THE CLOTTING OF FIBRINOGEN

II. FRACTIONATION OF PEPTIDE MATERIAL LIBERATED

by

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The work described in the preceding paper (BAILEY AND BETTELHEIM[1]) shows that the glutamic acid N-terminal groups lost from fibrinogen on clotting represent the tips of chains split off by thrombin, and the glycine N-terminal groups of fibrin represent the "scars" left behind on the main body of the protein molecule. This simple picture is inadequate, however, to account for the analytical data obtained, since the number of glycine end-groups found in fibrin is at least twice as great as the number of glutamic acid end-groups and the number of ε-amino groups of lysine in the peptide material liberated (BAILEY AND BETTELHEIM[1]). It is quite conceivable, of course, that besides splitting off the ends of some chains, thrombin also splits other bonds involving the amino groups of glycine in the interior of the molecule, without the concomitant liberation of peptide fragments. Examination of the peptide material liberated has shown, however, that it contains at least two distinct peptides, which have been called peptides A and B respectively. N-terminal glutamic acid is present in peptide A only. No N-terminal residue is detectable in peptide B, the presence of which is therefore capable of accounting, partly at least, for the excess of the number of glycine over glutamic acid end-groups. The present paper describes the isolation and some properties of the two peptides.

EXPERIMENTAL

Materials

Silicone-treated Hyflo Super-Cel. HOWARD AND MARTIN[2] treated kieselguhr with the vapour of dichlorodimethylsilane, but in the present work it has been found more convenient to use this reagent as a solution (5%) in cyclohexane. To ensure penetration of the liquid into the particles of Hyflo Super-Cel, the suspension was placed in a desiccator which was then briefly evacuated at the water pump. The treated Hyflo Super-Cel was filtered off, air-dried, washed with 50% methanol until no longer acid and finally dried at 110°.

Fibrinogen and *thrombin* were obtained as described previously (BAILEY AND BETTELHEIM[1]).

Methods

Preparation of peptide material. For paper electrophoresis, the peptide material was mostly prepared by the procedure using ammonium acetate as previously described (BAILEY AND BETTELHEIM[1]). However, because of the complications which arise due to modification of the peptide material during the removal of the ammonium acetate (see below), a procedure has also been developed whereby clotting can be carried out in the almost complete absence of salts. The fibrinogen was dialyzed thoroughly at 2° against 0.001 N NH_3 after which it was found possible to lower the pH cautiously as far as 7.2 with acetic acid before the protein began to

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precipitate. The clot formed from this solution underwent synaeresis normally, and the clot liquor was dried from the frozen state.

Paper electrophoresis. The apparatus used was essentially an adaptation of that described by DURRUM[3] capable of taking a paper 27 cm wide. It consisted of a perspex trough divided longitudinally into two compartments, into which platinum electrodes were sealed. The paper was hung over a glass support standing in the trough, the height being such that the exposed part of the paper was 35 cm in length. To prevent excessive evaporation, the whole was covered by a perspex top when in operation. The materials were applied to the dry paper at the position which formed the apex during the run; when protein was present, it was not allowed to dry completely on the paper before the run.

With this simple apparatus, the displacement observed does not correspond quantitatively to the electrophoretic mobilities of the substances, due to the electro-osmotic flow of liquid towards the cathode, and the flow up the paper on either side to replace that lost by evaporation (DURRUM[3]). The latter effect is not necessarily a disadvantage for purposes of separation because, acting in opposition to the electrophoretic effect, it apparently causes flattening of the spots.

Whatman No. 1 or No. 3 paper was used. When it was desired to elute material for further examination, the paper was chromatographically washed before use with 2 *N* acetic acid for 24 hours, followed by water for 24 hours.

Ninhydrin reaction. Ninhydrin was sprayed on papers as a 0.1 % solution in *n*-butanol saturated with water. Colour development with the peptides was better at 60° than at 105°. It was found that the reaction could be made considerably more sensitive and reliable for the peptides by buffering the paper beforehand with acetate at a pH near 5. This was especially the case after electrophoresis in buffers of low pH, following which the papers were sprayed with a solution of sodium acetate (1 % or 5 %, according to the amount of buffer on the paper) in 75 % ethanol. Air-drying then left them at the desired pH.

Sakaguchi reaction. ACHER AND CROCKER[4] have also used this reaction for the detection of arginine compounds on paper. The reagents used in the present work were based on those of MACPHERSON[5], but urea was omitted, and the concentration of hypobromite correspondingly reduced. Three solutions were made up as follows:

A. KOH (10 %) in 75 % ethanol.

B. α-naphthol (0.1 %) in 75 % ethanol.

C. 2 g bromine dissolved in 100 ml KOH (5 %), and diluted to 500 ml with water.

One volume of A and two volumes of B were mixed and used as a first spray, followed by a light spraying with solution C. The pink colour produced was stable for several days.

RESULTS

Separation by paper electrophoresis

A number of fractionation procedures were applied to the peptide material from fibrinogen without giving evidence of the presence of more than one component. Thus, the 2:4 dinitrophenyl-derivative (DNP-derivative) (BAILEY AND BETTELHEIM[1]) was submitted to chromatography on columns of silica gel, with 0.1 *N* HCl as the stationary phase, and *n*-butanol→chloroform (4:1 v/v) or methyl ethyl ketone as moving phases; good single bands of $R = 0.15$ and 0.10, respectively, were obtained. Chromatography of the peptide material itself on paper was not very successful; with phenol–ammonia–coal gas (CONSDEN, GORDON AND MARTIN[7]) a streak near the solvent front was obtained, while with butanol–acetic acid (PARTRIDGE[8]), though a discrete spot was formed, the R_F was low (0.03). Paper electrophoresis, however, worked well and, at pH 4.1, effected a clear separation into two components, which gave comparable amounts of colour with ninhydrin and ran true when re-submitted to electrophoresis under the same conditions (Fig. 1). The one moving faster towards the anode has been designated A, the other B. Good separation was obtained only within a rather narrow range of pH around 4.1, the difference in mobility between the two components decreasing as the pH was either raised or lowered (see below). In some runs carried out in a cold room at 2° instead of room temperature, it was found advantageous to raise the pH to 4.4.

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For preparative purposes, a volatile buffer of ammonium acetate (ionic strength 0.05, pH 4.1) was used so as to obtain the peptides in a salt-free state. The material was applied to Whatman No. 3 paper as a thin streak, and after electrophoresis at room temperature for 16 hours at 6.3 volts/cm, the bands were located by cutting out and developing a marker strip. After elution with water, the peptides were precipitated with ethanol (6 volumes) and ether (12 volumes), a few drops of an ethanolic solution of LiCl being first added to facilitate the formation of a discrete precipitate rather than a gum. The washed precipitates were dissolved in a little water and dried from the frozen state to constant weight. From clot liquor corresponding to 3.2 g fibrinogen, which was fractionated in 7 separate electrophoretic runs, the yields were 33.8 mg peptide A and 34.5 mg peptide B.

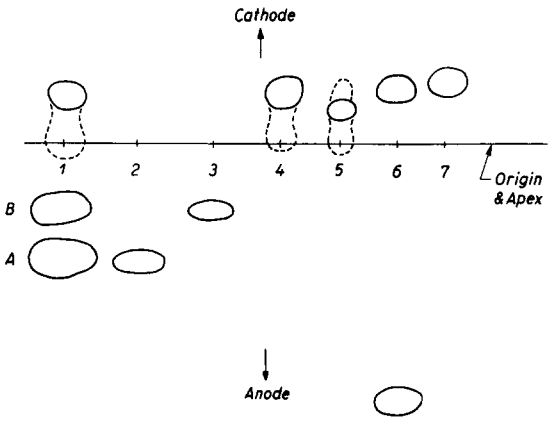

Fig. 1. Paper electrophoresis in sodium acetate buffer, pH 4.1, $\mu = 0.05$. 220 volts were applied over a 35 cm length of Whatman No. 1 paper for 16 hours. Developed with ninhydrin (glucose with aniline phthalate). 1, aqueous extract of freeze-dried ammonium acetate clot liquor corresponding to 30 mg fibrinogen. The material moving towards the cathode is unclotted protein. 2 and 3, water eluates of components A and B, respectively, from a previous run under the same conditions. 4, fibrinogen (1 mg). 5, thrombin (1 mg). 6, mixture of glycine and glutamic acid. 7, glucose.

Separation by column chromatography

The amount of material which can be handled at one time by means of paper electrophoresis is inconveniently small, and a different method of fractionation, utilizing partition chromatography, has therefore been developed

Preliminary experiments showed that satisfactory partition could be obtained between water and a variety of aliphatic solvents (*iso*butyric acid, and systems composed of *n*-butanol or *sec*-butanol used in conjunction with acetic or propionic acids), but the partition coefficients of peptides A and B did not differ strikingly. With phenol and collidine, however, peptide B passed into the organic phase to a much greater extent than peptide A. Using the former solvent on a reversed-phase partition column of Hyflo Super-Cel (HOWARD AND MARTIN[2]), it has been possible to develop a method which effects a separation clear-cut enough for the process to be carried out batch-wise. Both peptides, being acidic (see below), are held by the stationary phenol phase at low pH, while on raising the pH to neutrality only peptide A is eluted.

The phases were prepared by equilibrating phenol (1 part by weight) with 1.2% acetic acid (4 parts). Silicone-treated Hyflo Super Cel was mixed with half its weight of organic phase made into a slurry with the aqueous phase and packed into the column as described by PERRONE[6]. The fibrinogen, after purification by precipitation with ammonium sulphate, was clotted in the presence of that salt without further treatment. The clot liquor was deproteinized by the addition of trichloroacetic acid to a final concentration of 2.5%, then brought to 1% with respect to acetic acid

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and treated with just enough phenol to saturate it before applying, without preliminary concentration, to the column. The trichloroacetic acid was only slightly retarded, and the ammonium sulphate not at all, and both were removed by washing with the aqueous phase until the pH of the effluent reached that of the washing fluid. Peptide A was then eluted with the aqueous phase formed by equilibrating phenol (1 part) with 0.05 *N* ammonium acetate (4 parts), the washing again being continued until the pH of the effluent was as high as that of the eluant; this pH was 6.4, but is not critical, since peptide B was held on the column even when the pH was raised to 8.4 by addition of ammonia. Peptide B was brought off the column by washing off the organic phase with 50% methanol. The bulk of the phenol was removed from the fractions by distillation under reduced pressure in the presence of water, and the remainder by extraction with ether; peptide is lost into the ether phase unless most of the phenol is removed beforehand. Each peptide was then precipitated from concentrated aqueous solution with ethanol (6 volumes) and ether (12 volumes), dissolved in a little water and dried from the frozen state. In the biggest preparation which has been carried out, clot liquor from 16 g fibrinogen was fractionated on a column 5 cm in diameter and 8 cm high; the yields were 135 mg peptide A and 128 mg peptide B.

Some properties of peptides A and B

Both peptides are colourless, hygroscopic solids, dialyzable through cellophan and not precipitated by trichloroacetic acid at a concentration of 10%. They show no signs of undergoing any change which might be described as denaturation. Both are very readily soluble in aqueous media at all values of pH and ionic strength which have been used; in the dry state, they are intensely hygroscopic.

Constitution. The constituent amino acids of peptides A and B were identified by paper chromatography of acid hydrolysates, using phenol–ammonia–coal gas (CONSDEN, GORDON AND MARTIN[7]), butanol–acetic acid (PARTRIDGE[8]) and *tert.*-amyl alcohol–diethylamine (WORK[9]). The amino acids found are listed in Table I. No indication was found of the presence of cystine, methionine, *iso*leucine or histidine.

TABLE I

CONSTITUENT AMINO ACIDS OF PEPTIDES A AND B

Amino acid	*Peptide A*	*Peptide B*
Aspartic acid	+++	+++
Glutamic acid	+++	+++
Serine	++	?
Glycine	++++	++
Threonine	+	+
Alanine	absent	++
Tyrosine	absent	+
Valine	+	+
Leucine	+	+
Phenylalanine	+	+
Proline	++	++
Arginine	+	+
Lysine	absent	+

The number of +'s is intended to give some idea of the relative strengths of the spots produced with ninhydrin on two-dimensional chromatograms developed first with phenol-ammonia and then with butanol-acetic acid.

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The absence of sulphur-containing amino acids was confirmed by subjecting hydrolysates to oxidation with performic acid (TOENNIES AND HOMILLER[10]), after which treatment no cysteic acid and no methionine sulphoxide or sulphone could be detected. No tryptophan or carbohydrate could be found by the colour reactions with *p*-dimethylamino-benzaldehyde (SYNGE[11]) and orcin (TILLMANNS AND PHILIPPI[12]), respectively, and no phosphorus could be detected by the method of ALLEN[13].

Free amino groups. The unfractionated peptide material contains free α-amino groups of glutamic acid and ε-amino groups of lysine (BAILEY AND BETTELHEIM[1]). Upon applying the DNP technique to the separated components, peptide A gave rise to DNP-glutamic acid but no ε-N-DNP-lysine, whereas peptide B gave rise to ε-N-DNP-lysine but no detectable amount of an α-N-DNP-amino acid. A quantitative recovery experiment showed that substantially all the α and ε-amino groups of the peptide material could be accounted for by peptides A and B respectively (Table II).

TABLE II

RECOVERY OF FREE AMINO GROUPS OF CLOT LIQUOR IN PEPTIDES A AND B

Material	*DNP-glutamic acid (μ moles)*	*ε-N-DNP-lysine (μ moles)*
Clot liquor	0.60	1.20
Peptide A	0.50	< 0.10
Peptide B	< 0.10	1.15

2 samples of freeze-dried ammonium acetate clot liquor (BAILEY AND BETTELHEIM[1]) each corresponding to 300 mg fibrinogen were taken. One was treated with fluorodinitrobenzene (FDNB) directly, and insoluble DNP-protein removed before hydrolysis. The other was fractionated by paper electrophoresis at pH 4.1 (ammonium acetate buffer, $\mu = 0.05$), and the bands of A and B were cut out and treated with FDNB separately. All hydrolyses were for 8 hours in boiling 5.7 *N* HCl; no correction has been applied for destruction of DNP-amino acids during hydrolysis.

Various conditions were tried in attempts to detect a free α-amino group in peptide B. In some experiments, it was subjected to "denaturing" conditions (heating to 100° in water for 10 minutes, or treatment with hot 90% ethanol) before treatment with fluorodinitrobenzene (FDNB). Condensation with this reagent was carried out both in the presence of 1% $NaHCO_3$ (SANGER[14]) and of 1% trimethylamine (SANGER AND TUPPY[15]). Hydrolyses were mostly carried out in boiling 5.7 *N* HCl at 105° for 4 to 8 hours before extraction with ether, but since DNP-proline is rapidly destroyed under these conditions, hydrolysis in a sealed tube with 12 *N* HCl at 105° (PORTER AND SANGER[16]) was also tried. In no case could any yellow DNP-amino acid other than ε-N-DNP-lysine be detected, and it can therefore be stated with some confidence that peptide B contains no free α-amino group capable of reacting with FDNB.

Molecular weight. On the basis of the content of free amino groups (determined as DNP-glutamic acid and ε-N-DNP-lysine for peptides A and B respectively), the minimum molecular weight is of the order of 3,000 in each case. If it is true that only one residue each of N-terminal glutamic acid and of peptide lysine is liberated from one molecule of fibrinogen (see DISCUSSION), then the actual molecular weight must be the same as the minimum.

Iso-electric point. The iso-electric points of the isolated peptides were determined

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by paper electrophoresis in sodium citrate buffers at various pH values. Glucose was also put on the papers, and its displacement used as a correction for the amount of electro-osmosis taking place. The results are plotted in Fig. 2, which shows that the iso-electric points of peptides A and B are very close indeed to one another, both lying between 3.1 and 3.2. The reliability of the method was checked by using glutamic acid, for which an iso-electric point between 3.0 and 3.1 was found. This may be compared with literature values of 3.08 (SIMMS[17]), 3.22 (MIYAMOTO AND SCHMIDT[18]) and 3.24 (NEUBERGER[19]).

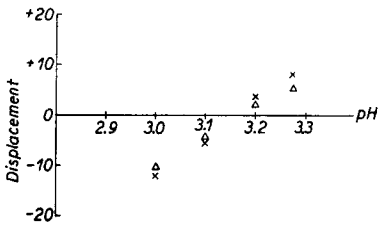

Fig. 2. Determination of iso-electric points of peptides A and B by paper electrophoresis. Ordinate: distance (mm) travelled relative to glucose (a positive value represents movement towards the positive electrode). Abscissa: pH of buffer solution (sodium citrate, $\mu = 0.05$). A potential difference of 220 volts was applied over a 35 cm length of Whatman No. 1 paper for 16 hours in each case. × Peptide A. △ Peptide B.

At pH values below the iso-electric point, for instance at pH 2.4, it again becomes possible to separate the two peptides by paper electrophoresis, peptide A now moving slightly more rapidly towards the cathode.

Inhibition of clotting. Slight inhibition of clotting by the peptides has been observed. Both peptides A and B, at final concentrations of 0.5%, approximately doubled the time required for citrated human serum to clot after recalcification. With a test system of purified fibrinogen and thrombin, it was possible to show a "progressive antithrombin" effect. Thus, unfractionated peptide material, added to a solution of thrombin in water to give a concentration of 0.2%, produced no immediate decrease in activity, but after standing at room temperature for 7 hours the test solution had lost 30% of its original activity, whereas a control solution had lost only 6%. This activity appears to reside in peptide A only, and was reduced rather than enhanced by the presence of heparin. Though the concentrations of peptides used in these experiments were higher than are ever likely to be attained physiologically, the effects observed were small, and it may be doubted whether they are of great importance *in vivo*.

The disappearance of α-amino groups

It has already been mentioned (BAILEY AND BETTELHEIM[1]) that, on taking the peptide material to dryness in the presence of ammonium acetate, α-amino groups of glutamic acid disappear. By means of paper electrophoresis, it has been possible to detect a substance which appears to be the form of peptide A which has lost its N-terminal glutamic acid residues. This material gives little or no colour with ninhydrin, and to locate it on paper a reaction not depending on free amino groups is necessary; the chlorine technique of RYDON AND SMITH[20] can be used, or, more conveniently, the Sakaguchi reaction for arginine (see section on *Methods*).

The amount of this material found was variable, being large when extensive destruction of free α-amino groups had taken place, such as when the ammonium acetate clot liquor was taken to dryness at 37°, and small when the temperature was kept low during this step. Furthermore, the formation of material resembling it in electrophoretic behaviour could be demonstrated when a solution containing peptides A and B and ammonium acetate was taken to dryness by warming. These

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facts constitute evidence that it is not a primary product of the clotting reaction, but material which has become modified during the isolation procedure.

The modified material is best detected by paper electrophoresis at low pH; thus, using ammonium formate buffer of ionic strength 0.03, pH 2.4, the distances travelled by the various peptides towards the cathode, after applying a potential gradient of 7.3 volts/cm for 12 hours at room temperature, were as follows: A, 5.2 cm; B, 4.5 cm; modified peptide, 2.3 cm. At higher pH, the modified peptide hardly separated from the other two; for instance, in ammonium acetate buffer of ionic strength 0.05, pH 5.1, other conditions being the same, the distances travelled towards the anode were A, 5.1 cm; B, 4.4 cm; modified peptide, 5.5 cm.

The disappearance of α-amino groups from the peptide material has been observed only when it is taken to dryness in the presence of salt; when salt-free it can be dried from the frozen state without detectable modification. Ammonium acetate is not specifically required, for the reaction has also been observed when the only salt present was sodium sulphate. The reaction does not take place on heating the aqueous solution near neutrality; when clot liquor at pH 6.0 was heated to 100° for periods of up to 90 minutes, no decrease in the amount of N-terminal glutamic acid, and no formation of modified peptide material, could be detected.

Small amounts of the modified material were prepared by elution from paper after electrophoresis in ammonium formate buffer at pH 2.4, and subsequent precipitation with ethanol and ether as described above for peptides A and B. The general amino acid composition resembled that of peptide A, showing, for instance, the same high content of glycine (*cf.* Table I). However, it also contained small quantities of alanine, tyrosine and lysine, which are constituents of peptide B but not of peptide A, though the amounts of these amino-acids present were considerably smaller than those in an equimolar mixture of the two peptides. For instance, the content of α-amino groups of lysine, determined as ε-N-DNP-lysine, corresponded to one equivalent per 20,000 g. No N-terminal residue could be detected by the DNP technique.

Relative rates of release of peptides A and B

Peptide B appears to be released more slowly during clotting than peptide A. An experiment was carried out in which an almost salt-free solution of fibrinogen (see section on *Methods*) was treated with an amount of thrombin such that the clotting time was 15 minutes, and clotting was complete in about 3 hours. Samples were removed 15, 40, 240 and 960 minutes after the addition of thrombin, unclotted protein was precipitated by the addition of two volumes of ethanol, and the supernatants concentrated and examined by paper electrophoresis at pH 4.1. The relative amounts of peptides A and B, as estimated roughly by the colours given with ninhydrin and with the Sakaguchi reagents, was 4:1, 2:1, 1:1 and 1:1 in the four samples, respectively.

DISCUSSION

Stoichiometry of the clotting reaction

The results presented here indicate clearly that at least two distinct types of peptide are liberated from bovine fibrinogen by thrombin. Peptide A carries all the N-terminal glutamic acid lost from fibrinogen on clotting. Peptide B is apparently

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devoid of an N-terminal residue, and its presence can therefore explain, partly at least, the discrepancy between the number of end-groups split off from fibrinogen and the number which appear in fibrin. The absence of lysine ε-amino groups from peptide A, and the difference between the two peptides in amino acid composition, leave no doubt that peptide B is quite distinct, and not merely peptide A modified by loss of its free α-amino group.

Since free α-amino groups of glutamic acid and ε-amino groups of lysine are characteristic of peptides A and B respectively, the amounts of these groups found in clot liquor give information about the number of molecules of each peptide released. According to the analytical data, one residue of N-terminal glutamic acid is derived from 265,000 g fibrinogen, and one residue of peptide lysine from 260,000 g (BAILEY AND BETTELHEIM[1]). Since the most likely value from physical measurements for the molecular weight of fibrinogen is 330,000 (EDSALL[21]) it is probable that one molecule of this protein gives rise on clotting to one molecule of each peptide. The amount of N-terminal glycine found in fibrin, one residue in 125,000 g, corresponds to 2 or 3 residues per molecule of fibrinogen. If the correct value is 2, then each end-group appearing can be correlated with a molecule of peptide split off. If the correct value is 3, then either thrombin splits a third bond in the interior of the molecule, without the concomitant release of a peptide, or a third peptide is liberated which has not been detected in the present work. This peptide would have to be devoid both of a free α-amino group and of a free ε-amino group of lysine, or it would have been detected by the DNP technique. No evidence has been obtained for the presence of such a substance in clot liquor.

Failure to detect an N-terminal residue in peptide B

Three possible explanations suggest themselves for the failure to detect an N-terminal residue in peptide B: folding of the chain in such a way as to make the α-amino group inaccessible to FDNB; masking of the amino group with some non-amino acid constituent; and the existence of cyclic chain. The first possibility appears unlikely in view of the relatively small size of the molecule, and the failure of denaturing agents to produce a reactive α-amino group. The second possibility has not been altogether excluded, but the third seems the most likely one.

From the demonstration that thrombin acts on synthetic substrates at bonds involving the carboxyl groups of arginine (SHERRY AND TROLL[22]), it is to be expected that both peptides A and B contain arginine in a C-terminal position. If this is so, then the case of peptide B is analogous to those of tropomyosin and tobacco mosaic virus, which contains C-terminal but apparently no N-terminal residues (LOCKER[23]; FRAENKEL-CONRAT AND SINGER[24]). Such a state of affairs could be explained by assuming the protein to be a "ring" carrying a "handle", *i.e.*, a cyclic chain branched at one point to carry a side chain ending in a C-terminal residue (BAILEY[25]).

Disappearance of α-amino groups

The mechanism by which amino groups of glutamic acid disappear from the peptide material remains largely unexplained. It is probably not analogous to pyrrolidone ring formation from glutaminyl or γ-glutamyl peptides in which the α-amino group is free for, unlike that reaction (VICKERY, PUCHER, CLARK, CHIBNALL AND WESTALL[26]; MELVILLE[27]) it does not occur on heating in neutral solution. The

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presence in the product of the reaction of small amounts of alanine, tyrosine and lysine, which are constituents of peptide B but not of peptide A, also indicates that the reaction is more complex.

Although the modified material formed has a lower mobility than peptide A at pH 2.4, its mobility is almost the same at pH 5.1, suggesting that carboxyl as well as amino groups disappear when it is formed. As far as the available evidence goes, it suggests that the α-amino groups of peptide A condense with carboxyl groups in the same or another molecule of peptide A, and also, though to a lesser extent, with carboxyl groups in peptide B. It seems unlikely that the modified material, as detected by paper electrophoresis, represents a single chemical substance. Except in that it is not enzymically catalysed, the reaction by which N-terminal glutamic acid residues disappear resembles somewhat the formation of "plastein" by the action of pepsin or trypsin on concentrated peptide mixtures, which has been shown in certain cases to be accompanied by a decrease in amino nitrogen (WASTENEYS AND BORSOOK[28]; VIRTANEN, KERKKONEN, HAKALA AND LAAKONEN[29]).

Role of electrostatic factors in clotting

The iso-electric point near 3.2 found for both peptides is strikingly low, and suggests that a change of electrostatic pattern on the fibrinogen molecule due to the splitting off of these acidic fragments may play a role in the clotting process. The forces principally responsible for holding the fibrin gel network together are probably hydrogen bonds (MIHALYI[30]), but electrostatic forces could be involved in the initial association of the monomer particles. The way in which they could do so has been discussed by FERRY, KATZ AND TINOCO[31].

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SUMMARY

1. The non-protein material released from fibrinogen by thrombin has been shown to contain at least two different peptides, which have been separated by paper electrophoresis and by column chromatography. Peptide A contains the N-terminal glutamic acid lost from fibrinogen on clotting. Peptide B contains no detectable N-terminal residue; its presence may therefore account for the fact that the number of glycine end-groups in fibrin is greater than the number of glutamic acid end-groups in fibrinogen.

2. Some properties of peptides A and B are described.

3. The N-terminal glutamic acid of peptide A shows a tendency to disappear, and at the same time there appears a new electrophoretic component in which no N-terminal residues are detectable.

RÉSUMÉ

1. L'action de la thrombine sur le fibrinogène laisse un matériel non protéique contenant au moins deux peptides différents qui ont été séparés par électrophorèse sur papier et par chromatographie sur colonne. Le peptide A contient l'acide glutamique N-terminal perdu par le fibrinogène lors de la coagulation. Le peptide B ne contient pas de résidu N-terminal décelable;

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sa présence peut, par conséquent, expliquer le fait que le nombre de groupes terminaux de glycine dans la fibrine est plus grand que le nombre de groupes terminaux d'acide glutamique dans le fibrinogène.

2. Nous décrivons ici quelques propriétés des peptides A et B.

3. L'acide glutamique N-terminal du peptide A montre une tendance à disparaître, et en même temps il apparaît un nouveau composant électrophorétique dans lequel on ne peut déceler aucun résidus N-terminaux.

ZUSAMMENFASSUNG

1. Es wurde gezeigt, dass das nicht proteinartige Material, welches aus Fibrinogen durch Thrombin in Freiheit gesetzt wird, mindestens zwei verschiedene Peptide enthält, die durch Papierelektrophorese und Säulenchromatographie getrennt wurden. Peptid A enthält die beim Gerinnen von Fibrinogen abgespaltene N-terminale Glutaminsäure. Peptid B enthält keine nachweisbaren N-terminalen Reste. Seine Gegenwart könnte deshalb die Tatsache erklären, dass die Zahl der Glycin-Endgruppen in Fibrin grösser als die Zahl der Glutaminsäure-Endgruppen in Fibrinogen ist.

2. Einige Eigenschaften der Peptide A und B wurden beschrieben.

3. Die N-terminale Glutaminsäure in Peptid A zeigt eine Tendenz zu verschwinden, während gleichzeitig eine neue elektrophoretische Komponent erscheint, in welcher keine N-terminalen Reste nachweisbar sind.

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