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Amino Acid Sequence Studies on Fibrinopeptides from Several Species

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N-Terminal amino acid analyses of fibrinogen from several mammalian species indicate that the fibrinogen molecule is built up of several peptide chains [1-5]. A common feature in mammalian fibrinogens so far investigated is the occurrence of the same number of N-terminal tyrosyl residues per unit weight of protein. These tyrosyl residues seem to have replaced N-terminal seryl residues in certain lower vertebrates.[6] As regards the other N-terminal chains demonstrated in different fibrinogens, an extreme qualitative variability has been noted among the N-terminal amino acids from different species.[4-6] During the transformation in different species of fibrinogen to fibrin, produced by thrombin, peptides (fibrinopeptides) are split off from the N-terminal parts of the molecule.[7-14] As a result of this limited proteolysis, N-terminal glycine residues are exposed in the different fibrins.[1-6] The N-terminal tyrosyl chains are not involved in this proteolysis.

The amino acid sequences of the fibrinopeptides exhibit marked species specificity[11], although it has recently been shown[12,14] that the C-terminal regions of bovine and human fibrinopeptides show interesting similarities. Thus a C-terminal sequence of six amino acid residues is identical in human and bovine fibrinopeptide A. The present study was undertaken in order to determine the extent of structural differences among fibrinopeptides from a variety of mammals. Complete or partial amino acid sequences for fibrinopeptides from pig, sheep, goat, reindeer, and rabbit are contained in this report.

Fibrinogen was prepared[15] from pooled citrated plasma from a number of animals from each species. Clotting was effected in all cases with purified bovine thrombin (about 200 NIH units/mg), and the peptides released were isolated as described previously[10]. The amino acid compositions of the various peptides were mainly determined by column chromatography[16]. The amino acid composition of pig and rabbit fibrinopeptides have been reported earlier[11]. Amino acid sequences of whole peptides and peptide fragments were determined by the phenylisothiocyanate method of Edman[17] in its three-stage form[18], and the phenyl thiohydantions (PTH) identified by paper chromatography.[19,20]. In order to determine the complete amino acid sequence by the stepwise degradation method, 7—10 μ-moles of the peptides were used. When only small amounts of peptides were available the step-wise degradations were supplemented by enzymatic and chemical degradations followed by amino acid analyses of acid (HCl) hydro-

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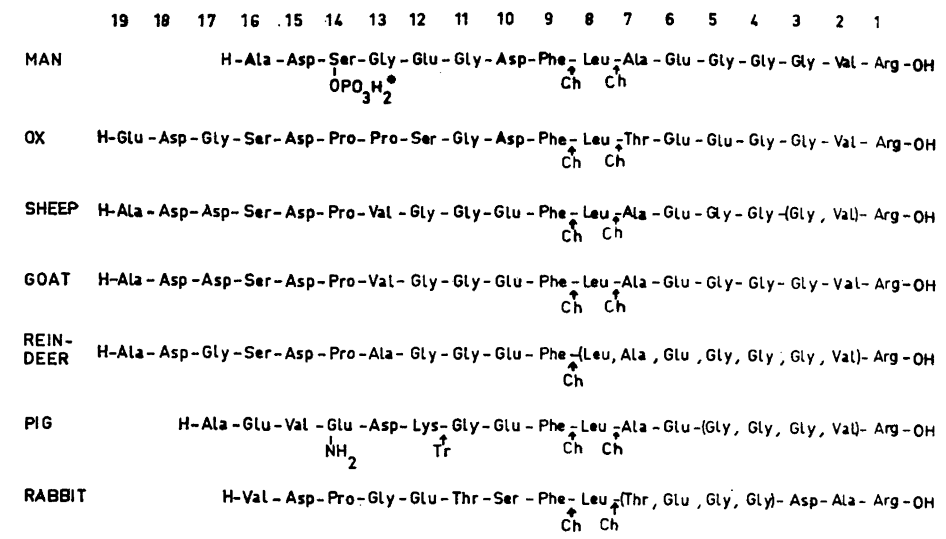

Fig. 1. Proposed amino acid sequences for seven mammalian fibrinopeptides A. The sequences of the human and ox peptides have been previously reported. [12,22] Partial sequences of the pig and rabbit peptides were also reported elsewhere,[11] in the latter report the glutamine at position 14 was reported as aspartic acid.

lysates in a two-dimensional paper electrophoresis-paper chromatographic system (0.1 M pyridine acetate, pH 4.1—butanol:acetic acid:water, 4:1:5). The presence of tyrosine-O-sulfate in certain peptides was established in the same system after alkaline hydrolysis in a sealed tube (0.2 M barium hydroxide, 100°, 20 h.[21]).

Postulated amino acid sequences or partial sequences are depicted in Fig. 1. As a comparison the earlier established sequences for ox [23,23] and human [12] fibrinopeptides have been included. Direct amino acid sequences of 16 residues in the sheep, 20 in the goat, 11 in the reindeer, 12 in the pig and 9 in the rabbit peptide A have hitherto been obtained. All peptides were also digested with crystalline chrymotrypsin. The enzyme splits mainly the Phe-Leu link. In the case of the pig A, trypsin splits the peptide at the Lys-Gly bond. The C-terminal sequence of the rabbit A-peptide was established by partial hydrolysis of the C-terminal chymotryptic fragment in 1 % acetic acid (100°, 20 h, pH 2.8). Only the bonds on either side of the aspartic acid residues were substantially split by this treatment. A dipeptide containing alanine and arginine was isolated from the hydrolysate. In view of the specificity of thrombin in other species this fragment is most probably C-terminal with the sequence Ala-Arg.

The sequences for fibrinopeptides B from pig, goat, sheep, and reindeer are listed in Fig. 2. As a comparison, the earlier established sequences for ox [23,24] and human [14], fibrinopeptides have been included. Direct amino acid sequences of 20 residues in the sheep, 6 in the goat, 19 in the pig and 8 in the reindeer have been obtained. Trypsin digests of pig peptide B yielded two components, due to the splitting of the Lys-Val linkage. The Arg-Pro bond was not susceptible to trypsin. Trypsin digestion of sheep, goat and reindeer B-peptides resulted in cleavage of both the Arg-Ala and Lys-Leu bonds. The C-terminal amino acid sequence in these peptides was deduced by partial hydrolysis of the C-terminal tryptic fragments in 1 % acetic acid as described above. Only the bonds on either side of the aspartic acid residue were detectably split by this treatment. From all species a dipeptide Ala-Arg, free aspartic acid and a tripeptide with varying amino acid composition, were recovered. The total amino acid composition of these fragments was consistent with that of the tryptic fragment of each species. In the case of the pig and the sheep, the sequence of the C-terminal region was afterwards confirmed by

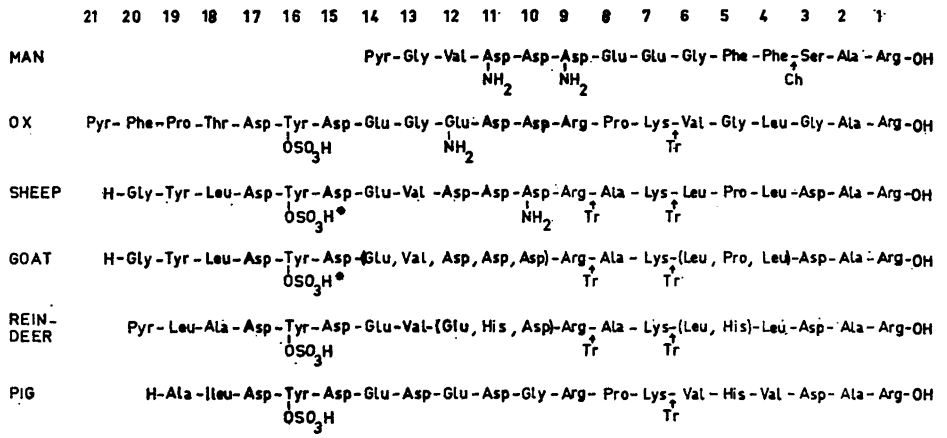

Fig. 2. Proposed amino acid sequences of six mammalian fibrinopeptides B. The sequences of the human and ox have been reported elsewhere, [14,23,24] as well as a part of the pig B[11]. Pyr = Pyrrolidone residue.

direct sequence with the Edman method. In the case of the reindeer, the tripeptide (His, Leu_2) was digested with carboxypeptidase A (Sigma), yielding a dipeptide containing leucine and histidine as well as free leucine. The most probable sequence of the dipeptide is Leu-His, since the peptide His-Leu would likely have been digested by the enzyme.

Each of the B-fibrinopeptides examined except the human contains one tyrosine-O-sulfate residue. The sheep and goat peptides contained one additional tyrosine residue. Attempts to cleave either of the bonds involving the tyrosyl residue with chymotrypsin failed. The position of the tyrosine-O-sulfate residue in the sheep and the goat can not be stated with certainty at present but regarding its position in the other species of the order Artiodactyla it is suggested that it is position 16 from the C-terminal end.

The N-terminal amino acid of the reindeer B-peptide was unaccessible to degradation by the Edman phenylisothiocyanate method. The possibility existed that the covered peptide ended in a pyrrolidone residue as has been suggested for the human [14], and bovine B-peptides [24]. As pyrrolidone rings in peptides have been shown to be split by mild alkaline hydrolysis, the peptide was digested with 1 N NaOH (20 h, room temp.) according to the method of Dekker *et al.* [25]. After the digestion, the peptide, which was apparently homogeneous on paper electrophoresis at pH 4.1, was subjected to step-wise degradation. Glutamic acid was found to be N-terminal and seven subsequent residues were established by step-wise degradation.

Two types of reindeer B-peptides have been isolated, B_1 and B_2. The B_1-peptide shown in Fig. 1 being the most frequent in the particular plasma pool used. The B_2-peptide, found only in one preparation, is more acidic, and preliminary amino acid analyses of tryptic fragments indicate that the histidine residue found in the N-terminal tryptic fragment of the B_1-peptide has been replaced by a glycine residue.

The amino acid sequences of the fibrinopeptides confirm the suggestion obtained from N-terminal analyses that a large degree of variability exists in the fibrinopeptides, and hence also in the fibrinogen molecules from various species. Certain areas of the peptides appear to be more susceptible to changes than others. The N-terminal regions of the A-peptides exhibit great variability. On the other hand, the C-terminal regions of the A-fibrinopeptides appear to be almost identical in several species.

In the B-peptides the variability is still more pronounced and it is only the C-terminal sequence Ala-Arg that is common to all species. Thus the C-terminal amino acid sequences common to the A peptides can not be the only structures directing thrombin action. It is, however, known that at least from bovine [9,10] and rabbit [26] fibrinogens, the B-peptide is released with a much lower initial rate than the corresponding A-peptide.

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Long-Chain Hydrocarbons in the Pollen of Rye

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The chemical composition of pollen grain has been subject to extensive investigations. Several amino acids, carbohydrates, lipids, vitamins, enzymes and even hormones and growth substances have been reported (*cf.* Ref. [1]). From the lipid fraction saturated hydrocarbons, higher alcohols, sterols, fatty acids and trace amounts of some ketone-containing components have been isolated [2-6].

Several hydrocarbons have been reported: a saturated hydrocarbon (probably nonacosane) in the pollen of "white flint corn" [2], a high content of heptacosane in the pollen of sugar beet [3], tricosane in the pollen of hazel (*Corylus avellana*) [4-5]. More recently Nilsson, Ryhage and von Sydow [6] investigated hydrocarbon mixtures from pollen of maize and alder (*Alnus glutinosa*). Their mass spectrometric analysis of the mixtures showed that maize pollen contained only pentacosane and heptacosane while alder contained heptacosane, nonacosane and traces of tricosane and pentacosane. Small peaks, in the C_{29} and C_{31} regions of the spectrum of maize pollen, were not believed to originate from saturated hydrocarbons or alcohols but possibly from unsaturated hydrocarbons.

The present study was devoted to hydrocarbons isolated from pollen of rye (*Secale cereale L.*). The chloroform-methanol extractable material amounted to 10.6 %