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Short Communications

On the Amino Acid Sequence of Bovine Fibrinopeptides

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Fibrinopeptides are split off from the fibrinogen of various species during its conversion to fibrin. Summaries of the results achieved in this field have recently been given by Scheraga and Laskowski [1] and by Blombäck [2]. From ox fibrinogen, at least two peptides, tentatively named A and B, are split off. These peptides have been isolated by different methods, and chemical studies on their amino acid and N-terminal composition have been performed in our laboratories, as well as in others [3-7]. These studies confirm that glutamic acid is N-terminal in peptide A, whereas peptide B seems to lack any N-terminal amino acid residue. With carboxypeptidase, however, arginine can be split off as C-terminal from both peptides A and B [8,9]. The sequence of the three first amino acid residues from the N-terminal end of peptide A has recently been described by Blombäck and Sjöquist [2]. Sequence studies of bovine peptides A and B have later been reported by Folk *et al.*[9]

Digestion of peptides A and B with a variety of proteolytic enzymes, *i. e.*, papain, chymotrypsin, subtilisin and trypsin, released several fragments which were separated either by high-voltage electrophoresis, or on ion-exchange columns of 2 % cross-linked Dowex-50 [5]. The N-terminal amino acids were determined with the phenylthiocarbamyl method of Edman, as applied by Sjöquist [10]. The amino acids were determined quantitatively according to the method of Sjöquist [7,11,12].

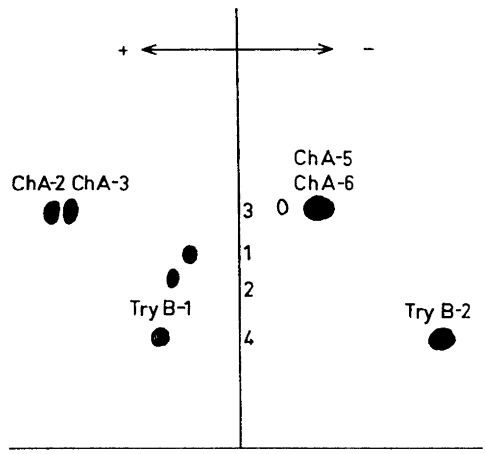

Fig. 1. Paper electrophoresis of peptides A and B after digestion with proteolytic enzymes. The experiments were carried out in 0.1 M pyridine adjusted to pH 4.1 with acetic acid. The potential gradient was approximately 20 V/cm. Each run lasted for about 3 hours.
1. Peptide B
2. Peptide A
3. Peptide A after digestion with chymotrypsin
4. Peptide B after digestion with trypsin

By means of chymotrypsin digestion, peptide A was split into four main fragments (Figs. 1 and 2). It can be seen from Table 1 that fragments ChA-2 and ChA-3 are similar in composition with respect to all amino acids except leucine (or *iso*leucine). Fragments ChA-5 and ChA-6 also differ only with regard to this particular amino acid. The latter peptides were not separated during electrophoresis at pH 4.1 in pyridine acetate buffer (Fig. 1). Separation could, however, be achieved by ion-exchange chromatography.

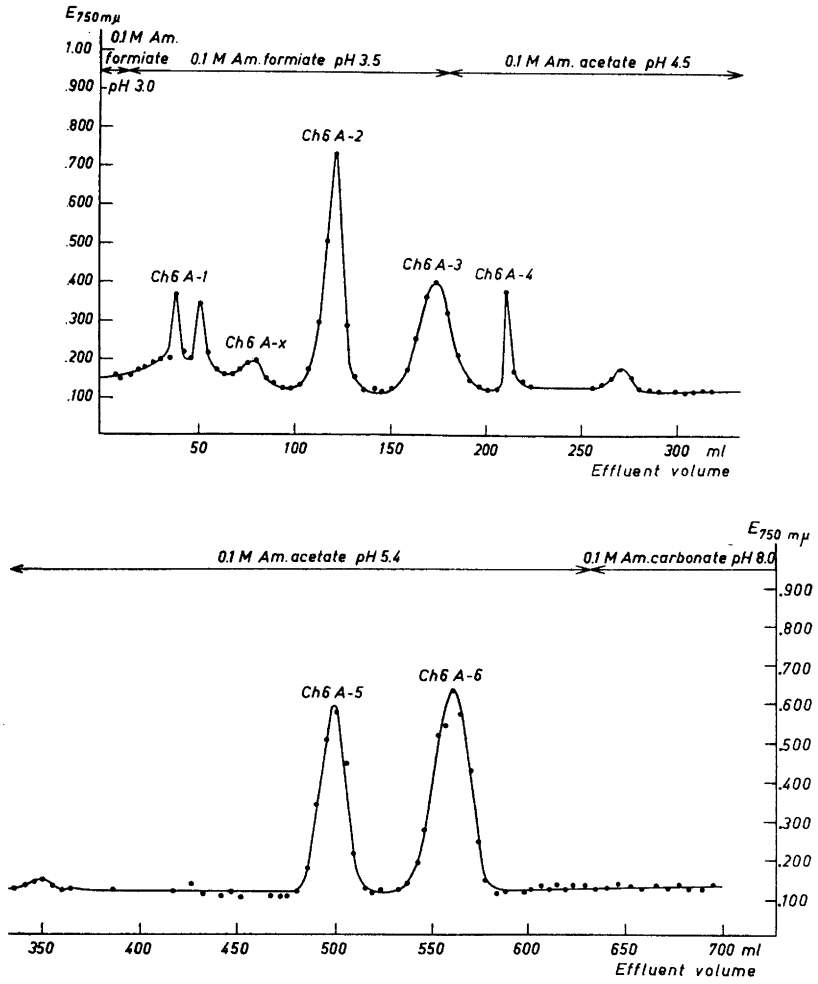

Fig. 2. Separation of peptides from a chymotryptic hydrolysate of peptide A by chromatography.

Assuming that peptide A consists of only one peptide chain, chymotrypsin had apparently split the chain on both sides of leucine (or *iso*leucine). The amino acid sequence determination of the fragments from the N-terminal end gave the following results: *ChA-2*: glu.asp.gly.ser.asp.asp., *ChA-3:* glu.asp.gly.ser.asp.asp., *ChA-5:* thr.glu.gly.gly.gly.val.arg., *ChA-6:* leu. (or ileu.) thr.glu.gly.gly.gly.val.arg. Since chymotrypsin has a specificity for peptide linkages near aromatic residues, the C-terminal residue in ChA-2 is most probably phenylalanine. An attempt was also made to investigate the C-terminal part of fragment ChA-2 and of the undigested A peptide, by means of the hydrazinolysis method of Akabori, as modified by Wallén and Sjöholm [14]. It was found that the C-terminal amino acid in fragment ChA-2 is phenylalanine, and that the residue next to the C-terminal amino acid is probably glycine. Furthermore, it was possible to confirm that the C-terminal sequence of the undigested A peptide is gly.val.arg. The amino acid

Table 1. Amino acid residues of peptide A and of peptides isolated from a chymotryptic digest of peptide A.

Amino acids	Number of residues						
	ChA-2	ChA-3	ChA-5	ChA-6	Peptide A	ChA-2+ ChA-6	ChA-3+ ChA-5
Arginine	0	0	1	1	1	1	1
Aspartic acid	3	3	0	0	3	3	3
Glycine	2	2	3	3	5	5	5
Glutamic acid	1	1	1	1	2	2	2
*Iso*leucine / Leucine	0	1	0	1	1	1	1
Phenylalanine	1	1	0	0	1	1	1
Proline	2	2	0	0	2	2	2
Serine	2	2	0	0	2	2	2
Threonine	0	0	1	1	1	1	1
Valine	0	0	1	1	1	1	1
Total amino acid residues	11	12	7	8	19	19	19

residues found in the fragments are in fairly good agreement with the residues found in the undigested peptide A (Table 1). From the N-terminal end, the following amino acid sequence was found in peptide A on direct determination: glu. asp.gly.ser.asp. However, when the sequence determination was performed on the undigested A peptide, an unidentified compound with the same R_F value as proline appeared in the second, third, fourth and fifth degradation steps. This compound is believed to be a side-reaction product formed during cyclization.

Digestion of peptide A with papain gave a fragment with the N-terminal sequence pro.pro.ser.gly. Thus, the studies so far seem to indicate that peptide A consists of a single peptide chain, with the following amino acid sequence: glu.asp.gly.ser.asp. asp.pro.pro.ser.gly.phe.leu.thr.glu.gly.gly. gly.val.arg.

Peptide B lacks an N-terminal residue which reacts with fluorodinitrobenzene, or which can form a phenylthiohydantoin derivative after coupling with phenyl*iso*thiocyanate. This indicates that at least this part of the molecule may be involved in a cyclic structure. Peptide B, however, has a C-terminal arginine residue as determined by means of carboxypeptidase or with the aforementioned hydrazinolysis method. This peptide has also been found to contain tyrosine-O-sulphate [5,13].

Peptide B could be split into two fragments by digestion with trypsin chymotrypsin or papain (Fig. 1). The different enzymes yielded different peptides, one of which was the main part of the original peptide, whereas the others contained fewer amino acid residues. The amino acid composition of the peptides (Try B-1 and Try B-2) obtained from a tryptic hydrolysate is shown in Table 2. The main fragment, Try B-1, containing tyrosine-O-sulphate, failed to give any N-

Table 2. Amino acid residues of peptide B and of peptides isolated from a tryptic hydrolysate of peptide B.

Amino acids	Number of residues			
	Try B-1	Try B-2	Peptide B	Try B-1+ Try B-2
Alanine	0	1	1	1
Arginine	1	1	2	2
Aspartic acid	4	0	4	4
Glutamic acid	3	0	3	3
Glycine	1	2	3	3
*Iso*leucine / Leucine	0	1	1	1
Lysine	1	0	1	1
Phenylalanine	1	0	1	1
Proline	2	0	2	2
Serine	0	0	0	0
Threonine	1	0	1	1
Tyrosine	1	0	1	1
Valine	0	1	1	1
Total amino acid residues	15	6	21	21

terminal residue. In the peptide Try B-2, valine is N-terminal. The amino acid sequence of this fragment is val.gly.leu. (or ileu.)gly.ala.arg. As is seen from Table 2, the residues found in the fragments are in fairly good conformity with the residues found in peptide B. In the preliminary report on the amino acid composition of peptide B (cf. Ref.[2]), threonine was stated to be absent. In subsequent analysis with a somewhat modified procedure [7], threonine was recovered from the hydrolysate of peptide B. This is in agreement with the original finding by Bettelheim [3].

For further elucidation of the structure of peptide B, digestion of this peptide by means of subtilisin has given promising results. After digestion with this enzyme, 6 or 7 small peptides were isolated by chromatography or by electrophoresis.

The complete paper on the amino acid sequence of bovine fibrinopeptides A and B will be published elsewhere.

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Synthesis of (—)-Methyl 2D, 4D,6D-Trimethylnonacosanoate and Identification of C_{32}-Mycocerosic Acid as a 2,4,6,8-Tetramethyloctacosanoic Acid

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A mass spectrometric study [1] of the methyl ester of mycocerosic acid isolated by Anderson and co-workers [2-4] from the lipids of tubercle bacilli, indicated a molecular weight of 494, corresponding to the methyl ester of a C_{32}-acid. The mass spectrum furthermore indicated the presence of methyl side chains at positions 2, 4, and 6. These methyl group positions were the same as deduced by Polgar [5] for the closely related or identical compound called mycoceranic acid [6,7]. The levo-rotation of mycoceranic acid and the optical rotations recorded by Polgar for the products obtained in the step-wise degradation suggested an all D optical configuration. For comparison we therefore synthesized (—)-methyl 2D,4D,6D-trimethylnonacosanoate. The synthesis was performed as follows.

n-Eicosylmalonic acid, m. p. 122.8—124.2°, was prepared in a yield of 90 % from ethyl *n*-docosanoate and ethyl oxalate [8]. The reaction of the acid chloride derived from (—)-6-methoxycarbonyl-3L, 5L-dimethylhexanoic acid [9] with the sodium derivative of di-(tetrahydropyranyl) *n*-eicosylmalonate [10] gave (+)-methyl 3D,5D-dimethyl-7-oxooctacosanoate, m. p. 42.8—43.6°, $[\alpha]_D^{25}$ +0.4° (c = 19.3) *,

* All optical rotations were measured in chloroform solution using a 1 dm tube.