were different between SP-22a and SP(SC)-2 among 22 amino acids.

The deduced structures of the new syringopeptins are shown in Fig. SP(SC)-1 bears a $\beta$-hydroxydodecanoyl residue instead of the $\beta$-hydroxydodecanoyl residue in SP(SC)-2 at the N-terminus. From analyses of syringomycin production genes syrB and syrD, strain SC1 shows significant evolutionary divergence from other syringomycin-producing isolates. The small but significant difference in the syringopeptin amino acids may reflect this divergence. The use of tandem mass spectra to analyze molecules of over 2000 daltons like the syringopeptins is rare. Our success might be due to the unique properties of these peptides, with hydrophobic linear peptides and hydrophilic lactone moieties. Our results show that tandem mass spectrometry can be useful for structural analyses of certain >2000-dalton peptides.

Experimental

P. syringae pv. syringae strain SC1 isolated from sugar cane was cultured in a potato medium at 26.5°C for 9 days as described in previous reports. The culture filtrate was acidified to pH 3 with acetic acid and then applied to a column of Amberlite XAD-7. The fraction eluted with 80% methanol containing 0.1% acetic acid was concentrated and applied to an SSC-OIS-H-5251 column in the HPLC system. The column was eluted with a linear gradient of 20% to 80% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 2.0 ml/min. The chromatogram was monitored by absorbance at 220 nm. The mass spectral analysis employed an SX102/102 tandem mass spectrometer (JEOL). The samples were mixed with glycine: thioglycerin (1:1) as a matrix and ionized by fast-atom bombardment. The ions were accelerated at 8 keV and introduced into the first mass spectrometer. Daughter ions in the tandem mass spectra were yielded by collision activation with helium and analyzed in the second mass spectrometer.

References

5) N. S. Iacobellis, P. Labernicocca, I. Grgurina, M. Simmaco, A.
A. Isogai et al.


