THE ACTION OF HUMAN THROMBIN ON HUMAN FIBRINOGEN

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When human fibrinogen is acted upon by human thrombin, two acidic peptides designated α and β can be isolated. In the present communication we wish to report the amino acid composition of these two peptides as well as the physiological activity of the β-peptide.

A highly purified human fibrinogen (97% clottable) was reacted with a human thrombin of high purity. After the thrombin action, the liquor was carefully separated from the resulting clot. The concentrated clot liquor was then passed through sephadex G-25, and two fractions were obtained. High voltage paper electrophoresis at pH 6.4 of one of the fractions showed the presence of two acidic arginine containing peptides. The faster and slower moving peptides were here designated α and β, respectively. These peptides were isolated by chromatography on DEAE-cellulose with a linear gradient between 0.2 M and 0.3 M ammonium bicarbonate buffering system at pH 7.8.

An automatic amino acid analyzer was utilized for determining the amino acid composition of these peptides after hydrolysis with 5.7 N hydrochloric acid for 24, 48 and 72 hours. The results are shown in Table I. The C-terminal amino acid residue of both the α and β-peptides, as determined by the use of a purified carboxypeptidase-B, was shown to be arginine. Thus in a manner analogous to an all bovine thrombin-fibrinogen reaction system, human thrombin has also hydrolyzed an arginyl-glycyl bond (Gladner et al., 1959), since glycine has been reported to be the

N-terminal in human fibrin (Blombäck and Yamashina, 1958). The elucidation of the sequence of the α and β-peptides, as well as other peptides isolated from this system, is now in progress.

Table I

Amino Acid composition of the α and β-peptides isolated from both a human thrombin-human fibrinogen system and a bovine thrombin-human fibrinogen system.

Amino Acid Composition	β		α	
	μ mole ratio on analysis	Number of Residues	μ mole ratio on analysis	Number of Residues
Aspartic Acid	0.111	2	0.101	2
Serine	0.048	1	0.051	1
Glutamic Acid	0.116	2	0.100	2
Glycine	0.252	5	0.248	5
Alanine	0.101	2	0.103	2
Valine	0.049	1	0.048	1
Leucine	0.051	1	0.051	1
Phenylalanine	0.052	1	0.049	1
Arginine	0.048	1	0.048	1
Phosphate	0	0	0.049	1

It was further observed that the β-peptide possesses physiological activity in that it potentiates the bradykinin-induced contration of isolated rat uterus. This observation is similar to the previously reported physiological activity of peptide-B from bovine cofibrin as observed in our laboratory (Gladner et al, 1963). However on an equal molar basis the human β-peptide appears to have a much greater potentiating effect than the above mentioned bovine counterpart. On the other hand, the α-peptide did not possess any physiological activity when assayed in the same system. Studies of various enzymatic and chemical modification of the β-peptide

as well as peptide-B of bovine cofibrin to relate chemical structure to the observed physiological activity are now in progress. These will be reported in a forth coming publication (Osbahr et al., 1963).

We also investigated the peptides released when fibrinogen of one species is clotted by thrombin from another species. Therefore human fibrinogen was clotted by bovine thrombin and the peptides released were isolated and their amino acid composition was determined. It was observed that the same α and β-peptides were released from human fibrinogen by both human and bovine thrombin. Blombäck et al., 1962 recently reported the peptides released from human fibrinogen by bovine thrombin.

The location of the phosphate group on the α-peptide was determined by hydrolyzing the peptide with Streptomyces griseus protease and performing an amino acid analysis on the hydrolysate. A comparison of the chromatogram with that of an acid hydrolyzed sample of the same peptide revealed that the serine peak present in the chromatogram of the acid hydrolyzed sample disappeared in the chromatogram of the enzyme hydrolyzed sample and a new peak appeared, which coincided with the peak obtained with O-phospho-serine. This therefore indicated that the phosphate in the α-peptide was organically bound in the α-peptide as O-phospho-serine.

Our experiments with the human fibrinogen-human thrombin system, indicate that the peptides released are the same as those released in the human fibrinogen-bovine thrombin system. Further work on this system is in progress.

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