Degradation of Human Fibrinopeptides in Serum

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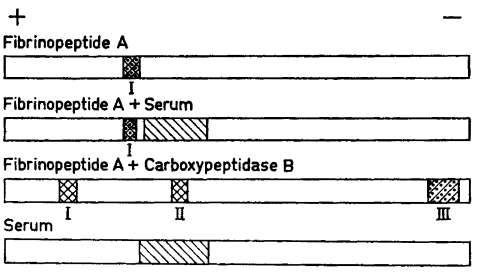
Fibrin formation is preceded by the release of fibrinopeptides from fibrinogen. Appreciable amounts of fibrinopeptides were thus expected to be present in blood serum. They may, however, rapidly degrade after their release from fibrinogen.

Fibrinopeptide B, lacking its C-terminal arginine, was recently isolated from clot supernatants of thrombin-treated purified fibrinogen from several species.[1] It was suggested that the peptide derivative was produced through enzymatic degradation by a contaminating exopeptidase of plasmatic origin. It is known from the work on blood kininases that blood contains carboxypeptidase B-like enzymes.[2]

The present investigation was undertaken in order to study the fate of fibrinopeptides in serum.

Human fibrinopeptides were prepared as described previously.[3] Fibrinopeptide A and B were dissolved in 0.1 M ammonium bicarbonate buffer, pH 8, to a concentration of 10 mg/ml. The peptides were treated with normal human serum as follows: 5 µl of serum were added to 45 µl of peptide solution. The mixtures were incubated for 4 and 24 h at 37°. Controls, containing only buffer and peptide, and buffer and serum, were run at the same time. Toluene was added as a preservative to the samples which were incubated for 24 h. The digests and the controls were submitted to paper electrophoresis in 0.1 M pyridine acetate buffer, pH 4.1. The components on the paper strips were identified with ninhydrin,[4] chlorination,[5] and Sakaguchi's reactions.[6] For elution experiments 15 µl serum and 135 µl peptide solution were incubated and the mixtures were applied to 20 cm wide paper strips. Guide strips for detection of the bands were cut out on each side of the strip. The bands on the rest of the strip were eluted with distilled water.

Peptide A was not susceptible to cleavage when incubated for 4 h (Fig. 1). However, after digestion for 24 h the peptide was split into three fragments (Fig. 2). Amino acid



H-ALA-ASP-SER-GLY-GLU-GLY-ASP-PHE-LEU-ALA-GLU-GLY-
-GLY-GLY-VAL-ARG-OH

Fig. 1. Electrophoretic picture of fibrinopeptide A after 4 h digestion with serum and carboxypeptidase B. Electrophoresis was run for 105 min in a horizontal apparatus (Analysteknik, Vallentuna, Sweden) in 0.1 M pyridine acetate buffer, pH 4.1. 3−7 µl sample/cm was applied on Whatman I paper. Voltage was 44 V/cm and current 30−50 mA. Below is shown the structure of fibrinopeptide A.[3] //// Ninhydrin reaction, \\\\ chlorination reaction, : : Sakaguchi's reaction.

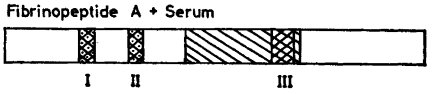
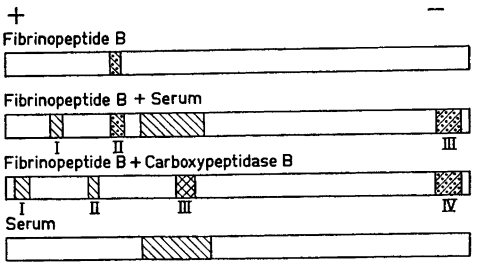


Fig. 2. Electrophoretic picture of fibrinopeptide A after 24 h digestion with serum. Electrophoresis was run for 5 h. Voltage was 55 V/cm and current 30−40 mA. Other details of the electrophoresis are given in Fig. 1. See legend to Fig. 1 for explanation of symbols.



ᶜGLU-GLY-VAL-ASN-ASP-ASN-GLU-GLU-GLY-PHE-PHE-SER-
-ALA-ARG-OH

Fig. 3. Electrophoretic picture of fibrinopeptide B after 4 h digestion with serum and carboxypeptidase B. Details of the electrophoresis are given in Fig. 1. Below is shown the structure of fibrinopeptide B.[3] See legend to Fig. 1 for explanation of symbols.

analysis (Technicon apparatus) after acid hydrolysis (5.7 N HCl, 110°, 22 h) of the eluted fractions showed the presence of all amino acids of peptide A in both fractions I and II. However, the amount of alanine in fraction I was lower and showed that only one alanine residue was present in this peptide. Fraction III was found to consist mainly of alanine (Table 1). The conclusion is that fraction I is identical with peptide Y [3] and fraction II is undigested peptide A. The N-terminal alanine residue of peptide A is thus split off by serum. No free arginine could be revealed on the strip.

Peptide B was split into three fractions after only 4 h digestion (Fig. 3). Amino acid analyses of the eluted fractions showed that fraction I was peptide B less C-terminal arginine, fraction II was unchanged peptide B, and fraction III contained arginine (Table 1). Digestion of peptide B for 24 h did not change the electrophoretic pattern significantly.

In order to check the susceptibility of the peptides towards pancreatic carboxypeptidase B, the following experiment was performed: 15 µl of a DFP-treated carboxypeptidase B solution containing 2 mg protein per ml (Worthington Biochem. Corp., N.J., Batch COB 6095) were added to 135 µl of the above peptide solutions. The mixtures were incubated for 4 h at 37°. Paper electrophoresis of the digest is shown in Figs. 1 and 3. No components were detectable on the strip containing buffer and enzyme. The bands were eluted

Table 1. Amino acid composition of the fractions of the fibrinopeptides.

Fibrinopeptide A after 24 h incubation with serum.

Fraction I				Fraction II				Fraction III	
amino acid	µmoles/mg	residue/ mole		amino acid	µmoles/mg	residue/ mole		amino acid	µmoles
Asp	0.84	1.9	(2)	Asp	1.05	2.1	(2)		
Ser	0.36	0.8	(1)	Ser	0.41	0.9	(1)	Gly	0.008
Glu	0.90	2.1	(2)	Glu	1.04	2.1	(2)	Ala	0.023
Gly	2.16	4.9	(5)	Gly	2.50	5.1	(5)		
Ala	0.47	1.1	(1)	Ala	1.00	2.0	(2)		
Val	0.43	1.0	(1)	Val	0.50	1.0	(1)		
Leu	0.43	1.0	(1)	Leu	0.50	1.0	(1)		
Phe	0.42	1.0	(1)	Phe	0.48	1.0	(1)		
Arg	0.43	1.0	(1)	Arg	0.49	1.0	(1)		

Fibrinopeptide B after 4 h incubation with serum.

Fraction I				Fraction II				Fraction III	
amino acid	µmoles/mg	residue/ mole		amino acid	µmoles/mg	residue/ mole		amino acid	µmoles
Asp	1.49	3.1	(3)	Asp	1.46	3.0	(3)	Gly	0.07
Ser	0.43	0.9	(1)	Ser	0.45	0.9	(1)	Arg	0.82
Glu	1.49	3.1	(3)	Glu	1.46	3.0	(3)		
Gly	1.01	2.1	(2)	Gly	1.01	2.1	(2)		
Ala	0.46	1.0	(1)	Ala	0.53	1.1	(1)		
Val	0.51	1.0	(1)	Val	0.50	1.0	(1)		
Phe	0.99	2.0	(2)	Phe	0.95	1.9	(2)		
				Arg	0.45	0.9	(1)		

and analyzed for amino acids as described above. It is evident that in both peptides the C-terminal arginine had been split off. The penultimate valine had in addition been partly released from peptide A (Fig. 1, fraction II). Several more fractions were disclosed in the digest of peptide B. Fractions I and II were the N-terminal nona- and hepta-peptide, respectively, and fraction III consisted of phenylalanine, serine, and alanine. These results indicate that the carboxypeptidase B preparation used was contaminated with carboxypeptidase A.

The susceptibility of the fibrinopeptides towards thrombin was investigated in a final experiment. 5 μl of a human thrombin solution containing 20 NIH-units/ml were added to 45 μl of the above peptide solutions in one experiment and 5 μl of a thrombin solution containing 200 NIH-units/ml were added in another experiment. The specific activity of the thrombin was 46 NIH-units per mg.[7] After 4 and 24 h of incubation, the samples were submitted to paper electrophoresis at pH 4.1. No other fractions than those corresponding to the original peptides could be detected on the paper strips. Thus thrombin is probably not responsible for the degradation of fibrinopeptides by serum.

The present results show that fibrinopeptides are degraded in serum by what appears to be exopeptidases of at least two different kinds, *i.e.* carboxypeptidases and aminopeptidases. The fact that under our conditions peptide B but not peptide A is degraded from the C-terminal end, may be explained by the structural differences, which exist in the two peptides. Peptide A is in contrast to peptide B degraded from the N-terminal end. This difference in susceptibility is to be expected as peptide B but not peptide A has a blocked N-terminal amino group. The degradation of fibrinopeptide A to fibrinopeptide Y in serum strongly suggests that the presence of Y-chains in human fibrinogen [3] is due to enzymatic degradation of the fibrinogen in blood rather than to a genetically determined variant of fibrinogen. The perspective that a plasma protein like fibrinogen may be degraded *in vivo* raises the question whether other proteins can also be degraded in a similar fashion. This may be of great importance in discussions on the origin of some isoenzymes.

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K_2BaCl_4, another Case of Extreme Ionic Conductivity in a Solid

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The phase diagram of the system KCl—$BaCl_2$ has been investigated by Gemsky,[1] Elchardus and Lafitte [2] and recently by Krohn.[3] In the system there is a congruently melting phase which appears around a composition of 33 mole % $BaCl_2$. (It is reasonable to believe that this solid phase has the composition K_2BaCl_4, at least at lower temperatures. Closely below the melting point it is possible that even non-stoichiometric ratios of KCl to $BaCl_2$ may occur, however). The phase is remarkable inasmuch as the corresponding liquidus line in the system is very flat. Several reasons for a flat liquidus line are conceivable, one being an extensive disorder in the solid. If this is the case, the transport properties of K_2BaCl_4 may show anomalies. Therefore an investigation of the electric conductivity of K_2BaCl_4 was undertaken.

Data on transport properties for compounds similar to K_2BaCl_4 appear in the