## Communications to the Editor

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# FURTHER INVESTIGATION OF PEPTIDE ANTIBIOTIC, HYPELCIN A: ISOLATION AND STRUCTURES OF HYPELCINS A-I, A-II, A-III, AND A-IV

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Four new antibiotics, hypelcins A-I, A-II, A-III, and A-IV were isolated from hypelcin A, previously thought to be a single compound, by high performance liquid chromatography and their structures have been established as (1-4), respectively, by fast atom bombardment mass spectrometry.

KEYWORDS —— peptide antibiotic; hypelcin; peptaibol; α-aminoisobutyric acid; fast atom bombardment mass spectrometry; peptide antibiotic HPLC; *Hypocrea peltata*

We have recently reported that the structures of hypelcin A (5)[1] and trichopolyns I and II,[2] are α-aminoisobutyric acid (Aib)-containing peptide antibiotics which were named peptaibols.[3] The peptaibols such as alamethicins,[4] suzukacillin,[5] trichotoxin,[6] emerimicins,[7] and zervamicins[8] are of considerable biological interest because they facilitate ion transport across membranes by a mechanism involving pore formation in the membranes.[9] Hypelcin A has an uncoupling activity[10] on rat liver mitochondria and prevents[1,11] the growth of *Lentinus edodes*, a Japanese edible mushroom. Hypelcin A was crystalline and uniform in thin layer chromatography. However, very recently we have found that hypelcin A is a mixture of more than four components, using high performance liquid chromatography (HPLC). We here report the isolation and structural elucidation of the hypelcins A I-IV (1-4).

Crude hypelcin A[11] (HPL chromatogram, Fig. 1) was purified by reversed-phase HPLC on Chemopak Nucreosil (60 cm x 8 mm i.d. x 2) using methanol-water-2-propanol (45:27:20) to yield hypelcins A-I, mp 265-266°C, $[\alpha]_D^{21}$ -17.0° ($c$=1.0, MeOH) and A-II, mp 254-256°C, $[\alpha]_D^{21}$ -16.0° ($c$=1.0, MeOH) as the major components, and hypelcins A-III, mp 256-258°C, and A-IV, mp 259-261°C as the minor components. The amino acid analysis of these compounds suggests that hypelcins A-I and A-II[12] are single compounds, but hypelcins A-III and A-IV still contain small amounts of other peptides (Table I). Molecular weights of 1927, 1913, 1941, and 1927 were assigned to the hypelcins A-I, A-II, A-III, and A-IV on the basis of their respective molecular ion species as they appeared on the positive ion fast atom bombardment (FAB) mass spectra,[13,14] Table I.

Hypelcin A-I has the same amino acid composition as hypelcin A. The presence of an acetyl group and twenty three amide carbonyl groups was revealed by,

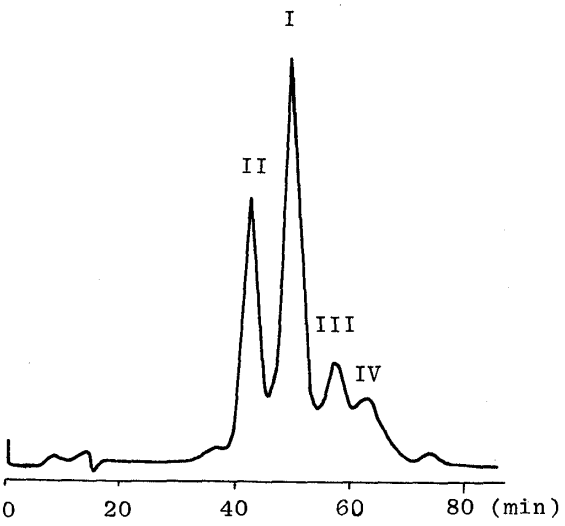


Fig. 1. HPLC of Hypelcin A

Conditions: column, TOYO SODA ODS 120A (30 cm x 7.8 mm i.d.); flow rate, 1.5 ml/min; eluant, methanol-water-2-propanol (45:25:20); detecter setting, UV 220 nm.

respectively, a signal at δ 2.05 ppm in the $^{1}$H-NMR spectrum and a number of carbon signals at δ 171-179 ppm in the $^{13}$C-NMR spectrum (100 MHz). The positive FAB mass spectrum of hypelcin A-I showed three acyl cleavage ion series indicating sequences Ia, Ib, and Ic, as shown in Figure 2. Sequences Ia and Ib have all the amino acids of hypelcin A-I. Furthermore, the sum of the molecular weights of sequences Ia and Ib agrees with that of hypelcin A-I and the presence of leucinol (Lol)[15] was confirmed by gas chromatography mass spectrometry[1,11] of *N,O*-ditrifluoroacetyl derivative from the hydrolysate. Hence, the structure of hypelcin A-I can be represented as (1).

The assignment of a structure (2) for hypelcin A-II is as follows:

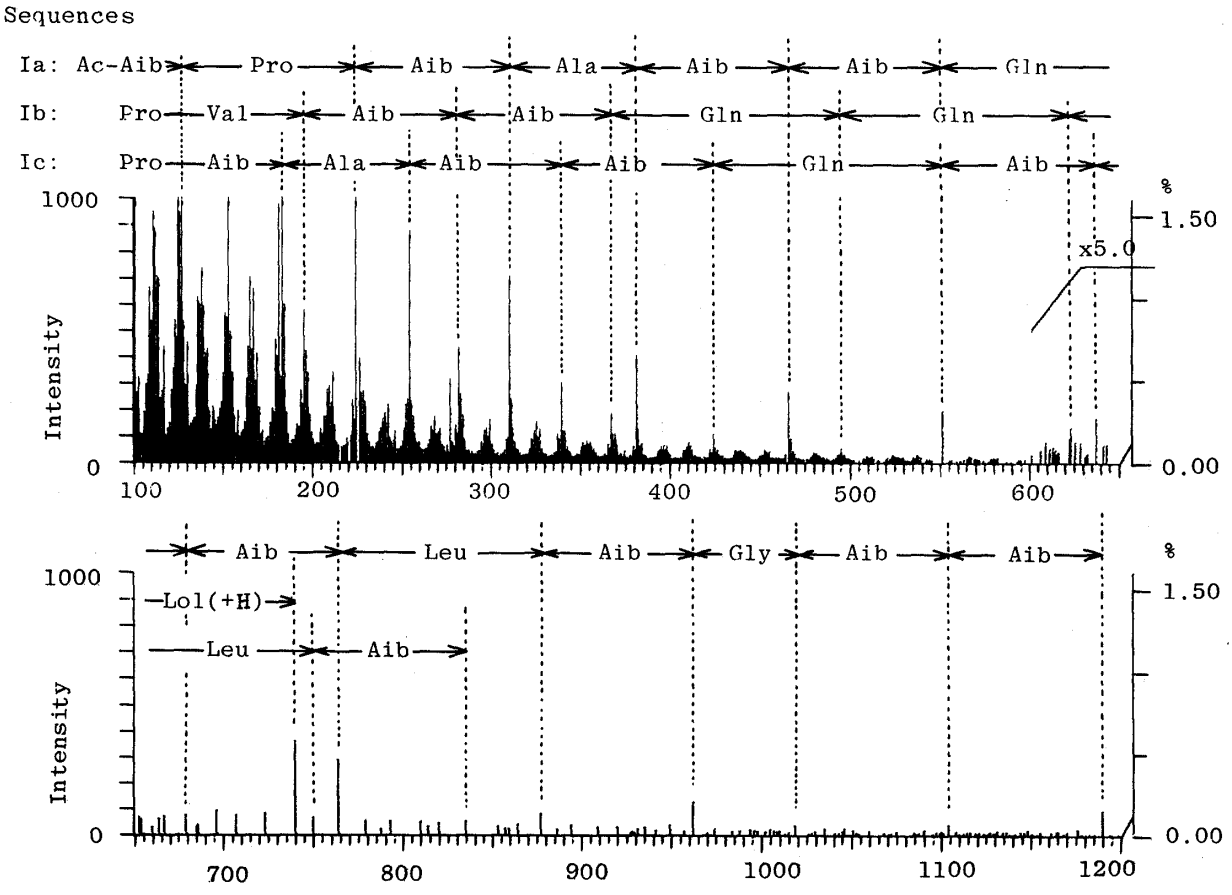


Fig. 2. FAB Mass Spectrum[16] of Hypelcin A-I

Table I. Amino acid composition and Molecular Ions of Hypelcins

| | Amino acid composition[a] | | | | | | | | Molecular ions[b] | | MW |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Glu | Pro | Aib | Ala | Gly | Val | Leu | Ile | $[M + Na]^+$ | $[M + K]^+$ | |
| A-I | 3 | 2 | 9-10 | 1 | 1 | 1 | 1 | 0 | 1950 | 1966 | 1927 |
| A-II | 3 | 2 | 8-9 | 2 | 1 | 1 | 1 | 0 | 1936 | 1952 | 1913 |
| A-III | 3 | 2 | 9-10 | 1 | 1 | 1 | 0.79 | 0.14 | 1964 | 1980 | 1941 |
| A-IV | 3 | 2 | 9-10 | 1 | 1 | 1 | 0.36 | 0.87 | 1950 | 1966 | 1927 |

[a]Determined by amino acids analysis, [b]Measured by FAB mass spectrometry.[14]

Table II. Diagnostic Ions Observed in FAB Mass Spectra[16] of the Hypelcins

| Series | Acyl cleavage ions, *m/z* | | | | | | | | | | | | | Series | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ac-1 | 2 | 3[a] | 4 | 5 | 6 | 7[b] | 8 | 9 | 10 | 11 | 12 | 13, | | 14 | 15 | 16 | 17 | 18 | 19 | Lol[c] |
| a Ia | 128 | 225 | 310 | 381 | 466 | 551 | 679 | 764 | 877 | 962 | 1019 | 1104 | 1189, | b Ib | 197 | 282 | 367 | 495 | 623 | 740 | |
| a IIa | 128 | 225 | 310 | 381 | 466 | 537 | 665 | 750 | 863 | 948 | 1005 | 1090 | 1175, | b IIb | 197 | 282 | 367 | 495 | 623 | 740 | |
| a IIIa | 128 | 225 | 310 | 381 | 466 | 551 | 679 | 764 | 877 | 962 | 1019 | 1104 | 1190, | b IIIb | 197 | 282 | 367 | 495 | 623 | 754[d] | |
| a IVa | 128 | 225 | 310 | 381 | 466 | 551 | 679 | 764 | 877 | 962 | 1019 | 1104 | 1189, | b IVb | 197 | 282 | 367 | 495 | 623 | 740 | |
| c Ic | | | 183 | 254 | 339 | 424 | 551 | 637 | 750 | 835 | | | | | | | | | | | |
| c IIc | | | 183 | 254 | 339 | 410 | 537 | 623 | | 821 | | 963 | | | | | | | | | |
| c IIIc | | | 183 | 254 | 339 | 424 | 551 | 637 | | | 892 | 977 | | | | | | | | | |
| c IVc | | | 183 | 254 | 339 | 424 | 551 | 637 | 750 | 835 | 892 | | | | | | | | | | |

[a]The ion peak *m/z* 183 in series c was formulated as H-Pro-Aib residue, [b]Acyl cleavage – H in the series c, [c]Acyl cleavage +H, [d]Unassinged amino alcohol.

| Hypelcin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A-I(1) | Ac- | Aib- | Pro- | Aib- | Ala- | Aib- | Aib- | Gln- | Aib- | Leu- | Aib- | Gly- | Aib- | Aib- | Pro- | Val- | Aib- | Aib- | Gln- | Gln- | Lol |
| A-II(2) | Ac- | Aib- | Pro- | Aib- | Ala- | Aib- | Ala- | Gln- | Aib- | Leu- | Aib- | Gly- | Aib- | Aib- | Pro- | Val- | Aib- | Aib- | Gln- | Gln- | Lol |
| A-III(3) | Ac- | Aib- | Pro- | Aib- | Ala- | Aib- | Aib- | Gln- | Aib- | Leu- | Aib- | Gly- | Aib- | Aib- | Pro- | Val- | Aib- | Aib- | Gln- | Gln- | $C_7H_{16}NO$ |
| A-IV(4) | Ac- | Aib- | Pro- | Aib- | Ala- | Aib- | Aib- | Gln- | Aib- | Ile- | Aib- | Gly- | Aib- | Aib- | Pro- | Val- | Aib- | Aib- | Gln- | Gln- | Lol |
| A(5) | Ac- | Aib- | Pro- | Aib- | Ala- | Aib- | Aib- | Gln- | Leu- | Aib- | Gly- | Aib- | Aib- | Aib- | Pro- | Val- | Aib- | Aib- | Gln- | Gln- | Lol |

the FAB mass spectrum shows a shift of ions above *m/z* 466 and 339 in the series a and c to positions 14 units lower than those of hypelcin A-I, respectively (Table II). These results indicate the replacement of Aib at position 6 in hypelcin A-I with an Ala residue in hypelcin A-II. Similary, the amino acid sequences of the minor peptides, hypelcins A-III (3) and A-IV (4), were deduced from FAB mass spectral data (Table II). The FAB mass spectral data of hypelcin A-III show that the Lol of hypelcin A-I is replaced by an unknown amino alcohol residue ($C_7H_{16}NO$). While, the FAB mass ions (Table II) of hypelcin A-IV appear to be very similar to those of hypelcin A-I, amino acid analysis shows that the major component of hypelcin A-IV is Ile instead of the Leu in hypelcin A-I. Consequently, the sequence of hypelcin A-IV can be explained by the replacement of Leu at position 9 in hypelcin A-I with the Ile residue.

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12) Although hypelcin A-II shows a single peak on HPLC, the carbonyl region of $^{13}$C-NMR indicats that hypelcin A-II contains small amounts of a similar peptide.
13) D. J. Surman and J. C. Vickerman, *J. Chem. Soc., Chem. Commun.*, 1981, 324; M. Barber, R. S. Bordoli, R. D. Sedwick, and A. N. Tayler, *ibid.*, 1981, 325.
14) Obtained on a JEOL JMS D-300 mass spectrometer employing 3 KeV xenon atom (from xenon ions charge exchanged with xenon gas) directed onto a matrix prepared from small amounts of a sample in MeOH, one drop of 1% NaI (or 1% KI) solution, and one drop of 1N HCl dispersed in triethanolamine on a tungsten ribbon. In the calibrations of molecular ions species (M + Na, M + K) exact mass numbers of the CsI ions were used.
15) The presence of Lol in hypelcins A-II and A-IV was also confirmed by gas chromatography-mass spectrometry of *N,O*-ditrifluoroacetyl derivative from their acid-hydrolysates.
16) Obtained on a JEOL JMS D-300 mass spectrometer employing 3 KeV xenon atom (from xenon ions charge exchanged with xenon gas) directed onto a matrix prepared from small amounts of a sample in MeOH and one drop of 1N HCl dispersed in glycerol on a tungsten ribbon.

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