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Studies on Fibrinopeptides from Mammals

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As has been shown in previous reports amino acid sequence studies of fibrinopeptides, released from fibrinogen by the action of thrombin, can give information on the specificity of thrombin action and on the phylogeny of the fibrinogen molecule.¹⁻⁹ Until now 22 animal species belonging to different orders have been investigated. Isolation of fibrinopeptides was made as described previously.^{10,3} Amino acid sequence analysis was done according to Edman.^{11-13, cf. 3}

Two main types of fibrinopeptides, A and B, are usually found. However, analogs of both A and B peptides can be isolated in smaller amounts. These analogs will be described and discussed in forthcoming papers. In Figs. 1 and 2 the amino acid sequences of fibrinopeptides A and B are shown.

Conclusions: Taken together with the results obtained in primates¹⁴ positions 1, 5, and 9 in the A-peptides have been stationary during mammalian evolution. This indicates that these amino acid residues might be of importance for directing thrombin action. Substantial support to this view comes from the recent

finding that only one of the four trypsin susceptible arginyl bonds in the gastrointestinal hormone secretin could be split by thrombin.¹⁵ At position 9 from this arginine residue, was also in secretin a phenylalanine residue located. Position 5 from the arginyl bond was occupied by leucine. It is believed that the narrow specificity of thrombin on fibrinogen is at least partially explained by the location of a phenylalanine residue in a certain space relationship to the thrombin susceptible arginyl bond.

In peptide B only the arginine residue in position 1 is common to all species. This peptide is, at least in all species investigated, cleaved off at a slower rate^{16,10,17, cf. 9} and is probably a result of secondary splitting by thrombin.

It is evident from the results that the data can be used for taxonomy of species and for obtaining phylogenetic data. These points will be discussed in detail in a complete forthcoming paper.

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FIBRINOPEPTIDE A

19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	
H-GLU-ASP-GLY-SER-ASP-PRO-PRO-SER-GLY-ASP-PHE-LEU-THR-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Ox
H-GLU-ASP-GLY-SER-ASP-PRO-ALA(SER)-GLY-ASP-PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Bison
H-ALA-ASP-GLY-SER-ASP-PRO-ALA-SER-SER-ASP-PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Red deer (C. elaphus)
H-ALA-ASP-GLY-SER-ASP-PRO-ALA-GLY-GLY-GLU-PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Reindeer
H-ALA-ASP-ASP-SER-ASP-PRO-VAL-GLY-GLY-GLU-PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Sheep, Goat
H-ALA-GLU-VAL-GLN-ASP-LYS-GLY-GLU-PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Pig
H-THR-ASP-PRO-ASP-ALA-ASP-LYS-GLY-GLU-PHE-LEU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Lama
H-THR-GLU-GLU-GLY-GLU-PHE-LEU-HIS-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Horse
H-THR-LYS-THR-GLU-GLU-GLY-GLU-PHE-ILU-SER-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Donkey
H-THR-ASN-SER-LYS-GLU-GLY-GLU-PHE-ILU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Dog, Fox
H-GLY-ASP-VAL-GLN-GLU-GLY-GLU-PHE-ILU-ALA-GLU-GLY-GLY-GLY-VAL-ARG-OH																			Cat
H-THR-ASP-VAL-LYS-GLU-SER-GLU-PHE-ILU-ALA-GLU-GLY-ALA-VAL(GLY-ARG)-OH																			Badger
H-THR-ASN-VAL-LYS-GLU-SER-GLU-PHE-ILU-ALA-GLU-GLY-ALA(ALA-GLY-ARG)-OH																			Mink
H-ALA-ASP-THR-GLY-THR-THR-SER-GLU-PHE-ILU(ASP, GLU, GLY, ALA, GLY, ILU, ARG)-OH																			Rat
H-THR-ASP-THR-GLU-PHE-GLU-ALA-ALA-GLY-GLY-GLY-VAL-ARG-OH																			Guinea pig
H-VAL-ASP-PRO-GLY-GLU-SER-THR-PHE-ILU-ASP-GLU-GLY-ALA-THR-GLY-ARG-OH																			Rabbit

Fig. 1. Amino acid sequence of fibrinopeptide A. The dog A peptide is partially recovered with a phosphorylated serine residue.¹⁸ The sequence of dog fibrinopeptide A deviates from that found by other investigators.¹⁸ Rabbit fibrinopeptide A deviates from that previously deduced^{5, 6, cf. 7} Complete sequences of ox,^{1, 19} reindeer, sheep, goat, pig, horse, donkey, dog A peptides have earlier been reported.⁵⁻⁷

FIBRINOPEPTIDE B

21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	
GLU-PHE-PRO-THR-ASP-TYR-ASP-GLU-GLY-GLN-ASP-ASP-ARG-PRO-LYS-VAL-GLY-LEU-GLY-ALA-ARG-OH																					Ox
(GLU, PHE, PRO, THR, ASP, TYR, ASP, GLU, GLY, GLU, ASP, ARG, PRO, LYS)-VAL-GLY-LEU-GLY-ALA-ARG-OH																					Bison
HIS-GLU-LEU-ALA-ASP-TYR-ASP-GLU-VAL-GLU-ASP-ASP-ARG-ALA-LYS(LEU, HIS)-LEU-ASP-ALA-ARG-OH																					Reindeer
H-GLY-TYR-LEU-ASP-TYR-ASP-GLU-VAL-ASP-ASP-ASN-ARG-ALA-LYS-LEU-PRO-LEU-ASP-ALA-ARG-OH																					Sheep, Goat
H-ALA-ILU-ASP-TYR-ASP-GLU-GLU-GLU-ASP-GLY-ARG-PRO-LYS-VAL-HIS-VAL-ASP-ALA-ARG-OH																					Pig
H-ALA-THR-ASP-TYR-ASP-GLU-GLU-GLU-ASP-ASP-ARG-VAL(LYS, VAL, ARG, LEU, ASP, ALA, ARG)-OH																					Lama
H-LEU-ASP-TYR-ASP-HIS-GLU-GLU-GLU-ASP-GLY-ARG-THR-LYS-VAL-THR-PHE-ASP-ALA-ARG-OH																					Horse
H-HIS-TYR-TYR-ASP-ASP-THR-ASP-GLU-GLU-ARG-ILU-VAL-SER-THR-VAL-ASP-ALA-ARG-OH																					Dog
(GLU, TYR, TYR, ASP, ASP, THR, ASP, GLU, GLU, ARG, ILU, VAL, SER, THR, VAL, ASP, ALA, ARG)-OH																					Fox
H-ILU-ILU-ASP-TYR-TYR-ASP-GLU-GLY-GLU-GLU-ASP-ASP-VAL-GLY-VAL-VAL-ASP(ALA, ARG)-OH																					Cat
H-ALA-THR-THR-ASP-SER-ASP-LYS-VAL-ASP(ILU, SER, LEU, ALA, ARG)-OH																					Rat
H-ALA-ASP-ASP-TYR-ASP-ASP-GLU-VAL-LEU-PRO-ASP-ALA-ARG-OH																					Rabbit

Fig. 2. Amino acid sequence of fibrinopeptide B. [GLU indicates pyroglutamyl residue.^{20, 21} Many of the tyrosine residues are present as tyrosine-O-sulfate residues.^{22, 10, 23, 24, 5} Sequence of reindeer B-peptide deviates from that previously suggested.⁵⁻⁸ Final sequence will await further investigation. N-terminal of reindeer B peptide is blocked. Complete sequences of ox,^{1, 21} sheep, goat and pig B peptides have earlier been reported.^{5, 6} In case of peptides with blocked N-terminal residue the amino acid sequences were deduced after analyses of fragments obtained with a variety of reagents including proteolytic enzymes.^{1, 3, 20, 5, 21}

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Gel Filtration with Buffers Containing Dextran

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It has been shown,^{1,2} that the solubility of a number of proteins decreases in dextran solutions ($M_w > 150\,000$). This decrease is dependent on the molecular size of the protein and the concentration of dextran. It was explained as a steric exclusion of the proteins from the polysaccharide solution.

By gel filtration³ substances are separated according to differences in molecular size. The gel can be regarded as a three-dimensional network of straight polymer fibres and the gel filtration process can be explained as a steric exclusion of solutes from this network.⁴ Laurent and Killander showed that there is good agreement between the available volume for certain proteins in a dextran solution and a dextran gel of the same concentrations.⁴ They concluded that the cross-linking in the gel does not have any essential influence on the exclusion property.

If a polymer is added to the buffer used in gel filtration experiments, it should have exclusion properties similar to the polymer gel. The available volume for a substance in the buffer phase should decrease as a result of the exclusion by the polymer solution and the partition coefficient between the gel and liquid phase should

increase. Thus the elution volume for a substance should change.

The purpose of this investigation was to examine the effect on the elution volume of a protein in gel filtration when the buffer solution contains a polymer.

The chromatographic tube was made of plexiglass and had adjustable plungers with porous membranes of polyethylene in the ends. After the tube had been packed with Sephadex G-200 (Pharmacia, Uppsala, Sweden) the plungers were adjusted to the size of the column (2×108 cm). A peristaltic pump was connected to the lower end of the tube giving a reverse flow. The transmission of the eluate at $254\text{ m}\mu$ was continuously registered in a 0.3 cm cuvette with an Uvicord absorptiometer (LKB, Stockholm, Sweden) connected to a recording potentiometer. The flow rate was approximately $3\text{ ml/cm}^2/\text{h}$ with 0.05 M phosphate buffer, pH 7.4, containing 0.1 M sodium chloride. To the buffer was added Dextran 150 (weight average molecular weight $153\,000$, Pharmacia, Uppsala, Sweden) to the final concentrations of 1 or 2% (w/v). The samples were applied in 1.0 ml volume. After each sample a small volume of 5% (w/v) sucrose in the corresponding buffer-dextran solution followed. The eluate was collected in small test tubes with a fraction collector. The content in the tubes was weighed and the volume was calculated. As partial specific volume for dextran the value 0.61 was used.⁵ The optical density at $280\text{ m}\mu$ in a 1.0 cuvette was measured with a Beckman DU spectrophotometer.

The void volume of the column was determined with 2.5 mg Blue Dextran $2,000$ (average molecular weight $2\,000\,000$, Pharmacia, Uppsala, Sweden). It was redetermined for each concentration of dextran used in the eluent. As test substance human serum albumin (AB Kabi, Stockholm, Sweden) was chosen. This albumin (55 mg) was separated into four fractions. The first three consisted of the polymer, dimer, and monomer fraction of albumin.⁶ The fourth fraction was of uncertain origin. Probably it consisted of a bacteriostatic agent added to the protein by the manufacturer. It was shown that this fraction and tritiated water emerge at the same place in the elution diagram. The elution volume of the fourth fraction was considered to correspond to the total volume of the column, which was in good agreement with direct measurements on the tube after the gel had been removed.