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# ACTIVITY OF ANGIOTENSIN-CONVERTING ENZYME INHIBITORS IN VENOMS OF CENTRAL ASIAN SNAKES

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*The inhibition of angiotensin-converting enzyme (ACE, K.F.3.4.15.1) by low-molecular-weight fractions of venoms from central Asian snakes was studied using N-(3-[2-furyl]acryloyl)-Phe-Gly-Gly (FAPGG) as substrate. Their activity is substantial in peptide fractions of poisons from snakes of the Viperidae and Crotalidae families and completely absent in cobra poison (Elapidae).*

**Key words:** snake venom, angiotensin-converting enzyme, inhibitors.

Peptides enriched in proline that effectively inhibit angiotensin-converting enzyme (ACE, synonyms: dipeptidyldipeptidase, carboxycatepsin, kininase II) are found in venom of the rattlesnakes *Agkistrodon halys blomhoffi*, *Bothrops jararaca*, and *B. jararacussu* [1-3]. Inhibition of ACE *in vivo* causes hypotension. Therefore, peptide inhibitors of the enzyme from snake venom are considered to be molecular models for designing new specific antihypertensive preparations [4].

Several peptide inhibitors of ACE were previously isolated pure from *Echis multisquamatus* Ch. venom and characterized [5]. In the present article, data for ACE inhibition by peptide fractions of venom from gyuza (*Vipera lebetina turanica* Ch., Viperidae), steppe viper (*Vipera ursini renardi*, Ch, Viperidae), common viper (*Vipera berus berus*, Viperidae), moccasin (*Agkistrodon halys halys*, Crotalidae), and cobra (*Naja oxiana* Eichwald, Elapidae) are examined.

Whole snake venom is known to contain enzymes in the high-molecular-weight fraction that interfere with ACE [6]. Therefore, the activity of the enzyme inhibitors (ACEI) was determined in peptide fractions of venoms that were produced by high-resolution chromatography on a calibrated column of Sephacryl S-100 HR 16/60 by separating them into peptides of different molecular weight. The results are presented in Fig. 1 and Table 1.

Thus, according to gel filtration, the low-molecular-weight fraction of gyuza venom contains peptides of mol. wt. 10.0-0.5 kDa, including peptides of 10.0, 6.6, and 1.5 kDa. The low-molecular-weight fraction of moccasin venom (8.0-0.5 kDa) contains peptides of 6.6, 4.5, and 1.1 kDa. The analogous fraction of common viper venom contains peptides of 9.0-0.5 kDa, including peaks at 8.9, 6.9, 4.6, and 2.1 kDa. The low-molecular-weight fraction of steppe viper venom has total peptides of 10.0-0.5 kDa with peptides of 8.3, 7.4, 5.5, 3.5, and 2.2 kDa. Finally, the peptide fraction of cobra venom (10.9-0.5 kDa) contains peptides of 8.7, 6.6, 5.7, 3.3, and 2.0 kDa and less.

It should be noted that relatively low-molecular-weight peptides (<1.0 kDa) do not appear as distinct peaks in the densitograms of these fractions because they lack aromatic amino acids. On the other hand, electrophoresis (under denaturing conditions) of peptide fractions of gyuza, moccasin, and steppe and common viper venoms did not isolate in them pure components of molecular weight >2.0 kDa. In other words, peptides with molecular weights 10.0-2.0 kDa in these fractions appear upon gel filtration as aggregates (Fig. 1, A, B, C, D). This differs from cobra venom (Fig. 1, E).

The nature of the inhibition was determined by exhaustive hydrolysis in HCl (5.7 N) or a mixture of proteolytic enzymes (trypsin, chymotrypsin, carboxypeptidase Y) of the low-molecular-weight venom fractions. In both instances the activity of the inhibitors in the fractions decreases by an average of 80%. This is consistent with their peptide nature. The remaining inhibitory activity (average 20%) may be due to the presence of an unhydrolyzed N-terminus residue of pyroglutamate (Pyr) in peptides that inhibits ACE by itself [7].

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TABLE 1. Inhibition by Peptide Fractions of Snake Venom for Angiotensin-Converting Enzyme

| Peptide fraction of snake venom | Mol. wt. of components, kDa | Fraction yield, % | IC-50, μg/ml |
|---|---|---|---|
| *Vipera lebetina turanica* Ch. | 10.0-0.5 | 12.6 | 129.1 |
| *Vipera berus berus* | 9.0-0.5 | 9.9 | 79.2 |
| *Vipera ursini renardi* Ch. | 10.0-0.5 | 24.1 | 178.6 |
| *Agkistrodon halys halys* | 8.0-0.5 | 9.4 | 53.0 |
| *Naja oxiana* Eichwald | 10.9-0.5 | 31.9 | - |

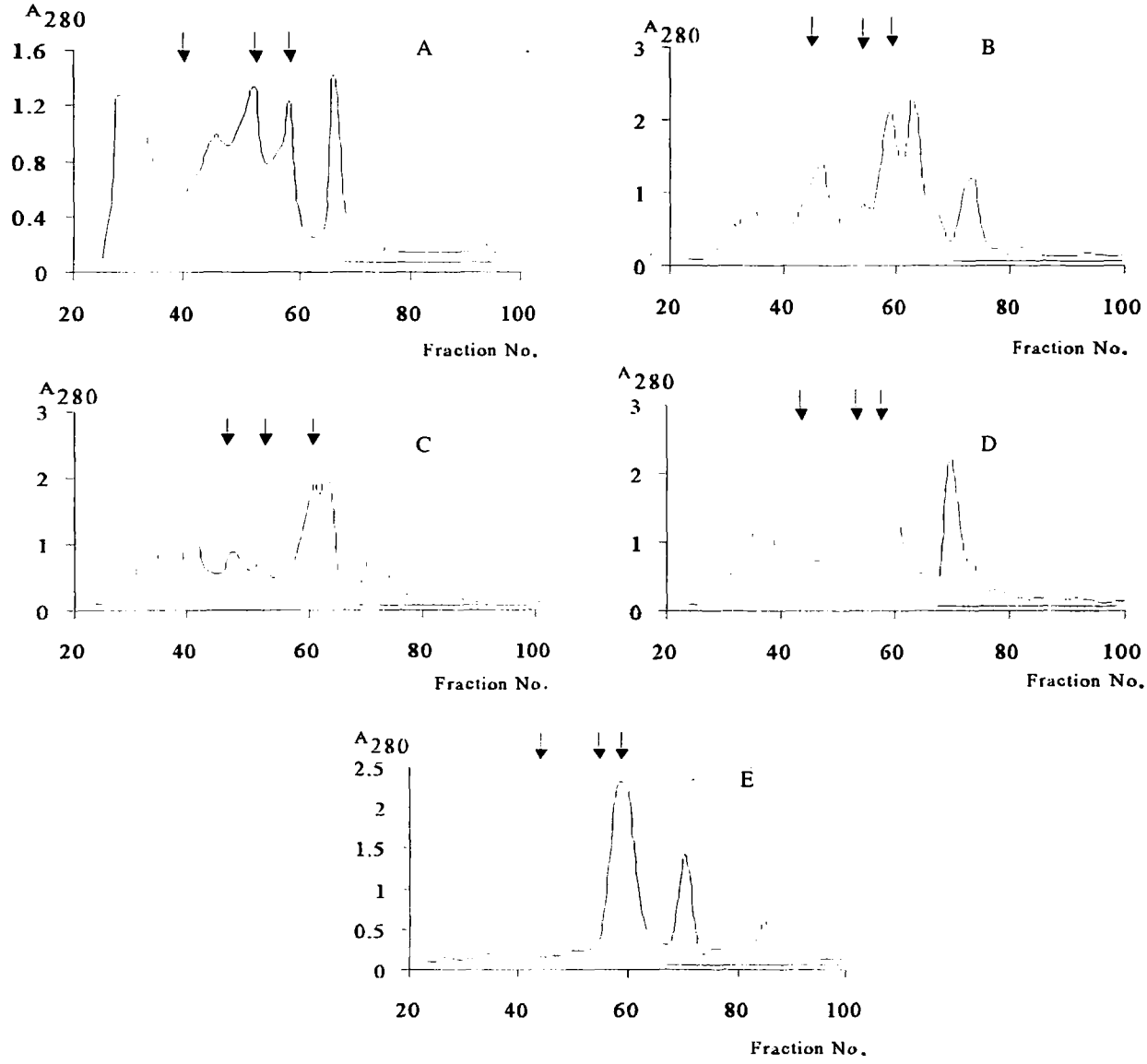


Fig. 1. Gel-filtration of venoms (50 mg) on Sephacryl 8-100 HR 16/60 column. The column (1.6×60 cm) was equilibrated with ammonium formate (0.01 M, pH 3.6). Arrows indicate the position of protein standards (ovalbumin, 43.0 kDa; chymotrypsinogen A, 25.0 kDa; ribonuclease A, 13.7 kDa). Fractions that were combined are shown. Gyuza venom (A), moccasin (B), common viper (C), steppe viper (D), cobra (E).

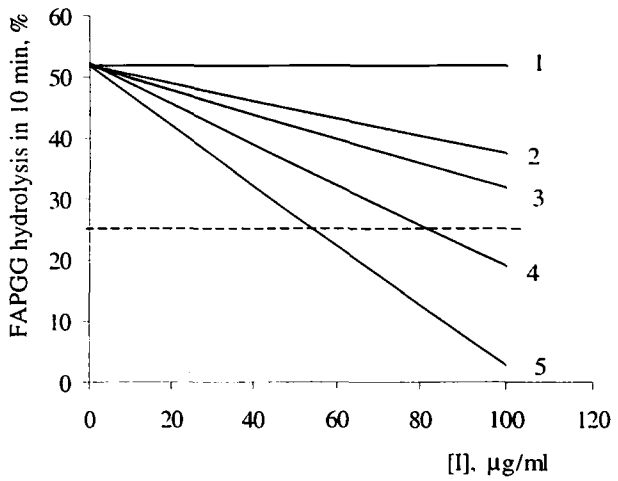


Fig. 2. Plot (regression lines) of ACE activity (% hydrolysis of FAPGG substrate) as a function of venom peptide-fraction concentration. Values of IC-50 calculated according to the linear regression equation are given in the text. Peptide fraction of venom from cobra (1), steppe viper (2), gyurza (3), common viper (4), moccasin (5). The dotted line represents 50% inhibition of enzyme activity.

The inhibition of ACE by the obtained peptide fractions of venom was estimated from the IC-50, i.e., the concentration of peptide at which enzyme activity is inhibited by 50% (Fig. 2 and Table 1).

Preliminary experiments demonstrated that low-molecular-weight fractions of venom do not contain proteinase, kininogenase, and kininase activity (i.e., do not interfere with ACE). Thus, they cannot influence the results of determining the ACE inhibitors.

The most active ACEI was the peptide fraction of moccasin venom (IC-50 53.0 μg/ml). Next come peptides from common viper (IC-50 79.2 μg/ml) and gyurza (IC-50 129.1 μg/ml). The activity of bovine-kidney ACE is 50% inhibited by 178.6 μg/ml of the peptide fraction from steppe viper venom. The low-molecular-weight fraction from cobra venom, despite the different peptide components, does not inhibit the enzyme at any concentration.

Comparison of the results for IC-50 with the activities of known ACEIs suggests the following. The most active pure peptides isolated from snake venom have IC-50 values in the range 1.2-50 μg/ml [8]. The activity of synthetic analogs, for example, captopril derivatives, is of the order of $10^{-8}$ M [9]. The IC-50 values of several peptides obtained from plants is 1.3-34 μg/ml [10]. In other words, we obtained highly active (comparable in activity) ACE inhibitors even in the peptide fractions of the venoms.

Thus, viper venom also contains inhibitors that are comparable in activity to those isolated from rattlesnake venom [4] and can be a source of these substances. Significant ACE inhibition was observed and characterized in the peptide fractions of venom from central Asian snakes. This makes them promising for isolation and subsequent structure—activity investigations and for use as molecular models for synthesizing specific antihypertensive preparations.

## EXPERIMENTAL

We used venoms of gyurza, steppe viper, common viper, moccasin, and cobra that were obtained from the Central Asian Regional Zoo and dried over $CaCl_2$; Sephadex (Pharmacia, Sweden); TSK-gel (Toyo-Soda, Japan); reagents for electrophoresis (Serva, Germany); the specific chromogenic ACE substrate N-(3-[2-furyl]acryloyl)-Phe-Gly-Gly (FAPGG) (Sigma, USA); and protein standards for gel filtration ovalbumin (43.0 kDa), chymotrypsinogen A (25.0 kDa), and ribonuclease A (13.7 kDa) (Pharmaica, Sweden).

**Peptide fractions** of snake venoms were obtained by chromatography on a high-resolution column of Sephacryl S-100

HR 16/60 in ammonium formate (0.01 M, pH 3.6) by separating the total peptides of different molecular weight. Eluate adsorption was measured at 280 nm using a Uvicord II spectrophotometer with flow cell. The column was calibrated and molecular weights of peptides were calculated using the formula $V_e - V_0/V_t - V_0$, where $V_e$ is the elution volume of the peptide, $V_0$ is the free volume of the column, and $V_t$ is the gel volume. The protein standards ovalbumin (43.0 kDa), chymotrypsinogen A (25.0 kDa), ribonuclease A (13.7 kDa), and $Tyr^8$-bradykinin (1.076 kDa) were used.

**ACE** was isolated from the outer layer of bovine kidney by the literature method [11].

**Principal kinetic parameters** of the isolated ACE were determined as before [12].

The ACE activity was measured by the literature method [13] using FAPGG (50 μM) in TRIS-HCl (0.05 M) at pH 7.5 containing NaCl (0.3 M) and $ZnCl_2$ (1 μM). The absorption decrease at 328 nm was monitored using an SF-46 spectrophotometer. In a typical instance, thermostatted (25° C) substrate (2 ml) in the spectrophotometer cuvette was treated with enzyme (20-40 μl) until the final concentration was 1-10 nM. The unit activity was taken as that amount of enzyme that catalyzes hydrolysis of substrate (1 μM) in 1 min under the above conditions. The reaction kinetics at the chosen substrate concentration are first-order in substrate. Hydrolysis reaches 98% in 10-30 min.

**Inhibition** by venom peptide fractions was estimated by their ability to block the action of ACE for FAPGG and was characterized as IC-50 values. The IC-50 values, i.e., concentrations (μg/ml), at which enzyme activity is 50% inhibited were calculated using inhibition curves (regression lines).

## REFERENCES


1. H. Kato and T. Suzuki, *Biochemistry*, **10**, No. 6, 972 (1971).
2. L. J. Greene, J. M. Stewart, and A. H. Ferreira, *Advan. Exp. Med. Biol.*, **8**, 81 (1970).
3. L. A. F. Ferreira, O. B. Henriques, I. Lebrun, M. B. C. Batista, B. C. Prezoto, A. S. S. Andreoni, R. Zelnik, and G. Habermehl, *Toxicon*, **30**, No. 1, 33 (1992).
4. K. F. Stocker, *Medical Use of Snake Venom Proteins*, CRC Press, Basel (1990), p. 46.
5. V. M. L'vov and L. Ya. Yukel'son, *Khim. Prir. Soedin.*, 435 (1995).
6. L. Ya. Yukel'son, V. M. L'vov, and N. Sultanalieva, *Khim. Prir. Soedin.*, 257 (1992).
7. A. C. O. Cintra, C. A. Vieira, and J. R. Giglio, *J. Protein Chem.*, **9**, 221 (1990).
8. M. A. Ondetti, N. J. Williams, E. F. Sabo, J. Pluscec, E. R. Weaver, and O. Kocy, *Biochemistry*, **19**, No. 22, 4033 (1971).
9. D. W. Cushman, H. S. Cheung, E. F. Sabo, and M. A. Ondetti, *Biochemistry*, **16**, 5484 (1977).
10. H. Wagner, G. Elbe, H. Lotter, and M. Guinea, *Pharm. Pharmacol. Lett.*, **1**, No. 1, 15 (1991).
11. Yu. E. Eliseeva, L. V. Pavlikhina, and V. N. Orekhovich, *Dokl. Akad. Nauk SSSR*, **217**, No. 4, 953 (1974).
12. V. M. L'vov and E. S. Sadykov, *Khim. Prir. Soedin.*, 368 (1999).
13. B. Holmquist, P. Bunning, and J. F. Riordan, *Anal. Biochem.*, **95**, 540 (1979).