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Degradation of Human Fibrinopeptides A and B in Blood Serum *in vitro*

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Human fibrinopeptides A and B were incubated in normal human serum. The derivatives of the fibrinopeptides were isolated with electrophoretic and chromatographic methods, and their amino acid composition was determined.

N-Terminal alanine and in part aspartic acid and serine, and C-terminal arginine were split off from fibrinopeptide A. C-Terminal arginine alone was split off from fibrinopeptide B.

The release of arginine from peptide A was slow and incomplete, whereas that of arginine from peptide B was rapid and complete.

Previous investigations with purified fibrinogen have shown that formation of fibrin is preceded by the release of two peptides from the fibrinogen molecule.[1–5] These peptides, fibrinopeptides A and B, have been obtained from fibrinogen of several species, and their structures have been determined.[6–8] It is probable that *in vivo* as well, fibrin formation is preceded by the release of fibrinopeptides. At present, little is known about the function, degradation or excretion of these peptides, but knowledge about the fate of the fibrinopeptides would be of interest especially for the understanding of states of intravascular coagulation.

It was recently shown that human fibrinopeptides were degraded when incubated with human serum *in vitro*.[9] In this paper, further degradation experiments with human fibrinopeptides are presented.

MATERIAL AND METHODS

Blood serum. Blood was drawn into glass tubes from the cubital vein of 7—10 healthy blood donors. After 1 h at room temperature, the serum was separated by centrifugation at 2000 *g*, pooled, dispensed in test tubes, and stored at −20° for up to 5 months.

Human fibrinopeptides A and B were prepared as described earlier.[10]

High-voltage electrophoresis was performed in a horizontal apparatus (Analysteknik, Sweden) on paper strips (Munktell 302) in 0.1 M pyridine acetate buffer, pH 4.1. Identification of the samples on the paper strips was made with ninhydrin reaction,[11] Sakaguchi reaction,[12] and chlorination reaction.[13]

Amino acid analyses were made with an auto-Analyzer (Technicon Instruments Corp., Chauncey, N.Y.). Prior to chromatography, the samples were hydrolyzed in 5.7 M hydrochloric acid at 110° for 22 h.

Arginine was determined with a manual modification of the method described by Catravas.[14] 0.3 ml of sample, standard, or buffer, 1.35 ml of 8-hydroxyquinoline solution (0.02 % in 3 M sodium hydroxide) were mixed. After 1 to 2 min, 1.2 ml of 0.1 % *N*-bromosuccinimide solution was added. The absorbance was measured within 1 h in a Zeiss spectrophotometer at 505 nm. The arginine concentration in the samples was calculated from a standard curve.

Phosphorus was determined as described by Brown.[15]

Quantitative ninhydrin reaction was made according to Moore and Stein.[16]

EXPERIMENTS AND RESULTS

Incubation of the fibrinopeptides in serum and isolation of the derivatives with electrophoresis. 1.5 mg of peptide A and B was dissolved each in 150 µl of serum, and incubated for 4 h at 37°. 150 µl of serum were used as blank. After incubation, the samples and the blanks were cooled to 0°, and the proteins were precipitated with 1.35 ml of 10 % chilled trichloroacetic acid. The precipitates were separated by centrifugation in the cold. The trichloroacetic

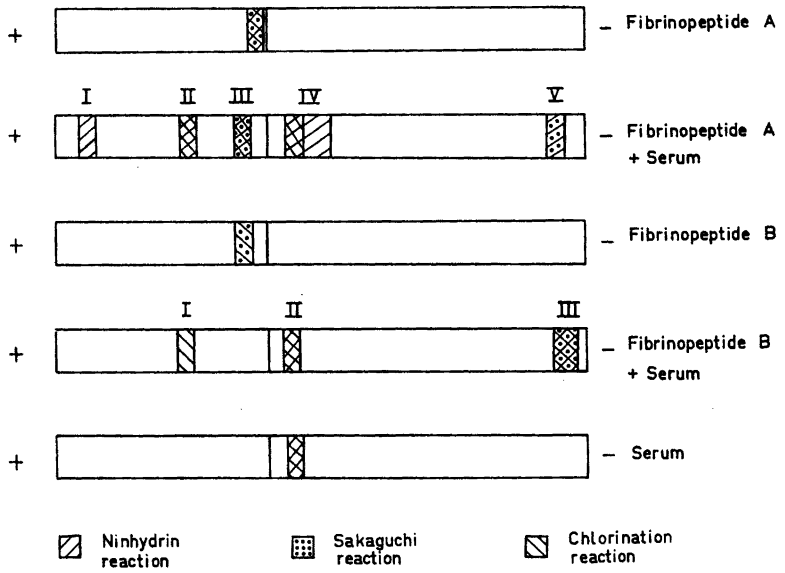

Fig. 1. Electrophoretic picture of the fibrinopeptides with and without incubation in serum. Structure [10] of fibrinopeptide A:

NH_2—Ala—Asp—Ser—Gly—Glu—Gly—Asp—Phe—Leu—Ala—Glu—Gly—Gly—
—Gly—Val—Arg—OH

Fibrinopeptide B:

Pyr—Gly—Val—ASN—Asp—ASN—Glu—Glu—Gly—Phe—Phe—Ser—Ala—Arg—
—OH.

Table 1 A. Amino acid composition of the electrophoretic fractions obtained after incubation of fibrinopeptide A in serum.

Amino acid	Fraction I	Control I	Fraction II		Fraction III		Fraction IV	Control IV	Fraction V	Control V
	μmoles	μmoles	μmoles/mg	residues/ mole	μmoles/mg	residues/ mole	μmoles	μmoles	μmoles	μmoles
Asp	0.066	0.002	1.11	2.0 (2)	0.41	1.9 (2)	0.009	0.004	0.004	0.004
Thr	—	—	—	—	—	—	0.014	0.010	—	0.002
Ser	0.006	—	0.46	0.8 (1)	0.16	0.7 (1)	0.050	0.010	0.006	0.005
Glu	0.005	—	1.19	2.1 (2)	0.43	2.0 (2)	0.049	0.030	0.005	0.007
Gly	0.009	0.004	2.69	4.7 (5)	1.00	4.7 (5)	0.071	0.015	0.009	0.009
Ala	—	—	0.61	1.1 (1)	0.24	1.1 (1)	0.444	0.022	—	0.005
Val	—	—	0.56	1.0 (1)	0.20	1.0 (1)	0.021	0.010	—	—
Leu	—	—	0.56	1.0 (1)	0.21	1.0 (1)	0.015	0.008	—	0.005
Phe	—	—	0.59	1.0 (1)	0.21	1.0 (1)	0.008	—	—	—
Arg	—	—	—	—	0.20	1.0 (1)	—	—	0.139	0.006

Table 1 B. Amino acid composition of the electrophoretic fractions obtained after incubation of fibrinopeptide B in serum.

Amino acid	Fraction I		Fraction II	Control II	Fraction III	Control III
	μmoles/mg	residues/mole	μmoles	μmoles	μmoles	μmoles
Asp	1.81	3.3 (3)	0.027	0.005	—	—
Thr	—	—	0.012	0.009	—	—
Ser	0.49	0.9 (1)	0.044	0.010	—	—
Glu	1.85	3.4 (3)	0.044	0.034	—	—
Gly	1.30	2.4 (2)	0.030	0.015	—	—
Ala	0.55	1.0 (1)	0.081	0.023	—	—
Val	0.61	1.1 (1)	0.035	0.012	—	—
Ile	—	—	0.004	0.004	—	—
Leu	—	—	0.008	0.006	—	—
Phe	1.24	2.3 (2)	0.015	0.004	—	—
Arg	—	—	—	—	0.297	0.005

acid was extracted from the supernatants with ether (2 ml × 6), and the samples were lyophilized. The residues were dissolved in 150 μl of distilled water. The residue from peptide B was sparingly soluble in water, and 5 μl of 0.1 M ammonium bicarbonate, pH 8.0, had therefore to be added to dissolve it. The samples were then submitted to high-voltage electrophoresis (44 V/cm, 80 mA) on 16 cm broad paper strips for 2 h. After the run, guide strips were cut out from each side of the papers, and the fractions obtained during electrophoresis were localized with ninhydrin, Sakaguchi, and chlorination reactions.

The results are shown in Fig. 1. It is evident that both peptides were degraded during incubation with serum. Peptide A produced several fractions, whereas only two new fractions were discernible in the digest of peptide B.

The electrophoretic bands from the rest of the paper were eluted with 2—3 ml of distilled water. As a check, elutions were also made from the papers with serum alone. The eluates were lyophilized, and the residues were analyzed for their amino acid composition. The results are shown in Tables 1 A and 1 B.

Fraction I from the experiment with peptide A consisted mainly of aspartic acid. As only traces of aspartic acid were present in the control, it is likely that the aspartic acid was derived from peptide A, and that it was the second amino acid from the N-terminal end of the peptide. The amino acid composition of fraction II corresponded reasonably well to that of peptide A less the N-terminal alanine and the C-terminal arginine. Analysis of fraction III showed that it consisted of peptide A less N-terminal alanine, *i.e.* peptide Y, described by Blombäck *et al.*[10] In fraction IV, several amino acids were detected in both the sample and the blank. The alanine content of the sample was, however, 20-fold that of the blank, and there were around five times as much glycine and serine in the sample as in the blank (Table 1 A). It is therefore probable that the alanine in this fraction was derived mainly from peptide A, due to release of the N-terminal alanine. The presence of serine and glycine in fraction IV might be the result of continuous digestion of the peptide from

the N-terminal end. Fraction V contained mainly arginine, whereas the arginine content of the blank was low.

Fraction I from the experiment with peptide B contained all the amino acids of peptide B in the expected proportions, except arginine (Table 1 B). Fraction I was thus probably peptide B degraded from its C-terminal end. Fraction II consisted of several amino acids, all in trace amounts. The amounts of aspartic acid, serine, alanine, valine, and phenylalanine were somewhat larger in the sample than in the blank, but still very low. This fraction has not been further studied. Fraction III contained arginine. The amount in the sample was much higher than that in the blank. It seems as if a complete splitting off of the C-terminal arginine had occurred during incubation of peptide B with serum.

Rate of release of C-terminal arginine from the fibrinopeptides. The incubation experiments suggested that C-terminal arginine was released more easily from peptide B than from peptide A. To compare the rate of release, the peptides were incubated with serum for different times, and the arginine released was determined.

In pilot experiments, 1 μmole each of peptide A and arginine, or peptide B and arginine, were dissolved in 0.5 ml of 0.1 M sodium acetate buffer, pH 5.0. The samples were applied to columns (1.5 cm × 0.12 cm², Dowex 50 × 2, 200—400 mesh),[17] equilibrated with 0.1 M sodium acetate buffer, pH 5.0. Flow rate was 1—2 ml/h. After washing with 3 × 2 ml of sodium acetate buffer, the arginine was eluted with 3 ml of 0.1 M sodium phosphate buffer, pH 7.0. Fractions of 0.5 ml were collected and analyzed for arginine. In another experiment, 0.5 ml of serum was applied to the column and eluted in the same way. The results of these experiments are summarized in Fig. 2. In contrast to arginine, the serum proteins and the fibrinopeptides were not adsorbed on the ion-exchange resin at pH 5.0, and there was no detectable component in serum at the position of arginine.

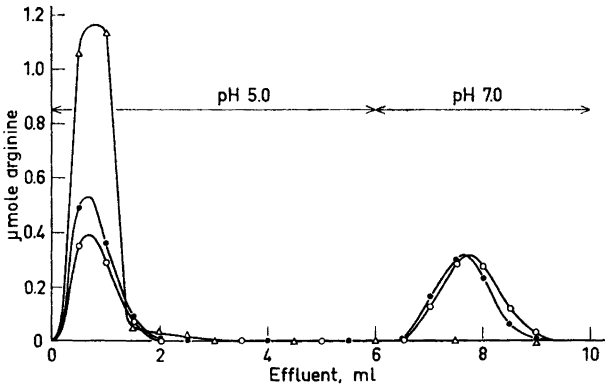

Fig. 2. Separation of fibrinopeptides and arginine on micro ion-exchange columns. ○ fibrinopeptide A and arginine; ● fibrinopeptide B and arginine; △ serum.

Table 2. Yield of arginine added to serum with and without incubation.

μmole arginine added	μmole arginine recovered	μmole arginine added	μmole arginine recovered
0.20 (no inc.)	0.17	1.00 (no. inc.)	1.06
0.20 » »	0.21	1.00 » »	1.05
0.40 » »	0.37	1.00 » »	1.13
0.40 » »	0.40	1.00 (30 min inc.)	1.21
0.60 » »	0.66	1.00 (1 h »)	1.25
0.60 » »	0.60	1.00 (2 h »)	1.17
0.80 » »	0.85	1.00 (4 h »)	1.00
0.80 » »	0.85	1.00 (8 h »)	1.04

The yield of arginine added to serum was also determined. As seen in Table 2, no significant destruction of arginine occurred during incubation with serum. The error of the method (S.D.), including incubation, separation and arginine determination, was calculated according to the formula $\sqrt{\sum d^2/2N}$ from 44 double determinations to be 0.04 μmole arginine.

0.5 μmole of peptide A and B, respectively, was dissolved in 0.2 ml of normal human serum, and incubated at 37° for between 10 min and 8 h. After incubation, 0.8 ml of 0.1 M sodium acetate buffer, pH 5.0, was added to the samples, which were then immediately frozen and stored at −20° for further analysis. Blanks with 0.2 ml of serum were run for each series of incubations. The incubation mixtures were applied to the same Dowex 50×2 columns as mentioned before. The columns were washed with 3×2 ml of sodium acetate buffer, pH 5.0, and the arginine was eluted with 3 ml of 0.1 M sodium phosphate buffer, pH 7.0, followed by further elution with 3×1 ml of phosphate buffer solution. The amount of arginine was determined in the first 3-ml fraction after the start of elution with phosphate buffer. The arginine content of the following 1-ml fractions was also determined. The arginine appeared, with a few exceptions, in the first 3-ml eluate.

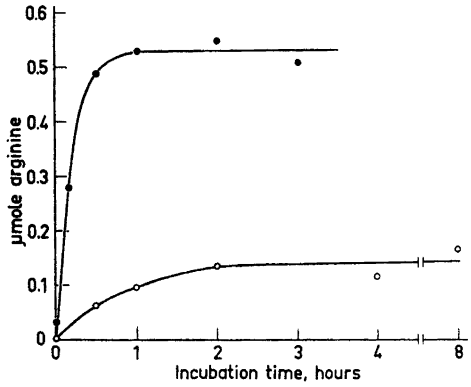

Fig. 3. Rate of arginine release from fibrinopeptides during incubation in serum. ○ fibrinopeptide A; ● fibrinopeptide B.

The results of the incubation experiments are shown in Fig. 3. Arginine was released slowly from peptide A, and only to about 30 %. From peptide B, arginine was split off far more rapidly. In 10 min, more than 50 % was split off, and after 30 min all the arginine had been released.

Incubation of the fibrinopeptides with serum and isolation of the derivatives with chromatographic methods. The fibrinopeptides and their derivatives, together with part of the serum constituents, were not adsorbed on the ion-exchange columns at pH 5.0. An attempt was therefore made to recover the fibrinopeptides and their degradation products from the material not adsorbed on the columns in the previous experiment.

The first 3 ml of eluate with acetate buffer, pH 5.0, from each chromatographic run of the digest of peptide A and B, respectively, were pooled. Totally 136 ml of peptide A solution and 126 ml of peptide B solution were obtained. The serum proteins were precipitated at 0° in 10 % trichloroacetic acid. The protein-free supernatants were collected by centrifugation in the cold, and the trichloroacetic acid was removed by extraction with chilled ether (5 × 180 ml). The water-phase was then evaporated to dryness *in vacuo*. The residues were dissolved in 11 ml of 0.05 M aqueous pyridine (peptide A fraction), or 0.1 M aqueous pyridine (peptide B fraction). Any undissolved material was separated by centrifugation. The supernatants were run through a Sephadex G-10 column (40 cm × 2 cm^2) equilibrated with 0.05 M or 0.1 M pyridine. Flow rate was 1 ml/min. Aliquots of the fractions were analyzed with the ninhydrin reagent after alkaline hydrolysis, and the conductivity was measured. Two ninhydrin-positive peaks appeared. The fractions in the first peak were collected and lyophilized. The residues were dissolved in 0.1 M ammonium formate buffer, pH 3.0, and applied to a column (90 cm × 1.5—2.5 cm^2, Dowex 50 × 2, 200—400 mesh) equilibrated with 0.1 M ammonium formate buffer, pH 3.0.[10] Elution was made with stepwise increase in pH. Flow rate was 16 ml/h. Aliquots of the fractions were analyzed with the ninhydrin reagent after alkaline hydrolysis. The chromatographic patterns are shown in Figs. 4 A and 4 B. The fractions of each peak were collected and lyophilized. The residual volatile buffer salts were removed by sublimation at 50° *in vacuo* over a cold trap. The samples were then subjected to high-voltage electrophoresis (44 V/cm, 30—45 mA). Fractions A—1, A—2, and A—3 were found to contain several bands, and were therefore subjected to a preparative electrophoretic run with elution of the different bands. The eluates were lyophilized, and analyzed for amino acid composition.

The results of the amino acid analyses are shown in Tables 3 A and 3 B. The fractions of the first peak in the chromatogram of peptide A, denoted as A—1, showed five bands in electrophoresis. Fraction A—1—1 was mainly aspartic acid, probably derived partly from the peptide. Aspartic acid was also present in large amounts in fraction A—1—2. Fraction A—1—4 might have represented the N-terminal penta-peptide of peptide A. The figures are, however, low and the analysis therefore uncertain.

The amino acid composition of fraction A—3—1 corresponded to peptide A less arginine and alanine, and A—3—2 to peptide A less arginine alone. Fraction A—3—3 contained some leucine. As judged from the amino acid analyses, the small fraction A—4 seemed to be unchanged peptide A. It appeared in a

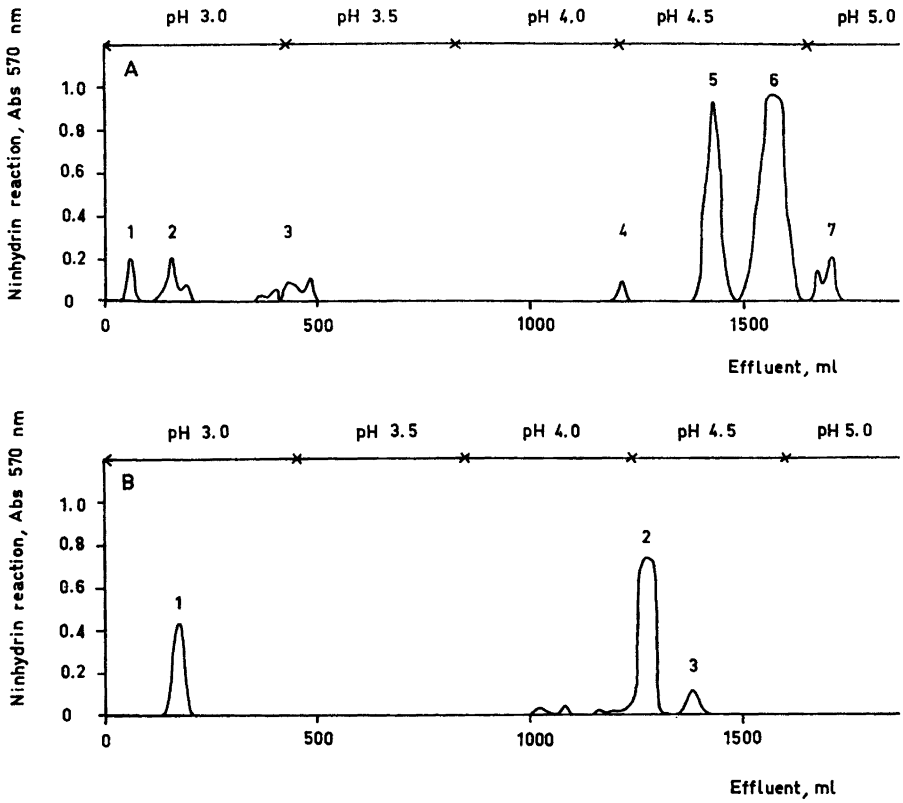

Fig. 4. Isolation of the fibrinopeptides and their derivatives by chromatography. A: fibrinopeptide A; B: fibrinopeptide B.

place in the chromatogram where the phosphorylated peptide A [10] could have been expected, but it did not contain any phosphorus. Fraction A—5 was peptide A less alanine alone, *i.e.* peptide Y, fraction A—6 was unchanged peptide A, and fraction A—7 was peptide A less alanine and aspartic acid.

The experiments thus give strong evidence for the degradation of peptide A from both the N- and C-terminal ends. As judged from the amino acid composition, the following derivatives are identified:

NH_2-Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-Arg-OH
NH_2-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-Arg-OH
NH_2-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-Arg-OH
NH_2-Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-OH
NH_2-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-OH

The presence of a large amount of unchanged peptide A was due to the samples with zero-time incubation, included in the experiment.

The main peaks in the chromatogram of peptide B were electrophoretically homogeneous. The first fraction, B—1, was — as judged from the amino acid

Table 3 A. Amino acid composition of the fractions obtained during isolation of serum-incubated fibrinopeptide A.

Amino acid	Fraction A−1−1	Fraction A−1−2		Fraction A−1−3		Fraction A−1−4		Fraction A−1−5	Fraction A−2−1	Fraction A−2−2	Fraction A−3−1	
	μmoles	μmole/mg	residues/ mole	μmole/mg	residues/ mole	μmole/mg	residues/ mole	μmoles	μmoles	μmoles	μmole/mg	residues/ mole
Asp	0.135	3.17	9.9 (10)	0.95	2.6 (3)	0.06	1.3 (1)	0.003	0.061	0.017	1.00	2.0 (2)
Thr	—	—	—	—	—	—	—	—	—	0.024	—	—
Ser	0.006	0.32	1.0 (1)	0.51	1.4 (1)	0.04	1.0 (1)	0.007	0.044	0.061	0.39	0.8 (1)
Glu	0.016	0.67	2.1 (2)	1.81	4.9 (5)	0.05	1.0 (1)	0.005	—	0.102	1.03	2.0 (2)
Gly	0.009	0.80	2.5 (3)	1.95	5.3 (5)	0.05	1.2 (1)	0.006	—	0.079	2.54	5.0 (5)
Ala	0.003	0.33	1.0 (1)	0.74	2.0 (2)	0.03	0.6 (1)	—	—	0.410	0.53	1.1 (1)
Val	—	—	—	0.36	1.0 (1)	—	—	—	—	0.033	0.49	1.0 (1)
Cys/2	—	—	—	—	—	—	—	—	—	0.006	—	—
Ile	—	—	—	—	—	—	—	—	—	—	—	—
Leu	—	—	—	0.38	1.0 (1)	—	—	—	—	—	0.49	1.0 (1)
Phe	—	—	—	0.48	1.3 (1)	—	—	—	—	—	0.51	1.0 (1)
Arg	—	—	—	—	—	—	—	—	—	—	—	—
Pro	—	—	—	—	—	—	—	—	—	0.063	—	—

	Fraction A−3−2		Fraction A−3−3	Fraction A−4		Fraction A−5		Fraction A−6		Fraction A−7	
	μmole/mg	residues/ mole	μmoles	μmole/mg	residues/ mole	μmole/mg	residues/ mole	μmole/mg	residues/ mole	μmole/mg	residues/ mole
Asp	0.65	2.0 (2)	—	0.75	1.8 (2)	1.08	2.0 (2)	0.97	1.8 (2)	0.46	1.0 (1)
Thr	—	—	—	—	—	—	—	—	—	—	—
Ser	0.26	0.8 (1)	0.004	0.33	0.8 (1)	0.46	0.8 (1)	0.45	0.8 (1)	0.26	0.6 (1)
Glu	0.61	1.9 (2)	—	0.79	1.9 (2)	1.06	1.9 (2)	0.82	1.5 (2)	0.88	1.9 (2)
Gly	1.47	4.7 (5)	0.005	1.95	4.7 (5)	2.72	4.9 (5)	2.67	4.9 (5)	2.10	4.5 (5)
Ala	0.73	2.3 (2)	—	0.72	1.7 (2)	0.58	1.0 (1)	1.14	2.1 (2)	0.49	1.1 (1)
Val	0.29	0.9 (1)	—	0.41	1.0 (1)	0.52	1.0 (1)	0.55	1.0 (1)	0.46	1.0 (1)
Cys/2	—	—	—	—	—	—	—	—	—	—	—
Ile	—	—	0.003	—	—	—	—	—	—	—	—
Leu	0.34	1.1 (1)	0.016	0.40	1.0 (1)	0.55	1.0 (1)	0.54	1.0 (1)	0.47	1.0 (1)
Phe	0.38	1.2 (1)	—	0.45	1.1 (1)	0.56	1.0 (1)	0.55	1.0 (1)	0.45	1.0 (1)
Arg	—	—	—	0.40	1.0 (1)	0.53	1.0 (1)	0.55	1.0 (1)	0.45	1.0 (1)

Table 3 B. Amino acid composition of the fractions obtained during isolation of serum-incubated fibrinopeptide B.

Amino acid	Fraction B—1		Fraction B—2		Fraction B—2	
	μmole/mg	residues/ mole	μmole/mg	residues/ mole	μmole/mg	residues/ mole
Asp	1.89	3.3 (3)	1.64	2.9 (3)	0.64	2.1 (2)
Thr	0.09	0.2 (0)	—	—	—	—
Ser	0.62	1.1 (1)	0.48	0.9 (1)	0.27	0.9 (1)
Glu	1.96	3.4 (3)	1.65	2.9 (3)	0.59	2.0 (2)
Gly	1.21	2.1 (2)	1.14	2.0 (2)	1.47	4.9 (5)
Ala	0.55	1.0 (1)	0.58	1.0 (1)	0.64	2.2 (2)
Val	0.59	1.0 (1)	0.57	1.0 (1)	0.30	1.0 (1)
Cys/2	—	—	—	—	—	—
Ile	—	—	—	—	—	—
Leu	—	—	—	—	0.30	1.0 (1)
Phe	1.23	2.1 (2)	1.05	1.9 (2)	0.29	1.0 (1)
Arg	—	—	0.53	0.9 (1)	0.31	1.0 (1)

composition — peptide B less arginine, and the other main fraction, B—2, was unchanged peptide B. The small fractions between B—1 and B—2 did not give material enough for analyses. Fraction B—3 had the same electrophoretic mobility and amino acid composition as peptide A. A repeated check of the purity of peptide B used for the experiment showed that it was contaminated, probably by small amounts of peptide A. As peptide B and probably also peptide B less arginine are sparingly soluble at pH 3, the yield of those peptides might be far from quantitative.

The following peptides were isolated from serum-incubated peptide B:

Glu-Gly-Val-Asp-Asp-Asp-Glu-Glu-Gly-Phe-Phe-Ser-Ala-Arg-OH
Glu-Gly-Val-Asp-Asp-Asp-Glu-Glu-Gly-Phe-Phe-Ser-Ala-OH

It could not be judged from the amino acid analyses wether there should be aspartic acid or asparagine. Release of C-terminal arginine seems thus to be the main degradation reaction in this particular experiment.

DISCUSSION

The fibrinopeptides are degraded in serum by exopeptidases of at least two different kinds, aminopeptidase and carboxypeptidase. The carboxypeptidase has similar specificity as carboxypeptidase B,[18] and is possibly identical with carboxypeptidase N described by Erdös,[19,20] No activity related to carboxypeptides A is found. As the purpose of the investigation was only to obtain knowledge about the degradation of fibrinopeptides in states of intravascular coagulation, no attempts were made to purify the enzymes involved.

The C-terminal arginine is released at different rates from peptide A and B. Although the reason is unknown, structural differences in the peptides may be of importance. The release of arginine from peptide B seems to occur

also in other species, since small amounts of peptide B less arginine have been isolated from fibrinogen in several of them.[21]

The difference in degradation from the N-terminal end of the peptides is easier to understand. Peptide B has no free amino group, but a pyroglutamic acid in N-terminal position,[22] and this structure could be expected to be resistant to the aminopeptidase in question. Peptide A has, however, a free amino group, and is liable to a stepwise degradation from the N-terminal end. At least alanine and aspartic acid are split off from peptide A. Some indications exist that also serine and possibly glycine might be split off to some extent. The rate of release of alanine from the N-terminal end of peptide A was not quantitatively studied, but — as judged from the isolation experiments — it is more rapid than the release of arginine from the C-terminal end of peptide A. The release of alanine seems to be complete under the conditions used.

The release of alanine is of particular interest in view of the fact that a fibrinopeptide with N-terminal aspartic acid, denoted as peptide Y, has been isolated from purified human fibrinogen, and shown to be peptide A less alanine.[10] The fibrinopeptides are situated in the N-terminal region of the fibrinogen molecule, and native human fibrinogen has, in addition to tyrosine and alanine, small amounts of aspartic acid in N-terminal position.[23–25] It is thus possible that not only peptides but also fibrinogen itself could be attacked by exopeptidases in blood.

Enhanced intravascular coagulation is a complex and often severe clinical condition. Of all the reactions involved in coagulation, the release of the fibrinopeptides from fibrinogen by thrombin is the best known. This proteolytic cleavage precedes fibrin formation. It is thus probable that increased amounts of fibrinopeptides or their derivatives occur in blood or urine in states of enhanced intravascular coagulation.

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