Chem. Pharm. Bull. 20(7)1579—1581(1972)

UDC 547.466.1.02.05 : 591.05

Comparative Studies on Angiotensins. II.[1] Structure of Rat Angiotensin and Its Identification by DNS-Method[2]

TAEKO NAKAYAMA, TERUMI NAKAJIMA,[3a] and HIROFUMI SOKABE[3b]

Faculty of Pharmaceutical Sciences, University of Tokyo[3a] and Department of Pharmacology, Toho University, School of Medicine[3b]

(Received January 8, 1972)

The structure of angiotensin I isolated from horse,[4] hog,[5] and human[6] is Asp[1]-Arg[2]-Val[3]-Tyr[4]-Ile[5]-His[6]-Pro[7]-Phe[8]-His[9]-Leu.[10] In bovine angiotensin I, isoleucine at position 5 is replaced by valine.[7] We have suggested that rat angiotensin may also be Ile[5]-angiotensin I by its chromatographic behavior, ratio of oxytocic to pressor activity, and susceptibility to proteases.[1]

The present paper reports a further study on structure of rat angiotensin. We have purified rat angiotensin and identified it as Ile[5]-angiotensin I by its amino acid composition and characteristics of its 1-dimethylaminonaphthalene-5-sulfonyl- (DNS-) derivatives.

Experimental

Starting Material[1,8]——Rat angiotensin was prepared by incubating the kidney extract with homologous plasma. Frozen kidney tissue (100 g) was homogenized with 140 ml of H_2O, and centrifuged. The supernatant fluid was dialyzed against 5.9 mM disodium ethylenediamine tetraacetic acid (EDTA) solution overnight, and acidified to pH 3.0 to remove angiotensinases. Plasma (960 ml) was obtained from 122 rats (Donryu strain) nephrectomized bilaterally 24 hr before, and also dialyzed overnight against 5.9 mM EDTA solution. Dialyzed plasma of 750 ml was incubated with 150 ml of kidney extract and 300 ml of 0.15 M sodium phosphate buffer at 37°, pH 7.4, for 10 min. The reaction was stopped by cooling the mixture to 0°, and by mixing it with 200 ml of Dowex 50W-X2(H^+).[9] The incubation mixture with the resin was applied to the column of 100 ml of Dowex 50W-X2(NH_4^+)(pH 6.0); washed succesively with 2000 ml of H_2O, 2000 ml of 0.2M ammonium acetate buffer (pH 6.0), 3000 ml of 10% (v/v) AcOH, and 6000 ml of H_2O; and eluted with each 2000 ml of 0.1 M diethylamine and 0.5M NH_4OH at 20—25°. The eluate was evaporated by rotary evaporators under 45° or lyophilized. Starting material of 631 μg in activity (equivalent to Asn[1]-Val[5]-angiotensin II) was obtained.

Synthetic Peptides——Asn[1]-Val[5]-angiotensin II was supplied by Ciba Pharmaceutical Products, Tokyo. Asp[1]-Ile[5]-angiotensins I and II, and Asp-Arg were the gifts of S. Sakakibara, Institute for Protein Research, Osaka University.

Determination of the Pressor Activity[1,8]——Activity of the materials was determined by their pressor action in the anesthetized rat, using synthetic Asn[1]-Val[5]-angiotensin II as the standard.

Dansylation[10]——Peptide of 10^{-9} to 10^{-10} mole was dissolved in 10 μl of 0.1 M triethylamine and 10 μl

1) Part I: T. Nakajima, T. Nakayama, and H. Sokabe, *Gen. Comp. Endocrinol.*, **17**, 3 (1971).
2) This work was presented at the 91st Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, Apr. 1971.
3) Location: *a) Hongo, Bunkyo-ku, Tokyo*; Present address: *Institutes of Pharmaceutical Sciences, Hiroshima University, School of Medicine. 2-3, Kasumi-cho 1 chome, Hiroshima*; *b) 21-16, Omori-nishi 5 chome, Ota-ku, Tokyo.*
4) L.T. Skeggs, Jr., K.E. Lentz, J.R. Kahn, N.P. Shumway, and K.R. Woods, *J. Exptl. Med.*, **104**, 193 (1956).
5) F.M. Bumpus, H. Schwarz, and I.H. Page, *Circulation*, **17**, 664 (1958).
6) K. Arakawa, M. Nakatani, A. Minohara, and M. Nakamura, *Biochem. J.*, **104**, 900 (1967).
7) D.F. Elliott and W.S. Peart, *Biochem. J.*, **65**, 246 (1957).
8) H. Nishimura and H. Sokabe, *Japan. Heart J.*, **5**, 494 (1968).
9) We did not add Dowex resin into the incubation mixture to suppress angiotensinases, because the resin also suppressed formation of angiotensin from the nephrectomized plasma.
10) Z. Tamura, T. Nakajima, T. Nakayama, J.J. Pisano, and S. Udenfriend, *Anal. Biochem.*, in press.

NII-Electronic Library Service

of 0.5% (w/v) DNS-Cl was added. The procedure was held in an ice bath. The mixture was kept for 16 hr in a refrigerator and the solvent was evaporated to dryness under nitrogen stream. Five μl of 90% (v/v) HCOOH and 50 μl of H_2O were added to the residue. The solution was kept 1—2 hr at room temperature, and then dried.

Result and Discussion

Purification of Rat Angiotensin

The crude active principle of 604 μg was purified further by gel filtration with Sephadex G-25 and ion exchange chromatography with SE-Sephadex and QAE-Sephadex (Chart 1). In each step of purification, activity was observed as a single peak. Recovery rate is shown

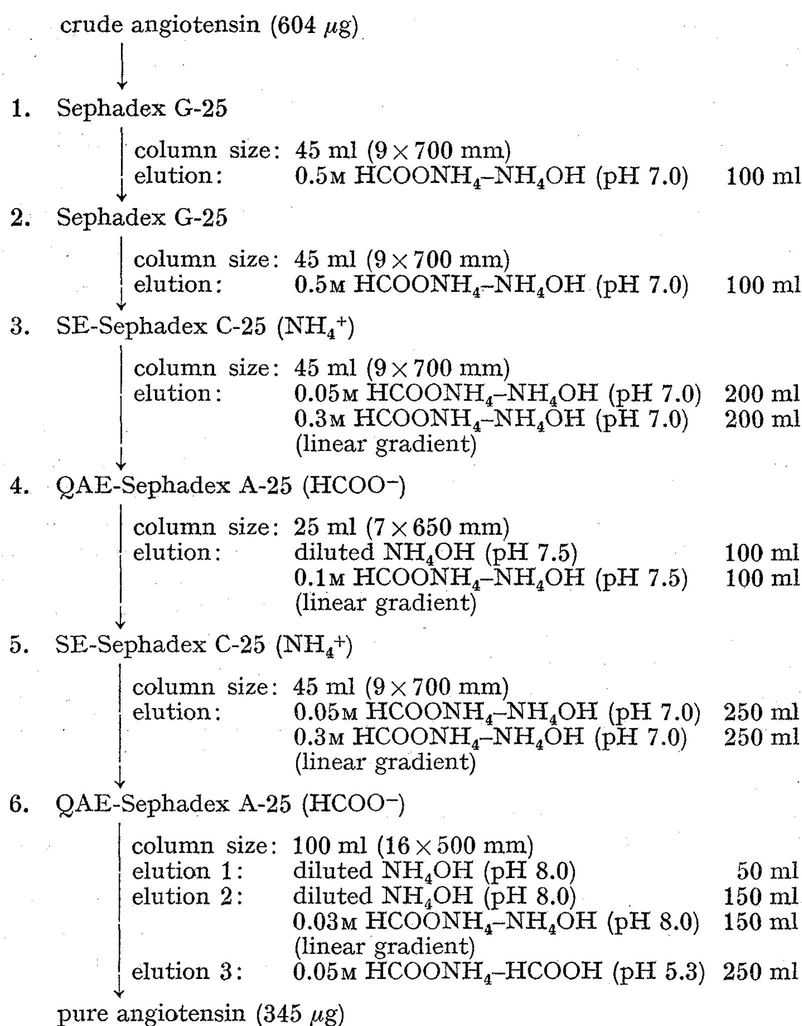

Chart 1. Purification of Rat Angiotensin

Table I. Recovery Rate of Rat Angiotensin

Step.	Activity (μg)	Recovery (%)
1. Sephadex G-25	459	76
2. Sephadex G-25	507	110
3. SE-Sephadex	450	89
4. QAE-Sephadex	400	89
5. SE-Sephadex	400	100
6. QAE-Sephadex	345	86

NII-Electronic Library Service

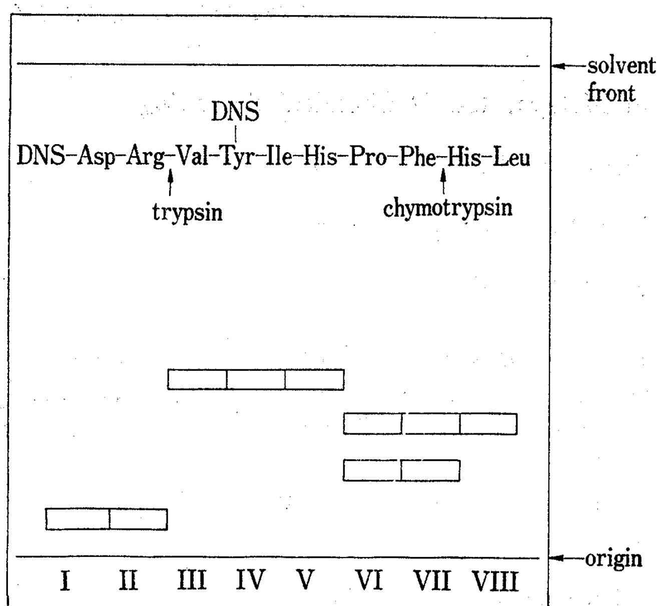

Fig. 1. Thin-Layer Chromatogram of DNS-Rat Angiotensin, DNS-Ile^5-Angiotensin I, and their Enzymatic Fragments

silica gel H plate
solvent system: n-BuOH: AcOH: H_2O (4: 1: 5)
I: DNS-rat angiotensin
II: DNS-Ile^5-angiotensin I
III: chymotryptic fragment of DNS-rat angiotensin
IV: chymotryptic fragment of DNS-Ile^5-angiotensin I
V: DNS-Ile^5-angiotensin II
VI: tryptic fragments of DNS-rat angiotensin
VII: tryptic fragments of DNS-Ile^5-angiotensin I
VIII: DNS-Asp-Arg

in Table I. A single spot was obtained in the DNS-material at the final step by thin-layer chromatography of Silica gel H.

Characterization of Rat Angiotensin

DNS-rat angiotensin on thin layer of Silica gel H was identical with N-DNS-Asp^1-O-DNS-Tyr^4-Ile^5-angiotensin I. Chymotryptic or tryptic peptides of DNS-rat angiotensin showed the same behaviors as those of N-DNS-Asp^1-O-DNS-Tyr^4-Ile^5-angiotensin I by thin-layer chromatography. These peptides were identical to N-DNS-Asp^1-O-DNS-Tyr^4-Ile^5-angiotensin II, N-DNS-Asp-Arg, and Val-O-DNS-Tyr-Ile-His-Pro-Phe-His-Leu (Fig. 1).

Rat angiotensin of 97.5 μg equivalent to Asn^1-Val^5-angiotensin II in pressor activity was hydrolyzed. It corresponds to 0.075 μ moles, assuming that pressor activity of angiotensin I is the same per unit weight as that of Asn^1-Val^5-angiotensin II used as the standard. This specific activity has been determined in our laboratory.[11] The following amino acid composition was obtained by an amino acid analyzer (Yanagimoto LC-2): Arg (1.2), Asp (1.2), His (2.1), Ile (1.1), Leu (1.0), Phe (1.1), Pro (1.0), Tyr (0.8), Val (1.2). The results accorded with those of synthetic Asp^1-Ile^5-angiotensin I. The amount of isolated rat angiotensin calculated from the amino acid analysis was about 0.042 μ moles which was 56% of recovery, when compared with the above figure (0.075 μ moles).

These results suggest that rat angiotensin, produced in a homologous system, can be deduced to the same as horse, hog, and human.

Acknowledgement We thank Dr. S. Sakakibara for suppling synthetic peptides, and Mrs. M. Sokabe, Mrs. H. Muramatsu, and Miss T. Horii for their help. Part of this investigation was supported by the Naito Foundation Research Grant.

11) Unpublished data.

NII-Electronic Library Service