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Primary Structures of Antibiotic Peptides, Trichocellins-A and -B from *Trichoderma viride*

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Abstract: *Trichocellins-A and -B are catecholamine-releasing peptides isolated from the fungus Trichoderma viride. Their amino acid sequences were determined by mass spectrometry and NMR spectroscopy. Trichocellins-A-I~VIII and -B-I and -II are each composed of 20 residues, having an N-terminal acetyl group and phenylalaninol as the C-terminal residue.*

Peptaibols are a family of peptides which form voltage-dependent ion channels in lipid bilayers[1] and, therefore, can provide insights into the structural principles of ion channel proteins. Peptaibols induce Ca^{2+}-dependent catecholamine secretion from bovine adrenal chromaffin cells.[2] We have isolated new catecholamine-releasing peptides, trichocellins (TC), from conidia of the fungus *Trichoderma viride*,[3] which is a strain producing large amounts of cellulase. These 20-residue peptides were classified as peptaibols like alamethicin[4] and trichosporin[5] because they contain an unusual amino acid, α-aminoisobutyric acid (Aib), in a high ratio, the N-terminal is blocked by an acetyl group, and the C-terminal residue is phenylalaninol (Pheol). In this paper, we describe the structural elucidations of TCs-A-I~VIII , and TCs-B-I and -II. TC-A-I and TC-A-II are identical with paracelsin B[6] and saturnisporin SA II.[7]

In the ^{1}H- and ^{13}C-NMR spectra of TCs, the signals of Aib and Pheol were recognized together with those of an acetyl group, which suggests that the N-terminal of TCs is acetylated. Thus, TCs were regarded as peptaibols. The configurations of optically active amino acids and Pheol were determined by HPLC according to the reported method,[8] which showed that Iva has the D-configuration while the other amino acids and Pheol have the L-configuration. TCs-A were negative to ninhydrin reagent and were not esterified with diazomethane. These facts indicate the absence of free amino and carboxy groups in the molecules. Thus, the three Glu residues detected by amino acid analysis arise from three Gln. On the other hand, TCs-B were negative to ninhydrin reagent but were esterified with diazomethane. In the ^{1}H-NMR spectrum of TC-B-II treated with diazomethane, a methoxy signal (δ_H 3.66) was observed, indicating that TCs-B contain one Glu residue in the molecules.

Pneumatically assisted electrospray[9] mass spectrometry of a main component, TC-B-II [Ac (1), Aib (8), Ala (3), Gln (2), Glu (1), Gly (1), isovaline (Iva, 1), Leu (1), Pheol (1), Pro (1) and Val (1)], showed multiply charged ions, $(M + 2H)^{2+}$ and $(M + 3H)^{3+}$, *m/z* 969.5 and 646.5, indicating that the molecular weight is 1936 ($C_{90}H_{148}N_{22}O_{25}$). In addition to the above ions, two notable fragment ions were recognized at *m/z* 789 and 1149. This observation suggests that TC-B-II cleaves preferentially at a labile Aib-Pro bond,[5b] as seen in the FAB-MS of peptaibols. The collision-induced dissociation (CID) spectrum of *m/z* 1149 ion

showed successive acylium ions assignable as shown in Fig. 1(a). To obtain the fragment ions lower than *m/z* 284, a CID experiment on the *m/z* 284 ion was performed and gave ions of *m/z* 199, 128 and 43 due to successive losses of Aib, Ala and Aib. Therefore, the structure of the N-terminal oligopeptide was elucidated to be as follows; Ac-Aib-Ala-Aib-Ala-Aib-Ala-Gln-Aib-Leu-Aib-Gly-Aib-Aib. On the other hand, the CID spectrum of the *m/z* 789 ion gave successive losses of Pheol, Gln, Glu, Iva (or Val) and Aib, leaving Pro + Val (or Iva, *m/z* 197) (Fig. 1(b)). The CID spectrum of the *m/z* 197 ion (not shown here) showed the a-type ion (*m/z* 70) arising from proline.[5b] Thus, the structure of the C-terminal oligopeptide was determined to be Pro-Val (Iva)-Aib-Iva (Val)-Glu-Gln-Pheol. The differentiation of isomeric amino acids, Iva and Val, was carried out by NOESY as shown in Fig. 2. The connectivities allowed us to locate Iva and Val at positions 17 and 15, respectively. Therefore, the primary structure of TC-B-II was obtained by connecting the two fragments between Aib[13] and Pro[14] : Ac-Aib-Ala-Aib-Ala-Aib-Ala-Gln-Aib-Leu-Aib-Gly-Aib-Aib-Pro-Val-Aib-Iva-Glu-Gln-Pheol.

The structures of TCs-B-I and -A-I~VIII were determined in the same manner as that of TC-B-II. The structures and characteristics of all TCs are shown in Tables 1 and 2, respectively.

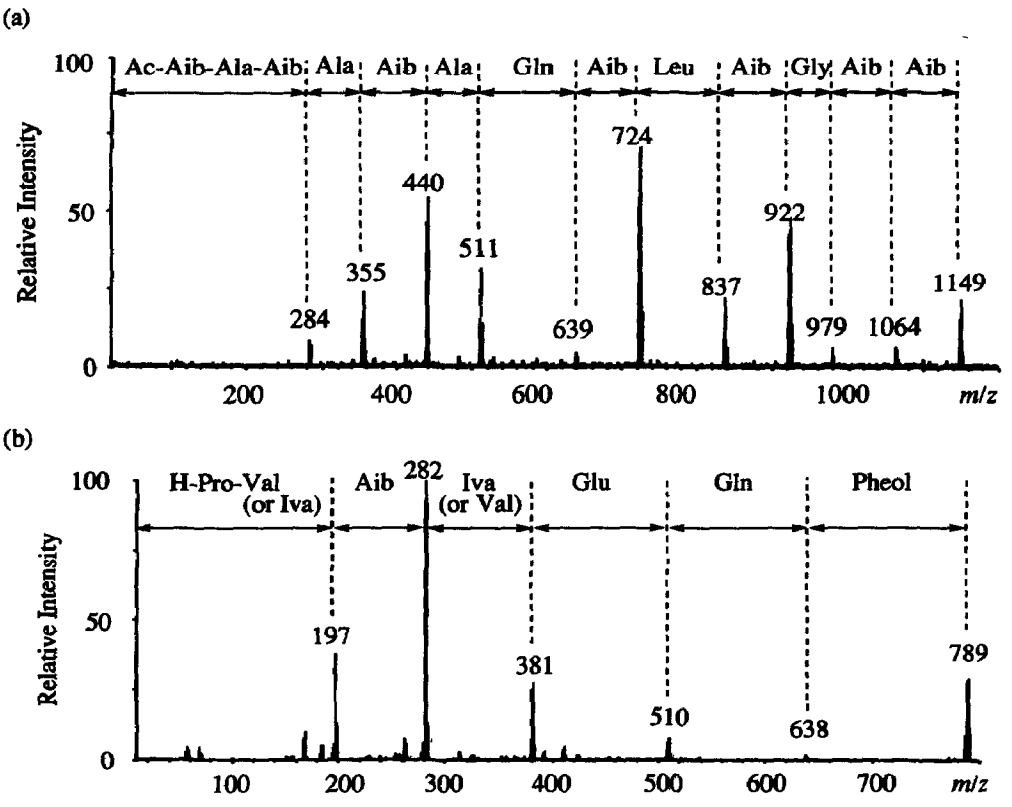

Fig. 1. Product Ion Spectra of the *m/z* 1149 (a) and 789 (b) Ions from Trichocellin-B-II.

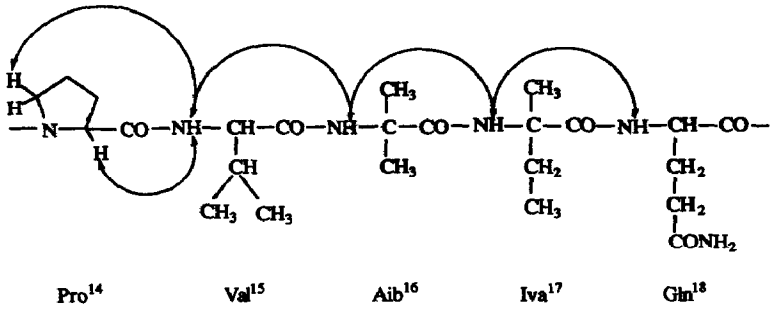

Fig. 2. Diagnostic NOE Connectivities Observed in the C-Terminal Part.

Table 1. Primary Structures of Trichocellins-A and -B.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A-I	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Leu-	Aib-	Gly-	Aib-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
A-II	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Leu-	Aib-	Gly-	Aib-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol
A-IIII	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Ile-	Aib-	Gly-	Aib-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
A-IV	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Ile-	Aib-	Gly-	Aib-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol
A-V	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Leu-	Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
A-VI	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Leu-	Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol
A-VII	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Ile-	Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
A-VIII	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Ile-	Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol
B-I	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Leu-	Aib-	Gly-	Aib-	Aib-	Pro-	Val-	Aib-	Aib-	Glu-	Gln- Pheol
B-II	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Ala-	Gln-	Aib-	Leu-	Aib-	Gly-	Aib-	Aib-	Pro-	Val-	Aib-	Iva-	Glu-	Gln- Pheol

Table 2. Characteristics for Trichocellins-A and -B.

	Formula	MW (nominal)	mp (°C)[a]	Molecular ellipticity [θ] (° cm^2 dmol^{-1})[b] 207 nm	221 nm
A-I	$C_{89}H_{147}N_{23}O_{24}$	1921	182—186	–290200	–247200
A-II	$C_{90}H_{149}N_{23}O_{24}$	1935	175—177	–318000	–277500
A-III	$C_{89}H_{147}N_{23}O_{24}$	1921	178—181	–312200	–275000
A-IV	$C_{90}H_{149}N_{23}O_{24}$	1935	182—185	–301200	–272600
A-V	$C_{91}H_{151}N_{23}O_{24}$	1949	225—228	–320300	–247700
A-VI	$C_{92}H_{153}N_{23}O_{24}$	1963	242—245	–321300	–255800
A-VII	$C_{91}H_{151}N_{23}O_{24}$	1949	260—263	–332000	–266600
A-VIII	$C_{92}H_{153}N_{23}O_{24}$	1963	253—255	–351700	–289700
B-I	$C_{89}H_{146}N_{22}O_{25}$	1922	181—184	–274800	–238600
B-II	$C_{90}H_{148}N_{22}O_{25}$	1936	189—192	–294900	–255600

[a] Uncorrected. [b] Measured in MeOH.

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