STUDIES ON FIBRINO-PEPTIDE

by

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Previous reports described the findings that N-terminal residues of glycine are set free in the fibrinogen molecule when acted upon by thrombin, and the number of end-groups liberated is proportional to the amount of fibrin formed in the clotting mixture[1,2,3]. It was also demonstrated that the action of thrombin is accompanied by a simultaneous liberation of non-protein N (trichloracetic acid-soluble N)[3–8], and a peptide—called fibrino-peptide—was found to appear at the same time[4,5,9,10]. Fibrino-peptide has been isolated[3,5] and shown to possess α-amino groups of glutamic acid in good agreement with the finding that the N-terminal residues of glutamic acid of fibrinogen cannot be found in fibrin[2,9,10].

The present note gives an account of some of our analytical studies on fibrino-peptide prepared from a bovine source as described previously[3,5]. It has already been reported[4] that the ultra-violet spectrum of the peptide in aqueous solution indicates the absence of tyrosine and tryptophane. One- and two-dimensional chromatograms of the peptide gave no indication of free amino-acids in the preparation. The acid hydrolysate of the peptide, however, revealed the presence of the amino acids shown in Fig. 1.

For further analysis the peptide was treated with 1-fluoro-2,4-dinitrobenzene by SANGER's method[11] and hydrolysed for 16 h with 5.7 *N* HCl, then extracted with ether and the yellow ether-soluble fraction separated on a pH 6.0 silica-gel column with ether. Thus dinitrophenyl-glutamic acid was obtained, indicating glutamyl or glutaminyl end-groups. Re-treatment of the hydrolysate with fluorodinitrobenzene and separation of the dinitrophenyl-amino acids on various silica-gel columns with various solvents[12] showed that the peptide contains the amino acids listed here, and judging by colorimetric estimations the approximate ratios stated were obtained.

Arginine	1
Lysine	1
Leucine (iso)	1
Phenylalanine	1
Valine	1
Alanine	1
Threonine	1
Serine	2
Glutamic acid	3
Aspartic acid	4
Glycine	4
Total number of residues	20

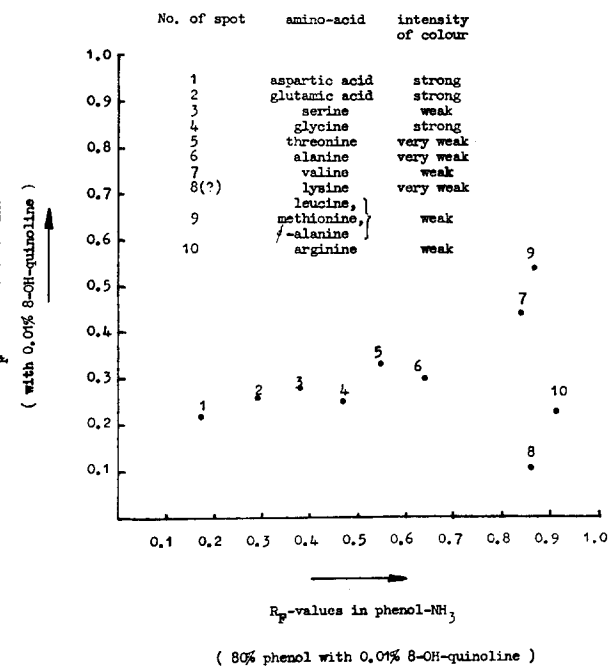

Fig. 1. Two-dimensional ascending chromatogram of acid-hydrolysed fibrino-peptide. Whatman No. 4 paper, room temperature.

The chemical analysis as such would give a minimum molecular weight of about 2200, but on the basis of the estimation of N-terminal residues of glutamic acid the value is nearer to 8000. The

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latter value, however, should be regarded with caution in view of the fact that such an estimation of the molecular weight presupposes that all the peptide chains are in the open form with their amino end-groups free to react with fluorodinitrobenzene; also the actual estimation itself may involve a relatively high degree of inaccuracy.

Fibrino-peptide thus appears to consist primarily of acidic and neutral amino acids, indicating that the part of the fibrinogen molecule from which the peptide is derived is richer in these than the rest of the protein. Fibrino-peptide was found to have an isoelectric point in the region of pH 3.3[9], and it would therefore represent at physiological pH highly charged negative centres in the fibrinogen molecule. During the enzymic phase of the clotting reaction[4]

$$\text{fibrinogen} \xrightarrow{\text{thrombin}} \text{fibrin} + \text{fibrino-peptide},$$

the release of the peptide would remove these strongly charged centres and would thereby help the particles to approach one other more easily in the process of clot formation.

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UNRELIABILITY OF THE DATA HITHERTO REPORTED ON THE DESOXYPENTOSENUCLEIC ACID CONTENT OF CELL NUCLEI DETERMINED BY MICROSPECTROPHOTOMETRY

by

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Recently efforts have been centred on the microspectrophotometric measurements of the DNA content of individual nuclei for various biological materials. Meanwhile, serious discrepancies have emerged among the results published from different laboratories, especially about the constancy *vs.* variability of the DNA content of individual diploid nuclei from the same species and sometimes even from the same type of cells[1,2,3,4]. Although such discrepancies may partially be due to factors involved in the FEULGEN reaction, in preparation of the material, or in reduction of the measured values[5,6], the most important source of error seems to be due to the use of photometric equipments[7,8,9] the optical system of which does not eliminate the little known "SCHWARZSCHILD-VILLIGER effect" (S–V effect), which has been foreseen theoretically and which proved experimentally to cause considerable error in the measurement of transmittance[10]. Especially with objects of such a high ab-