THE CLOTTING OF FIBRINOGEN

I. THE LIBERATION OF PEPTIDE MATERIAL

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The theory that the clotting of fibrinogen is a proteolytic process has been held in the past in a variety of forms. HAMMARSTEN[1] and HEUBNER[2] found that when their fibrinogen preparations were clotted, soluble protein remained behind in solution, and considered that thrombin might be a proteolytic enzyme splitting fibrinogen into insoluble fibrin on the one hand, and soluble "fibrinoglobulin" on the other. Purer fibrinogen, however, yielded no fibrinoglobulin on clotting (HUISKAMP[3]), showing that it was a contaminant of the earlier preparations of fibrinogen, and not a product of the clotting reaction. Later, it was found that thrombin preparations were able to cause not only the clotting of fibrinogen, but also subsequent dissolution of the clot (MILLS AND LING[4], PRESNELL[5], JAQUES[6]) suggesting that clotting represents merely an early stage in a process of extensive proteolysis. Consonant with this view was the ability of papain (EAGLE AND HARRIS[7]) and certain proteolytic snake venoms (EAGLE[8]) first to clot fibrinogen and then to redissolve the clot. HUDEMANN[9] and SEEGERS[10], however, were able to show that fibrinolysis was due to contamination of thrombin with one or more proteolytic enzymes from plasma. Highly purified thrombin cannot redissolve fibrin, except possibly in concentrations some 10,000 times greater than normally used to effect clotting (GUEST AND WARE[11]).

When fibrinolysis was eliminated, it was possible to recover a high proportion of the nitrogen of fibrinogen as fibrin (HUDEMANN[9], WARE, MURPHY AND SEEGERS[12]), and the hypothesis that thrombin is a proteolytic enzyme fell out of favour (see ASTRUP[13]).

Strong evidence has now been obtained that the clotting of fibrinogen is actually a proteolytic process of unusually high specificity. The evidence for this, comprising a study of the terminal groups of fibrinogen and fibrin, and an analysis of non-protein products formed during clotting, has already been reported in two preliminary communications (BAILEY, BETTELHEIM, LORAND AND MIDDLEBROOK[14]; BETTELHEIM AND BAILEY[15]) and is presented fully in this paper and the following one (BETTELHEIM[16]). Parallel work at Leeds (BAILEY, BETTELHEIM, LORAND AND MIDDLEBROOK[14]; LORAND AND MIDDLEBROOK[17]; LORAND[18]) has yielded results substantially in agreement with some of those reported here.

EXPERIMENTAL

Materials

Fibrinogen. This was prepared from fresh citrated ox plasma by 2 to 5 precipitations either with an equal volume of 2 *M* potassium phosphate buffer of pH 6.8 (JAQUES[19]) or with solid ammonium sulphate to 20% saturation, redissolving the precipitate in each case at an ionic strength of about 0.5. A final precipitation was usually carried out by adding ethanol at 0° to a final concentration of 10% (v/v). The purity of the products thus obtained appeared to be high, as indicated by the low content of non-clottable protein (less than 5%) and by the absence of foreign N-terminal groups (see below).

Fibrin. This was obtained by clotting the purified fibrinogen as described below.

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Thrombin. The purified preparation of human origin (MELLANBY[20]) appeared to be free from contamination with other proteolytic enzymes. Clots produced with it could be kept under sterile conditions for weeks without redissolving; and when it was incubated, under conditions similar to those used for clotting, with either rabbit myosin or ovalbumin, it caused no detectable liberation of N-terminal groups.

Carboxypeptidase. A five times recrystallized specimen was kindly provided by Dr. HANS NEURATH of the University of Washington, Seattle. In some experiments it was incubated before use with an excess of *diiso*propylphosphofluoridate (0.01 *M*) for 30 min at 37° to inhibit possible traces of chymotrypsin and trypsin in the preparation (JANSEN, FELLOWS NUTTING, JANG AND BALLS[21]), but the results were the same when this pretreatment was omitted.

Talc. This was obtained from Hopkins & Williams, Ltd. Before use, it had to be freed of fine particles which block the columns. Washing simply by decantation (SANGER[22]) was only partially successful, but when the suspension of talc in dilute HCl was boiled, the fine material was carried to the top as a scum and could be sucked off; it was necessary to repeat this procedure only once to obtain a product which gave good columns.

Methods

Analyses for N-terminal groups. The proteins were converted into their 2:4 dinitrophenyl (DNP) derivatives by treatment with 1:2:4 fluorodinitrobenzene (FDNB) as described by SANGER[23], and hydrolyses were carried out in boiling 5.7 *N* HCl. For quantitative estimation, the ether-extractable DNP-amino-acids were subjected to chromatography on columns of unbuffered silica gel. The use of two short columns with solvent systems giving large differences in R values was preferred to the use of one long column. The solvents were chosen from those given by SANGER[23]. The first column was developed with chloroform, in which the yellow artifacts of the DNP procedure (principally dinitrophenol and dinitroaniline) moved fast, bis-DNP-tyrosine and DNP-glycine moved in a band of $R = 0.4$ to 0.5, and DNP-glutamic acid remained near the top of the column, from which it was eluted with ether. The tyrosine and glycine derivatives were separated on an ethylene glycol-benzene column, on which bis-DNP-tyrosine moved at $R = 0.5$, while DNP-glycine remained near the top of the column and was eluted with ether. The separated α-N-DNP-amino acids were dissolved in 1% $NaHCO_3$ and estimated spectrophotometrically at 350 mμ; ε-N-DNP-lysine was estimated in *N* HCl at 390 mμ (SANGER[22]).

On the batch of silica gel used, the DNP-amino acids moved in chloroform at R values greater than those given by SANGER[23]; DNP-leucine, for instance, ran at $R = 1.3$, and was therefore not reliably separated from the unretarded yellow artifacts. To exclude the presence of the faster-moving DNP-amino acids, silica gel buffered at pH 7.0 (buffer B of BLACKBURN[24]) was used to retard them more. For purpose of detection and identification, use was also made of paper chromatography, especially the system employing *tert*-amyl alcohol and paper buffered with phthalate (BLACKBURN AND LOWTHER[25]).

Degradation of protein by carboxypeptidase. The fibrinogen used for these experiments was freshly precipitated by ethanol in the cold, and dissolved to give an approximately 1% solution in 3% ammonium acetate, the volatile salt being used in order to avoid interference with the subsequent chromatography. The fibrin could not be dissolved under conditions suitable for the action of the enzyme and had to be used in the form of solid particles; because of this fact, quantitative estimation of the liberated amino acids was not attempted. Before use, the gel was cut up as finely as possible in a Waring blendor, and washed with water and 3% ammonium acetate solution before being suspended in the latter medium. At the pH of the enzymic digestion (8.0), the particles were found to swell, taking up appreciable amounts of liquid, and it is probable that the carboxypeptidase was able to penetrate the interstices of the gel network, for it seemed to act reasonably well on the insoluble protein (*cf.* the permeability of fibrin film to haemoglobin and plasmin (FERRY AND MORRISON[26]). The results were not appreciably modified by heat-denaturing either protein before digestion. The pH of the protein samples was adjusted to 8.0 with ammonia, and crystalline carboxypeptidase was added to give a concentration of 0.05 mg per ml. Incubation was carried out at 37°, and was ended by inactivating the enzyme at 100° for 5 min. The digests were deproteinized where necessary by the addition of 3 volumes of ethanol, and aliquots were chromatographed on filter paper, using butanol-acetic acid (PARTRIDGE[27]) as solvent. Blanks in which either enzyme or substrate were omitted did not contain appreciable quantities of amino acids.

Preparation of clot liquor. Fibrinogen was dissolved to give a concentration of about 1% in NaCl, Na_2SO_4, potassium phosphate buffer or ammonium acetate solution of ionic strength 0.3 to 0.4. The pH was brought to 6.5–7.0 so as to obtain the "coarse" type of clot which undergoes synaeresis readily (FERRY AND MORRISON[26]). Enough thrombin was added to develop rigidity in about 5 minutes at room temperature, clotting being then complete in 1–2 hours. The time for which the reaction was allowed to proceed was not critical, and in some experiments the clotting system was allowed to stand overnight in the presence of toluene. To separate the fibrin

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from the clot liquor, the clot was treated for a short time in a Waring blendor, which served not only to cut up the fibrin, but also to induce synaeresis. The compact particles of fibrin could then be readily filtered off and pressed to squeeze out as much liquid as possible; 97% of the clot liquor can be recovered in this way.

RESULTS

N-terminal residues of fibrinogen and fibrin

The N-terminal groups of bovine fibrinogen are tyrosine and glutamic acid (BAILEY[28]). In order to confirm that both these end-groups really belong to fibrinogen and not to some impurity, preparations obtained by repeated salting-out were fractionally precipitated in the cold with ether, acetone and dioxan, and with ethanol in the presence of 6 *M* urea; no significant variation in end-group content was observed between the fractions obtained. Fibrin also contains tyrosine end-groups, but instead of glutamic acid it has N-terminal groups of glycine. In order to ensure maximum reactivity both proteins were always dissolved in 6 *M* urea before treatment with FDNB. This minimises the possibility that the glycine end-groups are not actually liberated during clotting, but merely rendered more accessible to the reagent (*cf.* PORTER[29]; BAILEY[28]). The small quantity of purified thrombin added to effect clotting contained negligible amounts of N-terminal groups.

The results of quantitative analyses are summarized in Table I. The chief source of uncertainty is the factor used to correct for the destruction of DNP-amino acids during hydrolysis. In the present work, this was estimated by determining recoveries of the appropriate DNP-amino acids after boiling under the actual conditions of the hydrolysis—*i.e.*, in 5.7 *N* HCl in the presence of DNP-fibrinogen or DNP-fibrin. This method allows for the effect of substances destroyed as hydrolysis proceeds, such as tryptophan, which has been shown to increase the destruction of several DNP-amino acids (THOMPSON[30]). The correction factor found for DNP-glycine is close to that determined after boiling in 5.7 *N* HCl alone, but the destruction of DNP-glutamic acid and bis-DNP-tyrosine was increased by the addition of DNP-protein appreciably above the values given by SANGER[23] and PORTER AND SANGER[31]. A further complication which was allowed for was the formation on hydrolysis of a yellow artifact derived from bis-DNP-tyrosine (*cf.* PORTER AND SANGER[31]), which runs close to DNP-glutamic acid on silica gel columns developed with chloroform.

The limited accuracy of the analyses, and the uncertainty surrounding the particle weight of fibrinogen, leave some doubt as to the number of N-terminal groups

TABLE I

N-TERMINAL RESIDUES OF FIBRINOGEN AND FIBRIN

N-terminal residue	*Period of hydrolysis (hours)*	*Correction factor**	*Weight of protein containing one mole N-terminal residue***	
			Fibrinogen	*Fibrin*
Glutamic acid	8	65	265,000	—
Tyrosine	8	65	150,000	150,000
Glycine	8	45	—	126,000

* % DNP-amino acid remaining after hydrolysis. See text.
** Weight of protein = 0.7 × weight of air dried DNP-protein.

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per molecule. Earlier estimates of the molecular weight, which gave values around 500,000 (NANNINGA[32]; STEINER AND LAKI[33]) have recently been revised downwards until the most likely value now appears to be 330,000 (see EDSALL[34]). Using this value, the most probable number of N-terminal groups would be two of tyrosine and one of glutamic acid per molecule of fibrinogen, and two of tyrosine and two or three of glycine in an approximately equal weight of fibrin.

The analytical figures given here may be compared with those obtained by LORAND AND MIDDLEBROOK[17]. The differences are probably due principally to the different methods used for estimating the breakdown of DNP-amino acids during hydrolysis.

C-terminal residues of fibrinogen and fibrin

A limited amount of information about C-terminal residues was obtained by the use of carboxypeptidase—a technique which has proved useful in some instances, though not of universal applicability (NEURATH AND SCHWERT[35]; NEURATH, GLADNER AND DAVIE[36]). When fibrinogen and fibrin were incubated with this enzyme, the amino acid produced in largest quantities was phenylalanine in each case; in addition, at least three other spots were found on the paper chromatograms, their R_F values corresponding to leucine and/or *iso*leucine, valine and/or methionine and/or tryptophan, and glutamic acid and/or threonine respectively. Qualitative differences were not observed when the incubation time was varied from 30–270 minutes, but greater amounts of the amino acids were found after the longer periods of enzyme action. The maximum yield of phenylalanine from fibrinogen was approximately one to two equivalents per mole of substrate. The yield from fibrin was one-half to one-third as much, but the general pattern of the amino acids liberated was indistinguishable from that obtained with fibrinogen. This suggests that the C-terminal residues susceptible to carboxypeptidase are the same in the two proteins, and that the lower yield from fibrin was due merely to the insolubility of the substrate. As far as can be detected by the carboxypeptidase technique, therefore, there is no change in C-terminal residues on clotting.

Fate of N-terminal glutamic acid

The N-terminal glutamic acid which is lost from fibrinogen on clotting does not disappear. It can be found after clotting in the non-protein fraction, which remains soluble after treatment with FDNB.

In a preliminary experiment, a whole clot, together with its clot liquor, was treated with FDNB; when the reaction was completed, the supernatant as well as the insoluble fraction was hydrolysed and examined for ether-extractable DNP-amino acids. An equal amount of fibrinogen was similarly treated to act as a control. Traces of several DNP-amino acids were found in the supernatants from both fibrinogen and fibrin, but all were identical in amount in the two cases with the striking exception of DNP-glutamic acid; much more of this was present in the supernatant from fibrin than in that from fibrinogen, and the difference between the two was equivalent to the amount which had disappeared from the insoluble fraction (Table II).

A further experiment showed that the substance which yields the extra DNP-glutamic acid is not free glutamic acid, but a peptide of some size containing glutamic

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TABLE II

FATE OF N-TERMINAL GLUTAMIC ACID DURING CLOTTING*

N-terminal residue	Amount found in DNP-insoluble fraction (μ moles)			Amount found in DNP-soluble fraction (μ moles)		
	Fibrinogen	Fibrin	Difference	Fibrinogen	Fibrin	Difference
Glutamic acid	0.50	0.14	0.36	0.21	0.71	0.50
Other amino acids	Not measured		—	0.64	0.71	0.07

* The amount of protein used was 0.145 g in each case. All fractions were hydrolyzed for 4 hours in boiling 5.7 *N* HCl; no correction factors have been applied. The discrepancy between the glutamic acid values in the third and sixth columns can be partly explained by the greater destruction of DNP-glutamic acid in the presence of a large amount of DNP-protein.

acid as its N-terminal residue; this follows from the fact that the DNP-derivative is not extractable from acid aqueous solution into ether, as DNP-glutamic acid is. The peptide was found entirely in the clot liquor, and not adsorbed on, or occluded within, the clot itself.

These results indicate clearly that, by the action of thrombin, peptide material is split off from the fibrinogen molecule. The appearance of glycine end-groups and the apparent disappearance of glutamic acid end-groups now both fit into one picture: the glycine end-groups represent the "scars" left behind on fibrin when the peptide material is split off. The fact that there are more glycine end-groups in fibrin than glutamic acid end-groups in fibrinogen is discussed in the following paper (BETTELHEIM[16]).

The conclusion that peptide material is liberated during the clotting of fibrinogen was reached independently by LORAND[18], who found that non-protein nitrogen amounting to 3 to 4% of the total nitrogen is formed during the reaction.

Preparation of peptide material as the DNP derivative

In preparing the peptide material, the principal problem is the removal of the large excess of inorganic salt used to bring the fibrinogen into solution before clotting. This salt cannot be dialyzed away, since the peptide material also passes rapidly through cellophan dialysis tubing. A convenient and rapid method has been developed for preparing the peptide material as its DNP-derivative, which can be entirely freed from salt by adsorption on talc and subsequent elution with ethanol (SANGER[22]).

The fibrinogen was dissolved before clotting with the aid of NaCl, Na_2SO_4 or potassium phosphate buffer, pH 6.8; an ammonium salt cannot be used, since it would interfere with the subsequent condensation with FDNB. After clotting, the clot liquor was concentrated by distillation under reduced pressure, and treated with FDNB under the conditions prescribed by SANGER[23]; unclotted protein was precipitated during this step, and was removed. The solution was now diluted with water, and excess FDNB removed by extraction ether. After lowering the pH to about 2 with HCl, the solution was again extracted with ether; these second ether extracts contained the DNP derivatives of any traces of free amino acids, while the DNP peptide material remained in the aqueous phase.

The adsorption on talc and subsequent elution are best done chromatographically. The column was made from a slurry of the washed talc in 0.01 *N* HCl. The acid

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solution of the DNP-peptide material was put on, and the column washed with water; the yellow material remained firmly adsorbed at the top, while the salt was not retarded and was removed by washing with water. For elution, acid ethanol (SANGER[22]) was found to be only moderately effective, but with 80% ethanol containing 0.3% ammonia all the yellow material came off rapidly.

When the alcohol eluate was evaporated to dryness under reduced pressure, the DNP-peptide material was obtained as an amorphous yellow solid, readily soluble in aqueous ethanol or dilute alkali, but only sparingly soluble in dilute acid. It dialyzed slowly through cellophan. On hydrolysis for 4–8 hours in boiling 5.7 *N* HCl, DNP-glutamic acid and ε-N-DNP-lysine were produced in approximately equimolar proportions, showing that the peptide material contains one free ε-amino group of lysine per N-terminal group. No ether-extractable DNP-amino acid other than DNP-glutamic acid could be detected.

Preparation of the peptide material in the free state

In order to prepare the peptide material in a salt-free state without converting it into the DNP derivative, clot liquors were prepared containing no salt other than ammonium acetate, which is volatile and was removed under reduced pressure.

Fibrinogen was freed of salts other than ammonium acetate by precipitating it with ethanol at 0° and redissolving in 3% ammonium acetate solution. After clotting with thrombin, the clot liquor was taken to dryness in a vacuum desiccator containing both H_2SO_4 and NaOH. A white residue remained, from which the peptide material was extracted with water, leaving behind some insoluble protein. When necessary, the solution was deproteinized by the addition of an equal volume of ethanol, or of trichloroacetic acid to a final concentration of 5%.

The conditions to which the peptide material was subjected during this procedure seem very mild, but nevertheless it was evidently not obtained in an unaltered form, for the amount of N-terminal glutamic acid found after taking to dryness was less than that expected. The quantity remaining depended on the temperature at which the ammonium acetate was removed. In one experiment, in which this step was performed at 37°, only 10% of the expected amount of glutamic acid end-groups remained. Real freeze-drying was not possible, because the material melted as the ammonium acetate concentration increased, but when the temperature was kept as low as possible by reducing the pressure to 0.1 mm Hg, 40–70% of the expected amount was found in a series of experiments. The number of free ε-amino groups of lysine, determined as ε-N-DNP-lysine, was always constant within the experimental error, the average amount obtained corresponding to one equivalent from 260,000 g fibrinogen. It thus appears that some reaction occurred during the removal of the ammonium acetate by which the free α-amino groups of glutamic acid disappeared. The nature of this reaction is discussed in the following paper (BETTELHEIM[16]).

The material obtained by the above procedure is a colourless hygroscopic solid, readily soluble in water. On cooling a solution in hot 90% ethanol, it separated in the form of spheroids. Its constitution is evidently rather complex, for in acid hydrolysates the following 13 amino acids could be identified by means of paper chromatography: aspartic acid, glutamic acid, serine, glycine, threonine, alanine, tyrosine, valine, leucine, phenylalanine, proline, arginine and lysine.

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Action of thrombin at low pH

By the action of thrombin at low pH (4.85 or 5.1), fibrinogen becomes modified in such a way that the clotting time on neutralization is shortened (LAKI AND MOMMAERTS[37]), but no clotting or even partial polymerization takes place, the viscosity and sedimentation diagrams remaining unchanged (LAKI[38]). Evidence has now been obtained that the proteolytic action of thrombin is fundamentally the same at low pH as in more alkaline solutions in which clotting occurs.

A solution of fibrinogen in 0.3 *M* KCl was adjusted to pH 4.85 and incubated for 16 hours at room temperature with an amount of thrombin sufficient to develop rigidity in 10 minutes in a similar solution at pH 6.8. The protein was then precipitated with ethanol to a final concentration of 20% and converted into the DNP derivative in the usual way, except that $NaHCO_3$ was not added until after the FDNB, so as to minimize the exposure of the protein to thrombin at alkaline pH.

From the supernatant, peptide material was isolated as the DNP-derivative as described above. Glycine end-groups were found in the protein, and DNP-glutamic acid and ε-N-DNP-lysine in approximately equal amounts were obtained from the DNP-peptide material. The action of thrombin was little more than half complete, however, as judged from the relative amounts of DNP-glutamic acid obtained from the peptide and protein fractions. This may have been due to inactivation or low activity of thrombin in the acid medium.

DISCUSSION

The specificity of thrombin

The results reported above show that thrombin acts proteolytically, but that it is unique amongst the known proteolytic enzymes in the very high specificity of its action. The fibrinogen molecule contains some 3,000 amino acid residues, but only 2 or 3 bonds are ruptured during clotting; no formation of extra end-groups was observed when clotting systems were allowed to stand for several hours after clotting was complete. Furthermore, no proteolysis at all was detected when thrombin was incubated with ovalbumin or rabbit myosin. The fibrinolytic activity of thrombin reported by GUEST AND WARE[11] was observed only with concentrations some 10,000 times greater than those normally used to effect clotting. However, it is interesting that thrombin appears to have an affinity for fibrinogen additional to that involved in its clotting activity (WAUGH AND LIVINGSTONE[39]); perhaps the enzyme has lost the ability to split bonds other than a few special ones, but retained an affinity for a wider variety.

The formation of N-terminal glycine residues during clotting indicates that thrombin attacks fibrinogen at bonds involving the amino groups of glycine. Information about the possible other participant in the susceptible bonds has come from the work of SHERRY AND TROLL[40], who have shown that plasmin-free thrombin attacks synthetic compounds at bonds involving the carboxyl groups of arginine; for instance, the trypsin substrate tosyl arginine methyl ester is split into tosyl arginine and methyl alcohol. Analogous compounds containing amino acids other than arginine were not attacked. There is thus evidence, albeit indirect, that thrombin splits arginylglycine links in fibrinogen. It is clear that in proteins most of the bonds involving the carboxyl groups of arginine are not split by thrombin.

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A preliminary experiment has shown that thrombin is strongly inhibited by di*iso*propylphosphofluoridate; this fact further enhances the analogy with trypsin (*cf.* JANSEN, FELLOWS NUTTING, JANG AND BALLS[21]).

Limited proteolysis

Besides the clotting of fibrinogen, a number of protein transformations are known which take place by proteolysis, but in no other case is the extent of proteolysis so sharply limited. In the conversion of ovalbumin into plakalbumin, for instance (LINDERSTRØM-LANG AND OTTESEN[41]), two or three bonds out of a total of about 400 are split some 10 times faster than any others. In the activation of chymotrypsinogen, the initial tryptic hydrolysis yielding π-chymotrypsin is rapidly followed by further proteolytic changes (JACOBSEN[42]; ROVERY, FABRE AND DESNUELLE[43]; GLADNER AND NEURATH[44]). The autocatalytic activation reactions of pepsinogen and trypsinogen are both followed by secondary hydrolysis, though in the latter case this can be inhibited by Ca^{++} (NORTHROP, KUNITZ AND HERRIOTT[15]; HERRIOTT[46]).

When an active enzyme is formed proteolytically from an inactive precursor, it is usually considered that "unmasking" of an active centre takes place, either by the unfolding of the molecule or by the splitting off of a part of it. Similarly, in the clotting of fibrinogen, it seems likely that aggregation occurs, in the first instance at any rate, at sites which are exposed when the peptide material is split off.

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SUMMARY

1. Estimations by the DNP technique indicate that the N-terminal residues of bovine fibrinogen are glutamic acid (1 mole per 265,000 g protein) and tyrosine (1 mole per 150,000 g), while those of fibrin are glycine (1 mole per 125,000 g) and tyrosine (1 mole per 150,000 g).

2. The pattern of amino acids liberated by carboxypeptidase is similar for fibrinogen and fibrin, phenylalanine being the main component in each case.

3. Peptide material is released from fibrinogen on clotting. The N-terminal glutamic acid which is present in fibrinogen but not in fibrin represents the tips of chains which are split off, while the N-terminal glycine of fibrin represents the "scars" left behind on the main body of the protein molecule.

RÉSUMÉ

1. Les résultats obtenus par la technique DNP indiquent que les résidus N-terminaux du fibrinogène de boeuf sont: l'acide glutamique (1 mole pour 265,000 g de protéine) et la tyrosine (1 mole pour 150,000 g de protéine), tandis que ceux de la fibrine sont: la glycine (1 mole pour 125,000 g de protéine) et la tyrosine (1 mole pour 150,000 g de protéine).

2. La composition du mélange d'acides aminés libérés par l'action de la carboxypeptidase sur le fibrinogène et sur la fibrine est similaire dans les deux cas, la phenylalanine en étant le composant principal.

3. Lors de la coagulation, le fibrinogène abandonne du matériel peptidique. L'acide glutamique N-terminal, qui est présent dans le fibrinogène, mais non dans la fibrine, représente les extrémités des chaînes qui sont scindées, tandis que la glycine N-terminale de la fibrine représente les "cicatrices" laissées sur le corps principal de la molécule protéique.

ZUSAMMENFASSUNG

1. Mit Hilfe der DNP-Methode durchgeführte Versuche ergaben, dass die N-terminalen Aminosäurereste in Rinder-Fibrinogen Glutaminsäure (1 Mol je 265,000 g Protein) und Tyrosin

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(1 Mol je 150,000 g) sind, während in Fibrin Glycin (1 Mol je 125,000 g) und Tyrosin (1 Mol je 150,000) gefunden wurden.

2. Die Zusammensetzung des durch Carboxypeptidase in Freiheit gesetztes Aminosäuregemisches ist für Fibrinogen und Fibrin ähnlich; Phenylalanin ist in beiden Fällen der Hauptbestandteil.

3. Beim Gerinnen wird aus Fibrinogen Peptid-Material in Freiheit gesetzt. Die in Fibrinogen, nicht aber in Fibrin vorhandene N-terminale Glutaminsäure bildet die Kettenenden, welche abgespalten werden, während das N-terminale Glycin in Fibrin den "Stamm" darstellt, der an dem Haupt-Körper des Proteinmoleküls zurückgelassen wird.

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