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PROTAMINES

THE PEPTIDES FROM THE THERMOLYSIN HYDROLYSIS OF STURINE B

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In a preceding paper, we gave the results of a study of the partial amino-acid sequence of sturine B [1]. To determine its complete sequence it was necessary to determine the number of arginine residues in blocks. For this purpose we have performed the hydrolysis of sturine B with thermolysin produced by the firm "Sigma" from Bacillus thermoproteolyticus Rokko.

Hydrolysis was performed in 0.02 M tris-hydrochloride buffer, pH 8.0, containing 0.005 M $CaCl_2$ (40°C, 10 h) at an enzyme–substrate ratio of 1:75 (by weight). The resulting mixture of peptides was separated by ion-exchange chromatography on a 1×25 cm column of carboxymethyl-Sephadex G-25 equilibrated with 0.05 M phosphate buffer, pH 6.15. For elution we used a step-exponential NaCl gradient. The peptides were purified additionally on Bio-Gel P2 (column 1 × 150 cm; elution with 0.02 N HCl).

The structures of the majority of the peptides were established on the basis of their amino-acid compositions and the determination of their N-terminal amino acids by the dinitrophenylation [2] and dansylation [3] methods and of their C-terminal amino acids with carboxypeptidases A and B [1] (Table 1).

From a trypsin hydrolyzate of sturine B [1] the peptide TI Ser-Ser-Arg-Pro-Glx-Arg has been isolated. The results of a comparison of the structure of this peptide with those on the amino-acid composition and the N- and C-terminal amino acids of peptide TmV, TmVI-1, and TmVI-2 made it possible to determine the primary structures of these peptides.

Because of the presence in the sturine B molecule of the sequences Arg-Ser-Ser and Arg-His-Gly, in which thermolysin hydrolysis affects all the bonds shown by arrows, the overlapping peptides TmIV-2, TmIV-1, TmV, TmVI-1, TmVI-2, TmIII, and TmI were obtained. A comparison of these results with those

TABLE 1

Peptide	Amino-acid composition (in residues)	Amino acid		Structure of the peptides
		N-terminal	C-terminal	
Tm1	Arg1, 9, Glyl, 0	Gly	Arg	$Gly\text{-}Arg_2$
Tm2	Arg 3, 8, Glyl, 0	Gly	Arg	$Gly\text{-}Arg_4$
Tm	His 0,9, Argl, 1,9, Gly 1,0	His	Arg	$His\text{-}Gly\text{-}Arg_2$
TmIV-1	Arg 4,7, Ser 0,9, Ala 1,0	Ala	Ser	$Ala\text{-}Arg_5\text{-}Ser$
TmIV-2	Arg 4,8, Ala, 1,0	Ala	Arg	$Ala\text{-}Arg_5$
TmV	Arg 6,6, Ser 1, 8, Pro 1,0, Glx 1,0	Ser	Arg	$Ser\text{-}Ser\text{-}Arg\text{-}Pro\text{-}Glx\text{-}Arg_6$
TmVI-1	His 0,9, Arg 6,8, Ser 1,7 Pro 1,0, Glx 1,0	Ser	His	$Ser\text{-}Ser\text{-}Arg\text{-}Pro\text{-}Glx\text{-}Arg_6\text{-}His$
TmVI-2	His 0,9, Arg 7,0, Ser 1,1 Pro 1,0, Glx 1,0	Ser	His	$Ser\text{-}Arg\text{-}Pro\text{-}Glx\text{-}Arg_6\text{-}His$

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of the determination of the C-terminal sequence of sturine B [1] enabled the complete amino-acid sequence of sturine B to be established: H-Ala-Arg_5-Ser-Ser-Arg-Pro-Glx-Arg_6-His-Gly-Arg_2-Gly-Arg_4-OH.

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SYNTHESIS OF A HEXAPEPTIDES RELATED TO ELEDOISIN

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In order to investigate the mechanism of the action of kinins, we have synthesized by a new method lysylphenylalanylisoleucylglycylleucylmethionine amide (I, 1-6, Table 1, Scheme) – a substance possessing a strong hypotensive and myotropic action [1]. In all cases (with the exception of E 5-6) for the formation of the peptide bond we used 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) [2] in solution in diformamide or ethanol (for H 1-6) in the presence of N-methylmorpholine. Compound E 5-6 was obtained as described by Lübke et al. [3]. After the transfer of the reaction products into ethyl acetate, the starting materials were separated by washing the solutions with sodium bicarbonate and potassium bisulfate.

The benzyloxycarbonyl and tert-butyloxycarbonyl groups were split off by treatment with solutions of hydrogen bromide and hydrogen chloride in acetic acid, respectively; the p-nitrobenzyl ester was cleaved by catalytic hydrogenolysis.

Compound E 2-4 was recrystallized from water, G 1-4 from 50% methanol, and H 1-6 from a mixture of dimethylformamide and water. The final product I 1-6 was purified by dissolution in water, filtration, and lyophilization of the filtrate; mp 241°C (decomp.) (240-243°C, decomp. [4]), $[\alpha]_D^{23}$ −16.3° (c 0.8; acetic acid) (−16.8° [4]). The results of amino-acid and elementary analyses agreed with the calculated figures. In the biological test* (contraction of the guinea-pig ileum), I 1-6 showed an activity more than twice as great as that of bradykinin, which agrees with literature information [4]. (See scheme.)

*The tests were performed by Z. P. Auna and V. E. Klusha.

TABLE 1. Electrophoretic and Chromatographic Constants of the Compounds Synthesized

Peptide	E_{His}*	Rf in the following systems†				
		1	2	3	4	5
B 3–4	0	0,45	0,95	0,98	0,85	0,96
C 3–4	0,71	0	0,10	0,50	0,50	0,50
D 2–4	0	0,40	0,95	0,98	0,90	0,85
E 2–4	0,56	0	0,35	0,90	0,75	0,80
F 1–4	0	0,18	0,95	0,90	0,85	0,96
G 1–4	0	0,02	0,90	0,97	0,86	0,88
H 1–6	0	0,02	0,90	0,90	0,86	0,88
I 1–6	0,76	0	0	0,70	0,55	0,20

*The electrophoretic mobility E_{His} was determined on type "S" [medium] paper (Leningrad Paper Mill No. 2) in 1 N acetic acid.

†For thin-layer chromatography on silica gel we used "Silufol" plates and the following solvent system: 1) chloroform–acetic acid (95 : 5); 2) chloroform–methanol–acetic acid (85 : 10 : 5); 3) n-butanol–acetic acid–pyridine–water (15 : 3 : 10 : 12); 4) n-propanol–concentrated aqueous ammonia (84 : 37); 5) n-butanol–isopropanol–water–monochloroacetic acid (65 ml : 15 ml : 20 ml : 3 g).

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