Pergamon

Tetrahedron Letters 41 (2000) 1429–1431

TETRAHEDRON LETTERS

Peptaivirins A and B, two new antiviral peptaibols against TMV infection

Bong-Sik Yun,[a] Ick-Dong Yoo,[a] Young Ho Kim,[b] Young-Sook Kim,[c] Sang-Jun Lee,[a] Kab-Sig Kim [c] and Woon-Hyung Yeo [c,*]

[a]*Korea Research Institute of Bioscience and Biotechnology, P.O. Box 115, Yusong, Taejon 305-600, South Korea*
[b]*School of Applied Biology and Chemistry, Seoul National University, Suwon 441-744, South Korea*
[c]*Korea Ginseng and Tobacco Research Institute, Yusong, Taejon 305-345, South Korea*

Received 4 November 1999; revised 29 November 1999; accepted 1 December 1999

Abstract

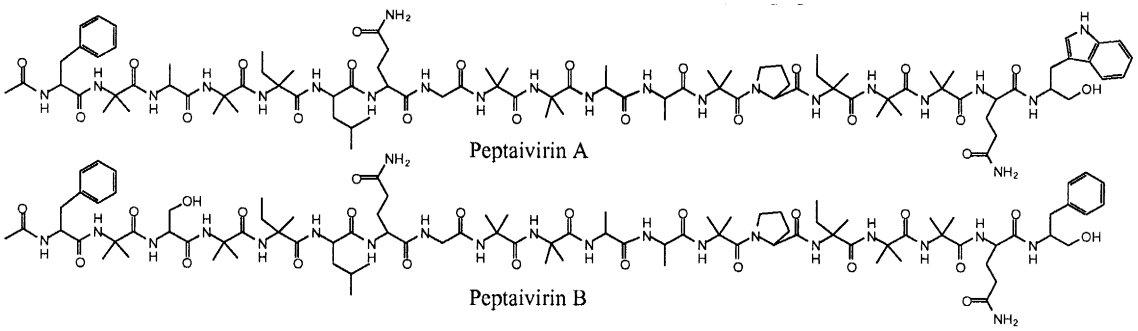
Two new peptaibols, peptaivirins A and B, have been isolated from the unidentified fungus KGT142 and their structures were assigned as AcPhe-Aib-Ala-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Trpol and AcPhe-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Pheol, respectively, on the basis of various spectroscopic analyses.

The peptaibols are characterized by structural features with an N-terminal acylated amino acid residue and a C-terminal amino alcohol on a lipophilic amino acid chain including many α,α-dialkylated amino acids, α-aminoisobutyric acid (Aib) and isovaline (Iva). Recently, several peptaibols have been isolated from *Trichoderma* spp. as antimicrobial substances.[1] In our search for antiviral agents against the tobacco mosaic virus (TMV) infection to the tobacco plant *Nicotiana tabacum* cv. Xanthi-nc, we have isolated two new active peptaibols, named peptaivirins A and B, from the solid culture of the unidentified fungus KGT142. We herein describe the isolation, structural elucidation and inhibitory effect on the TMV infection of both compounds.

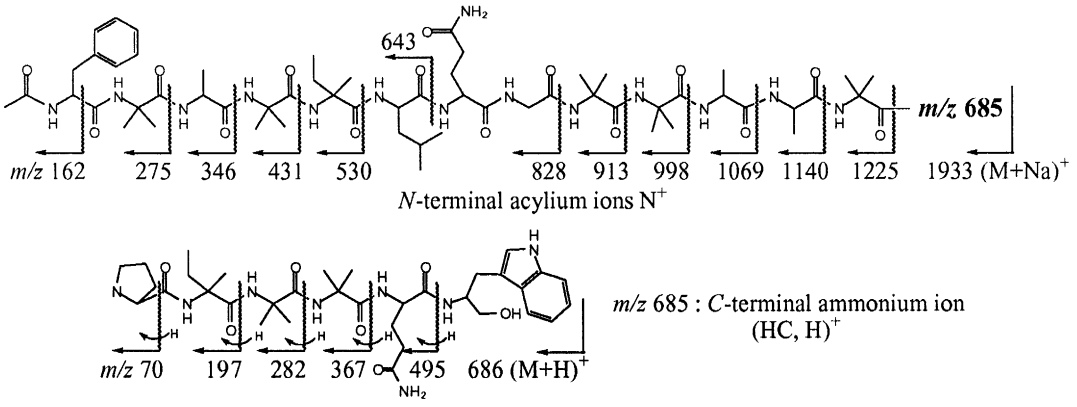
An ethanolic extract of the solid culture of the fungus KGT142 was purified by solvent partition, SiO_2 and ODS flash columns, reverse-phase TLC and HPLC consecutively to give peptaivirins A and B.[2]

* Corresponding author.

0040-4039/00/$ - see front matter
PII: S0040-4039(99)02308-4



Ninhydrin reaction of the hydrolysate of peptaivirin A suggested that this compound had a peptidic character. This was also supported by IR absorptions near 1660 and 1545 cm^{-1} attributable to amide carbonyl and amide NH groups, respectively. Amino acid analysis of the total acidic hydrolysate of peptaivirin A provided the following normal amino acid composition; Phe (1), Ala (3), Leu (1), Glx (2), Gly (1), Pro (1). In addition to ^{1}H and ^{13}C NMR spectra, the lipophilicity and molecular weight of *m/z* 1934 $(M+Na)^+$ suggested that peptaivirin A was a peptaibol class of antibiotics. ^{1}H NMR spectrum in DMSO-d_6 exhibited the peaks due to several Aibs at δ 1.25–1.43, α-protons at δ 3.5–4.3 and tryptophan, phenylalanine and a lot of exchangeable protons collapsed on shaking with D_2O in the low-field region between δ 6.5 to 10.8. The COSY and TOCSY spectra revealed the presence of Phe (1), Ala (3), Iva (2), Leu (1), Gln (2) but not Glu, Gly (1), Pro (1) and tryptophanol (Trpol, 1) in combination with HMBC. The molecular formula of $C_{92}H_{144}N_{22}O_{22}$ suggested from FAB-MS data and amino acid composition established the presence of seven Aibs in peptaivirin A. The sequence of 19 component amino acids was determined by tandem mass, which provided specific ions derived from the N-terminal at *m/z* 162, 275, 346, 431, 530, 643, 828, 913, 998, 1069, 1140 and 1225, along with a fragment ion peak at *m/z* 685 corresponding to a counterpart of *m/z* 1225, as shown in Scheme 1. The collision-induced dissociation (CID) spectrum of *m/z* 685 gave the fragment peaks at *m/z* 70, 197, 282, 367 and 495 to assign the C-terminal sequence as Pro-Iva-Aib-Aib-Gln-Trpol. Therefore, the structure of peptaivirin A was established to be a new 19-residue peptaibol with the sequence of AcPhe-Aib-Ala-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Trpol and confirmed by NOEs observed between amide protons in NOESY spectrum.



Scheme 1.

The structure of peptaivirin B was determined by direct comparison with CID mass spectrum of peptaivirin A. The mass spectrum of peptaivirin B gave the ion peaks at *m/z* 275, 362, 447, 546, 659, 844, 929, 1014, 1085, 1156 and 1241 corresponding to the N-terminal sequence, AcPhe-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib, which was very similar to peptaivirin A, the difference being replaced as Ala by Ser. The C-terminal sequence was established by the CID spectrum of *m/z* 646, a counterpart peak of *m/z* 1241, which exhibited the ion peaks at *m/z* 169, 197, 282, 367 and 495 consistent with the C-terminal sequence of Pro-Iva-Aib-Aib-Gln-Pheol. As a result, peptaivirin B was assigned as a new sequence of the peptaibol class, AcPhe-Aib-Ser-Aib-Iva-Leu-Gln-Gly-Aib-Aib-Ala-Ala-Aib-Pro-Iva-Aib-Aib-Gln-Pheol.

Inhibitory activity of peptaivirins A and B against TMV infection to the tobacco plant (Xanthi-nc) was estimated according to the method used by Kubo et al.[3] Peptaivirins A and B showed strong inhibitory effects of 74 and 79%, respectively, at a concentration of 10 μg/ml against TMV infection. The antiviral effect of peptaibols against TMV infection has been reported for the first time.

References

1. Duval, D.; Rebuffat, S.; Goulard, C.; Prigent, Y.; Becchi, M.; Bodo, B. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2147–2153. Rebuffat, S.; Goulard, C.; Bodo, B. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1849–1855. Auvin-Guette, C.; Rebuffat, S.; Prigent, Y.; Bodo, B. *J. Am. Chem. Soc.* **1992**, *114*, 2170–2174.
2. Ethanolic extract of the solid culture of the fungus KGT142 was partitioned between BuOH and H_2O, and the BuOH-soluble portion was subjected to a column of SiO_2 eluted with $CHCl_3$/MeOH stepwise. The active fractions were combined and chromatographed on ODS flash column by increasing the ratio of MeOH to water. The active eluate, 90% aq. MeOH fraction, was further purified with ODS–TLC developed with 90% aq. MeOH, followed by HPLC (ODS column, 62% aq. MeCN, 3.0 ml/min) to give peptaivirins.
3. Kubo, S.; Ikeda, T.; Imaizumi, S.; Takanami, Y.; Mikami, Y. *Ann. Phytopath. Soc. Jpn*. **1990**, *56*, 481–487.