Studies on Fibrinopeptides from Different Species

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Starting with the work of Bailey, Bettelheim, Lorand and Middlebrook [1] in 1951, it has now been fairly well established by these authors and by others that when bovine thrombin acts on bovine fibrinogen at least two different peptides (A and B) are released from the fibrinogen molecule revealing N-terminal glycyl residues in the fibrin (*cf.* Refs.[2-4]). Both peptides isolated from the supernatant contain arginine as the C-terminal amino acid residue [5,6]. The release of at least peptide A is followed stoichiometrically by the appearance of N-terminal glycine in the fibrin [3]. Thus peptide bonds involving arginylglycyl residues in fibrinogen are apparently hydrolyzed by thrombin.

With respect to synthetic substrates such as tosylarginine methyl ester the thrombin specificity seems to resemble that of trypsin [7]. Thrombin action differs, however, in many respects from that of trypsin [8,9] and in any case when acting on fibrinogen the proteolysis brought about by thrombin is much more limited than that by trypsin. Probably, the structure of the amino acid sequence in the vicinity of the peptide bonds hydrolyzed by thrombin or the secondary and tertiary structure of the fibrinogen molecule determines the narrow specificity of this enzyme.

Bovine thrombin also acts on fibrinogen from other species and species specific peptides seem to be released [10-13]. The characterization of these peptides may give information on the specific requirements for thrombin action. This paper gives preliminary data concerning the amino acid composition and amino acid sequences of some peptides released from human, pig and rabbit fibrinogen. The experiments which have been performed to establish the amino acid sequence of bovine peptides A and B have recently been reported elsewhere [3,6,14,15].

The different fibrinogens were mainly prepared according to a method previously described [16]. The coagulability of the fibrinogen preparations was 94—99 %. The peptides split off by bovine thrombin were isolated from the clot supernatant by column chromatography [17] on Dowex 50—X2.

From human fibrinogen two main peptides could be isolated and one of them was studied in detail. Also from each of pig and rabbit fibrinogen two main peptides could be isolated. The origin of other, smaller peptides observed on the chromatograms will not be discussed in this paper. All peptides containing tyrosine-O-sulphate have in this work been denoted as B-peptides in view of their similarity in this respect to bovine peptide B. The other peptides have tentatively been denoted as A-peptides.

The fact that the isolated peptides contain the N-terminal amino acids disappearing from the corresponding fibrinogen during the transformation to fibrin [10-12] establishes their relation to the proteolytic activity of thrombin.

The amino acid composition of the peptides (Table 1) was determined using the method recently reported [18,19]. As only small amounts of human, pig and rabbit peptides were accessible their content of cystine (or cysteine) was not determined. Peptides designated HP4-4 and KP10-A in Table 1 have not yet been analyzed for tryptophan. The other peptides do not contain tryptophan as judged from spectrophotometric readings in the ultra-violet. For comparison the amino acid residues of the bovine fibrinopeptides [6,15] are included in Table 1.

Although the amino acid compositions show remarkable differences some common features should be pointed out. Thus, all peptides in Table 1 have a high content of dicarboxylic amino acids. Furthermore, the peptides contain one or two arginine residues. In the bovine fibrinopeptides A and B the C-terminal position is occupied by arginine. The C-terminal amino acids in the other peptides have not yet been determined. It is also of interest that only one peptide, the pig peptide SP1-1, contains histidine.

Tyrosine has hitherto been found in fibrinopeptides from ox [20,17,21,4] and from rabbit fibrinogen [12]. It is also present in a fibrinopeptide from the pig. In all cases the hydroxyl group of tyrosine is esterified with sulphuric acid. The occurrence of tyrosine-O-sulphate in fibrinopeptides from

Table 1. Amino acid composition of fibrinopeptides from different species. R = residues

Species	Ox		Man		Pig				Rabbit			
Peptide	A	B	A HP 4–4		A SP 1–4		B SP 1–1		A KP 10–A		B KP 10–B	
Amino acid	R	R	μM/mg *	R	μM/mg *	R	μM/mg *	R	μM/mg *	R	μM/mg *	R
Alanine	0	1	1.05	2	0.76	2	0.62	2	0.42	1	0.78	2
Arginine	1	2	0.50	1	0.41	1	0.69	2	0.41	1	0.38	1
Aspartic acid	3	4	1.06	2	0.46	1	1.69	5	0.76	2	1.90	5
Glutamic acid	2	3	1.11	2	1.61	4	0.73	2	0.84	2	0.40	1
Glycine	5	3	2.57	5	1.60	4	0.37	1	1.24	3	—	0
Histidine	0	0	—	0	—	0	0.31	1	—	0	—	0
Leucine / *Iso*leucine	1	1	0.52	1	0.37	1	0.37	1	0.43	1	0.39	1
Lysine	0	1	—	0	0.40	1	0.36	1	—	0	—	0
Phenylalanine	1	1	0.51	1	0.35	1	—	0	0.46	1	—	0
Proline	2	2	—	0	—	0	0.36	1	0.40	1	0.40	1
Serine	2	0	0.40	1	—	0	—	0	0.42	1	—	0
Threonine	1	1	—	0	—	0	—	0	0.75	2	—	0
Tyrosine	0	1	—	0	—	0	0.32	1	—	0	0.42	1
Valine	1	1	0.53	1	0.82	2	0.74	2	0.41	1	0.42	1
Total number of residues	19	21		16		17		19		16		13

* Uncorrected for losses during acid hydrolysis. The figures refer to air-dry substances.

several species might indicate that this unique structural unit is of importance for the biological function of the protein. However, tyrosine-O-sulphate has been reported to be absent in peptide material from human fibrinogen [21]. In the peptide designated A from man tyrosine is absent (Table 1). The other main peptide present in the clot supernatant of human fibrinogen has, however, not been analyzed since it has not yet been obtained in homogeneous form.

Table 2 shows the amino acid sequences of the different peptides as determined with a modification of the phenylthiohydantoin method of Edman [15,22]. Except for bovine peptide A only partial sequences from the N-terminal end are known. All peptides with the possible exception of the bovine B-peptide seem to be made up of a single peptide chain. The partial sequences as well as the amino acid composition show clearly that these peptides are species specific.

The amino acid sequences of bovine peptides A and B have shown that the C-terminal part consists, in addition to arginine, mainly of neutral amino acid residues, whereas the dicarboxylic amino acids are displaced towards the N-terminal end of the molecule. Further elucidation of the amino acid sequences of the different peptides will show if this arrangement is a common feature.

It has been suggested that the pro · pro sequence in bovine A-peptide should be of importance in directing thrombin action [14]. This sequence, however, seems not to be a constant finding among fibrinopeptides as the peptide from man and one of the pig peptides does not contain proline.

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Table 2. N-terminal amino acid sequences of fibrinopeptides from different species.

	N-terminal amino acid or amino acid sequences of fibrinogen and peptides.
Ox	
Peptide A	H-Glu·Asp·Gly·Ser·Asp·Pro·Pro·Ser·Gly·Asp·Phe·Leu·Thr·Glu·Gly·Gly·Gly·Val·Arg-OH
	OSO_3H
Peptide B (partial sequence. No reactive N-terminal)	(Glu, Phe, Pro, Thr) Asp·Tyr·Asp·Glu·Gly·Glu·Asp·Asp·Arg·Pro·Lys·Val·Gly·Leu·Gly·Ala·Arg-OH
Fibrinogen	Glu. Tyr.
Man	
H P 4—4 (Peptide A)	Ala·Asp·Ser·Gly·
Fibrinogen	Ala. Tyr.
Pig	
S P 1—4 (Peptide A)	Ala·Glu·Val·Asp·
S P 1—1 (Peptide B)	Ala·Leu·Asp·Tyr (or Ileu) OSO_3H
Fibrinogen	Ala. Tyr.
Rabbit	
K P 10-A (Peptide A)	Val·Asp·Pro·Gly·Glu·
K P 10-B (Peptide B)	Ala·Asp·Asp·Tyr·Gly· OSO_3H
Fibrinogen	Val. Ala. Tyr.

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