On the Structure of Human Fibrinopeptides

BIRGER BLOMBÄCK*, MARGARETA BLOMBÄCK* and PEHR EDMAN

St. Vincent's School of Medical Research, Melbourne, N. 6, Vic. Australia

When human fibrinogen is transformed to fibrin three peptides are split off from the N-terminal ends of the molecule [1]. These peptides have tentatively been named A, AP and B. With the phenylisothiocyanate method [2] the complete amino acid sequence of the A peptide (Peak 3 peptide) as well as a partial sequence of the phosphorus containing AP-peptide (Peak 1 peptide) have been determined and the results reported elsewhere [1].

The amino acid sequence of peptide AP has now been shown to be identical with that of peptide A except for the presence of O-phosphoserine instead of serine in the third position from the N-terminal end [1,3]. The sequence, Asp-Ser-Gly, around the serine residue has a remarkable similarity to the sequence around the reactive serine residue found in a variety of proteolytic enzymes (*cf.* Ref. [4]).

The complete structure of the AP-peptide seems to be present in the fibrinogen itself before clotting as it has been shown that human fibrinogen contains covalently bound phosphorus which is partially released during clotting and recovered in the phosphopeptide [5].

A third human fibrinopeptide has tentatively been denoted peptide B. Considerable difficulties were encountered in isolating this peptide in pure form from the clot supernatant. In a previous note the isolation by chromatography of a Peak 2 peptide was reported [1]. This peptide gave on electrophoresis a single weakly ninhydrin positive spot, but was later found to be a complex composed of peptide A and B in a molar ratio of roughly 1:2. It was observed that the complex could be dissociated in acid solution. The Peak 2 peptide preparation was dissolved in 0.05 M pyridine to a concentration of 0.5–1 %, and the pH of the solution adjusted to pH 3–3.5 with formic acid. After standing in the cold (+ 4°) for at least 24 h a precipitate consisting of peptide B was formed. The precipitation was facilitated by intermittent scratching of the vessel wall with a glass rod. The precipitate was washed with cold 3 % (v/v) acetic acid. After reprecipitation the pure B-peptide was obtained. The B-peptide could also be obtained from a concentrated, salt-poor clot supernatant on acidifying to pH 3. However, in this case ethanol to a concentration of 50 % was added to facilitate the precipitation.

The purified B-peptide gave on electrophoresis in 0.1 M pyridine-acetate buffer, pH 4.1, a single spot, which was revealed by chlorination or with Sakaguchis α-naphthol reagent. It was ninhydrin negative. No N-terminal amino acid could be demonstrated with the phenylisothiocyanate method. Amino acid analysis [6] showed that the peptide consisted of 14 amino acid residues.

Fragmentation of the peptide with chymotrypsin, elastase, and papain gave several fragments which were submitted to amino acid and amino acid sequence analysis [2]. The results are summarized in Fig. 1. From these studies the C-terminal sequence of 10 amino acid residues could be preliminary deduced. It is interesting to note that the C-terminal sequence Ala-Arg of the human B-peptide is identical with that of the bovine B-peptide [7].

The remaining four amino acid residues from the N-terminal end of the B-peptide were found in a ninhydrin negative tetrapeptide, obtained by digestion with papain. No N-terminal amino acid could be demonstrated in the fragment. On paper electrophoresis at pH 4.1 the tetrapeptide moved somewhat slower than aspartic acid towards the anode. The peptide was disclosed by chlorination. After complete acid (HCl) hydrolysis the peptide was found to be composed of equimolar amounts of aspartic acid, glutamic acid, glycine and valine.

Next, the tetrapeptide (1 mg peptide in 0.6–1 ml of 1 % ammonium bicarbonate, pH 8) was treated [8] with carboxypeptidase A. The weight ratio enzyme to substrate was 1:20. Samples were taken after 1, 2, 4, and 24 h at 37°C. The samples were chromatographed [9] in phenol-borate buffer, pH 9.3. After 1 h only asparagine could be demonstrated on the chromatogram whereas after longer digestion times increasing amounts of valine as well appeared. The C-terminal sequence of the tetrapeptide therefore seemed to be valyl-asparagine. The sequence of the remaining two N-terminal residues was obtained by partial acid hydrolysis of the tetrapeptide. 1 mg peptide was dissolved in 100 μl of 5.7 N HCl

* Present address: Coagulation Research Laboratory, Chemistry Dept. II, Karolinska Institutet, Stockholm, Sweden.

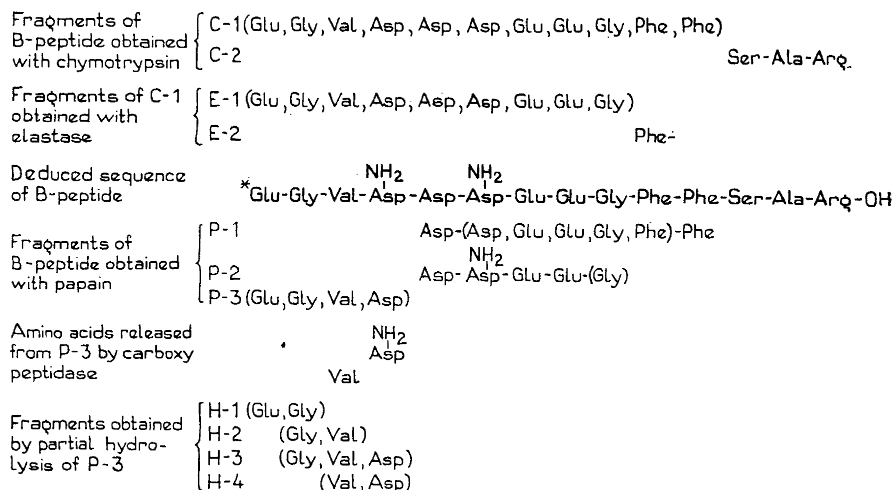

Fig. 1. Deduction of amino acid sequence in human fibrinopeptide B.

and heated for 20 min on the boiling water bath [10]. After evaporation of the HCl *in vacuo*, paper electrophoresis at pH 4.1 of the digest was performed. The bands obtained were eluted with water, hydrolysed in HCl and subjected to amino acid analysis by paper chromatography in phenol-borate buffer, pH 9.3. The amino acid compositions of some of the different fragments are listed in Fig. 1. From the results of the carboxypeptidase digestion and the partial hydrolysis the sequence for the N-terminal tetrapeptide seemed to be: Glu-Gly-Val-AspNH_2.

The amino group of the glutamic acid residue is, however, not free as it does not react with ninhydrin or phenylisothiocyanate. The possible occurrence of a N-acyl substituent was etherefor investigated. The occurrence of a high molecular weight N-acyl residue was, however, fairly improbable, since the quantitative amino acid analysis indicated that on a weight basis the amino acid residues accounted for 90 % of the weight of the peptide. On methanolysis [11] of peptide B (3 – 5 μ mole) no volatile methyl ester was obtained as would have been expected if the amino group had been blocked by, *e.g.*, formyl or acetyl residues. Furthermore, a spot test [12] for glycolic acid on the N-terminal fragment after elastase digestion of the B-peptide, gave negative result.

It was thought that hydrazinolysis of the N-terminal dipeptide would give further information about the N-terminal residue in peptide B. 3 mg of the tetrapeptide was treated for 24 – 48 h with carboxypeptidase and the digest subjected to electrophoresis in 0.1 M pyridine-acetate buffer, pH 4.1. Three bands were observed of which the two fast anodic were ninhydrin negative. The components were eluted from the paper. One band contained asparagine and valine and one undigested peptide. The fastest anodic component was a dipeptide which after acid hydrolysis gave glutamic acid and glycine. The dipeptide was then subjected to hydrazinolysis [13]. 0.2 – 0.4 mg of the dried dipeptide was dissolved in 100 μl of anhydrous hydrazine and heated for 10 – 26 h at 100°C. After evaporation of excess hydrazine over H_2SO_4 *in vacuo* the samples were dissolved in 30 – 50 μl of water. Glycine was the only free amino acid that could be demonstrated in the hydrazinolysate by paper chromatography [9] in phenol-borate buffer, pH 9.3. Thus in the dipeptide glycine seems to be C-terminal. Three spots were revealed by paper chromatography in pyridine-aniline-water (9:1:4) [13] or lutidine-water (5:3) [14] followed by staining of the chromatogram with ammoniacal $AgNO_3$. One of the spots had the same R_F value as pyroglutamic acid hydrazide whereas the others had lower R_F values. Also in paper electrophoresis at pH 4.1 three hydrazide spots were disclosed on staining with ammoniacal $AgNO_3$. One of the components had the same mobility as pyroglutamic acid hydrazide, whereas the others had a greater cathodic mobility. The slower of the latter was found to be a glutamic acid hydrazide; the fastest glycine hydrazide. The identification was based

on the fact that after acid hydrolysis glutamic acid and glycine, respectively, were revealed. No acetyl hydrazide could be demonstrated in the hydrazinolysates of the dipeptide either by chromatography or electrophoresis. As a control N-acetyl glutamic acid was subjected to hydrazinolysis. Acetyl hydrazide was disclosed in this sample both by chromatography and electrophoresis, but no pyroglutamic acid hydrazide. The results of the hydrazinolysis give further proof that the N-terminal glutamic acid residue is not N-acetylated.

Although the picture of the hydrazides in the hydrazinolysate of the dipeptide is complex the results do not exclude the possibility that a pyroglutamic acid residue might be N-terminal in human peptide B. Further work will clarify this point.

We are much indebted to Miss Jan Searle for skilful assistance, and to Miss M. Gilbo and Mr. A. Broad, Commonwealth Serum Laboratories, Melbourne, for the amino acid analyses. Pyroglutamic acid hydrazide and acetyl hydrazide were kindly supplied by K. Narita, Ozaka University.

1. Blombäck, B., Blombäck, M., Edman, P. and Hessel, B. Nature **193** (1962) 883.
2. Edman, P. *Acta Chem. Scand.* **4** (1950) 283.
3. Blombäck, B., Blombäck, M., Edman, P. and Hessel, B. (1963) *In preparation.*
4. Sanger, F. *Proc. Chem. Soc. (London)* March 1963, p. 76.
5. Blombäck, B., Blombäck, M. and Searle, J. *Biochim. Biophys. Acta* **74** (1963) 148.
6. Spackman, D. H., Stein, W. H. and Moore, S. *Anal. Chem.* **30** (1958) 1190.
7. Sjöquist, J., Blombäck, B. and Wallén, P. *Arkiv Kemi* **16** (1960) 425.
8. Harris, J. I. *Biochem. J.* **71** (1959) 451.
9. Levy, A. L. and Chung, D. *Anal. Chem.* **25** (1953) 396.
10. Naughton, M. A., Sanger, F., Hartley, B. S. and Shaw, D. C. *Biochem. J.* **77** (1960) 149.
11. Ludowieg, J. and Dorfman, A. *Biochim. Biophys. Acta* **38** (1960) 212.
12. Feigl, F. *Spot test in organic analysis,* Elsevier 1960, p. 377.
13. Akabori, S., Ohno, K. and Narita, K. *Bull. Chem. Soc. Japan* **25** (1952) 214.
14. Narita, K. and Ohta, Y. *Bull. Chem. Soc. Japan.* **32** (1959) 1023.

Received April 8, 1963.

The Fatty Acid Composition of Filbert Oil

AAGE JART

Organisk-kemisk Laboratorium, The Technical University, Copenhagen, Denmark

Filbert nuts are all of the species *Corylus avellana*. Most of the more recent investigations [1-5] indicate oleic acid to be the main constituent of the oil accompanied by smaller amounts of linoleic acid and saturated fatty acids. In contrast, however, Fang and Bullis [6] in 1949 reported a high content of eicosenoic acid (15–20 %), together with a small amount of dococenoic acid (0.4 %).

In the present investigation, filbert nuts grown in Hellerup, Denmark, in 1961 were used. The methyl ester fraction of the fatty acids was examined by gas-liquid chromatography. The calculated fatty acid composition (in weight per cent of the fatty acid mixture) was as follows:

	%
Palmitic acid	5.0
Palmitoleic acid	0.1
Stearic acid	1.4
Oleic acid	77
Linoleic acid	16.0
Linolenic acid	0.3
Arachidic acid	0.1
Eicosenoic acid	0.1

As apparent from these data the filbert oil is of the olive oil type. Obviously, eicosenic acid is only a trace constituent of the present filbert oil. Probably, the double bond is located at C–11, though the chromatographic data do not provide any rigorous proof. Acids with 22 carbon atoms are absent, or present in at most 0.05 %.

Although considerable variations may occur in the fatty acid composition of seeds within botanical varieties of the same species, it seems doubtful that *Corylus avellana* should contain such high quantities of eicosenoic acid as reported [6]. The American authors utilized a 48-inch glass helix packed column operated at a pressure of 2 mm for the ester fractionation. According to the experience of the present