

## THE NATURE OF THE PEPTIDES RELEASED FROM CANINE FIBRINOGEN

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When canine fibrinogen is acted upon by canine thrombin, three large acidic peptides can be isolated. In this communication, we wish to report the amino acid composition and to propose sequences for two of these peptides.

Purified canine fibrinogen was reacted with canine thrombin and the liquor was carefully separated from the resulting clot. The clot liquor was concentrated and then deproteinized by the addition of trichloroacetic acid at 0°C. The last traces of trichloroacetic acid were removed from the supernatant containing the peptides by a cold continuous liquid-liquid extraction with ether. The peptide mixture was separated and purified by chromatography on DEAE-cellulose with a linear gradient between 0.225 M and 0.300 M potassium acetate buffer at pH 4.5. Three peptides were isolated. Two of these peptides were hydrolyzed with 5.7 N hydrochloric acid at 110°C. for 24, 48 and 72 hours and analyzed on an automatic amino acid analyzer. The results are shown in Table I. These two peptides contain the same sixteen amino acid residues; however, they differ in mobility on high voltage paper electrophoresis at pH 3.5. The peptide which moved more slowly toward the cathode contained one mole of phosphate per mole of peptide, whereas the faster moving peptide was free of phosphate. The phosphate was deter-

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mined to be covalently bound to the serine residue of the peptide as O-phospho-serine (Osbaehr *et al.*, 1963A). The two canine peptides are thus analogous to the two human peptides  $\alpha$  and  $\beta$  (Osbaehr *et al.*, 1963B) which have identical amino acid analysis and differ by the substitution in the  $\alpha$ -peptide of O-phospho-serine for serine. Therefore, we designate the canine peptide which possesses phosphate as the  $\alpha$ -peptide and the peptide without phosphate as the  $\beta$ -peptide. Table I shows a comparison of the amino acid analysis of the  $\alpha$  and  $\beta$ -peptides of both canine and human origin. The human and the canine peptides both contain sixteen amino acids. The canine peptides have one less aspartic acid and one more glutamic acid than their human counterpart. Leucine in the human peptides is replaced by isoleucine in the canine peptides. The canine peptides have one less glycine and alanine than do the human peptides, but they contain one threonine and one lysine, which are not present in the human peptides. Aside from these differences, the canine  $\alpha$  and  $\beta$ -peptides possess a striking resemblance to their respective human peptides. The peptides of human and canine species are the only ones investigated to date which contain O-phospho-serine. The peptides examined previously, such as the bovine (Folk *et al.*, 1960), were shown to contain tyrosine-O-sulfate.

The C-terminal amino acid residue of both the  $\alpha$  and  $\beta$ -canine peptides was shown to be arginine as determined by the use of a purified carboxypeptidase-B. Once again thrombin exhibits its unique specificity of hydrolyzing an arginyl-glycyl bond (Blombäck *et al.*, 1963) in which arginine contributes its C-terminal. The N-terminal residue of the canine peptides was shown to be threonine as determined by the phenyl isothiocyanate method (Edman, 1950).

Table I

The amino acid composition of the  $\alpha$  and  $\beta$ -peptides isolated from canine and human systems.

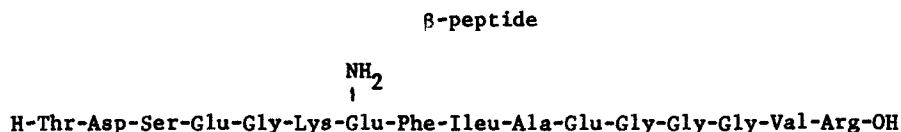
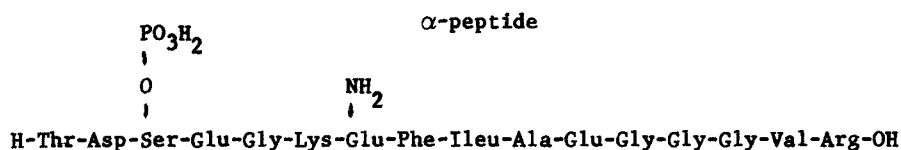
<u>Amino Acid Composition</u>	<u>Canine</u>				<u>Human</u>	
	<u><math>\alpha</math></u>		<u><math>\beta</math></u>		<u><math>\alpha</math></u>	<u><math>\beta</math></u>
	<u><math>\mu</math> mole ratio</u>	<u>Number of Residues</u>	<u><math>\mu</math> mole ratio</u>	<u>Number of Residues</u>	<u>Number of Residues</u>	
Aspartic Acid	0.254	1	0.250	1	2	2
Threonine	0.240	1	0.211	1	-	-
Serine	0.228	1	0.202	1	1	1
Glutamic Acid	0.752	3	0.611	3	2	2
Glycine	1.010	4	0.840	4	5	5
Alanine	0.258	1	0.213	1	2	2
Valine	0.248	1	0.230	1	1	1
Isoleucine	0.260	1	0.193	1	-	-
Leucine	-	-	-	-	1	1
Phenylalanine	0.250	1	0.190	1	1	1
Lysine	0.248	1	0.186	1	-	-
Arginine	0.244	1	0.181	1	1	1
Phosphate	0.245	1	0	0	1	0
Ammonia	0.508	2	0.402	2	-	-

The proposed amino acid sequences of the  $\alpha$  and  $\beta$ -canine peptides as determined by the use of proteolytic enzymes and the Edman method are shown in Figure 1.

A detailed report concerning the sequences will be presented in a forth coming publication. The third peptide from the canine system is currently being characterized.

Figure I

Proposed amino acid sequences of the  $\alpha$  and  $\beta$ -canine peptides.

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