FURTHER INVESTIGATION OF THE PEPTIDES FROM CANINE FIBRINOGEN

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Received October 3, 1966

A previous communication (Osbahr, et al., 1964) reported some aspects of the peptides released from canine fibrinogen by bovine thrombin. In this communication, we wish to elaborate further on these peptides and discuss the effect of canine, bovine and human thrombins upon the canine fibrinogen. Three peptides were isolated following the action of canine thrombin upon canine fibrinogen.

The canine fibrinogen was purified by the method of Laki, 1951, and further purification could be obtained by ion exchange chromatography on DEAE-cellulose (Osbahr, A. J., Patsy, S. M., 1966). After thrombin action, three major peptides were isolated by ion exchange chromatography on a 100X1 cm. column of Dowex (50-X2) with a stepwise gradient of pyridine formate, ammonium formate, and ammonium bicarbonate buffers pH 4-10. Purity of the peptides was determined by high voltage paper electrophoresis at pH's of 3.5 and 6.4, 150 V/cm. and combinations of chromatography in butanol-acetic acid-water (4:1:5), phenol-water-cresol (2:6:4) and isopropanol-water-formic acid (3:5:1). At times paper chromatography followed by high voltage paper electrophoresis was utilized to ascertain the purity of the peptides. The amino acid composition, after hydrolysis in 5.7 N hydrochloric acid at 110°C. for 24, 48 and 72 hrs. of the three peptides, is reported in Table I. Corrections were

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applied for incomplete hydrolysis and amino acid destruction.

Table I

Amino acid composition of the peptides from canine fibrinogen by canine thrombin

Amino acid composition	PEPTIDES I μmoles	I # of residues	II μmoles	II # of residues	III μmoles	III # of residues
Aspartic acid	0.254	1	0.250	1	1.310	4
Threonine	0.240	1	0.211	1	0.587	2
Serine	0.228	1	0.202	1	0.340	1
Glutamic acid	0.752	3	0.611	3	0.981	3
Glycine	1.010	4	0.840	4	--	--
Alanine	0.258	1	0.213	1	0.312	1
Valine	0.248	1	0.230	1	0.616	2
Isoleucine	0.260	1	0.193	1	0.306	1
Tyrosine	--	--	--	--	0.572	2
Phenylalanine	0.250	1	0.190	1		
Lysine	0.248	1	0.186	1	--	--
Histidine	--	--	--	--	0.281	1
Arginine	0.244	1	0.181	1	0.593	2
Ammonia	0.508	2	0.402	2	0.482	1
Phosphate	0.245	1	0	0		

Carboxypeptidase-B was utilized to determine the C-terminal residue of these peptides and in all cases stoichiometric amounts of arginine were released and no further release of amino acids occurred. The application of the DNP method to determine the N-terminal amino acids of canine fibrin revealed 3-4 μmoles of DNP-glycine per μmole of canine fibrin, as well as 2 μmoles of tyrosine. Thrombin again exhibits its unique specificity for an arginyl-glycyl bond. Peptide III was subjected to the Edman method (1950), as well as enzymatic cleavage by proteolytic enzymes as summarized in Table II.

Table II

Fragments Released from Peptide III by the use of Proteolytic Enzymes.

Peptide	Enzymes Used	Fragment	Amino acid composition
III	Chymotrypsin	C-1	(tyr., his.)
		C-2	($tyr._2$, his.)
		C-3	($thr._2$, ser., $asp._4$, $glu._3$, ileu., $val._2$, ala., $arg._2$)
III	Trypsin	T-1	($tyr._2$, his., $asp._3$, $glu._3$, thr., arg.)
		T-2	(ser., $val._2$, ileu., thr., asp., ala., arg.)

Peptide	Enzyme (used)	Time course of appearance of amino acids following enzyme addition — time hrs. 6 (μmoles)	12 (μmoles)	24 (μmoles)	48 (μmoles)	72 (μmoles)	96 (μmoles)
III (C-2)	C*ase A	tyr. 0.05	tyr. 0.08 his. 0.01	tyr. 0.15 his. 0.04	tyr. 0.18 his. 0.07	tyr. 0.24 his. 0.10	
III (C-3) †	C*ase A	ala. 0.06	ala. 0.08 asp. 0.01	ala. 0.15 asp. 0.04	ala. 0.15 asp. 0.08 val. 0.02	ala. 0.16 asp. 0.09 val. 0.06 thr. 0.02	
III (T-1)	C**ase B	arg. 0.04	arg. 0.08	arg. 0.10	arg. 0.15	arg. 0.16	
III (T-1) †	C*ase A	-	-	glu. 0.05	glu. 0.09	glu. 0.15	glu. 0.19	glu. 0.26
III (T-2) †	C*ase A	ala. 0.05	ala. 0.07 asp. 0.01	ala. 0.17 asp. 0.05 val. 0.01	ala. 0.18 asp. 0.09 val. 0.04	ala. 0.18 asp. 0.10 val. 0.07 thr. 0.02	ala. 0.18 asp. 0.11 val. 0.09 thr. 0.05 ser. 0.01

* Carboxypeptidase A
** " B
† less arg. C-terminal

The sequences of the peptides released from canine fibrinogen by canine thrombin are shown in Figure 1.

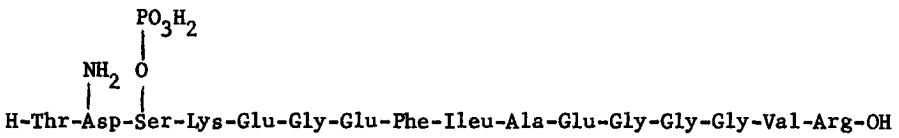
From Figure 1, it can be seen that peptides I and II, which were originally called α and β respectively, bear a close resemblance (at least in the C-terminal portion) to the A series of peptides as reported by Folk, et al. (1959) and Blömback and Doolittle (1963) for other species. In addition, the proposed structure of peptide III is similar to the B series with two arginines, one at C-terminal and one

within the molecule; however, the tyrosine of the canine peptide III does not appear to contain an anion as does, for example, the B peptide of the bovine species. Oddly enough, one of the canine peptides that resembles the A series does contain an anion in the form of a phosphate group covalently bound, not to a tyrosine, but to the hydroxyl of the serine.

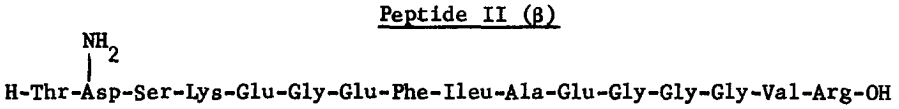
Figure 1

Final Amino Acid Sequences of Peptides I and II

Peptide I (α)



Peptide II (β)



Peptide III (Proposed Sequence)

H-His-Tyr-Tyr-Asp-Asp (Thr,Asp) Glu-Glu-Glu-Arg-Ileu-Val-Ser-Thr-Val-Asp-Ala-Arg-OH

Whether canine fibrinogen was clotted with canine, bovine, or human thrombin, the same three peptides appeared to be released. The relative rates of migration on high voltage electrophoresis were identical in each case as were the amino acid analyses of the isolated peptides. Further investigation of the peptides released from the canine fibrinogen by the various thrombins is in progress so that a better comparison can be made.

References

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