Chinese Chemical Letters Vol. 13, No. 2, pp 147– 150, 2002
http://www.imm.ac.cn/journal/ccl.html

A Novel Polypeptide from *Cervus elaphus* Linnaeus

Liang WENG[1], Qiu Li ZHOU[2], Takashi IKEJIMA[1], Ben Xiang WANG[1]*

[1]Research Centre of New Drug, Affiliated Hospital of Changchun College of Traditional Chinese Medicine, Changchun 130021
[2]Institute of Biological Engineering, Norman Bethune University of Medical Sciences, Changchun 130021

Abstract: A novel polypeptide having stimulant effect on some cell proliferation was isolated from the velvet antler (*Cervus elaphus* Linnaeus). The velvet antler polypeptide consists of a single chain of 32 amino acid residues. Amino acid sequence of the polypeptide was identified as: VLSAADKSNVKAAWGKVGGNAPAFGAEALLRM.

Keywords: Velvet antler, peptide, amino acid sequence.

As an unique physiological property, velvet antler (VA) can grow up rapidly. Therefore some investigations proposed that there are certain growth factors related to growing of cartilage, bone, epidermis, and other tissues in VA[1-3]. We previously reported that a fraction containing several active polypeptides from VA of sika deer (*Cervus nippon* Temminck) was isolated and this fraction exerted a fracture-healing activity[4]. In this report, we will present the primary structure of the novel polypeptide from VA of red deer (*Cervus elaphus* Linnaeus).

Experimental

Collection and Extraction

Whole fresh velvet antlers were obtained from red deer (*Cervus elaphus* Linnaeus) produced in Jilin Province of China in June 1997, stored at –80°C. The raw materials (1 kg) were cut into small pieces (approx. 1 cm^3) and washed with cold distilled water (approx. 4°C) to remove the blood. The pieces were ground with 5 L of precold acetic acid solution (pH 3.5) using a colloidal mill. The supernatant was obtained by centrifugation (8,500 rpm for 20 min), and further 95% ethanol was added to the supernatant to make the final ethanol concentration of 65% (v/v), then stood for 3 h at 4°C to settle the precipitate, then centrifuged again. The supernatant was evaporated under reduced pressure at 55°C, the residue was dissolved in distilled water. The

* E-mail: cctcmwbx@public.cc.jl.cn

water-soluble fraction was partitioned by an ultrafilter (Millipore, model 142 MM) to collect partially purified polypeptide with molecular weight 3 ~ 10 kDa and lyophilized.

This polypeptide (2 g dry weight) was dissolved in 200 mL 5 mmol acetic acid-sodium acetate (HAc-NaAc) buffer, pH 3.5, and applied to CM-Sepharose Fast Flow column (Φ 50 × 200 mm) pre-equilibrated with same buffer. The first protein peak appeared when it was eluted with 5 mmol/L HAc-NaAc, pH3.5 at 10 mL/min flow rate. The second peak appeared after addition of 0.5 mol/L buffer (pH 4.0) and the third one appeared at pH 4.0 (buffer 1 mol/L). The second fraction showed the highest mitogenic activity and was collected, dialysed against distilled water and lyophilized. The above freeze-dried material (200 mg dry weight) was dissolved in 5 mL of 2 mmol/L HAc-NaAc pH 4.0, and applied to Sephadex G-50 column (Φ 20 × 1000 mm) which had been pre-equilibrated with the same buffer. The samples were eluted with the same buffer at 1 mL/min flow rate. Fractions with mitogenic effect on epidermal cell were combined, dialysed against distilled water and lyophilized. Preparative HPLC was further performed using a Shimadzu SPD-6AV system equipped with UV-vis spectrophotometric detector. Portions up to 2 mg of the fractions were repeatedly injected on a 250 × 10 (i. d.) mm Spherisorb column (C_6, 5 μm, 300 Å) and eluted with H_2O-Isopropyl alcohol, 33:67 (0.05% v/v of trifluoroacetic acid was added in the mobile phase) at 2 mL/min flow rate. Analysis and final purification of velvet antler polypeptide was carried out using a 250 × 4.6 (i. d.) mm Spherisorb column (C_6, 5μm, 300 Å) and eluted with the same mobile phase. The final yield of velvet antler polypeptide was 14.8 mg (0.0148 ‰w/w fresh velvet antler).

Sequence Determination

Peptide samples were hydrolyzed at 105°C for 24 h with 6 mol/L HCL containing 0.1% phenol and the hydrolysate was analyzed with a Beckman model 6300 automatic amino acid analyzer. Molecular mass was determined by a LDI-1700 MALDI-TOF mass spectrometry. Amino acid sequence was determined by sequential Edman degradation using a protein/peptide sequencer (PE, Inc., model ABI-491A).

Results and Discussion

Fractionation of the acid water extract (2 g) of whole fresh VA (1,000 g) from *Cervus elaphus* Linnaeus by a combination of acid water extraction, ethanol precipitation and ultrafiltration concentrated its proliferation-promoting constituents into a peptide-enriched fraction. The final purification of this material was performed by chromatography on column CM-Sepharose Fast Flow, followed by chromatography on Sephadex G-50, then by repeating reversed-phase HPLC (C_6), provided 14.8 mg of velvet antler polypeptide (VAPP).

The VAPP was isolated as a amorphous white solid. The mobility of VAPP by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and its staining by Coomassie brilliant blue (a single band of M_r approximately 3 kDa) suggested that it contained polypeptide(s). Acid hydrolysis analysis indicated that this polypeptide was

composed of 32 amino acids, without cysteine. The preliminary analysis showed that the full sequence of VAPP was determined by Edmen degradation as illustrated in **Figure 1**. The estimated value of the molecular mass (3200.7 Da) was detrmined from the amino acid sequence. It is 16.0 Da less than that determined by mass spectroscopy (3215.8 Da). This difference may be attributed to oxidation of the residue methionine (+16 Da) at C-terminus of the native polypeptide (**Table 1**).

Figure 1 Amino acid sequence of VAPP

Table 1 The amino acid composition and molecular weight of the VAPP

animo acid	mol. wt of VAPP Da	VA PP[c] molar ratio of amino acid	amount of amino acid
Asp/Asn (D/N)		2.80	1D 2N
Thr (T)		0.19	
Ser (S)		2.02	2
Glu/Gln (E/Q)		1.08	1E
Pro (P)		1.75	1
Gly (G)		4.96	4
Ala (A)		7.31	8
Cys[a] (C)			
Val (V)		2.87	3
Met (M)		1.00	1
Ile (I)		0.05	
Leu (L)		1.96	3
Tyr (Y)		0.00	1
Phe (F)		1.11	1
Arg (R)		0.93	3
Lys (k)		2.75	
His (H)		0.12	1
Trp[b] (W)		1	
No aa:s		32	
	3200.7 (calcd.)[d]		
	3215.8 (MS)[e]		

[a]Cysteine was determined as cysteic acid with a separate sample following oxidation with performic acid. [b]Tryptophan was determined photometrically through separate measurements by utilizing the HPLC on-line spectra obtained from the diode-array detection. The presence of tryptophan was then estimated from the relative intensity of UV absorbance at 280 *vs* 250 nm. [c]For the VAPP, the residues from amino acid analysis are listed to the left and the residues from sequencing to the right. [d]Calculated with average masses and with the total sum from amino acid composition. [e]Mass was determined by MALDI-TOF MS with an experimental accuracy of ±0.1% (see experimental section).

Acknowledgment

We thank Dr. L.G. Xu at Shanghai Institute of Biochemistry, Chinese Academy of Sciences for amino acid sequence analyses. We also thank Dr. Y. P. Ji at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences for providing us the MALDI-TOF MS data.

References

1. J. M. Suttie, P. D. Gluckman, J. H. Butler, *et al.*, *Endocrinology*, **1985**, *116*, 846.
2. K. M. Ko, T. T. Yip, S. W. Tsao, *et al.*, *J. Gen. Comp. Endocrinol.*, **1986**, *63*, 431.
3. Y. C. Kong, K. M. Ko, T. T. Yip, *et al.*, *Acta Zoologica Sin.*, **1987**, *33*, 301.
4. Q. L. Zhou, Y. J. Guo, L. J. Wang, *et al.*, *Acta Pharmacol. Sin.*, **1999**, *20*, 279.

Received 11 June, 2001